6-(SUBSTITUTED METHYLENE)PENEMS, POTENT BROAD SPECTRUM INHIBITORS OF BACTERIAL β-LACTAMASE

V. CHIRAL 1,2,3-TRIAZOLYL DERIVATIVES

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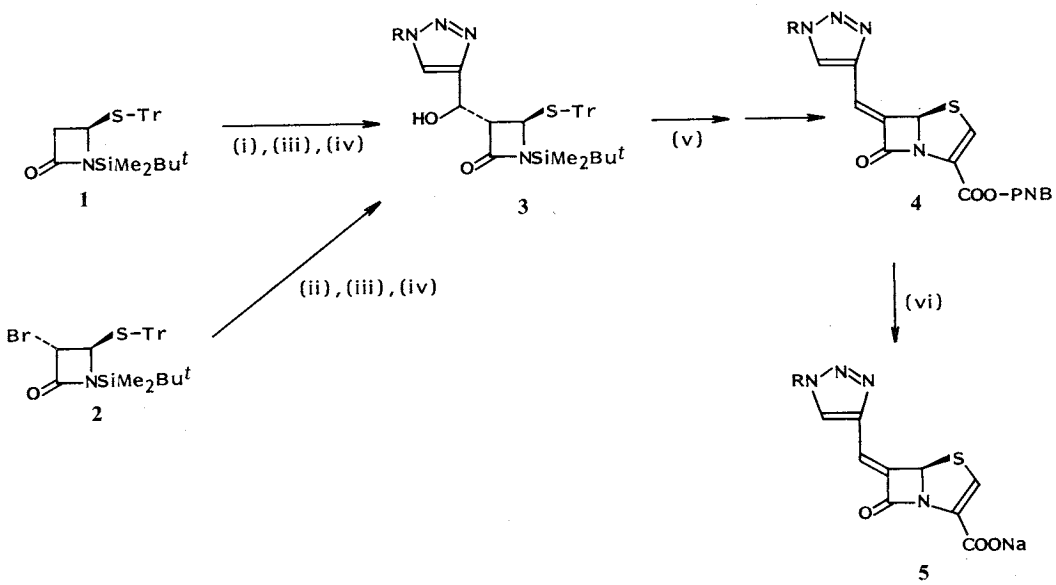
Structure-activity relationships in a series of (5*R*)-6-triazolylmethylene penems with potent β-lactamase inhibitory activity are described. In most cases, their *in vitro* synergistic activity with amoxycillin is superior to that of clavulanic acid, sulbactam and tazobactam (YTR 830). Against an *Escherichia coli* TEM-1 infection in mice, the compounds showed a broad range of potencies; an optimum polarity was found, however, which gave maximum potency.

Earlier papers in this series[1~4] have described the synthesis and biological properties of a series of racemic 6-(substituted methylene)penems. Of particular interest was the triazolylmethylene penem (**5b**), and this paper outlines some further work on a series of triazolyl derivatives with a chiral centre at C-5.

Chemistry

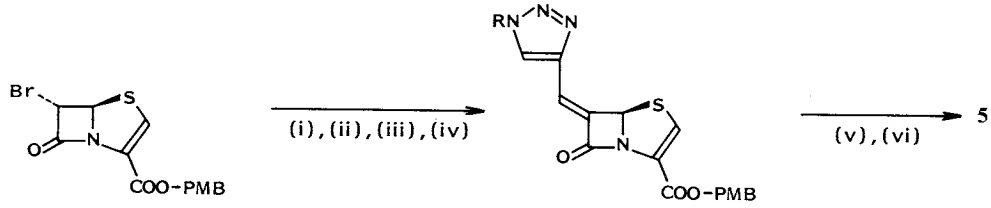
The (5*R*,6*Z*)penems (**5**) were prepared using two routes. Route A (Scheme 1) has been described for the preparation of **5b**[5] and is shown schematically in full elsewhere[6]: it requires a 1,2,3-triazolecarboxylic

Scheme 1.



Reagents: (i) lithium diisopropylamide, THF, −70°C; (ii) *n*-BuLi, THF, −70°C; (iii) **8**; (iv) $NaBH_4$, THF, EtOH; (v) see ref 6; (vi) 5% Pd-C, H_2, aq dioxan, $NaHCO_3$. Tr=triphenylmethyl.

Scheme 2.



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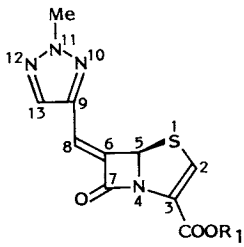
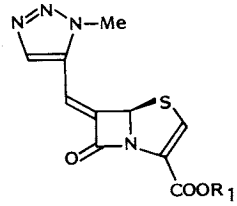
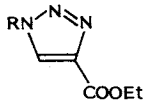
Reagents: (i) Ph_2NLi, THF, $-70°C$; (ii) **9**; (iii) Ac_2O; (iv) Zn, NH_4Cl, $(CH_3)_2NCH_2CH_2N(CH_3)_2$-2HCl, DMF; (v) $AlCl_3$, anisole, CH_2Cl_2, $-40°C$; (vi) aq Na_2HPO_4.



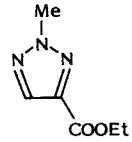
4c R_1 = PNB
5c R_1 = Na



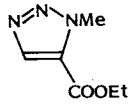
4d R_1 = PNB
5d R_1 = Na



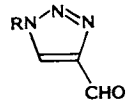
8a, 8b, 8e~8x



8c



8d



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ester (**8**) and either of the chiral azetidinones (**1** or **2**). Route B (Scheme 2) has also been described for the preparation of **5b**[6~9]; it requires a 1,2,3-triazolecarboxaldehyde (**9**) and the (6*S*)-bromopenem (**6**)[9].

The triazole esters (**8b~8e**) were prepared by alkylation of **8a**. The methyl group positions in **8b~8d** were determined as follows: **8b** was identified by comparison with an authentic sample[5]; **8c** and **8d** were progressed by route A to penems (**4c** and **4d**) and the structures identified by NOE studies. In **4d**, a strong NOE between 10-*N*-methyl and 8-CH was observed, but in **4c** no NOE to 11-*N*-methyl

Table 1. Structures of triazole esters, penems and intermediates.

R	R
a H	**n** $CH_2COOPNB$
b CH_3	**p** CH_2COONa
e DMT	**q** $N(CH_3)_2$
f Et	**r** OCH_3
g Allyl	**s** OH
h *n*-Pr	**t** OPMB
j Cyclopropyl	**u** CH_2CH_2OH
k CH_2CF_3	**v** $CH_2CH_2OSi(CH_3)_2Bu^t$
l CH_2COOCH_2Ph	**w** $(CH_2)_3OH$
m $CH_2COOSiPh_2Bu^t$	**x** $(CH_2)_3OSi(CH_3)_2Bu^t$

This table refers to compounds **3**, **4**, **5**, **7**, **8** and **9** (**a**, **b** and **e~x**). For **c** and **d**, see separate structures. The DMT group in **e** is written in the N-12 position for convenience, although its position is unknown.

Table 2. Summary of biological activity of three isomeric *N*-methyl triazolylmethylene penem derivatives.

Class	I_{50} (μg/ml)					Amoxycillin MIC (μg/ml) in the presence of 1 μg/ml of inhibitor					Relative potency *in vivo*
	E.cl. Ia	*P.m.* II	*E.co.* TEM-1 III	*K.p.* IV	*E.co.* OXA-1 V	*E.cl.* Ia	*P.m.* II	*E.co.* TEM-1 III	*K.p.* IV	*E.co.* OXA-1 V	*E.co.* TEM-1
5b	0.001	0.007	0.001	0.001	0.001	1	2	2	2	2	1.00
5c	0.019	0.010	0.002	0.003	0.002	64	1	2	4	2	<0.05
5d	0.055	3.500	0.017	0.032	0.002	128	>512	64	64	8	<0.05
Clavulanic acid	>50.0	0.020	0.036	0.019	0.780	>512	16	8	4	>512	0.20
Sulbactam	2.8	0.08	1.9	10.0	3.2	256	64	128	64	>512	<0.05
Tazobactam	0.02	0.02	0.02	0.1	1.15	256	16	8	16	>512	0.20
Amoxycillin alone						512	>512	>512	256	>512	<0.05

Class: Enzyme classification based on RICHMOND and SYKES[18].

Abbreviations: *E.cl.*, *Enterobacter cloacae*; *P.m.*, *Proteus mirabilis*; *E.co.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*.

could be seen (for numbering, see structure **4c**). Triazole esters (**8f**, **8g**, **8j**~**8l**, **8q**~**8s**, **8u** and **8w**) were prepared by an extension of the method of STOJANOVIC and ARNOLD[10], by which ethyl α-formyldiazoacetate was condensed with amines, 1,1-dimethyl hydrazine and oxyamines. Hydroxy derivatives (**8s**, **8u** and **8w**) required protection as **8t**, **8v** and **8x**. Triazole (**8l**) reacted with the anion from **1** mainly at the benzyl ester; it was therefore converted into **8m**.

Triazole aldehydes (**9t**, **9v** and **9x**) were obtained from **8t**, **8v** and **8x** by a standard reduction-oxidation sequence. Penem esters (**4e**, and **7v** and **7x**) required deprotection to **4a**, and **7u** and **7w** before de-esterification. In the cases of **4n** and **7t** the de-esterification conditions served to remove both protecting groups. De-esterification of **4g** also resulted in alkyl group hydrogenation to give **5h**.

Biology

Table 2 shows the effects on biological activity of altering the position of the methyl group on the triazole ring. An N-10† methyl substituent (**5d**) resulted in a marked loss of synergistic activity with amoxycillin, as expected from the previously established structure-activity relationships in the 5-membered heterocyclic series[3], and also resulted in poor *in vivo* activity relative to the *N*-12-methylated derivative (**5b**). The *N*-11-methylated derivative (**5c**) showed reduced synergistic activity against the Class 1 β-lactamase of *Enterobacter aerogenes*, but synergistic activity against all other β-lactamases was similar to that seen with **5b**. The good *in vitro* activity of **5c** against the TEM-1 enzyme was not evident *in vivo*, however.

Thus, the *N*-12-methyl derivative (**5b**) was clearly the most active of the three, and a number of compounds were synthesised which contained other small alkyl groups at the *N*-12 position (Table 3). There was very little difference in the synergistic activity with amoxycillin shown by these compounds, and all twelve proved better broad spectrum synergists than clavulanic acid, sulbactam or tazobactam (YTR 830). This increased potency was most noticeable against organisms producing the Class 1 or OXA-1 enzyme. All derivatives, except perhaps the parent (**5a**), were reasonably stable to human kidney homogenate, and none showed excessively high binding to human serum. When tested *in vivo* against an *Escherichia coli* TEM-1 infection in mice, however, these compounds revealed a very broad range of

† For numbering, see structure **4c**.

Table 3. Biological activity of N-12-substituted triazolylmethylene penems.

Class	Amoxycillin MIC (μg/ml) in the presence of 1 μg/ml of inhibitor					Human serum binding (%)	Human kidney stability (%)	Relative potency *in vivo*
	E.cl. Ia	*P.m.* II	*E.co.* TEM-1 III	*K.p.* IV	*E.co.* OXA-1 V			
5a	2	16	2	2	8	79	45	0.64
5b	2	4	1	2	4	72	61	1.00
5f	2	4	2	2	4	72	72	0.28
5h	32	2	2	4	8	81	67	0.04
5j	4	2	4	4	8	80	82	0.17
5k	4	64	8	2	4	78	67	0.14
5p	32	8	4	2	8	87	72	0.43
5q	2	4	8	8	4	73	69	0.13
5r	4	2	4	2	2	74	56	0.43
5s	4	16	8	8	16	72	90	<0.03
5u	4	4	2	2	4	56	69	1.00
5w	16	4	4	2	4	59	74	0.50
Clavulanic acid	>512	16	8	4	>512	20	100	0.20
Sulbactam	256	64	128	64	>512		100	<0.03
Tazobactam	256	16	8	16	>512	20	NT	0.20
None	512	>512	>512	256	>512	17	100	<0.03

Class: Enzyme classification according to RICHMOND and SYKES[18].
NT: Not tested.
Abbreviations: See footnote in Table 2.

potencies with only 7 out of 12 showing a greater potency than clavulanic acid, and only one compound (**5u**) showing potency equal to that of **5b**. The log k′ parameter[11] can be used as a measure of the polarity of these derivatives, and a plot of log k′ against log relative potency gave a curve (Fig. 1) which could best be described using the Bilinear model of KUBINYI[12]. The equation for this curve was found to be:

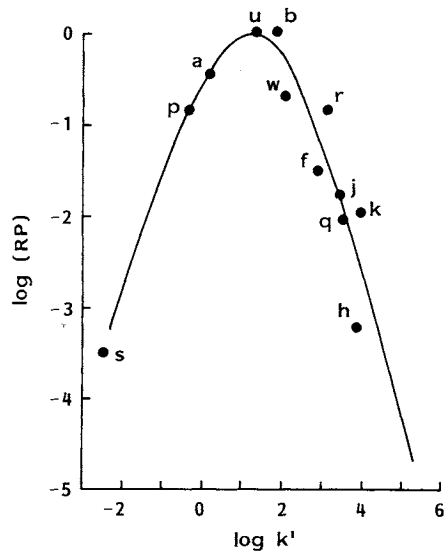
log relative potency

$$= 1.52 \log k' - 3.29 \log(0.29k' + 1) + 0.34$$

(multiple r-square

$$= 0.895; \text{ std. dev. of regression} = 0.44)$$

Fig. 1. Correlation between polarity (log k′) and relative potency.



The *N*-12-methyltriazolyl derivative (**5b**) is one of two compounds falling within the area of maximal potency on the curve, and the biological activity of this derivative is described in more detail in a separate publication[13].

Experimental

β-Lactamase inhibition studies were carried out on isolated enzyme preparations as previously

described[14].

MICs were determined in microtitre plates by serial dilution of amoxycillin in broth, followed by addition of inhibitor (1 μg/ml) and organism (approx 2×10^6 cfu/ml) as previously described[14].

Serum binding was carried out using the method previously described[4].

Stability to human kidney homogenate was determined at 37°C as described previously[4]. Since compounds were chiral, treatment with *Bacillus cereus* II enzyme was not necessary.

The 50% curative dose (CD_{50}) determinations were performed in mice. The organism (*E. coli* E96) was suspended in 3% hog gastric mucin +1% carboxymethylcellulose at $100 \times LD_{50}$, and 0.5 ml of suspension was injected ip into groups of five mice. Compounds were administered subcutaneously at 2 mg/kg with varying doses of amoxycillin at 1 and 5 hours post-infection. Survivors were recorded over a 4-day period. The CD_{50} of amoxycillin in the presence of inhibitor was calculated using log probit analysis. Compound **5b** was used as control in every experiment, and the data were normalised by conversion to relative potencies thus:

$$CD_{50} \text{ of compound } \mathbf{5b}/CD_{50} \text{ of test compound.}$$

Log k' was calculated from the reverse phase HPLC Rt's using the method of MIYAKE *et al.*[11].

All compounds were chromatographically pure as shown by TLC on Merck Silica gel 60 F_{254} plates. Chromatography of intermediates was carried out using Merck Silica gel 60, eluting with EtOAc-hexane mixtures, and of sodium salts using either Bio-Gel P-2 (route A) or Diaion HP-20SS (route B). HPLC was carried out using Beckman equipment with an Ultrasphere ODS column, eluting with MeOH-pH 7.4 phosphate buffer. Instrumentation for IR, NMR, UV and mass spectra and mp's is as described in previous papers of this series[1~4]. Optical rotations of all chiral compounds were measured on a Perkin-Elmer 141 polarimeter. All new compounds gave satisfactory IR, NMR, MS and/or microanalysis, and UV where applicable.

Azetidinone (**1**) ($[\alpha]_D^{20}$ −55°C (*c* 1, $CHCl_3$)) was prepared from (4*R*)-4-tritylthioazetidinone[15] using a silylation procedure described for the corresponding racemic compound[16]. Bromoazetidinone (**2**) has been described[5]. Triazole (**8a**) was prepared by the method of KLEIN *et al.*[17].

Full experimental details are in the patent literature for both routes A[5] and B[9]. A minor modification to route A was that KF-MeOH treatment of **3m** caused double desilylation, but re-esterification with NaH-PNB bromide and continuation of the synthesis produced (**4n**). In route B some (*E*)-isomer was produced along with **7**; this was removed either chromatographically or by crystallisation of **7**. Geometries were assigned as already described[7].

Ethyl *N*-Methyl-1,2,3-triazole Carboxylates (**8b**~**8d**)

Triazole (**8a**) (10 g, 71 mmol) in DMF (120 ml) was ice-cooled and treated with K_2CO_3 (6 g, 43.5 mmol) and MeI (4.67 ml, 75 mmol). After stirring 24 hours at room temperature, the DMF was evaporated and the residue taken up in EtOAc-water. The EtOAc was further washed with $Na_2S_2O_3$ soln and water, dried ($MgSO_4$) and evaporated. Chromatography separated the three isomeric *N*-methyl compounds: **8b** (1.99 g, 18%, Rf 0.15 in EtOAc-hexane, 2:3), **8c** (4 g, 36%, Rf 0.76) and **8d** (3.2 g, 29%, Rf 0.66) were identified as described in the chemistry section.

For **8b**: ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, CH_2CH_3), 4.20 (3H, s, N–CH_3), 4.44 (2H, q, CH_2CH_3), 8.19 (1H, s, NCH=).

For **8c**: ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, CH_2CH_3), 4.30 (3H, s, N–CH_3), 4.45 (2H, q, CH_2CH_3), 8.09 (1H, s, NCH=).

For **8d**: ^{1}H NMR ($CDCl_3$) δ 1.41 (3H, t, CH_2CH_3), 4.36 (3H, s, N–CH_3), 4.45 (2H, q, CH_2CH_3), 8.16 (1H, s, NCH=).

Ethyl *N*-(4,4'-Dimethoxytrityl)-1,2,3-triazole-4-carboxylate (**8e**)

Triazole (**8a**) (7.05 g, 50 mmol) in THF (200 ml) was ice-cooled and treated with Et_3N (6.95 ml, 50 mmol), 4-dimethylaminopyridine (200 mg) and 4,4'-dimethoxytrityl (DMT) chloride (17 g, 50 mmol). After stirring at room temperature for 1 hour, the mixture was diluted with EtOAc, washed with 0.5 N HCl and brine, dried and evaporated. Chromatography provided the major isomer, of unknown regiochemistry (15.9 g, 72%), as microcrystals (EtOAc-hexane): MP 123~125°C. ^{1}H NMR ($CDCl_3$) δ 1.35

(3H, t, J=11 Hz, CH_2CH_3), 3.79 (6H, s, 2×O–CH_3), 4.40 (2H, q, J=11 Hz, CH_2CH_3), 6.7~7.5 (13H, m, Ar-H), 8.17 (1H, s, –NCH=).

Anal Calcd for $C_{26}H_{25}N_3O_4$: C 70.4, H 5.6, N 9.5.
Found: C 70.1, H 5.8, N 9.3.

Protection of Triazoles (**8s**, **8u** and **8w**)

Ethyl 1-*p*-Methoxybenzyloxy-1,2,3-triazole-4-carboxylate (**8t**)

Triazole (**8s**) (6 g, 38.2 mmol) in DMF (40 ml) was treated with diazabicycloundecene (DBU) (7.45 ml, 50 mmol) and a solution of *p*-methoxybenzyl (PMB) bromide (prepared from PBr_3 and PMB alcohol 50 mmol and used crude) in DMF (10 ml). After 1 hour the soln was diluted with EtOAc, washed with 0.2 N HCl and water, dried and evaporated. Chromatography and crystallisation (EtOAc-hexane) gave colourless plates (9.5 g, 90%): MP 102~103°C. ^{1}H NMR ($CDCl_3$) δ 1.38 (3H, t, J=11 Hz, CH_2CH_3), 3.85 (3H, s, OCH_3), 4.44 (2H, q, J=11 Hz, CH_2CH_3), 5.51 (2H, s, OCH_2Ar), 6.99 (2H, d, J=7 Hz, Ar-H), 7.39 (2H, d, J=7 Hz, Ar-H), 7.84 (1H, s, –NCH=).

Anal Calcd for $C_{13}H_{15}N_3O_4$: C 56.3, H 5.4, N 15.2.
Found: C 56.4, H 5.7, N 15.5.

Ethyl 1-(2-*tert*-Butyldimethylsilyloxyethyl)-1,2,3-triazole-4-carboxylate (**8v**)

Triazole (**8u**) (23.9 g, 129 mmol) in DMF (250 ml) was treated with Et_3N (36.1 ml, 260 mmol), 4-dimethylaminopyridine (2 g) and *tert*-butyldimethylsilyl chloride (37.6 g, 260 mmol). After 3 hours the mixture was diluted with EtOAc, washed with 0.2 N HCl and water, dried and evaporated. Crystallisation (EtOAc-hexane) gave colourless plates (31 g, 80%): MP 51~52°C. ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, s, *tert*-Bu), 1.40 (3H, t, J=11 Hz, CH_2CH_3), 4.00 (2H, t, J=7 Hz) and 4.57 (2H, t, J=7 Hz) (OCH_2CH_2N), 4.44 (2H, q, J=11 Hz, CH_2CH_3), 8.22 (1H, s, –NCH=).

Anal Calcd for $C_{13}H_{25}O_3Si$: C 52.2, H 8.4, N 14.1.
Found: C 52.4, H 8.5, N 14.2.

Compound (**8x**) was similarly prepared.

^{1}H NMR ($CDCl_3$) δ 0.91 (9H, s, *tert*-Bu), 1.40 (3H, t, J=11 Hz, CH_2CH_3), 2.22 (2H, m, $CH_2CH_2CH_2$), 3.68 (2H, t, J=9 Hz) and 4.62 (2H, t, J=9 Hz) ($CH_2CH_2CH_2$), 4.45 (2H, q, J=11 Hz, CH_2CH_3), 8.23 (1H, s, –NCH=).

Anal Calcd for $C_{14}H_{27}N_3O_3Si$: C 53.7, H 8.6, N 13.4.
Found: C 53.8, H 8.8, N 13.5.

Ethyl 1-(*tert*-Butyldiphenylsilyloxycarbonylmethyl)-1,2,3-triazole-4-carboxylate (**8m**)

Benzyl ester (**8l**) (10 g, 34.6 mmol) in THF (200 ml) with 10% Pd-C (1 g) was shaken under H_2 at atmospheric pressure until H_2 uptake stopped (15 minutes). The catalyst was filtered off and the filtrate ice-cooled, treated with Et_3N (5.3 ml, 38 mmol) and *tert*-butyldiphenylsilyl chloride (10 ml, 38 mmol) and left 20 minutes. The solvent was evaporated, the residue taken up in EtOAc and filtered. The filtrate was evaporated to about 80 ml and left to crystallise to provide colourless needles (13.9 g, 92%): MP 157~159°C.

IR ν_{max} (Nujol) cm^{-1} 1742, 1730, 1225; ^{1}H NMR ($CDCl_3$) δ 1.07 (9H, s, *tert*-Bu), 1.37 (3H, t, J=11 Hz, CH_2CH_3), 4.42 (2H, q, J=11 Hz, CH_2CH_3), 5.34 (2H, s, NCH_2), 7.3~7.8 (10H, m, Ar-H), 8.28 (1H, s, –NCH=).

Anal Calcd for $C_{23}H_{27}N_3O_4Si$: C 63.2, H 6.2, N 9.6.
Found: C 62.9, H 6.3, N 9.6.

1-*p*-Methoxybenzyloxy-1,2,3-triazole-4-carboxaldehyde (**9t**): Typical Triazole-4-carboxaldehyde Synthesis

Reduction of Ester to Alcohol: Ester (**8t**) (3 g, 10.8 mmol) in THF (40 ml) was refluxed with $LiBH_4$ (0.5 g) for 30 minutes. The mixture was ice-cooled, quenched with excess 5% aqueous citric acid and extracted with EtOAc. The extract was dried and evaporated. Chromatography and crystallisation (EtOAc-hexane) gave colourless plates of 1-PMB-1,2,3-triazol-4-methanol (2.14 g, 84%): MP 74~75°C.

IR ν_{max} (Nujol) cm^{-1} 3240 (br, OH), 1618, 1590, 1518, 1260; ^{1}H NMR ($CDCl_3$) δ 3.80 (3H, s, OCH_3), 4.23 (1H, br s, OH), 4.68 (2H, br s, CH_2OH), 5.37 (2H, s, OCH_2Ar), 6.91 (2H, d, J=7 Hz, Ar-H), 7.2~7.4

(3H, m, Ar-H and –NCH=).

Anal Calcd for $C_{11}H_{13}N_3O_3$: C 56.2, H 5.5, N 17.9.
Found: C 55.9, H 5.7, N 17.6.

Oxidation of Alcohol: The above triazolemethanol (1.3 g, 5.5 mmol) in CH_2Cl_2 (25 ml) was stirred vigorously with pyridinium dichromate (**9g**) for 3 hours, diluted with EtOAc (300 ml) and filtered through Celite. Evaporation of the filtrate and chromatography gave **9t** (0.59 g, 46%), which crystallised (EtOAc - hexane) as colourless plates: Melting range 87~95°C.

IR ν_{max} (Nujol) cm^{-1} 1695, 1617, 1519, 1260; ^1H NMR ($CDCl_3$) δ 3.82 (3H, s, OCH_3), 5.48 (2H, s, OCH_2Ar), 6.93 (2H, d, J=7 Hz, Ar-H), 7.29 (2H, d, J=7 Hz, Ar-H), 7.76 (1H, s, –NCH=), 10.04 (1H, s, CHO).

Anal Calcd for $C_{11}H_{11}N_3O_3$: C 56.7, H 4.7, N 18.0.
Found: C 56.6, H 4.3, N 18.0.

DMT Removal from Penem (**4e**)

Penem (**4e**) (0.57 g, 0.83 mmol) in CH_2Cl_2 (20 ml) and PrOH (20 ml) was ice-cooled and treated dropwise over 10 minutes with formic acid (25 ml). CH_2Cl_2 (30 ml) and water (100 ml) were added, followed by solid $NaHCO_3$ until basic. The organic layer was separated, dried and evaporated. Chromatography and crystallisation (EtOAc - hexane) provided (**4a**) as yellow microcrystals (0.21 g, 66%): MP 159~162°C; $[\alpha]_D^{20}$ +466° (c 0.5, DMSO); IR ν_{max} (Nujol) cm^{-1} 3260, 1790, 1785, 1770, 1715, 1610, 1560, 1520; ^1H NMR (DMSO-d_6) δ 5.40 (2H, ABq, OCH_2Ar), 6.71 (1H, s, 5-CH), 7.39 (1H, s, 8-CH), 7.72 (2H, d, Ar-H), 7.90 (1H, s, 2-CH), 8.2~8.4 (3H, m, 13-H and Ar-H), 15.70 (1H, br s, NH); UV λ_{max}^{EtOH} ($E_{1cm}^{1\%}$) nm 280 (21,350).

Anal Calcd for $C_{16}H_{11}N_5O_5S$: C 49.9, H 2.9, N 18.2, S 8.3.
Found: C 49.9, H 2.9, N 18.2, S 8.1.

Desilylation of Penem (**7v**)

Penem (**7v**) (18.4 g, 35 mmol) in THF (110 ml) and AcOH (90 ml) was treated with tetrabutylammonium fluoride in THF (70 ml, 1 M) and stirred 3 hours. The soln was diluted with EtOAc, washed with water and aqueous $NaHCO_3$, dried and evaporated. The solid was briefly (2 minutes) boiled with EtOAc - $CHCl_3$ (1 : 1) (100 ml), left 2 minutes and the crystals filtered off to provide pure (5*R*), (6*Z*) penem (**7u**), (5.05 g, 35%) as yellow needles: MP 137~140°C; $[\alpha]_D^{20}$ +420° (c 0.5, DMSO).

IR ν_{max} (Nujol) cm^{-1} 3500 (br, OH), 1778, 1695; UV λ_{max}^{EtOH} nm (ε) 288 (25,100); ^1H NMR (DMSO) δ 3.7~3.9 (5H, m, OCH_3 and CH_2CH_2OH), 4.47 (2H, t, J=5 Hz, CH_2CH_2OH), 5.10 (1H, t, J=5 Hz, OH), 5.16 (2H, s, OCH_2Ar), 6.68 (1H, d, J=0.7 Hz, 5-CH), 6.95 (2H, d, J=8 Hz, Ar-H), 7.3~7.4 (3H, m, Ar-H and 8-CH), 7.74 (1H, s, 2-CH), 8.45 (1H, s, 13-CH).

Anal Calcd for $C_{19}H_{18}N_4O_5S$: C 55.1, H 4.4, N 13.5, S 7.7.
Found: C 55.1, H 4.3, N 13.7, S 7.7.

NOE Study of Penem Esters (**4c** and **4d**)

Penem (**4c**) showed: ^1H NMR (DMSO-d_6) δ 4.23 (3H, s, N–CH_3), 5.40 (2H, ABq, OCH_2Ar), 6.65 (1H, d, J=1 Hz, 5-CH), 7.35 (1H, d, J=1 Hz, 8-CH), 7.71 (2H, d, Ar-H), 7.90 (1H, s, 2-CH), 8.06 (1H, s, 13-CH), 8.26 (2H, d, Ar-H). Irradiation of 5-CH produced NOE's to 2-CH and 13-CH; irradiation of 8-CH produced an NOE to 13-CH; irradiation of 13-CH produced NOE's to 5-CH and 8-CH; no NOE was seen to N-CH_3.

Penem (**4d**) showed: ^1H NMR (DMSO-d_6) δ 4.18 (3H, s, N–CH_3), 5.42 (2H, ABq, OCH_2Ar), 6.93 (1H, s, 5-CH), 7.57 (1H, s, 8-CH), 7.72 (2H, d, Ar-H), 7.98 (1H, s, 2-CH), 8.07 (1H, s, 13-CH), 8.26 (2H, d, Ar-H).

Irradiation of N-CH_3 produced an NOE to 8-CH; irradiation of 5-CH produced an NOE to 13-CH; these NOE's were also seen in the reverse direction. The absence of an NOE to N-CH_3 in **4c** and its presence in **4d** verifies these structures. Also, the fact that in **4c** there are NOE's from 13-CH to both 5-CH and 8-CH indicates relatively free rotation of the triazole group; in **4d** the absence of an NOE from 13-CH to 8-CH and from N–CH_3 to 5-CH indicates a relatively fixed triazole position due to steric hindrance by the N–CH_3.

Deprotection of Penem Esters (**4** and **7**) to Sodium Salts (**5**)

Route A

A general deprotection of penem *p*-nitrobenzyl (PNB) esters is available[2,5]. The following were prepared by this method:

5a: $[\alpha]_D^{20}$ +382° (*c* 0.45, H_2O); IR ν_{max} (KBr) cm^{-1} 1739, 1680, 1654, 1583, 1558; UV $\lambda_{max}^{H_2O}$ nm (ε) 280 (14,050), 354 (1,250); ^1H NMR (D_2O) δ 6.58 (1H, s, 5-CH), 7.03 (1H, s, 2-CH), 7.25 (1H, s, 8-CH), 8.04 (1H, s, 13-CH); FAB-MS (matrix thioglycerol) *m/z* 295 (M+Na).

5b: Data under route B.

5c: $[\alpha]_D^{21}$ +521° (*c* 0.8, H_2O); IR ν_{max} (KBr) cm^{-1} 1756, 1685, 1601, 1552; UV $\lambda_{max}^{H_2O}$ nm (ε) 284 (19,850), 368 (1,500); ^1H NMR (D_2O) δ 4.19 (3H, s, N–CH_3), 6.48 (1H, d, *J*=1 Hz, 5-CH), 7.01 (1H, s, 2-CH), 7.12 (1H, d, *J*=1 Hz, 8-CH), 7.83 (1H, s, 13-CH).

5d: $[\alpha]_D^{20}$ +327° (*c* 0.8, H_2O); IR ν_{max} (KBr) cm^{-1} 1761, 1600, 1551; UV $\lambda_{max}^{H_2O}$ nm (ε) 284 (16,880), 370 (1,400); ^1H NMR (D_2O) δ 4.12 (3H, s, N–CH_3), 6.58 (1H, d, *J*=1 Hz, 5-CH), 7.04 (1H, s, 2-CH), 7.25 (1H, br s, 8-CH), 7.83 (1H, s, 13-CH).

5f: $[\alpha]_D^{24}$ +429° (*c* 1.0, H_2O); IR ν_{max} (KBr) cm^{-1} 1763, 1688, 1601, 1552; UV $\lambda_{max}^{H_2O}$ nm (ε) 283 (18,400), 363 (1,700); ^1H NMR (D_2O) δ 1.50 (3H, t, CH_2CH_3), 4.44 (2H, q, CH_2CH_3), 6.54 (1H, d, *J*=1 Hz, 5-CH), 7.01 (1H, s, 2-CH), 7.15 (1H, d, *J*=1 Hz, 8-CH), 8.18 (1H, s, 13-CH).

5h: $[\alpha]_D^{20}$ +431° (*c* 0.5, H_2O); IR ν_{max} (KBr) cm^{-1} 1760, 1685, 1600, 1552; UV $\lambda_{max}^{H_2O}$ nm (ε) 282 (21,710), 368 (1,830); ^1H NMR (D_2O) δ 0.84 (3H, t, *J*=7.4 Hz, $CH_2CH_2CH_3$), 1.8~2.0 (2H, m, $CH_2CH_2CH_3$), 4.39 (2H, t, *J*=7.4 Hz, $CH_2CH_2CH_3$), 6.62 (1H, d, *J*=1 Hz, 5-CH), 7.03 (1H, s, 2-CH), 7.20 (1H, d, *J*=1 Hz, 8-CH), 8.19 (1H, s, 13-CH).

5j: $[\alpha]_D^{23}$ +410° (*c* 1.1, H_2O); IR ν_{max} (KBr) cm^{-1} 1761, 1686, 1601, 1552; UV $\lambda_{max}^{H_2O}$ nm (ε) 284 (20,100), 362 (1,650); ^1H NMR (D_2O) δ 1.1~1.3 (4H, m, cyclopropyl-H), 3.8~4.0 (1H, m, cyclopropyl-H), 6.40 (1H, d, *J*=1 Hz, 5-CH), 7.01 (1H, s, 2-CH), 7.09 (1H, d, *J*=1 Hz, 8-CH), 8.16 (1H, s, 13-CH).

5k: $[\alpha]_D^{19}$ +277° (*c* 1.0, H_2O); IR ν_{max} (KBr) cm^{-1} 1760, 1690, 1603, 1552; UV $\lambda_{max}^{H_2O}$ nm (ε) 277 (16,710), 366 (1,360); ^1H NMR (D_2O) δ 5.32 (2H, q, *J*=8.5 Hz, CH_2CF_3), 6.59 (1H, s, 5-CH),7.03 (1H, s, 2-CH), 7.19 (1H, s, 8-CH), 8.39 (1H, s, 13-CH).

5p: $[\alpha]_D^{19}$ +369° (*c* 0.8, H_2O); IR ν_{max} (KBr) cm^{-1} 1749, 1609, 1553; UV $\lambda_{max}^{H_2O}$ nm (ε) 283 (19,800), 362 (1,900); ^1H NMR (D_2O) δ 5.07 (2H, s, CH_2COONa), 6.63 (1H, d, *J*=1 Hz, 5-CH), 7.04 (1H, s, 2-CH), 7.23 (1H, d, *J*=1 Hz, 8-CH), 8.17 (1H, s, 13-CH).

5q: $[\alpha]_D^{18}$ +332° (*c* 0.7, H_2O); IR ν_{max} (KBr) cm^{-1} 1755, 1600, 1552; UV $\lambda_{max}^{H_2O}$ nm (ε) 282 (16,210), 366 (1,350); ^1H NMR (D_2O) δ 3.01 (6H, s, $N(CH_3)_2$), 6.59 (1H, d, *J*=0.8 Hz, 5-CH), 7.04 (1H, s, 2-CH), 7.16 (1H, d, *J*=0.8 Hz, 8-CH), 8.28 (1H, s, 13-CH).

5r: $[\alpha]_D^{20}$ +433° (*c* 0.8, H_2O); IR ν_{max} (KBr) cm^{-1} 1762, 1688, 1599, 1553; UV $\lambda_{max}^{H_2O}$ nm (ε) 279 (18,690), 370 (1,660); ^1H NMR δ (D_2O) 4.28 (3H, s, OCH_3), 6.56 (1H, d, *J*=1 Hz, 5-CH), 7.03 (1H, s, 2-CH), 7.12 (1H, d, *J*=1 Hz, 8-CH), 8.31 (1H, s, 13-CH).

5s: $[\alpha]_D^{19}$ +492° (*c* 1.0, H_2O); IR ν_{max} (KBr) cm^{-1} 1745, 1676, 1597, 1555; UV $\lambda_{max}^{H_2O}$ nm (ε) 211 (8,710), 296 (11,310); ^1H NMR (D_2O) δ 6.54 (1H, d, *J*=1 Hz), 7.02 (1H, s, 2-CH), 7.08 (1H, d, *J*=1 Hz, 8-CH), 7.59 (1H, s, 13-CH); FAB-MS (matrix glycerol) *m/z* 311 (M+Na).

Route B

Typical De-esterification: Sodium (5*R*),(6*Z*)-6-[1-(2-Hydroxyethyl)-1,2,3-triazole-4-ylmethylene]-penem-3-carboxylate (**5u**)

Penem ester (**7u**) (3 g, 7.25 mmol) in CH_2Cl_2 (225 ml) was added dropwise over 10 minutes to a solution of $AlCl_3$ (2.43 g, 18.3 mmol) in CH_2Cl_2 (18 ml) and anisole (72 ml) at −40°C under argon. The resulting suspension was stirred a further 10 minutes and poured into aqueous Na_2HPO_4 (250 ml of 0.5 M), which was stirred vigorously 15 minutes and filtered through Celite, washing through with water. The layers of the filtrate were separated, the aqueous washed with ether and evaporated to low volume. Chromatography (Diaion HP-20SS, eluent water) and freeze-drying provided the title compound as a yellow solid (1.45 g, 63%): $[\alpha]_D^{20}$ +431° (*c* 0.9, H_2O); IR ν_{max} (KBr) cm^{-1} 1756, 1688, 1599; UV $\lambda_{max}^{H_2O}$ nm (ε) 283 (21,260), 366 (1,880); ^1H NMR (D_2O) δ 3.98 (2H, t, *J*=5 Hz), 4.56 (2H, t, *J*=5 Hz), 6.61 (1H, d, *J*=0.6 Hz, 5-CH), 7.04 (1H, s, 2-CH), 7.21 (1H, d, *J*=0.6 Hz, 8-CH), 8.24 (1H, s, 13-CH); FAB-MS (matrix thioglycerol) *m/z* 339 (M+Na), 317 (M+H).

Also prepared by route B: **5b**: $[\alpha]_D^{20}$ +508° (*c* 1.0, H_2O); IR ν_{max} (Nujol) cm^{-1} 1750, 1687, 1664, 1588, 1559; UV $\lambda_{max}^{H_2O}$ nm (ε) 282 (24,600); ^1H NMR (D_2O) δ 4.13 (3H, s, NCH_3), 6.63 (1H, d, J=0.7 Hz, 5-CH), 7.06 (1H, s, 2-CH), 7.22 (1H, d, J=0.7 Hz, 8-CH), 8.17 (1H, s, 13-CH).

5w: $[\alpha]_D^{19}$ +418° (*c* 0.8, H_2O); IR ν_{max} (KBr) cm^{-1} 1760, 1685, 1600, 1554; ^1H NMR (D_2O) δ 2.11 (2H, quintet, J=6.5 Hz, $CH_2CH_2CH_2$), 3.55 and 4.50 (2×2H, 2t, J=6.5 Hz, $CH_2CH_2CH_2$), 6.52 (1H, d, J=1 Hz, 5-CH), 7.00 (1H, s, 2-CH), 7.14 (1H, d, J=1 Hz, 8-CH), 8.18 (1H, s, 13-CH); UV $\lambda_{max}^{H_2O}$ nm (ε) 282 (20,000), 366 (1,750).

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