Novel C-2 Substituted Carbapenem Derivatives

Part I. Synthesis and Biological Activity of Non-aromatic Heterocyclic Derivatives

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(Received for publication May 28, 1996)

A new series of carbapenems, having a saturated or partially unsaturated heterocycle at C-2, has been synthesised. The *in vitro* antibacterial activity of these compounds and their stability to human dehydropeptidase-1 (DHP-1) are described. The stereochemistry of the C-2 side-chain and the presence of a double bond in the heterocycle were shown to have significant effects on the stabilities of the compounds to DHP-1.

Although carbapenem antibiotics generally exhibit a potent, broad spectrum of antibacterial activity, they are susceptible to hydrolysis by the enzyme, dehydropeptidase-1 (DHP-1). In the case of the therapeutic agent imipenem (**1**)[1], this problem is overcome by co-administration with cilastatin, an inhibitor of DHP-1.

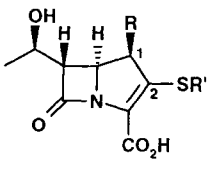
Stability may also be imparted by modification of the carbapenem nucleus. For example, substitution at C-1 with a β-methyl group[2] has resulted in compounds that combine chemical and metabolic stability with enhanced antibacterial potency, as exemplified by meropenem (**2**)[3].

Directly linked aryl groups at C-2 have also been shown to improve stability to DHP-1[4].

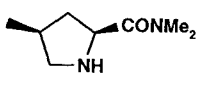
Our recent interests have focused on identifying a carbapenem suitable for the treatment of community acquired infections and which is also stable to the DHP-1 enzyme. Herein we report the chemistry and biological properties of a range of novel C-2 heterocyclic carbapenems.

Chemistry

A range of non-aromatic ketones (**3**)[5] was synthesised as precursors to the novel C-2 substituents and converted to the corresponding silylenol ethers (**4**) by trapping the enolates with *t*-butyldimethylsilyl triflate[6] (Scheme 1).



(1) R = H R' = $CH_2CH_2NHCH{=}NH$

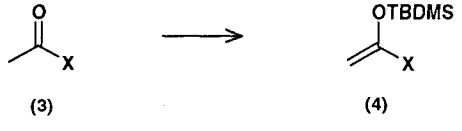
(2) R = Me R' = 

Lewis acid mediated condensation of these silylenol ethers (**4a**~**f**) with the 4-acetoxyazetidinone (**5**)[7] gave the C-4 functionalised azetidinones (**6a**~**f**) following *N*-desilylation. Elaboration to the bicyclic carbapenem was achieved using established intramolecular Wittig cyclisation methodology[8]. Acid mediated desilylation of the C-8 hydroxyl group and subsequent palladium(0) catalysed cleavage of the allyl esters (**8a**~**f**) yielded the C-2 heterocyclic carbapenems (**9a**~**f**) (Scheme 2).

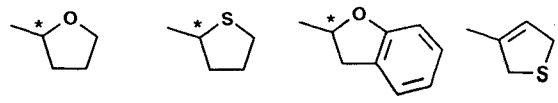
Diastereoisomers of the tetrahydrothiophen-2-yl and 2,3-dihydrobenzofuran-2-yl derivatives were separated after coupling to the azetidinone (**5**). Their stereochemistries were assigned by comparison with the NMR spectra of the tetrahydofuran-2-yl analogues (**6a**) and (**6b**) which were prepared from chiral ketones (**3a**) and (**3b**)[5].

Incorporation of a β-methyl group at the C-1 position of the carbapenem nucleus has been reported to improve the stability of compounds to the DHP-1 enzyme[2]. Hence, the 1β-methyl C-2 tetrahydrofuran-2-yl car-

Scheme 1. Synthesis of sililenol ethers.



(3) (4)

X= 

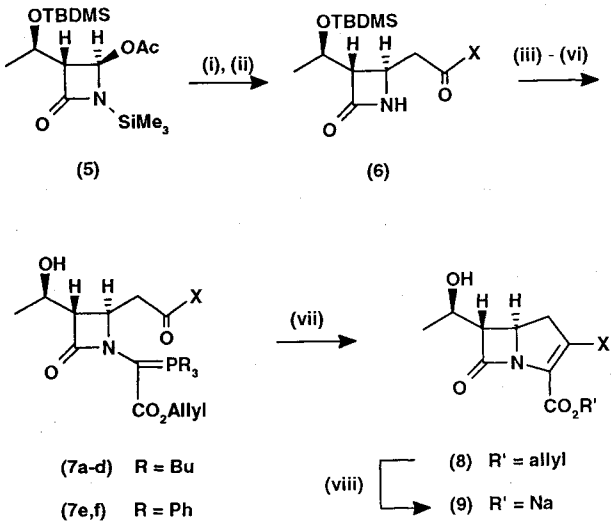
(a) (S)-isomer (c) (S)-isomer (e) (S)-isomer (g)

(b) (R)-isomer (d) (R)-isomer (f) (R)-isomer

bapenems (**12a**) and (**12b**) were synthesised in order to compare them with their C-1 unsubstituted counterparts (**9a**) and (**9b**). Treatment of the pyridyl thioester (**10**)[9] with the lithium organocuprate species (**13**) gave the tetrahydrofuran ketone (**11**) (Scheme 3). This was converted to the carbapenem diastereoisomers (**12a**) and (**12b**) using the Wittig methodology previously described (Scheme 3).

Carbapenems incorporating a partially unsaturated heterocycle at C-2 presented a greater synthetic challenge. Reaction of C-4 acetoxyazetidinone (**5**) with 1-(2,5-dihydrothiophen-3-yl)-1-(*t*-butyldimethylsilyloxy)-ethene (**4g**) afforded only a poor yield of the desired C-4 alkylation product (**6g**). The major product, was the Diels Alder adduct (**14**), analogous to products described by MEYERS *et al.*[10]. Elaboration of azetidinone (**6g**) to the phosphorane (**15**) proceeded satisfactorily, but cyclisation necessitated protection of the hydroxyethyl side-chain as a trimethylsilyl ether (**16**). This increased the rate of the intramolecular Wittig reaction and reduced degradation during thermolysis. A *p*-methoxybenzyl ester was employed rather than allyl in order to avoid the possibility of coordination of palladium to the double bond in the C-2 substituent during ester deprotection. Concomitant removal of the trimethylsilyl and *p*-methoxybenzyl ester groups to give carbapenem

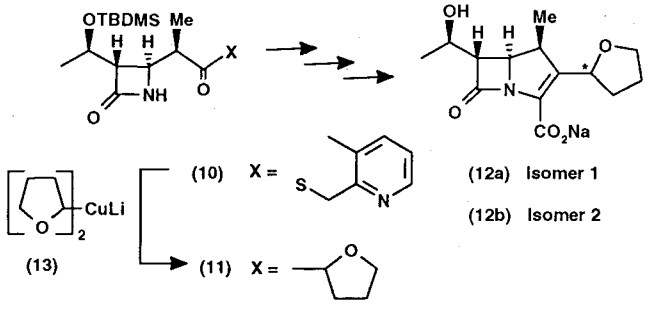
Scheme 2. Synthesis of carbapenems *via* intramolecular Wittig cyclisation.



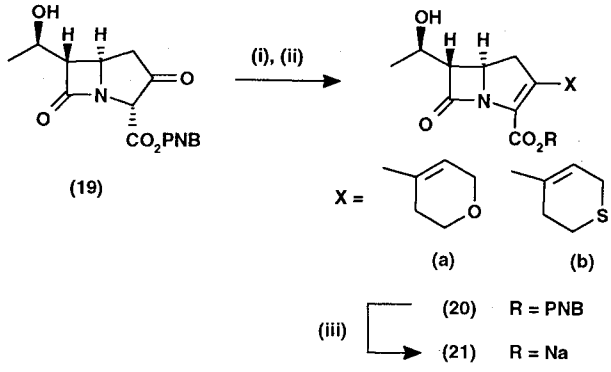
Reagents and conditions: (i) $ZnCl_2$, (4), CH_2Cl_2; (ii) pyridinium *p*-toluenesulphonate, THF; (iii) allyl glyoxylate, benzene or toluene, Δ; (iv) $SOCl_2$, 2,6-lutidine, THF; (v) PPh_3, 2,6-lutidine or PBu_3, DMF; (vi) 5 M HCl, MeOH; (vii) toluene, Δ; (viii) $Pd(Ph_3)_4$, PPh_3, sodium 2-ethylhexanoate, $EtOAc/CH_2Cl_2$.

For X refer to Scheme 1.

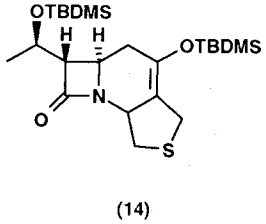
Scheme 3. Synthesis of 1β-methyl substituted carbapenems.



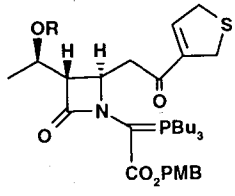
Scheme 4. Introduction of C-2 substituents *via* cross-coupling reactions.



Reagents and conditions: (i) $^iPr_2NH$, Tf_2O, THF, $-20°C$; (ii) Me_3SnX, $Pd_2(dba)_3$, Ph_3As, $ZnCl_2$, LiCl, THF; (iii) Zn, THF/0.35 M phosphate buffer.

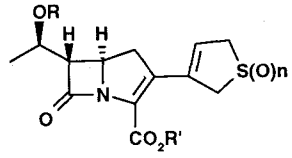


(14)



(15) R = H

(16) R = TMS



(17a) R = TMS, R' = PMB, n = 0

(17b) R = TMS, R' = PMB, n = 2

(18a) R = H, R' = Na, n = 0

(18b) R = H, R' = Na, n = 2

Table 1. Summary of antibacterial activity and stability to DHP-1.

Compound	*E.c.*	*H.i.*	*M.c.* MIC (μg/ml)	*S.a.*	*S.p.*	Stability to DHP-1 (%)
Imipenem (**1**)	0.12	0.25	0.06	0.06	0.06	66*
Meropenem (**2**)	≤0.03	0.13	0.03	0.03	1	85, 88*
9a	0.13	0.13	≤0.06	≤0.06	≤0.06	74
9b	0.13	0.25	≤0.06	≤0.06	0.25	19
9c	0.5	≤0.06	≤0.06	≤0.06	0.25	80
9d	0.13	≤0.06	≤0.06	≤0.06	0.25	37
9e	16	≤0.06	≤0.06	≤0.06	0.12	55
9f	2	≤0.06	≤0.06	≤0.06	0.12	12
12a	4	NT	≤0.06	≤0.06	1	77*
12b	0.25	0.5	≤0.06	0.06	0.13	13*
18a	0.13	0.25	≤0.06	≤0.06	0.13	80, 73*
18b	0.13	4	0.25	0.25	1	71
21a	≤0.06	0.25	≤0.06	≤0.06	≤0.06	77*
21b	0.25	0.25	≤0.06	0.13	≤0.06	77*

Abbreviations: *E.c.*, *Escherichia coli* DCO (TEM 1 β-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: Percentage carbapenem remaining after incubation with human DHP-1 pure enzyme* or human kidney homogenate at 37°C for 60 minutes.

(**18a**) was achieved by treatment with aluminium trichloride.

Oxidation of the dihydrothiophene derivative (**17a**) with *m*-chloroperoxybenzoic acid gave the corresponding sulphone (**17b**). Subsequent removal of the protecting groups provided the carbapenem (**18b**).

Unsaturated substituents can also be introduced directly at C-2 *via* carbon-carbon bond formation[11]. Thus, the dihydropyranyl and dihydrothiopyranyl analogues (**20a**) and (**20b**) were synthesised using palladium catalysed cross-coupling reactions between the requisite heterocyclic stannane and the enol triflate derived from the readily available β-keto ester (**19**)[12]. Triphenylarsine proved to be a versatile ligand for these transformations[13]. Deprotection of the *p*-nitrobenzyl esters (**20a**) and (**20b**) was achieved without double bond reduction using zinc in THF/phosphate buffer[14] to afford carbapenems (**21a**) and (**21b**) (Scheme 4).

Biological Evaluation

The antibacterial activities and susceptibilities to human DHP-1 of these new carbapenems in comparison with values for imipenem and meropenem are reported in Table 1. Compounds were evaluated *in vitro* against a range of important target organisms associated with community acquired infections.

In general, the novel C-2 heterocyclic carbapenems were highly potent against the target organisms. They exhibited useful Gram-negative antibacterial activity and good activity against Gram-positive organisms.

The stereochemistry of the C-2 heterocycle was found to impart a significant effect on the stability of the compound to DHP-1. The C-2 tetrahydrofuran-2-yl (**9a**) and (**9b**), tetrahydrothiophen-2-yl (**9c**) and (**9d**) and 2,3-dihydrobenzofuran-2-yl derivatives (**9e**) and (**9f**) all exhibited the same relationship between stereochemistry and relative DHP-1 stability, the (*S*) diastereoisomer being the more stable. Molecular modelling studies on the tetrahydrothiophen-2-yl diastereoisomers (**9c**) and (**9d**) indicated that the (*S*) isomer tended to favour conformations with the bulk of the tetrahydrothiophene ring on the β-face of the carbapenem nucleus. In contrast, the less stable (*R*) isomer favoured conformations with the bulk of the ring on the α-face. This is in agreement with the observation that a β-methyl group at the C1 position can lead to increased stability[2].

Interestingly, no increase in stability to DHP-1 was observed by the introduction of a 1β-methyl group to the C-2 tetrahydrofuran-2-yl compounds, (**12a**) and (**12b**). Although this is in contrast to results reported for thienamycin[2], it is in accord with literature reports that the incorporation of a 1β-methyl group does not necessarily always result in improved DHP-1 stability[15]. However, as in the 1-unsubstituted series, one diastereoisomer was considerably more stable than the other.

The presence of a double bond conjugated to the carbapenem (**18a**, **18b**, **21a** and **21b**) resulted in compounds exhibiting reasonable stabilities to DHP-1 hydrolysis, albeit less stable than meropenem (**2**).

In conclusion, the novel C-2 heterocyclic carbapenems described herein exhibited good antibacterial activity, especially against Gram-positive organisms. Of the diastereoisomers, the (*S*)-isomer was considerably more stable to DHP-1 than the (*R*)-isomer. Introduction of a double bond into the heterocycle also improved DHP-1 stability. However, none of the carbapenems achieved the level of stability to DHP-1 exhibited by meropenem (**2**).

Experimental

IR spectra were recorded in CH_2Cl_2 solution unless otherwise specified and were recorded on Perkin Elmer 983 or Philips PU9706 spectrometers. UV spectra were recorded on a Beckman DU spectrophotometer in EtOH solution. NMR spectra were recorded on a Bruker AC250 spectrometer in the solvents specified. Mass spectral data were recorded on a VG ZAB1F or a VG Trio-2 spectrometer in electron impact (EI), chemical ionisation using NH_3 gas (CI), electrospray (ESI-MS) or fast atom bombardment (FAB) mode, as specified.

Chromatography was performed on Merck silica 60, <230 or 230~400 mesh. Salts were purified using Dianion HP-20SS resin.

1-*t*-Butyldimethylsilyloxy-1-[(*RS*)-2,3-dihydrobenzofuran-2-yl]ethene (**4e** and **4f**)

t-Butyldimethylsilyl trifluoromethanesulphonate (6.58 ml, 28.64 mmol) in dichloromethane (5 ml) was added dropwise to a solution of (*RS*)-2-acetyl-2,3-dihydrobenzofuran (**3e**) and (**3f**) (3.87 g, 23.89 mmol) and triethylamine (5.0 ml, 35.87 mmol) in dichloromethane (120 ml) at 0°C. The solution was stirred for 1.5 hours, then diluted with dichloromethane, washed with water, dried over magnesium sulphate, concentrated *in vacuo* and chromatographed on silica gel eluting with 10% ethyl acetate in hexane to give a racemic mixture of the title compounds (**4e**) and (**4f**) as a pale yellow oil (6.28 g, 95%); MS (Found: M^+ 276.1549. $C_{16}H_{24}O_2Si$ requires *M* 276.1546); IR ν_{max} (CH_2Cl_2) cm^{-1} 3056, 2931, 2857, 1639, 1594, 1480, 1326, 1231 and 1031; ^1H NMR ($CDCl_3$) δ 0.15 (3H, s), 0.20 (3H, s), 0.81 (9H, s), 3.19 (1H, dd, J=15.6, 7.1 Hz), 3.32 (1H, dd, J=15.6, 9.6 Hz), 4.19 (1H, d, J=1.4 Hz), 4.44 (1H, d, J=0.9 Hz), 5.03 (1H, dd, J=9.6, 7.1 Hz), 6.70 (2H, m) and 7.09 (2H, m).

Compounds **4a**, **4b**, **4c**, **4d** and **4g** were prepared using a similar procedure.

(3*S*,4*R*)-3-[(*R*)-1-(*t*-Butyldimethylsilyloxy)ethyl]-4-[2-oxo-2[(*S*)-2,3-dihydrobenzofuran-2-yl]ethyl]-2-azetidinone (**6e**)

A solution of (**4e**) and (**4f**) (6.28 g, 22.75 mmol) in dichloromethane (50 ml) was added to a solution of (3*R*,4*R*)-4-acetoxy-3-[(*R*)-*t*-butyldimethylsilyloxy)-ethyl]-1-trimethylsilyl-2-azetidinone (**5**)[7] (7.5 g, 20.89 mmol) in dichloromethane (100 ml). To this mixture was added a solution of zinc chloride (21 ml of a 1 M solution in diethylether, 21.0 mmol) dropwise over 10 minutes. After stirring for 16 hours at room temperature, the mixture was diluted with dichloromethane and washed successively with water, saturated aqueous sodium hydrogen carbonate and brine. The solution was dried over magnesium sulphate and the solvent evaporated *in vacuo* to yield a yellow gum. The gum was dissolved in tetrahydrofuran (100 ml) and water (10 ml) and treated with pyridinium *p*-toluenesulphonate (50 mg). After stirring for 2 hours at room temperature, the mixture was diluted with ethyl acetate, washed with water (×2), dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by chromatography on silica gel eluting with 30% ethyl acetate in hexane to yield the title compound (**6e**) as a pale yellow oil (2.05 g, 25%); MS (Found: M^+ 389.2025. $C_{21}H_{31}NO_4Si$ requires *M* 389.2022); IR ν_{max} (CH_2Cl_2) cm^{-1} 3410, 2955, 2856, 1763, 1719, 1480, 1375 and 1143; ^1H NMR ($CDCl_3$) δ 0.07 (6H, s), 0.88 (9H, s), 1.22 (3H, d, J=6.2 Hz), 2.77 (1H, dd, J=18.7, 10.1 Hz), 2.79 (1H, dd, J=5.1, 2.3 Hz), 3.24 (1H, dd, J=18.7, 3.2 Hz), 3.27 (1H, dd, J=16.1, 6.4 Hz), 3.52 (1H, dd, J=16.1, 10.8 Hz), 3.98 (1H, m), 4.18 (1H, m), 5.07 (1H, dd, J=10.8, 6.4 Hz), 5.95 (1H, br s), 6.90 (2H, m) and 7.19 (2H, m). Also obtained was the (*R*)-isomer (**6f**) as a colourless oil (1.87, 23%); MS (Found: M^+ 389.2018. $C_{21}H_{31}NO_4Si$ requires *M* 389.2022); IR ν_{max} (CH_2Cl_2) cm^{-1} 3410, 2930, 2856, 1763, 1718, 1480, 1462 and 1375; ^1H NMR ($CDCl_3$) δ 0.02 (3H, s), 0.04 (3H, s), 0.82 (9H, s), 1.19 (3H, d, J=6.3 Hz), 2.70 (1H, dd, J=5.1, 2.3 Hz), 2.92 (1H, dd, J=18.6, 9.9 Hz), 3.12 (1H, dd, J=18.6, 3.4 Hz), 3.30 (1H, dd, J=16.1, 6.4 Hz), 3.50 (1H, dd, J=16.1, 10.8 Hz), 3.92 (1H, m), 4.13 (1H, m), 5.06 (1H, dd, J=10.8, 6.4 Hz), 6.01 (1H, br s), 6.90 (2H, m) and 7.18 (2H, m).

Compounds **6a**, **6b**, **6c**, **6d** and **6g** were prepared using a similar procedure.

Allyl 2-[(3*S*,4*R*)-3-[(*R*)-1-Hydroxyethyl]-4-[2-oxo-2-[(*S*)-2,3-dihydrobenzofuran-2-yl]ethylazetidin-2-on-1-yl]-2-triphenylphosphoranylidene Acetate (**7e**)

Allyl glyoxylate (770 mg, 5.83 mmol) in benzene (30 ml) was heated at reflux for 1 hour using Dean and Stark apparatus. The solution was cooled to room temperature and treated with (**6e**) (2.05 g, 5.27 mmol). The mixture was stirred for 16 hours at room temperature, concentrated and then purified by chromatography on silica gel eluting with 20 and 30% ethyl acetate in hexane to yield allyl (*RS*)-2-hydroxy-2-[(3*S*,4*R*)-3-[(*R*)-1-*t*-butyldimethylsilyl-oxyethyl]-4-[2-oxo-2-[(*S*)-2,3-dihydrobenzofuran-2-yl]ethylazetidin-2-on-1-yl]acetate (2.46 g, 93%); IR ν_{max} (CH_2Cl_2) cm^{-1} 3513, 2955, 2856, 1763, 1480 and 1374; MS *m/z* (CI, +ve ion, ammonia) 504 (MH^+), 521 (MNH_4^+).

Thionyl chloride (260 ml, 3.56 mmol) was added dropwise to the hydroxy compound (1.20 g, 2.39 mmol) and 2,6-lutidine (420 ml, 3.61 mmol) in tetrahydrofuran (40 ml) at −10°C. After stirring for 20 minutes, the

reaction mixture was filtered through a pad of celite, and the filtrate evaporated *in vacuo*. Toluene was added and re-evaporated (×2) to yield allyl-(*RS*)-2-[(3*S*,4*R*)-3-[(*R*)-1-*t*-butyldimethylsilyloxyethyl]-4-[2-oxo-2-[(*S*)-2,3-dihydro-benzofuran-2-yl]ethylazetidin-2-on-1-yl]-2-chloro-acetate.

The crude chloro compound was dissolved in dioxan (12 ml) and treated with triphenylphosphine (2.51 g, 9.58 mmol) at room temperature. The solution was concentrated to approximately half of its volume and was then treated with 2,6-lutidine (309 ml, 2.65 mmol). After stirring for 3.5 hours the mixture was diluted with ethyl acetate, washed successively with 5% aqueous citric acid, brine, saturated aqueous sodium hydrogen carbonate solution and brine, dried over magnesium sulphate and then concentrated *in vacuo*. The residue was purified by chromatography on silica gel eluting with ethyl acetate to yield allyl 2-[(3*S*,4*R*)-3-[(*R*)-1-*t*-butyldimethylsilyloxyethyl]-4-[2-oxo-2-[(*S*)-2,3-dihydrobenzofuran-2-yl]ethylazetidin-2-on-1-yl]-2-triphenylphosphoranylidene acetate (1.17 g, 66%); IR ν_{max} (CH_2Cl_2) cm^{-1} 2930, 2856, 1740, 1615, 1480 and 1105; MS *m/z* (FAB, +ve ion, glycerol) 748 (MH^+), 770 (MNa^+).

For the preparation of **7a**~**d** tributylphosphine was used instead of triphenylphosphine and 2,6-lutidine.

A solution of the phosphorane (1.15 g, 1.54 mmol) in methanol (50 ml) was treated with hydrochloric acid (14 ml, 2 M) and stirred for 3 hours at room temperature. The pH was adjusted to 7 by the addition of saturated aqueous sodium hydrogen carbonate solution, and then the methanol was evaporated *in vacuo*. The product was extracted into ethyl acetate (×3) and the combined organic solution washed with brine, dried over magnesium sulphate and concentrated *in vacuo* to give the title compound (**7e**) as a yellow foam (950 mg, 97%); IR ν_{max} (CH_2Cl_2) cm^{-1} 3495, 1744, 1616, 1480, 1438, 1233 and 1106; MS *m/z* (FAB, +ve ion, glycerol) 634 (MH^+), 656 (MNa^+).

Compounds **7a**, **7b**, **7c**, **7d**, **7f** and **7g** were prepared using a similar procedure.

Allyl (5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(*S*)-2,3-dihydrobenzofuran-2-yl]carbapen-2-em-3-carboxylate (**8e**)

A solution of the phosphorane (940 mg, 1.48 mmol) (**7e**) in toluene (200 ml) was heated at reflux for 1 hour. The reaction mixture was cooled, concentrated *in vacuo* and the residue purified by chromatography on silica gel eluting with ethyl acetate to yield the title compound (**8e**) (467 mg, 89%); MS (Found: M^+ 355.1422. $C_{20}H_{21}NO_5$ requires *M* 355.1419); IR ν_{max} (CH_2Cl_2) cm^{-1} 3601, 1780, 1719, 1479, 1372, 1332, 1281, 1230, 1200 and 1098; ^1H NMR ($CDCl_3$) δ 1.30 (3H, d, J=6.3 Hz), 1.70 (1H, d, J=4.9 Hz, exch), 2.75 (1H, dd, J=18.8, 8.5 Hz), 2.96 (1H, dd, J=16.0, 7.3 Hz), 3.15 (1H, dd, J=6.6, 2.7 Hz), 3.17 (1H, dd, J=18.8, 10.2 Hz), 3.61 (1H, dd, J=16.0, 9.9 Hz), 4.15~4.27 (2H, m), 4.70~4.89 (2H, m), 5.26~5.48 (2H, m), 5.99 (1H, m), 6.23 (1H, dd, J=9.9, 7.3 Hz), 6.79~6.90 (2H, m) and 7.10~7.19 (2H, m).

Compounds **8a**, **8b**, **8c**, **8d** and **8f** were prepared using a similar procedure.

Sodium (5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(*S*)-2,3-dihydrobenzofuran-2-yl]carbapen-2-em-3-carboxylate (**9e**)

A solution of the carbapenem (200 mg, 0.56 mmol) (**8e**) in 1 : 1 ethyl acetate - dichloromethane (6 ml) was treated successively with triphenylphosphine (15 mg, 0.057 mmol), a solution of sodium 2-ethylhexanoate (93 mg, 0.56 mmol) in ethyl acetate (2 ml), then tetrakis(triphenylphosphine)palladium(0) (22 mg, 0.019 mmol). After stirring for 10 minutes the mixture was concentrated *in vacuo* and the residue triturated with diethyl ether. The product was purified by HP20SS chromatography eluting with water, then 1, 2, 5 and 10% THF in water. Fractions containing the product (HPLC analysis) were combined, concentrated and freeze-dried to give the title compound (**9e**) (132 mg, 70%); UV λ_{max} (H_2O) nm (ε) 276 (8463); IR ν_{max} (KBr) cm^{-1} 3423, 1759, 1596, 1400 and 1224; ^1H NMR (D_2O) δ 1.21 (3H, d, J=6.4 Hz), 2.71 (2H, d, J=9.2 Hz), 3.04 (1H, dd, J=16.2, 6.5 Hz), 3.34 (1H, dd, J=5.9, 2.9 Hz), 3.54 (1H, dd, J=16.2, 9.9 Hz), 4.10~4.23 (2H, m), 6.25 (1H, dd, J=9.9, 6.5 Hz), 6.70~6.91 (2H, m) and 7.15~7.30 (2H, m); MS *m/z* (FAB, +ve ion, glycerol) 338 (MH^+).

Compounds **9a**, **9b**, **9c**, **9d** and **9f** were prepared using a similar procedure. The spectral data for **9a**, **9b**, **9c**, **9d** and **9f** are listed in Table 2.

(3*S*,4*R*)-4-{(2*R*)-1-Oxo-1-[(*RS*)-tetrahydrofuran-2-yl]-propan-2-yl}-3-[(*R*)-1-*t*-butyldimethylsilyloxyethyl]-azetidin-2-one (**11**)

A solution of (2-tetrahydrofuranyl)tri-*n*-butylstannane[16] (3.94 g, 10.9 mmol) in dry tetrahydrofuran (20 ml) under argon was cooled to −70°C and a 1.26 M solution of *n*-butyllithium in hexane (8.67 ml, 10.9 mmol) was added. The mixture was stirred for 15 minutes and then transferred *via* a double-ended needle into a flask containing a solution of copper(I) bromide-dimethylsulphide complex (1.12 g, 5.45 mmol) in dry THF (20 ml) containing dimethylsulphide (10 ml) under argon, and cooled to −70°C. The mixture was stirred in the cold for 30 minutes to give a solution of the copper complex (**13**). This solution of the copper complex was transferred *via* a double-ended needle into a flask containing a solution of (3*S*,4*S*)-3-[(*R*)-*t*-butyldimethylsilyloxyethyl]-4-{(*R*)-1-[(3-methylpyridin-2-ylmethylthio)carbonyl]ethyl}-azetidin-2-one (**10**)[9] (2.09 g, 4.95 mmol) in dry THF under argon, and cooled to −70°C. The mixture was stirred in the cold for 4.5 hours. Saturated aqueous ammonium chloride solution (30 ml) was then added by syringe, and the mixture was then poured into ethyl acetate (250 ml)/saturated aqueous ammonium chloride solution (100 ml). After separation the aqueous layer was re-extracted with ethyl acetate (3 × 75 ml). The combined organic layers were dried over magnesium sulphate,

filtered through Kieselguhr and evaporated *in vacuo*. The residue was chromatographed on silica gel eluting with CH_2Cl_2, followed by CH_2Cl_2/ethyl acetate mixtures to give the title compound (**11**) (950 mg, 54%); MS (Found: M^+ 355.2186. $C_{18}H_{33}NO_4$ requires M 355.2179); IR ν_{max} (CH_2Cl_2) cm^{-1} 3409, 1762 and 1712; ^1H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.87 (9H, s), 1.1~1.21 (6H, m), 1.82~2.0 (3H, m), 2.1~2.22 (1H, m), 2.87 (dd, J=4.9, 1.8 Hz), 2.98 (dd, J=3.6, *ca.* 1 Hz) (combined integrals 2.87, 2.98 signals 1H), 3.19~3.35 (1H, m), 3.80~3.98 (3H, m), 4.16 (1H, pentet, J *ca.* 6 Hz), 4.30~4.40 (1H, m), 5.86 (br s) and 5.95 (br s) (combined integral 5.86 and 5.95 signals 1H). The NMR spectrum indicated that the diastereoisomeric ratio was *ca.* 13:9.

Preparation of compounds **12a** and **12b** was carried out by a method similar to that described for **9e**. The isomers were separated by chromatography on silica gel after conversion to the *n*-tributylphoshorane. The spectral data for **12a** and **12b** are listed in Table 2.

4-Methoxybenzyl 2-[(3*S*,4*R*)-3-[(1*R*)-1-(Trimethylsilyloxy)ethyl]-4-[2-oxo-2-(2,5-dihydrothiophen-3-yl)-ethylazetidin-2-on-1-yl]-2-triphenylphosphoranylidene Acetate (**16**)

4-Methoxybenzyl 2-[(3*S*,4*R*)-3-[(1*R*)-1-hydroxyethyl]-4-[2-oxo-2-(2,5-dihydrothiophen-3-yl)ethylazetidin-2-on-1-yl]-2-triphenylphosphoranylidene acetate (**15**) (340 mg, 0.46 mmol) (prepared using an analogous method to that of **7e** except that 4-methoxybenzyl glyoxylate was used instead of allyl glyoxylate) in dichloromethane (15 ml) was cooled to 0°C and treated with trimethylsilyl chloride (140 ml, 1.10 mmol) and triethylamine (154 ml, 1.10 mmol). After stirring for 1 hour at 0°C, the reaction mixture was diluted with ethyl acetate, washed successively with dilute aqueous sodium hydrogen carbonate and brine, dried over magnesium sulphate and the solvent evaporated to yield the crude title compound (**16**) (370 mg, 97%); IR ν_{max} (CH_2Cl_2) cm^{-1} 2961, 1737, 1668, 1630, 1612, 1514, 1374 and 1174; MS m/z (EI) 691 (M^+), (CI, +ve ion, ammonia) 692 (MH^+).

4-Methoxybenzyl (5*R*,6*S*)-6-[(1*R*)-1-(Trimethylsilyloxy)ethyl]-2-(2,5-dihydrothiophen-3-yl)carbapen-2-em-3-carboxylate (**17a**)

A solution of the crude phosphorane (**16**) (350 mg, 0.51 mmol) in toluene (100 ml) was heated at reflux for 1 hour. The reaction mixture was cooled, concentrated and the residue purified by chromatography on silica gel eluting with 10 and 20% ethyl acetate in hexane yielding the title compound (**17a**) (125 mg, 52%); IR ν_{max} (CH_2Cl_2) cm^{-1} 2960, 1779, 1718, 1612, 1516, 1302 and 1174; ^1H NMR ($CDCl_3$) δ 0.12 (9H, s), 1.27 (3H, d, J=6.2 Hz), 3.02~3.08 (2H, m), 3.13 (1H, dd, J=6.6, 2.9 Hz), 3.62~3.77 (3H, m), 3.80 (3H, s), 4.02~4.22 (3H, m), 5.20 (2H, s), 5.99 (1H, m), 6.88 (2H, d, J=8.7 Hz) and 7.36 (2H, d, J=8.7 Hz); MS m/z (EI) 473 (M^+), (CI, +ve ion, ammonia) 474 (MH^+).

Sodium (5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-(2,5-dihydrothiophen-3-yl)carbapen-2-em-3-carboxylate (**18a**)

Anisole (2.5 ml) was added to a solution of aluminium chloride (138 mg, 1.04 mmol) in dichloromethane (2 ml) at −40°C. The solution was cooled to −60°C, and treated with a solution of the carbapenem ester (**17a**) (120 mg, 0.25 mmol) in dichloromethane (5 ml). After stirring for 40 minutes at −60°C, the mixture was treated with a solution of sodium hydrogen carbonate (255 mg, 3.03 mmol) in phosphate buffer (1.8 ml of 0.2 M Na_2HPO_4 and 1.2 ml of 0.2 M NaH_2PO_4). The thick white suspension was stirred for 30 minutes at room temperature and then filtered through celite. The filtrate was washed with dichloromethane (×2) and the aqueous solution purified by HP20SS chromatography eluting with water. Fractions containing the product (HPLC analysis) were combined, concentrated and freeze-dried to give the title compound (**18a**) (8 mg, 10%); UV λ_{max} (H_2O) nm (ε) 292 (6611); IR ν_{max} (KBr) cm^{-1} 3462, 1752, 1602, 1395, 1249 and 1133; ^1H NMR (D_2O) δ 1.25 (3H, d, J=6.4 Hz), 2.98 (1H, dd, J=16.2, 9.9 Hz), 3.10 (1H, dd, J=16.2, 8.3 Hz), 3.42 (1H, dd, J=5.9, 2.9 Hz), 3.62~3.77 (3H, m), 4.00~4.25 (3H, m) and 5.89 (1H, m); MS m/z (IS +ve ion, MeCN/aq NH_4OAc) 304 (MH^+), 326 (MNa^+).

Compound **18b** was prepared using a similar procedure. The spectral data for compound **18b** is listed in Table 2.

4-Methoxybenzyl (5*R*,6*S*)-6-[(*R*)-1-Trimethylsilyloxyethyl]-2-(2,5-dihydrothiophen-3-yl-1,1-dioxide)carbapen-2-em-3-carboxylate (**17b**)

4-Methoxybenzyl (5*R*,6*S*)-6-[(*R*)-1-trimethylsilyloxyethyl]-2-(2,5-dihydrothiophen-3-yl)carbapen-2-em-3-carboxylate (**17a**) (30 mg, 0.063 mmol) was dissolved in dichloromethane (2 ml) and cooled to 0°C. Sodium hydrogen carbonate (11 mg, 0.131 mmmol) was added, followed by the dropwise addition of a solution of *m*-chloroperoxybenzoic acid (27 mg, 0.125 mmol) in dichloromethane (2 ml). The mixture was stirred at 0°C for 30 minutes and then diluted with ethyl acetate. The organic solution was washed with dilute aqueous sodium hydrogen carbonate solution (×2), water and brine, dried over magnesium sulphate and concentrated *in vacuo*. The residue was purified by chromatography on silica gel eluting with 50% ethyl acetate in hexane to yield the title compound (**17b**) (26 mg, 82%); IR ν_{max} (CH_2Cl_2) cm^{-1} 2960, 1784, 1719, 1613, 1516, 1319, 1252 and 1132; ^1H NMR ($CDCl_3$) δ 0.07 (9H, s), 1.21 (3H, d, J=6.2 Hz), 3.05 (1H, d, J=10.0 Hz), 3.07 (1H, d, J=8.9 Hz), 3.14 (1H, dd, J=6.3, 3.0 Hz), 3.70~3.79 (3H, m), 3.74 (3H, s), 4.07~4.25 (3H, m), 5.16 (2H, s), 5.98 (1H, m), 6.84 (2H, d, J=8.7 Hz) and 7.33 (2H, d, J=8.7 Hz); MS m/z (EI) 505 (M^+), (CI, +ve ion, ammonia) 506 (MH^+), 523 (MNH_4^+).

Table 2. Spectral data for carbapenem sodium salts.

Compound	IR (KBr) β-lactam cm^{-1}	^1H NMR (D_2O unless stated)
9a	1754	(d_6-DMSO) 1.13 (3H, d, J=6.3 Hz), 1.4~1.6 (1H, m), 1.7~2.1 (3H, m), 2.65 (1H, s), 2.69 (1H, s), 2.98 (1H, dd, J=6.9, 2.6 Hz), 3.5~3.95 (4H, m), 4.95 (1H, br s, exch) and 5.41 (1H, t, J=7.6 Hz)
9b	1754	(d_6-DMSO) 1.13 (3H, d, J=6.2 Hz), 1.4~2.1 (4H, m), 2.6~2.8 (2H, m), 2.95 (1H, dd, J=6.9, 2.6 Hz), 3.5~3.9 (4H, m), 4.93 (1H, d, J=4.9, exch) and 5.47 (1H, t, J=7.5 Hz)
9c	1752	1.35 (3H, d, J=6.0 Hz), 1.82 (1H, m), 2.01 (1H, m), 2.25 (2H, m), 2.87~3.11 (3H, m), 3.22 (1H, dd, J=17.5, 9.5 Hz), 3.41 (1H, dd, J=6.0, 2.5 Hz), 4.20 (1H, dt, J=9.0, 2.5 Hz), 4.28 (1H, q, J=6.0 Hz) and 5.24 (1H, t, J=7.0 Hz)
9d	1750	1.34 (3H, d, J=6.0 Hz), 1.87 (1H, m), 2.00 (1H, m), 2.22 (2H, m), 2.99 (3H, m), 3.18 (1H, dd, J=18.0, 8.5 Hz), 3.42 (1H, m), 4.18 (1H, m), 4.27 (1H, m) and 5.13 (1H, t, J=7.0 Hz)
9f	1757	1.26 (3H, d, J=6.4 Hz), 2.67 (1H, d, J=9.7 Hz), 2.69 (1H, d, J=8.6 Hz), 3.11 (1H, dd, J=16.2, 6.3 Hz), 3.35 (1H, dd, J=5.9, 2.8 Hz), 3.54 (1H, dd, J=16.2, 9.8 Hz), 4.08~4.25 (2H, m), 6.28 (1H, dd, J=9.8, 6.3 Hz), 6.82~6.99 (2H, m) and 7.17~7.30 (2H, m)
12a	1744	1.24 (3H, d, J=7.3 Hz), 1.36 (3H, d, J=6.4 Hz), 1.84 (1H, dq, J=12.2, 8.6 Hz), 1.98~2.11 (2H, m), 2.36~2.43 (1H, m), 3.41 (1H, dq, J=9.5, 7.4 Hz), 3.47 (1H, dd, 6.2, 2.6 Hz), 3.88 (1H, q, J=7.3 Hz), 4.05 (1H, q, J=7.3 Hz), 4.20 (1H, dd, J=2.5, 9.6 Hz), 4.30 (1H, quintet, J=6.2 Hz) and 5.14 (1H, t, J=8.2 Hz)
12b	1732	1.13 (3H, d, J=7.2 Hz), 1.27 (3H, d, J=6.3 Hz), 1.36~1.43 (1H, m), 1.73~2.0 (2H, m), 2.12~2.21 (1H, m), 3.20 (1H, m), 3.36 (1H, dd, J=6.3, 2.5 Hz), 3.7~3.9 (2H, m), 4.10 (1H, dd, J=9.2, 2.4 Hz), 4.20 (1H, m) and 5.18 (1H, approx t, J=*ca.* 7.2 Hz)
18b	1753	1.26 (3H, d, J=6.4 Hz), 3.13 (2H, m), 3.47 (1H, dd, J=5.8, 3.0 Hz), 3.92~4.34 (6H, m) and 6.04 (1H, s)
21b	1738	1.27 (3H, d, J=6.4 Hz), 2.25~2.42 (1H, m), 2.51~2.68 (1H, m), 2.78 (2H, t, J=5.8 Hz), 2.85 (1H, dd, J=16.6, 9.8 Hz), 3.10 (1H, dd, J=16.7, 8.5 Hz), 3.20~3.33 (2H, m), 3.42 (1H, dd, J=6.0, 2.7 Hz), 4.10~4.30 (2H, m) and 5.88~5.97 (1H, m)

4-Nitrobenzyl (5*R*,6*S*)-2-(5,6-Dihydro-2H-pyran-4-yl)-6-[(*R*)-hydroxyethyl]carbapen-2-em-3-carboxylate (**20a**)

Diisopropylamine (90 ml, 0.64 mmol) was added to a solution of 4-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-oxocarbapenam-3-carboxylate (**19**)[12] (200 mg, 0.57 mmol) in THF (7 ml), cooled to −70°C under argon, followed after 5 minutes by trifluoromethanesulphonic anhydride (105 μl, 0.62 mmol). The triflate solution was then allowed to stir with cooling for 30 minutes. Triphenylarsine (18 mg, 0.059 mmol) was added to a solution of tris(dibenzylideneacetone)palladium(0) (26 mg, 0.028 mmol) in THF (3 ml). After stirring for 5 minutes the catalyst was added to the crude triflate solution at −70°C (rinsed in with THF (2 ml)). Zinc chloride (1.15 ml, 1 M in ether, 1.15 mmol) and solid anhydrous lithium chloride (49 mg, 1.15 mmol) were then added followed by 4-trimethyl-stannyl-5,6-dihydo-2H-pyran[5] (154 mg, 0.62 mmol) in THF (7 ml). The cooling bath was removed and the reaction mixture stirred for 2 hours. Concentration and chromatography on silica gel eluting with 70% ethyl acetate in hexane gave the title compound (**20a**) (138 mg, 58%); MS (Found: M^+ 414.1435. $C_{21}H_{22}N_2O_7$ requires M 414.1427); IR ν_{max} (CH_2Cl_2) cm^{-1} 3326, 1777 and 1732; ^1H NMR ($CDCl_3$) δ 1.37 (3H, d, J=6.3 Hz), 1.7 (1H, br s), 2.16 (1H, m), 2.44 (1H, m), 3.07 (2H, d, J=9.8 Hz), 3.21 (1H, dd, J=6.7, 2.8 Hz), 3.78 (2H, m), 4.22 (4H, m), 5.26 (1H, d, J=13.7 Hz), 5.44 (1H, d, J=13.7 Hz), 5.89 (1H, m), 7.64 (2H, d, J=8.7 Hz) and 8.23 (2H, d, J=8.7 Hz); MS m/z (EI) 414.

Compound **20b** was prepared using a similar procedure.

Sodium-(5*R*,6*S*)-2-(5,6-dihydro-2H-pyran-4-yl)-6-[(*R*)-1-hydroxy-ethyl]carbapen-2-em-3-carboxylate (**21a**)

A suspension of 4-nitrobenzyl (5*R*,6*S*)-2-(5,6-dihydro-2H-pyran-4-yl)-6-[(*R*)-hydroxyethyl]carbapen-2-em-3-carboxylate (**20a**) (138 mg, 0.33 mmol) in THF-0.35 M phosphate buffer (1:3, pH 6) (5 ml) was treated with activated zinc (1.4 g) and the mixture rapidly stirred for 30 minutes. The mixture was filtered through celite and the residue thorougly washed with water. After adjusting the pH of the filtrate to pH 6.5 it was washed with ethyl acetate and concentrated to approximately 10 ml. The crude solution was chromatographed on HP20SS eluting with water then 1~10% THF in water to afford the title compound (**21a**) (28 mg, 28%); UV λ_{max} (H_2O) nm (ε) 290 (7354); IR ν_{max} (KBr) cm^{-1} 3433, 1749 and 1734; ^1H NMR (D_2O) δ 1.24 (3H, d, J=6.3 Hz), 2.12 (1H, br d, J=16.9 Hz), 2.54 (1H, br d, J=16.9 Hz), 2.90 (1H, dd, J=16.4, 9.9 Hz), 3.05 (1H, dd, J=16.4, 8.4 Hz), 3.39 (1H, dd, J=5.6, 2.5 Hz), 3.73 (1H, m), 3.86 (1H, dt, J=11.3, 5.0 Hz), 4.20 (4H, m) and 5.71 (1H, m); MS m/z (FAB, +ve ion, thioglycerol) 302 (MH^+).

Compound **21b** was prepared using a similar procedure. The spectral data for **21b** is listed in Table 2.

Biology

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 compound stability to human DHP-1

The test compound (0.5 mmol solution in 0.02 M MOPS at pH 7.0) (0.08 ml) was challenged with either human kidney homogenate or pure human 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.

Acknowledgements

The authors wish to thank Dr. I. S. BENNETT for microbiological results, Mr. J. W. TYLER, Mr. G. D. RISBRIDGER and Mr. E. A. CUTMORE for NMR, MS, IR and UV spectra.

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