Novel C-2 Substituted Carbapenem Derivatives

Part II. Synthesis and Structure-activity Relationships of Isoxazolin-2-yl, Isoxazolidin-2-yl and 2-Pyrazolin-2-yl Carbapenems Generated Using 1,3-Dipolar Cycloaddition Chemistry

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(Received for publication May 28, 1996)

A series of carbapenems containing novel C-2 semisaturated heterocyclic substituents were synthesised by 1,3 dipolar cycloaddition reactions of nitrile oxides, nitrile imines and a nitrone to 2-vinylcarbapenem. The isoxazoline and isoxazolidine compounds showed potent antibacterial activity but moderate stability to human dehydropeptidase 1 (DHP-1). Stability to DHP-1 was improved by methyl substitution in the isoxazoline ring, but at the expense of antibacterial activity. The pyrazolines exhibited excellent stability to DHP-1, but reduced potency against Gram-negative organisms.

In the preceding paper[1], we described the synthesis, antibacterial activity and stability to human DHP-1 of a novel series of carbapenems containing non-aromatic heterocyclic substituents at the C-2 position. This report describes the use of 1,3 dipolar cycloaddition chemistry to synthesise new carbapenems with saturated and semi-saturated heterocyclic substituents at C-2 and their biological properties.

Chemistry

Although several synthetic routes to carbapenems have been reported, we wanted to develop a methodology that would allow the facile synthesis of several C-2 heterocyclic analogues from a common late stage intermediate. To this end we investigated the possibility of using cycloaddition chemistry to construct heterocyclic systems by reaction of 1,3 dipoles with carbapenem dipolarophiles. 1,3 Dipolar cycloaddition to β-lactam dipolarophiles was demonstrated in studies on nitrone[2] and nitrile oxide[3] cycloaddition to 3-vinyl cephems producing 3-(isoxazolidin-5-yl) and 3-(isoxazolin-5-yl)cephalosporins. Indeed, a pro-drug of one of the resulting 3-(isoxazolidin-5-yl)cephalosporin showed promise as an orally active therapeutic agent[4]. Cycloaddition reactions have also been carried using cephem attached nitrile oxide[5], nitrile imine and azide[6] 1,3 dipolarophiles. Very recently nitrone cycloaddition to 2-vinyl carbapenem as dipolarophile has been used to generate carbapenems with an isoxazolidine ring at C-2, which showed potent broad spectrum antibacterial activity[7]. 2-Vinyl carbapenem (**2**) was synthesised as reported[8] by treatment of ketone **1** with triflic anhydride followed by Stille coupling of the resulting triflate with trimethylvinylstannane (Scheme 1).

Reaction of **2** with benzonitrile oxide, generated *in situ* by treatment of chlorobenzaldoxime with triethylamine, at room temperature afforded C-2 carbapenem isoxazoline with complete regioselectivity as a mixture of 2 diastereomers. The diastereomers, which were separated by silica gel chromatography, were obtained in approximately 1 : 1 ratio. The stereochemistry at C-5′ could not be determined by NMR, nor could a crystalline compound be obtained of sufficient quality for X-ray structure determination. Previous work on the 3-(isoxazolin-5-yl)cephalosporins, for which the structure of a Δ^2 isomer was determined by X-ray crystallography, established that the less polar isomers and more polar isomers have *S* and *R* configurations at C-5′ respectively. By analogy, we assumed that the same relationship between polarity and C-5′ configuration applied to the structurally similar 2-(isoxazolin-5-yl)carbapenems and have tentatively assigned the stereochemistry accord-

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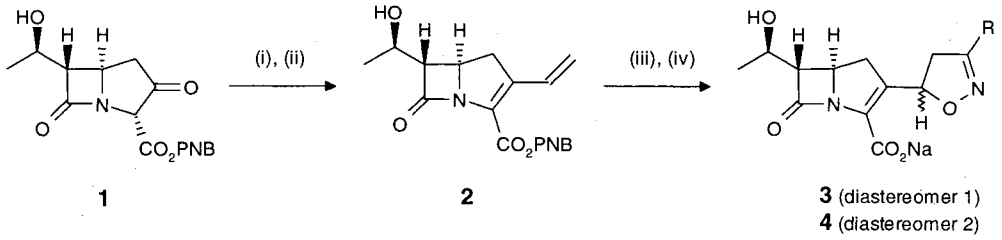
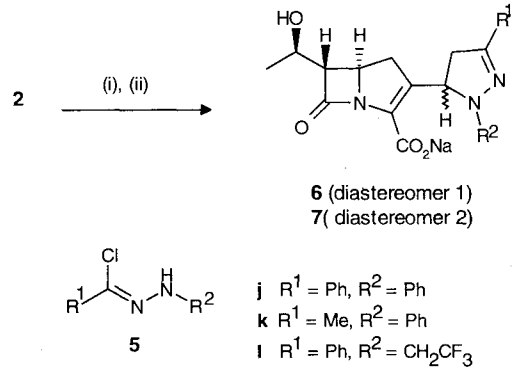
Scheme. 1. Synthesis of isoxazolines.



1 **2** **3** (diastereomer 1)
4 (diastereomer 2)

Reagents and conditions: i) Tf_2O, NEt_3; ii) $AsPh_3$, $Pd(dba)_2$, trimethylvinyl stannane; iii) RCClNOH, NEt_3, dioxane, separate diastereomers; iv) H_2, Pd/C, HP20SS.

Scheme 2. Synthesis of pyrazolines.



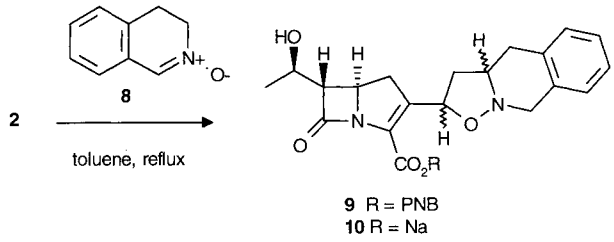
6 (diastereomer 1)
7(diastereomer 2)

5

j R^1 = Ph, R^2 = Ph
k R^1 = Me, R^2 = Ph
l R^1 = Ph, R^2 = CH_2CF_3

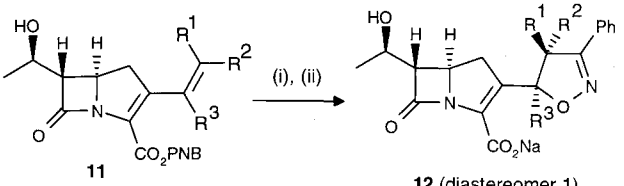
Reagents and conditions: i) **5**, NEt_3, dioxane, separate diastereomers; ii) H_2, Pd/C, HP20SS.

Scheme 3. Synthesis of isoxazolidine.



9 R = PNB
10 R = Na

Scheme 4. Synthesis of 4- and 5-methylisoxazolines.



11

m R^1 = Me, R^2,R^3 = H
n R^2 = Me, R^1,R^3 = H
o R^3 = Me, R^1,R^2 = H

12 (diastereomer 1)
13 (diastereomer 2)

Reagents and conditions: i) PhCClNOH, NEt_3, dioxane, separate diastereomers; ii) H_2, Pd/C, HP20SS.

ingly, although proof of this would require crystallographic structure determination. Hydrogenolysis of the 4-nitrobenzyl group followed by HP20SS purification gave the two diastereomeric 2-isoxazolinyl carbapenem salts **3** and **4**. A number of other 2-isoxazolinyl carbapenems were prepared in similar manner.

Reaction of **1** with nitrile imines to give pyrazoline derivatives was also examined (Scheme 2). Nitrile imines were generated *in situ* by dropwise addition of triethylamine to chlorohydrazones **5**. Overnight reaction gave the 2-(pyrazolin-5-yl)carbapenems as a diastereomeric mixture which was separable by silica gel chromatography. The yields obtained were similar to those obtained by nitrile oxide cycloaddition, and the ratio of the less polar to the more polar isomer was approximately 2:1. The corresponding sodium salts **6** and **7** were obtained as before.

Nitrone cycloaddition to **2** was investigated using isoquinoline *N*-oxide **8** (Scheme 3). Although **8** is one of the most reactive nitrones, as dipoles nitrones are inherently less reactive dipoles than nitrile oxides and nitrile imines and cycloaddition to **2** required 5 hours reflux in toluene giving the cycloadduct **9** as a mixture of inseparable diastereoisomers. In this case the cycloadduct was formed much less cleanly than with nitrile oxides and nitrile imines and this is presumably due to the more forcing cycloadditon conditions which may have caused degradation of the carbapenem nucleus. Deprotection of **9** as before gave a diastereomeric mixture of carbapenem **10**.

Modelling studies indicated that some 4- and 5-isoxazoline ring substituents on the isoxazolin-5-yl carbapenems could occupy the same space as a 1β-methyl substituent, and thus may confer stability to DHP-1. In order to test this hypothesis, several 2(4- and

5-methyl isoxazolin-5-yl)carbapenems were synthesised by benzonitrile oxide cycloaddition to methyl substituted dipolarophiles (Scheme 4). The precursor dipolarophiles **11** were synthesised in moderate yield from **1** by Stille reaction with the appropriate vinylstannane[8].

Discussion

The *in vitro* antibacterial activity of the synthesised compounds against selected Gram-negative and Gram-positive bacteria, including pathogens common in community acquired infections, and stabilities to DHP-1 are shown in Table 1 and compared with those of imipenem and meropenem. In general all the compounds displayed potent antibacterial activities, particularly against Gram-positive organisms. In the monosubstituted isoxazoline series (**3**, **4**), the activity against Gram-positive organisms was independent of the substituent, although potency against Gram-negative organisms, particularly against *Escherichia coli*, was reduced with larger substituents, with the sterically demanding 2,4,6 trimethylphenyl derivatives (**3g**, **4g**) showing the lowest activities. However, the introduction of polarity restored activity against *E. coli*, with 2- and 3- pyridyl compounds (**3e**, **3f**, **4e**, **4f**) exhibiting significantly better activity than phenyl compounds (**3d**, **4d**). There appeared to be little significant difference in potency against Gram-positive organisms, between the diastereomers but diastereomer 2 (**4**) consistently showed a slight improvement in activity against Gram-negatives over diastereomer 1 (**3**). The 4- and 5-methyl substituted isoxazolines (**12**, **13**) exhibited similar Gram-positive activities to the unsubstituted compounds (**3d**, **4d**) but equivalent or poorer Gram-negative activity. For the 5-methyl series, activity against *Haemophilus influenzae* was much reduced for both diastereomers, although there was only a slight

Table 1. Summary of antibacterial activity and stability to DHP-1 of C-2 heterocyclyl carbapenems.

Compound	*E.c.*	*H.i.*	*M.c.*	*S.a.*	*S.p.*	Stability to DHP-1*	
			MIC (μg/ml)			Spect.	HPLC
Imipenem	0.12	0.25	0.06	0.06	0.06	15.1	
Meropenem	<0.03	0.13	0.03	0.03	1.0	1.0	88
3a	0.25	0.5	0.06	0.06	0.06	35	
3b	0.25	0.25	0.06	0.06	0.25		71
3c	4	0.5	0.06	0.06	0.25	36	
3d	8	NT	0.06	0.06	0.06		65
3e	0.25	0.25	0.06	0.06	0.06	100	
3f	0.5	0.25	0.06	1	0.06	13	67
3g	>64	4	0.25	0.06	0.06	40	
3h	32	2	0.06	0.06	0.06		57
3i	32	0.06	0.06	0.06	0.06	82	
4a	0.13	0.25	0.06	0.06	0.06	69	
4b	0.13	0.13	0.06	0.06	0.13		<10
4c	2	0.5	0.25	0.06	1	45	
4d	4	0.06	0.06	0.06	0.13		29
4e	0.25	0.06	0.06	0.06	0.06	37	
4f	0.5	0.13	0.06	0.13	0.06	35	32
4g	64	1	0.06	0.06	0.13	60	
4h	16	1	0.06	0.06	0.06		<10
4i	64	0.13	0.06	0.06	0.06	20	
6j	>64	16	0.13	0.06	0.06		>98
6k	64	0.5	0.06	NT	0.13	<0.3	
6l	64	4	<0.06	0.13	<0.06	NT	
7j	>64	16	0.25	0.25	1		>90
7k	64	0.5	0.06	NT	0.25	0.5	0.5
7l	>64	8	0.25	<0.06	0.5	NT	
10	16	0.25	0.06	0.06	NT		64
12m	>64	1	0.25	0.06	0.06	12	56
12n	>64	0.25	0.25	0.06	0.25	6.1	73
12o	16	1	0.06	NT	0.13	<0.3	
13m	8	0.03	NT	0.03	0.03	9.3	49
13n	32	0.25	0.06	0.06	0.25	11	56
13o	4	1	0.06	NT	0.06	0.3	

Abbreviations: *E.c.*, *Escherichia coli* DCO (TEM 1β-lactamase); *H.i.*, *Haemophilus influenzae* WM493 (β-lactamase −ve); *M.c.*, *Moraxella catarrhalis* Ravasio (β-lactamase +ve); *S.a.*, *Staphylococcus aureus* Russell (β-lactamase +ve); *S.p.*, *Streptococcus pneumoniae* PU7. NT, not tested.

* Stability to DHP-1: Rates of hydrolysis by purified human DHP-1 enzyme at 37°C, relative to meropenem (spectrophotometric assay) or percentage carbapenem remaining after incubation with human DHP-1 at 37°C for 60 minutes (HPLC assay). See experimental section for assay details.

reduction in potency against *E. coli*. Again diastereomer 2 (**13**) exhibited slightly better Gram-negative potencies than diastereomer 1 (**12**). In particular the *cis* 4-methyl substituted 3-phenylisoxazolin-2-yl diastereomer 2 (**13m**) showed improved overall antibacterial potency over diastereomer 1 (**12m**). The isoxazolidine (**10**) had similar antibacterial potency to phenyl isoxazoline (**3d**, **4d**).

The pyrazoline series displayed similar activities against Gram-positive organisms but reduced activity against Gram-negative organisms compared to the isoxazoline series. For all the pyrazolines activity against *E. coli* was very much reduced, but the reduction in *H. influenzae* potency appeared to be related to the size of the *N*-substituent *i.e.* methyl (**6k**, **7k**) > 2,2,2-trifluoroethyl (**6l**, **7l**) > phenyl (**6j**, **7j**). In contrast to the isoxazoline series, the activity against Gram-positive organisms of diastereomer 1 (**6**) was generally slightly better than diastereomer 2 (**7**), and there was little difference between the diastereomers in activities against Gram-negative organisms.

None of the monosubstituted isoxazolines possessed stability to DHP equivalent or superior to meropenem. As with antibacterial activity the nature of the isoxazoline 3-substituent appeared to have little effect on stability to DHP-1. However, there did appear to be a significant difference in stability to DHP-1 between the diastereomers with diastereomer 1 having greater stability than diastereomer 2. Interestingly, (*S*) diastereomers have been found to possess greater stability to DHP-1 than (*R*) diastereomers for C-2 tetrahydrofuran-2-yl and tetrahydrothiophen-2-yl carbapenems[1], and this is consistent with the tentative assignment of (*S*)-stereochemistry to diastereomer 1 of the isoxazoline series. Methyl substitution at position 4 of the isoxazoline appeared to have little effect on the stability of diastereomer 1 (**12m** and **12n** *vs.* **3d**) although a significant improvement was noted for diastereomer 2 (**13m** and **13n** *vs.* **4d**). Thus, 4-substitution reduced the difference in stability to DHP-1 between the diastereomers. However, orientation of the methyl group (*cis* or *trans*) to the ring junction did not significantly alter stability to DHP-1. The only isoxazoline compounds that did show better stability than meropenem were the 5-methyl isoxazolines **12o** and **13o**, with similar stability to DHP-1 demonstrated for both diastereomers. The isoxazolidine **10** showed poorer stability than meropenem, however the 1,3 disubstituted pyrazoline series did exhibit stability to DHP-1 equivalent or superior to meropenem and as with the isoxazolines, diastereomer 1 (**6**) was more stable than diastereomer 2 (**7**).

In summary it has been shown that 1,3 dipolar cycloaddition chemistry can be used to prepare a number of C-2 semisaturated heterocyclic carbapenems which have potent broad spectrum antibacterial activities. C-2 carbapenem isoxazolines showed the most potent antibacterial activities but their stabilities to DHP-1 were poorer than meropenem. Attempts to improve stability by introduction of substituents at C-5 did produce stable compounds but at the expense of reduced activity against Gram-negative organisms. The pyrazoline series also showed good stability to DHP-1 but had less Gram-negative potency than the monosubstituted isoxazolines. The reduction in Gram-negative activity appeared to be related to the steric demands of the substituents on the C-2 heterocycle which were not directly responsible for stability to DHP-1.

Experimental

^{1}H NMR spectra were recorded on a Bruker AC250 spectrometer. IR spectra were measured in solution or a KBr disc using Philips PU9706 spectrometer. UV spectra were recorded on a Beckman DU spectrophotometer in ethanol solution. Mass spectral data were recorded on a VG ZAB1F or VG Trio-2 spectrometer in electron impact (EI), chemical ionisation using ammonia gas (CI), electrospray (ESI-MS) or fast atom bombardment (FAB) mode. Chromatography was performed on Merck silica 60, 230~400 mesh.

General Procedure for Nitrile Oxide Cycloadditions

Generation of Nitrile Oxides from Chlorooximes (Method A)

Triethylamine (187 ml, 1.3 mmol) in dichloromethane (1 ml) was added dropwise over 0.5 hour to a stirring suspension of *p*-nitrobenzyl (5*R*, 6*S*)-6-[(*R*)-1-hydroxyethyl]-2-vinylcarbapen-2-em-3-carboxylate (0.40 g, 1.1 mmol) and chlorooximidoacetamide (165 mg, 1.3 mmol) in 1:1 dichloromethane-dioxane (10 ml) under argon. After 0.5 hour a further 1 Eq of aminocarbonyl nitrile oxide generated *in situ* as above was added and the mixture was stirred for 1 hour. The reaction mixture was evaporated to dryness and ethyl acetate (100 ml) was added and the mixture was washed with water (50 ml). The aqueous phase was extracted with ethyl acetate (100 ml) and the combined organic extracts were dried and evaporated under reduced pressure. The residue was chromatographed (silica gel, 1:2~1:4 hexane-ethyl acetate and 100% ethyl acetate eluent) and gave *p*-nitrobenzyl (5*R*, 6*S*)-6-[(*R*)-1-hydroxyethyl]-2-(3-aminocarbonylisoxazolin-5-yl)carbapen-2-em-3-carboxylate as a single diastereomer 1 (135 mg, 27%), a mixture of diastereomers (152 mg, 31%) and a single diastereomer 2 (105 mg, 21%). Diastereomer 1 (Rf 0.44, 9:1 dichloromethane-methanol): ν_{max} (KBr) 3449, 3336, 2972, 1767, 1721, 1684, 1668, 1598, 1520, 1440, 1379, 1341, 1288, 1207, 1102 and 1061 cm^{-1}; δ_H (d_6-acetone) 1.25 (3H, d, J=6.2 Hz), 3.01 (1H, dd, J=10.3, 18.6 Hz), 3.15 (dd, J=8.1, 17.9 Hz) and 3.16 (dd, J=8.2, 18.6 Hz) [2H], 3.41 (1H, dd, J=3.2, 6.1 Hz), 3.48 (1H, dd, J=11.7, 18.0 Hz), 4.06~4.37 (2H, m), 5.38 (1H, d, J=13.9 Hz), 5.56 (1H, d, J=14.0 Hz), 6.23 (1H, dd, J=8.1, 11.6 Hz), 6.79 (1H, br s), 7.15 (1H, br s), 7.83 (2H, d, J=8.7 Hz) and 8.27 (2H, d, J=8.8 Hz); *m/z* (EI) 444 (M^+, <1), 400 (3), 339 (9), 315 (11), 285 (11), 264 (10),

239 (14), 220 (15), 203 (10), 178 (22), 153 (21), 136 (55), 117 (45), 89 (60), 77 (71) and 44 (100).

Diastereomer 2 (Rf 0.38, 9:1 dichloromethane-methanol): ν_{max} (KBr) 3449, 3394, 2984, 1769, 1707, 1637, 1597, 1448, 1340, 1288, 1261, 1204, 1134 and 1101 cm^{-1}; δ_H (d_6-acetone) 1.27 (3H, d, J=6.1 Hz), 3.05 (2H, dd, J=0.9, 9.4 Hz), 3.15 (1H, dd, J=8.1, 17.9 Hz), 3.40 (1H, dd, J=3.1, 6.0 Hz), 3.54 (1H, dd, J=11.5, 17.9 Hz), 4.11~4.32 (2H, m), 5.37 (1H, d, J=14.0 Hz), 5.56 (1H, d, J=13.9 Hz), 6.26 (1H, dd, J=8.0, 11.5 Hz), 6.80 (1H, br s), 7.19 (1H, br s), 7.83 (2H, d, J=8.7 Hz) and 8.27 (2H, d, J=8.8 Hz); m/z (EI) 444 (M^+, <1), 400 (8), 383 (5), 339 (5), 314 (3), 287 (23), 264 (15), 220 (18), 203 (11), 178 (18), 153 (20), 136 (80), 121 (12), 89 (65), 77 (70) and 44 (100).

Generation of Nitrile Oxides from Oximes (Method B)

Pyridine (18 ml, 0.22 mmol) then acetaldoxime (40 mg, 0.68 mmol) was added to a suspension of *N*-chlorosuccinamide (90 mg, 0.68 mmol) in chloroform (2 ml) and stirred for 10 minutes. To the resulting clear solution was added *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-vinylcarbapen-2-em-3-carboxylate (200 mg, 0.56 mmol) in one portion followed by a solution of triethylamine (86 ml) in chloroform dropwise over 0.5 hour and the mixture was stirred for a further 0.5 hour. A further 1 Eq of methyl nitrile oxide generated *in situ* as above was added and the mixture stirred for 0.5 hour. The resulting yellow solution was evaporated to dryness and the residue was dissolved in dichloromethane (50 ml) and washed with water (30 ml). The aqueous phase was extracted with dichloromethane (50 ml) and the combined organic extracts were dried and evaporated under reduced pressure. The residue was chromatographed (silica gel, 1:2~1:9 hexane-ethyl acetate) to give *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-(3-methyl-isoxazolin-5-yl)carbapen-2-em-3-carboxylate as a single diastereomer 1 (72 mg, 32%) and as a mixture of diastereomers (32 mg, 14%) and a single diastereomer 2 (51 mg, 22%).

Diastereomer 1 (Rf 0.17, 5:1 ethyl acetate-hexane): ν_{max} (KBr) 3431, 2966, 2926, 1777, 1718, 1627, 1522, 1436, 1382, 1347, 1197 and 1103 cm^{-1}; δ_H ($CDCl_3$) 1.35 (3H, d, J=6.3 Hz), 2.01 (3H, s), (1H, dd, J=8.3, 18.7 Hz), 3.09 (1H, dd, J=7.4, 17.0 Hz), 3.19 (1H, dd, J=2.9, 6.4 Hz), 3.28 (1H, dd, J=10.1, 18.9 Hz), 3.72 (1H, dd, J=11.2, 17.0 Hz), 4.21~4.33 (2H, m), 5.24 (1H, d, J=13.7 Hz), 5.49 (1H, d, J=13.7 Hz), 6.01 (1H, dd, J=8.0, 11.1 Hz), 7.65 (2H, d, J=8.7 Hz) and 8.23 (2H, d, J=8.7 Hz); m/z (EI) 415 (M^+, 5), 358 (8), 314 (12), 279 (10), 235 (12), 222 (45), 191 (15), 178 (100), 152 (28), 136 (57), and 106 (65); (Found M^+ 415.1382. $C_{20}H_{21}O_7N_3$ requires 415.1379).

Diastereomer 2 (Rf 0.13, 5:1 ethyl acetate-hexane): ν_{max} (KBr) 3421, 2963, 2925, 1777, 1717, 1624, 1609, 1521, 1436, 1382, 1346, 1283, 1196 and 1100 cm^{-1}; δ_H ($CDCl_3$) 1.34 (3H, d, J=6.3 Hz), 2.02 (3H, s), 2.74 (1H, ddd, J=1.0, 7.8, 17.5 Hz), 2.92 (1H, ddd, J=1.0, 10.2, 19.5 Hz), 3.12 (1H, ddd, J=0.9, 8.6, 19.6 Hz), 3.25 (1H, dd, J=3.0, 6.2 Hz), 3.37 (1H, ddd, J=1.0, 11.1, 17.5 Hz), 4.17~4.32 (2H, m), 5.24 (1H, d, J=13.8 Hz), 5.47 (1H, d, J=13.8 Hz), 6.01 (1H, dd, J=7.6, 11.1 Hz), 7.65 (2H, d, J=8.7 Hz) and 8.24 (2H, d, J=8.7 Hz); m/z (EI) 415 (M^+, 5), 358 (10), 333 (12), 314 (13), 279 (10), 235 (15), 222 (53), 191 (20), 178 (100), 152 (37), 136 (70), 120 (22) and 106 (81); (Found M^+ 415.1385. $C_{20}H_{21}O_7N_3$ requires 415.1379).

Cycloaddition with Stable Nitrile Oxides (Method C)

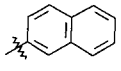
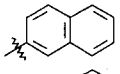
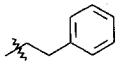
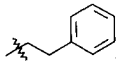
To a stirred solution of *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-vinylcarbapen-2-em-3-carboxylate (250 mg, 0.70 mmol) in dichloromethane (4 ml) and dioxane (6 ml) was added a solution of 2,4,6-trimethylbenzonitrileoxide (112 mg, 0.70 mmol) in dioxane (2 ml). The mixture was stirred for 16 hours and worked up as method A. The diastereomers were separated using normal phase prep. HPLC on Silica gel 60 eluting with 8% ethyl acetate/92% dichloromethane. Rt 16 minutes yielding less polar *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[3-(2,4,6-trimethylphenyl)isoxazolin-5-yl]carbapen-2-em-3-carboxylate diastereomer 1 (129 mg, 22%) and Rt 18 minutes yielding more polar *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[3-(2,4,6-trimethylphenyl)isoxazolin-5-yl]carbapen-2-em-3-carboxylate diastereoisomer 2 (69 mg, 12%).

Diastereomer 1 ν_{max} (KBr) 3434, 1779, 1720, 1624, 1522 and 1346 cm^{-1}; δ_H ($CDCl_3$) 1.37 (3H, d, J=6.3 Hz), 1.75 (1H, d, J=4.6 Hz, exch), 2.23 (6H, s), 2.29 (3H, s), 2.86~2.98 (2H, m), 3.22 (1H, dd, J=6.7, 2.9 Hz), 3.35~3.56 (2H, m), 4.25~4.35 (2H, m), 5.25 and 5.50 (2H, ABq, J=13.7 Hz), 6.18 (1H, dd, obsc), 6.90 (2H, s), 7.64 (2H, d, J=8.7 Hz) and 8.22 (2H, d, J=8.7 Hz); m/z (CI, +ve ion, ammonia) 520 (MH^+) and 538 (MNH_4^+). Found (M^+) 519.2015. $C_{28}H_{29}N_3O_7$ requires 519.2007. Diastereomer 2 ν_{max} (CH_2Cl_2) 3432, 1776, 1721, 1608, 1526 and 1346 cm^{-1}; δ_H ($CDCl_3$) 1.37 (3H, d, J=6.3 Hz), 1.70 (1H, d, J=4.9 Hz, exch), 2.23 (6H, s), 2.29 (3H, s), 2.93 (1H, dd, J=17.7, 8.3 Hz), 3.05~3.25 (2H, m), 3.29 (1H, dd, J=6.3, 3.0 Hz), 3.54~3.65 (1H, dd, J=17.7, 11.2 Hz), 4.20~4.35 (2H, m), 5.25 and 5.50 (2H, ABq, J=13.7 Hz), 6.13 (1H, dd, obsc), 6.91 (2H, s), 7.65 (2H, d, J=8.7 Hz) and 8.22 (2H, d, J=8.7 Hz); m/z (CI, +ve ion, ammonia) 520 (MH^+) and 538 (MNH_4^+). Found M^+ 519.2004. $C_{28}H_{29}N_3O_7$ requires 519.2007.

General Procedure for Nitrile Imine Cycloadditions (Method D)

To a stirred solution of *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-vinylcarbapen-2-em-3-carboxylate (110 mg, 0.31 mmol) in dichloromethane (3 ml) and dioxane (3 ml) was added a solution of 1-(α-chlorobenzylidene)-2-phenylhydrazine (106 mg, 0.46 mmol) in dichloromethane (2 ml) followed by the dropwise addition of triethylamine (64 μl, 0.46 mmol). The mixture was stirred for 3 hours, the solvent evaporated *in vacuo*

Table 2. Spectral data for compounds **3** and **4a**~**i**.

Compound	R	CA[a]	IR[b]	^{1}H NMR[c]
3a	$CONH_2$	A	1757	1.28 (3H, d, J=6.4 Hz), 2.90 (2H, d, J=9.3 Hz), 3.09 (1H, dd, J=7.9, 18.0 Hz), 3.42 (1H, dd, J=2.9, 5.9 Hz), 3.52 (1H, dd, J=11.7, 18.2 Hz), 4.18~4.32 (2H, m) and 6.33 (1H, dd, J=7.9, 11.6 Hz)
4a	$CONH_2$	A	1759	1.27 (3H, d, J=6.4 Hz), 2.89 (2H, d, J=9.2 Hz), 3.17 (1H, dd, J=7.8, 18.0 Hz), 3.43 (1H, dd, J=2.9, 5.9 Hz), 3.53 (1H, dd, J=11.6, 18.0 Hz), 4.14~4.28 (2H, m) and 6.33 (1H, dd, J=7.8, 11.6 Hz)
3b	Me	B	1749	1.25 (3H, d, J=6.4 Hz), 1.99 (3H, s), 2.84 (d, J=9.2 Hz) and 2.87 (ddd, J=0.8, 7.1, 18.0 Hz) [3H], 3.32 (ddd, J=1.1, 11.0, 18.0 Hz) and 3.36 (dd, J=3.0, 5.9 Hz) [2H], 4.12~4.27 (2H, m), 6.04 (1H, dd, J=7.0, 11.0 Hz)
4b	Me	B	1752	1.25 (3H, d, J=6.4 Hz), 1.99 (3H, s), 2.82 (d, J=8.9 Hz) and 2.84 (d, J=9.8 Hz) [2H], 2.95 (1H, dd, J=7.0, 17.9 Hz), 3.35 (dd, J=11.4, 17.5 Hz) and 3.38 (dd, J=2.9, 5.9 Hz) [2H], 4.09~4.24 (2H, m), 6.06 (1H, dd, J=6.8, 11.0 Hz)
3c	$CHMe_2$	B	1756	1.23 (3H, d, J=7.0 Hz), 1.34 (3H, d, J=6.4 Hz), 2.82 (1H, septet, J=7.0 Hz), 2.89~2.92 (2H, m), 3.00 (1H, dd, J=6.3, 17.8 Hz), 3.41~3.48 (2H, m), 4.22~4.32 (2H, m), 6.14 (1H, dd, J=6.3, 11.0 Hz)
4c	$CHMe_2$	B	1752	1.16 (3H, d, J=6.4 Hz), 2.7~2.91 (3H, m), 3.03 (1H, dd, J=6.2, 17.8 Hz), 3.34~3.46 (2H, m), 4.13~4.24 (2H, m), 6.10 (1H, dd, J=6.2, 10.9 Hz)
3d		A	1760	1.23 (3H, d, J=6.4 Hz), 2.83 (dd, J=8.7, 17.7 Hz) and 2.86 (dd, J=9.5, 17.7 Hz) [2H], 3.31 (dd, J=7.0, 17.5 Hz) and 3.33 (dd, J=2.9, 5.9 Hz) [2H], 3.77 (1H, dd, J=11.1, 17.5 Hz), 4.13~4.25 (2H, m), 6.26 (1H, dd, J=6.9, 11.1 Hz), 7.42~7.57 (3H, m), 7.64~7.73 (2H, m)
4d		A	1754	1.24 (3H, d, J=6.4 Hz), 2.80 (1H, dd, J=9.7, 17.8 Hz), 2.92 (1H, dd, J=8.4, 17.9 Hz), 3.39 (dd, J=2.9, 5.9 Hz) and 3.40 (dd, J=6.8, 17.3 Hz) [2H], 3.78 (1H, dd, J=11.0, 17.5 Hz), 4.09~4.24 (2H, m), 6.28 (1H, dd, J=6.7, 11.0 Hz), 7.42~7.59 (3H, m), 7.65~7.75 (2H, m)
3e		A	1747	1.26 (3H, d, J=6.4 Hz), 2.90 (2H, d, J=9.3 Hz), 3.32 (1H, dd, J=6.1, 2.9 Hz), 3.38 (1H, dd, J=17.7, 7.3 Hz), 3.80 (1H, dd, J=17.7, 11.3 Hz), 4.18~4.29 (2H, m), 6.36 (1H, dd, J=11.3, 7.3 Hz), 7.56 (1H, m), 7.88~8.00 (2H, m) and 8.58 (1H, d, J=4.9 Hz)
4e		A	1755	1.27 (3H, d, J=6.4 Hz), 2.86 (1H, dd, J=17.9, 9.9 Hz), 2.98 (1H, dd, J=17.9, 8.5 Hz), 3.40 (1H, m), 3.48 (1H, dd, J=17.7, 7.1 Hz), 3.80 (1H, dd, J=17.7, 11.2 Hz), 4.12~4.28 (2H, m), 6.36 (1H, dd, J=11.2, 7.1 Hz), 7.54 (1H, m), 7.85~8.00 (2H, m) and 8.56 (1H, d, J=4.8 Hz)
3f		A	1756	1.09 (3H, d, J=6.1 Hz), 2.50 (1H, obs.), 2.72 (1H, dd, J=16.7, 8.3 Hz), 3.03 (1H, dd, J=6.5, 2.5 Hz), 3.22 (1H, dd, J=17.3, 7.4 Hz), 3.53 (1H, dd, J=17.3, 11.2 Hz), 3.82~3.96 (2H, m), 4.98 (1H, br s, exch), 6.60 (1H, dd, J=11.2, 7.4 Hz), 7.48 (1H, dd, J=8.0, 4.9 Hz), 8.07 (1H, br d, J=8.0 Hz), 8.62 (1H, br d, J=4.9 Hz) and 8.84 (1H, br s)
4f		A	1755	1.13 (3H, d, J=6.2 Hz), 2.61 (2H, d, J=9.3 Hz), 3.07 (1H, dd, J=6.4, 2.7 Hz), 3.30 (1H, dd, J=17.2, 7.7 Hz), 3.58 (1H, dd, J=17.2, 11.2 Hz), 3.82~3.96 (2H, m), 5.08 (1H, br s, exch), 6.65 (1H, dd, J=11.2, 7.7 Hz), 7.48 (1H, dd, J=7.7, 4.9 Hz), 8.06 (1H, br d, J=7.7 Hz), 8.62 (1H, dd, J=4.9, 1.6 Hz) and 8.84 (1H, d, J=1.6 Hz)
3g		C	1760	1.28 (3H, d, J=6.4 Hz), 2.22 (3H, s), 2.24 (3H, s), 2.29 (3H, s), 3.06 (1H, dd, obsc), 3.17 (1H, dd, J=18.5, 8.4 Hz), 3.41 (1H, dd, J=5.9, 2.9 Hz), 3.56~3.68 (2H, m), 4.21~4.32 (2H, m), 6.31 (1H, dd, obsc) and 7.05 (2H, s)
4g		C	1755	1.28 (3H, d, J=6.4 Hz), 2.22 (6H, s), 2.29 (3H, s), 3.05 (1H, dd, obsc), 3.15~3.30 (2H, m), 3.28 (1H, dd, obsc), 3.64 (1H, dd, obsc), 4.18~4.30 (2H, m), 6.31 (1H, dd, obsc) and 7.05 (2H, s)
3h		A	1761	1.22 (3H, d, J=6.4 Hz), 2.86 (1H, dd, J=9.5, 5.4Hz.), 3.32~3.41 (3H, m), 3.75 (1H, dd, J=17.5, 11.1 Hz), 4.17~4.22 (2H, m), 6.27 (1H, dd, J=11.1, 7.0 Hz) and 7.61~8.04 (7H, m)
4h		A	1754	1.23 (3H, d, J=6.3 Hz), 2.88 (1H, dd, obsc), 3.35~3.50 (3H, m), 3.79 (1H, dd, obsc), 4.10~4.25 (2H, m), 6.32 (1H, dd, obsc) and 7.61~8.06 (7H, m)
3i		A	1756	1.39 (3H, d, J=6.4 Hz), 2.30 (1H, dd, J=17.9, 8.3 Hz), 2.57 (1H, dd, J=17.9, 10.1 Hz), 2.80~3.21 (5H, series of m), 3.35 (1H, dd, J=5.7, 3.0 Hz), 3.42 (1H, dd, J=18.1, 11.2 Hz), 4.13 (1H, m), 4.31 (1H, m), 6.12 (1H, dd, J=11.2, 6.2 Hz) and 7.32~7.52 (5H, m)
4i		A	1752	1.19 (3H, d, J=6.3 Hz), 2.19 (1H, dd, J=18.2, 9.9 Hz), 2.62 (1H, dd, J=18.2, 8.3 Hz), 2.72~3.10 (5H, series of m), 3.25~3.43 (2H, m), 4.03 (1H, m), 4.23 (1H, m), 6.06 (1H, dd, J=10.9, 5.9 Hz) and 7.30~7.46 (5H, m)

[a] CA Cycloaddition method (see experimental).
[b] IR KBr disc absorbance of β-lactam carbonyl.
[c] D_2O, 250 MHz, δ ppm.

and the residue re-dissolved in dichloromethane. The organic solution was washed with saturated sodium hydrogen carbonate, dried over magnesium sulphate and the solvent evaporated *in vacuo*. The residue was purified by chromatography on silica gel eluting with 30% ethyl acetate in hexane to yield *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[(1,3-diphenyl)pyrazolin-5-yl]carbapen-2-em-3-carboxylate as two separate diastereoisomers; less polar diastereoisomer (A) (81 mg, 47%) and more polar diastereoisomer (B) (40 mg, 23%).

Diastereoisomer (A); ν_{max} (CH_2Cl_2) 3602, 1780, 1719, 1597, 1525 and 1349 cm^{-1}; δ_H ($CDCl_3$) 1.26 (3H, d, J=6.2 Hz), 1.63 (1H, d, J=4.7 Hz, exch), 2.76 (2H, d, J=9.3 Hz), 3.00 (1H, dd, J=17.3, 6.2 Hz), 3.13 (1H, dd, J=6.0, 2.9 Hz), 3.71 (1H, dd, J=17.3, 12.2 Hz), 4.02~4.26 (2H, m), 5.32 and 5.62 (2H, ABq, J=13.7 Hz), 6.01 (1H, dd, J=12.2, 6.2 Hz), 6.88 (1H, m), 7.08 (2H, m), 7.23 (2H, m), 7.39 (3H, m), 7.71 (4H, m) and 8.23 (2H, d, J=8.7 Hz); *m/z* (CI, +ve ion, ammonia) 553 (MH^+). Found M^+ 552.1996. $C_{31}H_{28}N_4O_6$ requires 552.2009.

Diastereoisomer (B); ν_{max} (CH_2Cl_2) 3601, 1782, 1720, 1597, 1525 and 1333 cm^{-1}; δ_H ($CDCl_3$) 1.22 (3H, d, J=6.4 Hz), 1.58 (1H, d, J=4.8 Hz, exch), 2.61 (1H, dd, J=19.3, 8.5 Hz), 2.85 (1H, dd, J=19.3, 10.1 Hz), 3.00 (1H, dd, J=6.0, 3.0 Hz), 3.08 (1H, dd, J=17.2, 6.0 Hz), 3.78 (1H, dd, J=17.2, 12.2 Hz), 4.05~4.26 (2H, m), 5.33 and 5.58 (2H, ABq, J=13.8 Hz), 6.08 (1H, dd, J=12.2, 6.0 Hz), 6.84 (1H, m), 7.08 (2H, m), 7.22 (2H, m), 7.38 (3H, m), 7.69 (4H, m) and 8.26 (2H, d, J=8.7 Hz). Found M^+ 552.2007. $C_{31}H_{28}N_4O_6$ requires 552.2009.

p-Nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-(1,4,5,9b-tetrahydro-2H-isoxazolo[3.2.a]isoquinolin-2-yl)carbapen-2-em-3-carboxylate (**9**)

Isoquinoline *N*-oxide (41 mg, 0.28 mg) was added to a stirred suspension of *p*-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-vinylcarbapen-2-em-3-carboxylate (100 mg, 0.28 mmol) in dioxan (1 ml) under an argon atmosphere and the mixtrue was heated to 50°C for 7 hours. The resulting orange solution was flash chromatographed on silica gel eluting with 4:1, 3:1, 2:1 and 1:1 toluene-acetone to give the title compound as a mixture of diastereomers (71 mg, 50%); ν_{max} (KBr) 3451, 2970, 2932, 1773, 1718, 1607, 1521, 1454, 1346, 1283, 1194 and 1111 cm^{-1}; δ_H ($CDCl_3$) 1.35 (d, J=6.2 Hz) and 1.36 (d, J=6.3 Hz) [3H], 2.19~3.48 (9H, m), 4.20~4.32 (2H, m), 4.54 (t, J=8.2 Hz) and 4.58 (t, J=8.8 Hz) [1H], 5.20 (1H, d, J=13.7 Hz), 5.44 (d, J=13.8 Hz) and 5.45 (d, J=13.7 Hz), [1H], 5.77 (dd, J=5.4, 9.4 Hz) and 5.79 (dd, J=3.9, 9.3 Hz) [1H], 7.61 (d, J=8.7 Hz) and 7.62 (d, J=8.7 Hz) [2H], 7.62 (2H, d, J=8.7 Hz); *m/z* (FAB, +ve ion, glycerol) 506 (MH^+).

General Procedure for Hydrogenolysis

p-Nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-(3-phenylisoxazolin-5-yl)carbapen-2-em-3-carboxylate diastereomer A (60 mg, 0.12 mmol) and palladium catalyst (3% on carbon, 10 mg) in THF (2 ml) and phosphate buffer (0.2 M, pH 7, 1 ml) was hydrogenated (1 atm) at room temperature for 5 minutes, and the catalyst was removed by filtration through a celite pad and washed with water (50 ml). Sodium bicarbonate (4 mg, 48 μmol) was added and the mixture was evaporated to remove THF, and then extracted with ethyl acetate (50 ml). The aqueous phase was concentrated *in vacuo* to *ca.* 5 ml and chromatographed on HP20SS eluting with water/THF and freeze dried sodium (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-(3-phenylisoxazolin-5-yl)carbapen-2-em-3-carboxylate diastereomer 1. was hydrogenated for 10 minutes in the same manner as in Example 1b to give sodium (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-(3-phenylisoxazolin-5-yl)carbapen-2-em-3-carboxylate diastereomer 1 **3d** (39 mg, 85%); ν_{max} (KBr) 3417, 2958, 1760, 1597, 1444, 1398, 1354, 1242, 1223 and 1142 cm^{-1}; δ_H (D_2O) 1.23 (3H, d, J=6.4 Hz), 2.83 (dd, J=8.7, 17.7 Hz) and 2.86 (dd, J=9.5, 17.7 Hz) [2H], 3.31 (dd, J=7.0, 17.5 Hz) and 3.33 (dd, J=2.9, 5.9 Hz) [2H], 3.77 (1H, dd, J=11.1, 17.5 Hz), 4.13~4.25 (2H, m), 6.26 (1H, dd, J=6.9, 11.1 Hz), 7.42~7.57 (3H, m), 7.64~7.73 (2H, m); λ_{max} (H_2O) 271.5 nm (ε 15089), 208 (ε 16109); *m/z* (FAB, +ve ion, glycerol) 365 (MH^+), 387 (MNa^+).

Determination of MIC

Antibacterial activity was determined by a broth microdilution technique in microtitre plates using Hamilton AT+liquid handling technology, and defined as the minimum inhibitory concentration (MIC in μg/ml) needed to inhibit growth of the micro-organism. Mueller-Hinton Broth (Difco) was used as the growth medium; for growth of the more fastidious micro-organisms (*S. pneumoniae*, *H. influenzae* and *M. catarrhalis*) this was supplemented with sterile heat-inactivated donor horse serum (ICN Biomedicals)-5%; hematin (Sigma)-0.02 mg/ml and NAD (-nicotinamide adenine dinucleotide, Sigma)-0.08 mg/ml (all final concentrations). Overnight broth cultures were added to give a final concentration of 5×10^5 cfu/ml. Plates were incubated at 37°C for 18 hours.

Determination of Stability to DHP-1

(i) HPLC Method

The test compound (0.5 mmol solution in 0.02 M MOPS at pH 7.0) (0.08 ml) was challenged with pure DHP-1 enzyme (0.08 ml of 2.5 μg/ml solution) at 37°C. Samples were removed at 0, 30, 60, and 90 minute time points and analysed by reverse phase HPLC. The hydrolysis of the carbapenem was monitored by integration of the area under the peak for the test compound. The results were submitted to a statistical evaluation programme which allowed for the calculation of the percentage of test compound remaining intact after 60 minutes. Control experiments were conducted for each test compound to check its stability in buffer alone.

Table 3. Spectral data for compounds **6** and **7j~l** and **10**.

Compound	CA[a]	IR[b]	^{1}H NMR[c]
6j	D	1752	1.14 (3H, d, J=6.3 Hz), 2.40 (1H, dd, J=17.7, 10.8 Hz), 2.68 (1H, dd, J=17.7, 7.9 Hz), 3.08~3.24 (2H, m), 3.66 (1H, dd, J=17.7, 11.9 Hz), 3.81 (1H, m), 4.09 (1H, m), 6.02 (1H, dd, J=11.9, 4.8 Hz), 6.95 (1H, m), 7.13 (2H, m), 7.34 (2H, m), 7.46 (3H, m) and 7.76 (2H, m)
7j	D	1757	1.17 (3H, d, J=6.2 Hz), 2.32 (1H, dd, J=17.9, 8.3 Hz), 2.65 (1H, dd, J=17.9, 10.9 Hz), 2.92 (1H, m), 3.36 (1H, dd, J=18.0, 4.5 Hz), 3.77 (1H, dd, J=18.0, 11.3 Hz), 4.00~4.21 (2H, m), 6.32 (1H, dd, J=11.3, 4.5 Hz), 7.01 (1H, m), 7.25~7.63 (7H, m), and 7.82 (2H, m)
6k	D	1753	1.21 (3H, d, J=6.4 Hz), 2.12 (3H, s), 2.49 (1H, dd, J=17.8, 10.0 Hz), 2.72~2.90 (2H, m), 3.28~3.41 (2H, m), 3.90 (1H, m), 4.18 (1H, m), 5.81 (1H, dd, J=11.2, 5.5 Hz), 6.93~7.08 (3H, m) and 7.37 (2H, m)
7k	D	1756	1.19 (3H, d, J=6.4 Hz), 2.11 (3H, s), 2.36 (1H, dd, J=18.1, 8.1 Hz), 2.77 (1H, dd, J=18.1, 10.0 Hz), 2.78~3.00 (2H, m), 3.35 (1H, dd, J=18.1, 11.3 Hz), 4.01~4.19 (2H, m), 6.05 (1H, dd, J=11.3, 5.3 Hz) and 6.92~7.39 (5H, m)
6l	D	1756	1.22 (3H, d, J=6.4 Hz), 2.90 (2H, d, J=9.2 Hz), 3.01 (1H, dd, J=16.9, 11.5 Hz), 3.32~3.44 (2H, m), 3.58~3.89 (2H, m), 4.16~4.26 (2H, m), 5.18 (1H, t, J=10.7 Hz), 7.48 (3H, m) and 7.67 (2H, m)
7l	D	1757	1.23 (3H, d, J=6.4 Hz), 2.80~3.11 (3H, m), 3.29~3.43 (2H, m), 3.74~3.92 (2H, m), 4.10~4.22 (2H, m), 5.23 (1H, t, J=10.5 Hz), 7.43 (3H, m) and 7.65 (2H, m)
10	E	1753	1.28 (3H, d, J=6.4 Hz), 2.60 (t, J=8.6 Hz) and 2.61 (t, J=8.1 Hz) [2H], 2.94~3.24 (5H, m), 3.43 (1H, dd, J=2.8, 6.1 Hz), 4.17~4.25 (2H, m), 4.60 (t, J=8.5 Hz) and 4.61 (t, J=8.5 Hz) [1H], 5.87 (t, J=6.7 Hz) and 5.89 (t, J=7.5 Hz) [1H], 7.25 (4H, m)

[a] CA Cycloaddition method (see experimental).
[b] IR KBr disc absorbance of β-lactam carbonyl.
[c] D_2O, 250 MHz, δ ppm.

Table 4. Spectral data for compounds **12** and **13m~o**.

Compound	CA[a]	IR[b]	^{1}H NMR[c]
12m	A	1757	1.18 (3H, d, J=6.4 Hz), 1.32 (3H, d, J=7.2 Hz), 2.68 (1H, dd, J=8.5, 17.7 Hz), 2.81 (1H, dd, J=9.8, 17.7 Hz), 3.26 (1H, dd, J=2.8, 5.9 Hz), 3.68 (1H, dt, J=*ca.* 7.2, 3.7 Hz), 4.08~4.19 (2H, m), 5.87 (1H, d, J=3.6 Hz), 4.47~7.51 (3H, m), 7.67~7.72 (2H, m)
13m	A	1759	1.24 (3H, d, J=6.4 Hz), 1.34 (3H, d, J=7.3 Hz), 2.73 (1H, dd, J=*ca.* 10, 17.9 Hz), 2.91 (1H, dd, J=8.4, 18.0 Hz), 3.38 (1H, dd, J=2.8, 6.0 Hz), 3.80 (1H, m), 4.07~4.22 (2H, m), 5.94 (1H, d, J=4.2 Hz), 7.5~7.6 (3H, m), 7.7~7.8 (2H, m)
12n	A	1758	1.10 (3H, d, J=7.4 Hz), 1.29 (3H, d, J=6.4 Hz), 2.85 (1H, dd, J=7.3, 18.3 Hz), 3.20 (1H, dd, J=10.0, 18.5 Hz), 3.38 (1H, dd, J=2.8, 6.0 Hz), 4.10~4.33 (2H, m), 6.1 (1H, d, J=10.1 Hz), 7.45~7.60 (3H, m) and 7.68~7.80 (2H, m)
13n	A	1753	1.10 (3H, d, J=7.4 Hz), 1.30 (3H, d, J=6.4 Hz), 2.90 (1H, dd, J=9.3, 18.8 Hz), 3.12 (1H, dd, J=8.3, 18.5 Hz), 3.45 (1H, dd, J=2.7, 5.8 Hz), 4.15~4.30 (2H, m), 6.20 (1H, d, J=9.9 Hz), 7.50~7.60 (3H, m) and 7.68~7.75 (2H, m)
12o	A	1751	1.29 (3H, d, J=6.4 Hz), 1.70 (3H, s), 2.92 (1H, dd, J=8.9, 17.8 Hz), 3.09 (1H, dd, J=9.9, 17.5 Hz), 3.40 (1H, dd, J=2.8, 6.0 Hz), 3.66 (2H, ABq), 4.12~4.24 (2H, m), 7.47~7.59 (3H, m) and 7.67~7.74 (2H, m)
13o	A	1753	1.38 (3H, d, J=6.4 Hz), 1.77 (3H, s), 3.15 (2H, d, J=9.1 Hz), 3.54 (1H, dd, J=3.1, 6.1 Hz), 3.70 (2H, ABq), 4.26 (2H, dq, J=3.0, 9.0 Hz), 4.29 (1H, dq, J=6.2, 6.2 Hz), 7.55~7.67 (3H, m) and 7.73~7.81 (2H, m)

[a] CA Cycloaddition method (see experimental).
[b] IR KBr disc absorbance of β-lactam carbonyl.
[c] D_2O, 250 MHz, δ ppm.

(ii) Spectrophotometric Method

The test compounds were challenged with the pure DHP-1 enzyme as described above. Rates of hydrolysis were recorded on a UV spectrophotometer at time points between 0 and 90 minutes. Absorbance changes were converted to concentration changes by prior analysis of wavelength scans for each test compound (concentration as above) in the presence of *Bacillus cereus* metallo-β-lactamase (100 ng). Assumptions made are that each compound is completely hydrolysed by the β-lactamase, that the absorbance changes caused by each enzyme are the same, and that the rate of change of the hydrolysis by metallo-β-lactamase is linear. The standard compound, meropenem, is assayed in each screen and the results quoted as hydrolysis rates relative to meropenem. Control experiments were conducted for each test compound to check its stability in buffer alone.

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