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 (5R)-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 series1-4) 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 (5R,6Z)penems (5) were prepared using two routes. Route A (Scheme 1) has been described for the preparation of 5b5) and is shown schematically in full elsewhere6): 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) NaBH₄, THF, EtOH; (v) see ref 6; (vi) 5% Pd · C, H₂, aq dioxan, NaHCO₃. Tr=triphenylmethyl.
ester (8) and either of the chiral azetidinones (1 or 2). Route B (Scheme 2) has also been described for the preparation of 5b-9), it requires a 1,2,3-triazolecarboxaldehyde (9) and the (6S)-bromo-penem (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; 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-\(^{13}\)-methyl and 8-CH was observed, but in 4c no NOE to 11-\(^{13}\)-methyl

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

<table>
<thead>
<tr>
<th>R</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>a H</td>
<td>n CH(_2)COOPNB</td>
</tr>
<tr>
<td>b CH(_3)</td>
<td>p CH(_2)COONa</td>
</tr>
<tr>
<td>c DMT</td>
<td>q N(CH(_3))(_2)</td>
</tr>
<tr>
<td>d Et</td>
<td>r OCH(_3)</td>
</tr>
<tr>
<td>e Allyl</td>
<td>s OH</td>
</tr>
<tr>
<td>f n-Pr</td>
<td>t OPMB</td>
</tr>
<tr>
<td>g Cyclopropyl</td>
<td>u CH(_2)CH(_2)OH</td>
</tr>
<tr>
<td>h CH(_2)CF(_3)</td>
<td>v CH(_2)CH(_2)OSi(CH(_3))(_2)Bu'</td>
</tr>
<tr>
<td>i CH(_2)COCH(_2)Ph</td>
<td>w (CH(_2))(_2)OH</td>
</tr>
<tr>
<td>j CH(_2)COOSiPh(_2)Bu'</td>
<td>x (CH(_2))(_3)OSi(CH(_3))(_2)Bu'</td>
</tr>
</tbody>
</table>

This table refers to compounds 3, 4, 5, 7, 8 and 9 (a, b and e-8). 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.

<table>
<thead>
<tr>
<th>Class</th>
<th>( I_{S0} ) (µg/ml)</th>
<th>Amoxycillin MIC (µg/ml) in the presence of 1 µg/ml of inhibitor</th>
<th>Relative potency in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.cl. Ia</td>
<td>P.m. II</td>
<td>E.co. TEM-1 III</td>
</tr>
<tr>
<td>5b</td>
<td>0.001</td>
<td>0.007</td>
<td>0.001</td>
</tr>
<tr>
<td>5c</td>
<td>0.019</td>
<td>0.010</td>
<td>0.002</td>
</tr>
<tr>
<td>5d</td>
<td>0.055</td>
<td>3.500</td>
<td>0.017</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulbactam</td>
<td>2.8</td>
<td>0.020</td>
<td>0.036</td>
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<tr>
<td>Tazobactam</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Amoxycillin alone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Class: Enzyme classification based on Richmond and Sykes18).
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 Arnold10), by which ethyl a-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\textsuperscript{t} 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\textsuperscript{9}, and also resulted in poor in vitro activity relative to the N-12-methylated derivative (5b). The N-11-methylated derivative (5c) showed reduced synergistic activity against the Class 1 \( \beta \)-lactamase of Enterobacter aerogenes, but synergistic activity against all other \( \beta \)-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 I 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

\textsuperscript{t} For numbering, see structure 4c.
Table 3. Biological activity of N-12-substituted triazolylmethylene penems.

<table>
<thead>
<tr>
<th>Class</th>
<th>E. cl. 1a</th>
<th>P. m. II</th>
<th>E. co. TEM-1 III</th>
<th>K. p. IV</th>
<th>E. co. OXA-1 V</th>
<th>Human serum binding (%)</th>
<th>Human kidney stability (%)</th>
<th>Relative potency in vivo</th>
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<tr>
<td>5a</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>79</td>
<td>45</td>
<td>0.64</td>
</tr>
<tr>
<td>5b</td>
<td>2</td>
<td>4</td>
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<td>2</td>
<td>4</td>
<td>72</td>
<td>61</td>
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<td>5f</td>
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<td>4</td>
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<td>2</td>
<td>4</td>
<td>72</td>
<td>72</td>
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<tr>
<td>5h</td>
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<td>2</td>
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<td>4</td>
<td>8</td>
<td>81</td>
<td>67</td>
<td>0.04</td>
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<td>4</td>
<td>8</td>
<td>80</td>
<td>82</td>
<td>0.17</td>
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<td>78</td>
<td>67</td>
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<td>8</td>
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<td>69</td>
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<td>2</td>
<td>2</td>
<td>74</td>
<td>56</td>
<td>0.43</td>
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<tr>
<td>5s</td>
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<td>8</td>
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<tr>
<td>5u</td>
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<td>2</td>
<td>4</td>
<td>56</td>
<td>69</td>
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<td>5w</td>
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<td>4</td>
<td>2</td>
<td>4</td>
<td>59</td>
<td>74</td>
<td>0.50</td>
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<tr>
<td>Clavulanic acid</td>
<td>&gt;512</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>&gt;512</td>
<td>20</td>
<td>100</td>
<td>0.20</td>
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<tr>
<td>Sulbactam</td>
<td>256</td>
<td>64</td>
<td>&gt;128</td>
<td>64</td>
<td>&gt;512</td>
<td>100</td>
<td>NT</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Tazobactam</td>
<td>256</td>
<td>16</td>
<td>8</td>
<td>&gt;16</td>
<td>&gt;512</td>
<td>20</td>
<td>NT</td>
<td>0.20</td>
</tr>
<tr>
<td>None</td>
<td>512</td>
<td>&gt;512</td>
<td>&gt;512</td>
<td>256</td>
<td>&gt;512</td>
<td>17</td>
<td>100</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

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 Sykes18). NT: Not tested.
Abbreviations: See footnote in Table 2.

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

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' parameter11) 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 Kubinyi12). The equation for this curve was found to be:

\[
\log \text{relative potency} = 1.52 \log k' - 3.29 \log(0.29k' + 1) + 0.34
\]

(multiple r-square
= 0.895; std. dev. of regression = 0.44)

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 publication13).

Experimental

β-Lactamase inhibition studies were carried out on isolated enzyme preparations as previously
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 x 10^6 cfu/ml) as previously described. Serum binding was carried out using the method previously described. Stability to human kidney homogenate was determined at 37°C as described previously. 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 x 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:

\[
\frac{\text{CD}_{50} \text{ of compound 5b}}{\text{CD}_{50} \text{ of test compound}}
\]

\[\log k'\] was calculated from the reverse phase HPLC Rt's using the method of Miyake et al. 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. 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.

\textbf{Azetidinone (1) ([\alpha]_D^{25} - 55°C (c 1, CHCl_3)) was prepared from (4\text{R})-4-tritylthioazetidinone using a silylation procedure described for the corresponding racemic compound.} Bromoazetidinone (2) has been described. Triazole (8a) was prepared by the method of Klein et al. Full experimental details are in the patent literature for both routes A and B. 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.

\textbf{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 Mel (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: \[\text{^1}H \text{ NMR (CDCl}_3\] \[\delta 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: \[\text{^1}H \text{ NMR (CDCl}_3\] \[\delta 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: \[\text{^1}H \text{ NMR (CDCl}_3\] \[\delta 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=)

\textbf{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. \[\text{^1}H \text{ NMR (CDCl}_3\] \[\delta 1.35\]
(3H, t, J = 11 Hz, CH₂CH₃), 3.79 (6H, s, 2 × O–CH₃), 4.40 (2H, q, J = 11 Hz, CH₂CH₃), 6.7–7.5 (13H, m, Ar-H), 8.17 (1H, s, –NCH=).

Anal Calcd for C₂₆H₂₅N₃O₄: 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-Methoxybenzylxylo-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 PBr₃ 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. ¹H NMR (CDCl₃) δ 1.38 (3H, t, J = 11 Hz, CH₂CH₃), 3.85 (3H, s, OCH₃), 4.44 (2H, q, J = 11 Hz, CH₂CH₃), 5.51 (2H, s, OCH₂Ar), 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 C₁₃H₁₅N₃O₄: 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 Et₃N (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. ¹H NMR (CDCl₃) δ 0.88 (9H, s, tert-Bu), 1.40 (3H, t, J = 11 Hz, CH₂CH₃), 4.00 (2H, t, J = 7 Hz) and 4.57 (2H, t, J = 7 Hz) (OCH₂CH₂N), 4.44 (2H, q, J = 11 Hz, CH₂CH₃), 8.22 (1H, s, –NCH=).

Found: C 52.4, H 5.5, N 14.2.

Compound (8x) was similarly prepared.

¹H NMR (CDCl₃) δ 0.91 (9H, s, tert-Bu), 1.40 (3H, t, J = 11 Hz, CH₂CH₃), 2.22 (2H, m, CH₂CH₂CH₂), 3.68 (2H, t, J = 9 Hz) and 4.62 (2H, t, J = 9 Hz) (CH₂CH₂CH₂), 4.45 (2H, q, J = 11 Hz, CH₂CH₃), 8.23 (1H, s, –NCH=).

Anal Calcd for C₁₄H₂₇N₃O₃Si: 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 (81) (10 g, 34.6 mmol) in THF (200 ml) with 10% Pd-C (1 g) was shaken under H₂ at atmospheric pressure until H₂ uptake stopped (15 minutes). The catalyst was filtered off and the filtrate ice-cooled, treated with Et₃N (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 νmax (Nujol) cm⁻¹ 1742, 1730, 1225; ¹H NMR (CDCl₃) δ 1.07 (9H, s, tert-Bu), 1.37 (3H, t, J = 11 Hz, CH₂CH₃), 4.42 (2H, q, J = 11 Hz, CH₂CH₃), 5.34 (2H, s, NCH₂), 7.3–7.8 (10H, m, Ar-H), 8.28 (1H, s, –NCH=).


1-p-Methoxybenzylxylo-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 LiBH₄ (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 νmax (Nujol) cm⁻¹ 3240 (br, OH), 1618, 1590, 1518, 1260; ¹H NMR (CDCl₃) δ 3.80 (3H, s, OCH₃), 4.23 (1H, br s, OH), 4.68 (2H, br s, CH₂OH), 5.37 (2H, s, OCH₂Ar), 6.91 (2H, d, J = 7 Hz, Ar-H), 7.2–7.4
Oxidation of Alcohol: The above triazole methanol (1.3 g, 5.5 mmol) in CH₂Cl₂ (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 νmax (Nujol) cm⁻¹ 1695, 1617, 1519, 1260; ¹H NMR (CDCl₃) δ 3.82 (3H, s, OCH₃), 5.48 (2H, s, OCH₂Ar), 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 C₁₁H₁₁N₃O₃: C 56.7, H 4.7, N 18.0.

Found: C 56.6, H 4.3, N 18.0.

DME Removal from Penem (4e)

Penem (4e) (0.57 g, 0.83 mmol) in CH₂Cl₂ (20 ml) and PrOH (20 ml) was ice-cooled and treated dropwise over 10 minutes with formic acid (25 ml). CH₂Cl₂ (30 ml) and water (100 ml) were added, followed by solid NaHCO₃ 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; [α]D +466° (c 0.5, DMSO); IR νmax (Nujol) cm⁻¹ 3260, 1790, 1785, 1770, 1715, 1610, 1560, 1520; ¹H NMR (DMSO-d₆) δ 5.40 (2H, ABq, OCH₂Ar), 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-Hand Ar-H), 15.70 (1H, br s, NH); UV λmax (E₄cm⁻¹) nm 280 (21,350).

Anal Calcd for C₁₅H₁₅N₄O₅S: 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 NaHCO₃, dried and evaporated. The solid was briefly (2 minutes) boiled with EtOAc - CHCl₃ (1 : 1) (100 ml), left 2 minutes and the crystals filtered off to provide pure (5R, 6Z) penem (7u), (5.05 g, 35%) as yellow needles: MP 137~140°C; [α]D +420° (c 0.5, DMSO).

IR νmax (Nujol) cm⁻¹ 3500 (br, OH), 1778, 1695; UV λmax (E₄cm⁻¹) nm (e) 285 (25,100); ¹H NMR (DMSO) δ 3.7~3.9 (5H, m, OCH₃ and CH₂CO₂OH), 4.47 (2H, t, J = 5 Hz, CH₂CO₂OH), 5.10 (1H, t, J = 5 Hz, OH), 5.16 (2H, s, OCH₂Ar), 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).


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-d₆) δ 4.23 (3H, s, N-CH₃), 5.40 (2H, ABq, OCH₂Ar), 6.65 (1H, d, J = 1 Hz, 5-CH), 7.35 (1H, d, J = 1 Hz, 8-CH), 7.71 (2H, d, Ar-H), 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-CH₃.

Penem (4d) showed: ¹H NMR (DMSO-d₆) δ 4.18 (3H, s, N-CH₃), 5.42 (2H, ABq, OCH₂Ar), 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-CH₃ 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-CH₃ 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-CH₃ to 5-CH indicates a relatively fixed triazole position due to steric hindrance by the N-CH₃.
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^\circ (c 0.45, H_2O); IR v_{max} (KBr) cm^{-1} 1739, 1680, 1654, 1583, 1558; ^1H NMR (D_2O) \delta 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^{20} +521^\circ (c 0.8, H_2O); IR v_{max} (KBr) cm^{-1} 1756, 1685, 1601, 1552; ^1H NMR (D_2O) \delta 4.19 (3H, s, N-CH\text{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^\circ (c 0.8, H_2O); IR v_{max} (KBr) cm^{-1} 1761, 1600, 7.25 (1H, s, 2-CH), 7.25 (1H, s, 2-CH), 7.12 (1H, d, J = 1 Hz, 8-CH), 7.83 (1H, s, 13-CH).\]

5f: \([\alpha]_D^{20} +429^\circ (c 1.0, H_2O); IR v_{max} (KBr) cm^{-1} 1763, 1688, 1601, 1552; ^1H NMR (D_2O) \delta 1.50 (3H, t, CH_2CH_2C\#3), 4.44 (2H, q, C\#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^\circ (c 0.5, H_2O); IR v_{max} (KBr) cm^{-1} 1760, 1685, 1601, 1552; ^1H NMR (D_2O) \delta 0.84 (3H, t, /= 7.4Hz, CH_2CH_2C\#3), 1.8-2.0 (2H, m, CH_2CH_2CH_3), 4.39 (2H, t, /= 7.4Hz, CH_2CH_2C\#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^{20} +410^\circ (c 1.1, H_2O); IR v_{max} (KBr) cm^{-1} 1756, 1688, 1601, 1552; ^1H NMR (D_2O) \delta 5.32 (2H, q, /= 8.5Hz, 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^{20} +369^\circ (c 0.8, H_2O); IR v_{max} (KBr) cm^{-1} 1749, 1609, 1553; UV \lambda_{max}^{\text{nm}} (c 283 (19,800), 362 (1,900); ^1H NMR (D_2O) \delta 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^{20} +332^\circ (c 0.7, H_2O); IR v_{max} (KBr) cm^{-1} 1755, 1600, 1552; UV \lambda_{max}^{\text{nm}} (c 282 (16,210), 366 (1,350); ^1H NMR (D_2O) \delta 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^\circ (c 0.8, H_2O); IR v_{max} (KBr) cm^{-1} 1762, 1688, 1599, 1553; UV \lambda_{max}^{\text{nm}} (c 279 (18,690), 370 (1,660); ^1H NMR \delta (D_2O) 4.28 (3H, s, OCH_3), 6.56 (1H, d, J = 1 Hz, 5-CH), 7.03 (1H, s, 8-CH), 7.12 (1H, d, J = 1 Hz, 8-CH), 8.31 (1H, s, 13-CH).\]

5s: \([\alpha]_D^{20} +492^\circ (c 1.0, H_2O); IR v_{max} (KBr) cm^{-1} 1745, 1676, 1597, 1555; UV \lambda_{max}^{\text{nm}} (c 211 (8,710), 296 (11,310); ^1H NMR (D_2O) \delta 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 (5R),(6Z)-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 for another 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 (Daiion HP-20SS, eluent water) and freeze-drying provided the title compound as a yellow solid (1.45 g, 63%): \([\alpha]_D^{20} +431^\circ (c 0.9, H_2O); IR v_{max} (KBr) cm^{-1} 1763, 1688, 1601, 1552; UV \lambda_{max}^{\text{nm}} (c 284 (19,850), 368 (1,500); ^1H NMR (D_2O) \delta 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); FAB-MS (matrix thioglycerol) m/z 295 (M + Na).\]
Also prepared by route B: 5b: \([x]_D^{20} +508^\circ (c 1.0, H_2O); IR v_{max} (Nujol) \text{ cm}^{-1} 1750, 1687, 1664, 1588, 1559; UV \lambda_{max}^\text{nm} (\epsilon) 282 (24,600); ^1H NMR (D_2O) \delta 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: \([x]_D^{20} +418^\circ (c 0.8, H_2O); IR v_{max} (KBr) \text{ cm}^{-1} 1760, 1685, 1600, 1554; ^1H NMR (D_2O) \delta 2.11 (2H, quintet, J=6.5 Hz, CH_2CH_2CH_2), 3.55 and 4.50 (2 x 2H, 2t, J=6.5Hz, C7/2CH2C/H2), 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}^\text{nm} (\epsilon) 282 (20,000), 366 (1,750).

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