2-(HETEROATOM-SUBSTITUTED)METHYL PENEMS. IV.[1] OXYGEN DERIVATIVES

*Marco Alpegiani, Angelo Bedeschi, Franco Giudici, Ettore Perrone, Giuseppina Visentin, Franco Zarini, and Giovanni Franceschi**

Farmitalia Carlo Erba, Research & Development, Infectious Diseases Dep., Via dei Gracchi 35, Milano, Italy

Abstract — The synthesis of "2-CH_2X" penems wherein X is an oxygen atom part of an acyloxy, N-substituted carbamoyloxy, alkoxy or aryloxy residue is described, with emphasis to procedures which exploit a common 2-hydroxymethylpenem precursor. Correlations are attempted between chemical structure of the X moiety and antibacterial activity of obtained compounds.

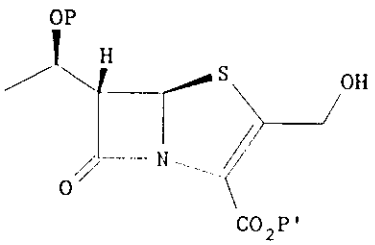
The subtle influence exerted by the C_2-functionalization of penems on their antibacterial properties has fostered chemists to synthesize hundreds of compounds differing for the sidechain at this position.[2] Structural analogies with carbapenems and cephalosporins, the carbocyclic and homo counterparts of penems, suggested an attentive investigation in the class of 2-alkylthio and 2-(heteroatom-substituted)methyl derivatives. Recent papers from our laboratories dealt with "2-CH_2X" penems where X is sulphur[3,4] or nitrogen.[1] This work describes the 2-oxymethylpenems, allocated into three main subclasses, depending on the oxygen being part of an ester (2~25, Scheme I), a carbamate (26~41, Scheme II), or an ether (43~66, Scheme III). For brevity, only the most direct synthetic access will be reported, whenever possible exploiting the pivotal 2-hydroxymethyl intermediate,[5] variously protected at the C_3-carboxyl and C_8-hydroxyl (1a,a',a'').

2-(Acyloxymethyl)penems. Compound 3c, bearing the acetoxymethyl sidechain featured by the natural cephalosporin C and by some commercial hemisynthetic cephalosporins (e.g., cefotaxime) was the first target in our programme. Its *in vitro* activity,[6] one order of magnitude higher than that displayed by the reference compound (68, Table III), encouraged the synthesis of analogs (Scheme I). Most of the modifications here described were performed in the attempt to improve stability towards human serum esterases (through bulkier acyl residues[7]: 4c, 5c, 11c), penetration across the outer membrane of Gram-negative bacteria (introduction of charged residues: 13c, 23~25), activity against *Pseudomonas* spp. (basic groups: 23), and intrinsic antibacterial potency (screening of different sidechains, including the aminothiazolyl-*Z*-methoxyiminoacetic[8] group: 8c). The method of choice was the mild Mitsunobu-Volante procedure,[9] involving treatment of a suitably protected carbinol (1a,a') and the appropriate carboxylic acid (1~1.2 mol equiv., THF, a few min) with a slight excess of preformed triphenylphosphine - diethyl azodicarboxylate complex (TPP-DEAD). Accordingly, the fully protected penems 2~5a,7a,10a,16a' were isolated in yields ranging from 75% to 95% after flash chromatography. Other methods from 1a included direct acylation with acid chlorides (12a: allyloxyoxalyl chloride, NEt_3, CH_2Cl_2, 69%; 14a: ethyl chlorocarbonate, NEt_3, CH_2Cl_2, 85%) and ketene addition (6a: diketene, cat. amount of NEt_3, CH_2Cl_2, 1 h, 45%).

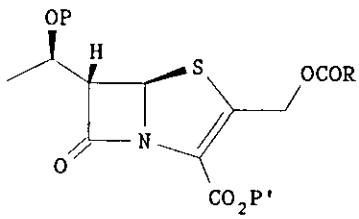
Scheme I

2-(Acyloxymethyl)penems

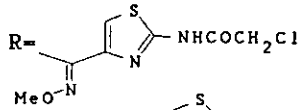
a	P= $SiMe_2t$-Bu	P'= allyl
b	P= H	P'= allyl
c	P= H	P'= Na
a'	P= $SiMe_2t$-Bu	P'= *p*-NB
b'	P= H	P'= *p*-NB

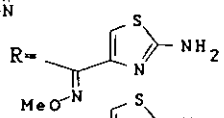


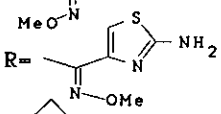
1 a, a', b

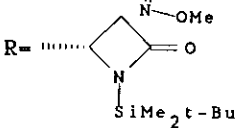


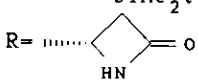
2 a,b,c R= H
3 a,b,c R= Me
4 a,b,c R= *n*-Pr
5 a,b,c R= Ph
6 a,b,c R= CH_2COMe

7 a 

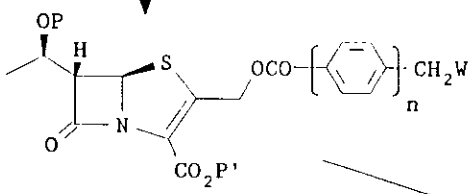
8 a,b,c 

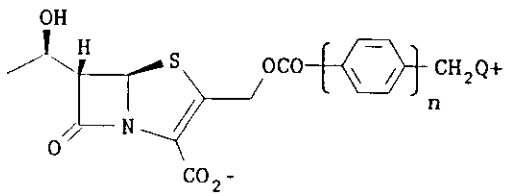
9 a 

10 a 

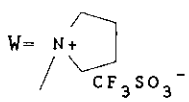
11 b,c 

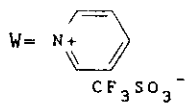
12 a,b R= $CO_2CH_2CH{=}CH_2$
13 c R= CO_2Na
14 a,b,c R= OEt
15 a R= OMe



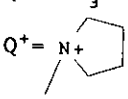


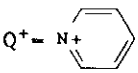
16 a',b' W= $NHCO_2p$-NB n= 0
17 a W= $OSiPh_2t$-Bu n= 0
18 a W= $OSiPh_2t$-Bu n= 1
19 a W= OH n= 0
20 a W= OH n= 1

21 a,b  n= 0

22 a,b  n= 1

23 Q^+= NH_3^+ n= 0

24 Q^+=  n= 0

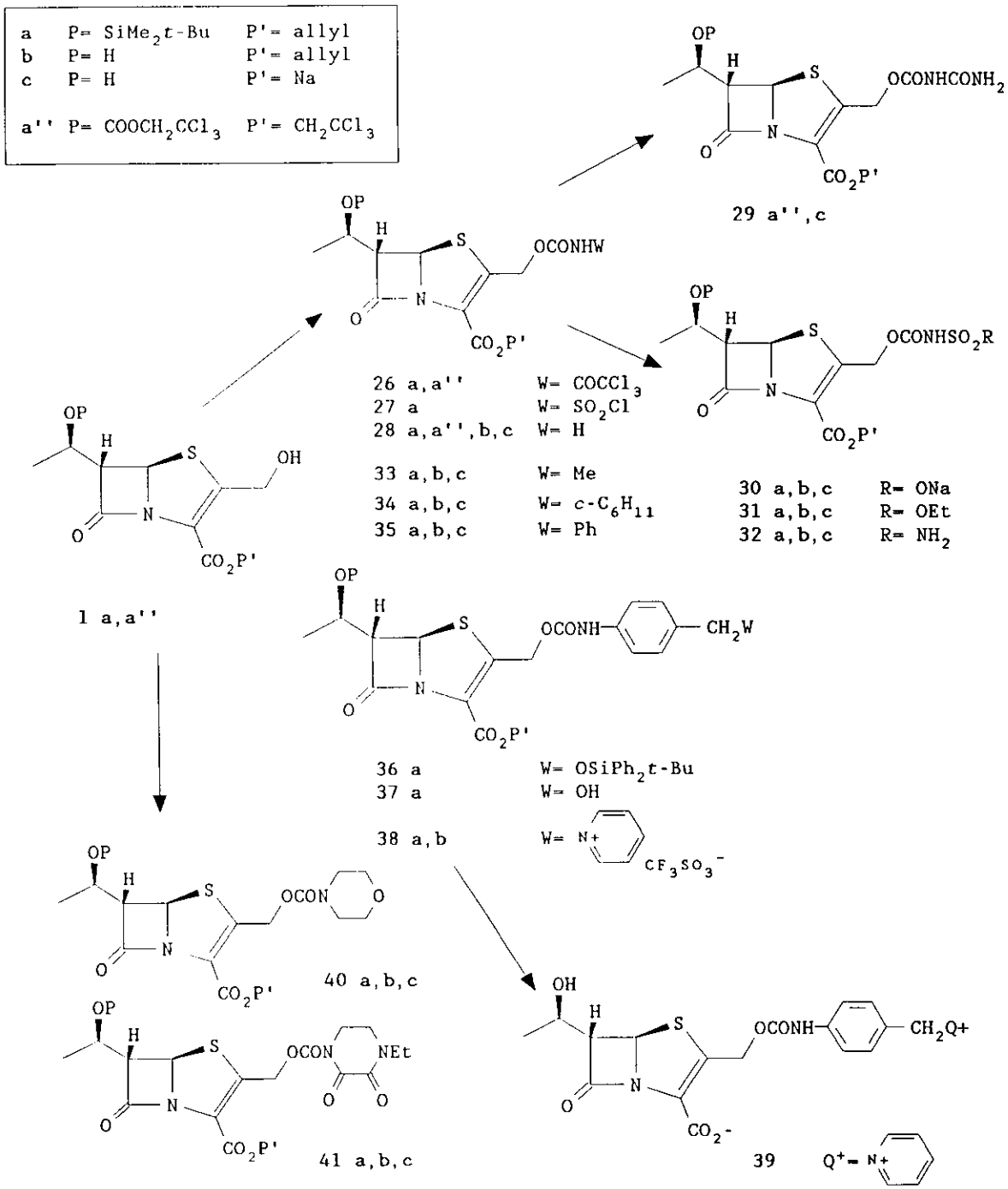
25 Q^+=  n= 1

Functional protecting groups in the acyl residue (10a, 12a, 16a') were selected in order to allow concomitant deblocking, with the exception of *N*-chloroacetyl in 7a, which required an extra deprotection step (thiourea 4 mol equiv., EtOH, 6 h; 85%). Partial oxime isomerization (*Z*:*E* = 3:1) accompanied dechloroacetylation; on prolonging the reaction time, the *E* (*anti*) isomer 9a was exclusively isolated.
The quaternary ammonium derivatives 21a, 22a were obtained from the novel carbinol intermediates 19a and 20a (3 mol equiv. of tertiary amine, 1.5 equiv. of triflic anhydride, CH_2Cl_2, -50°C; quenching with 4% aq. HCl and EtOAc extraction), in turn prepared from 1a by Mitsunobu condensation with silylated glycolic[10] and *p*-hydroxymethylbenzoic acid[11] (84% and 98%, respectively), followed by selected unmasking of the primary hydroxyl of obtained 17a, 18a ($Bu_4NF \cdot 3H_2O$, HOAc-THF, 1 h, 56% and 90%).
Removal of C_8-hydroxyl and C_3-carboxyl protecting groups was achieved by procedures by now customary in penem chemistry. Thus, prolonged (12~24 h) exposure to the fluoride reagent afforded 2~6b, 8b, 14b, 16b', 21b, 22b (65~85%) from the corresponding *tert*-butyldimethylsilyl ethers. The bis-silylated compound 10a was deprotected to 11b (60%). Compound 12a afforded a mixture of the expected product 12b (34%) and the deacylated penem 1b (25%), owing to competitive fluoride attack at the oxalic ester moiety. Palladium-mediated transallylation ($Pd(PPh_3)_4$ 0.2 mol equiv., PPh_3, CH_2Cl_2, 15 min) with sodium 2-ethylhexanoate (1.2 mol equiv.) afforded the sodium salts 2~6c, 8c, 11c, 14c and the disodium salt 13c, isolated as white lyophiles (60~92%) after reverse-phase chromatography (LiChroprep C-18, H_2O-MeCN). Substitution of acetic acid for sodium 2-ethylhexanoate in the deallylation of 21b, 22b gave zwitterions 24 and 25. Compound 23 was obtained by reductive debenzylation of 16b' (Fe/NH_4Cl, aq. THF; 29%).

2-(Carbamoyloxymethyl)penems. The carbamoyloxymethylpenem 28c (code-named FCE 22101),[5] structurally related to cephamycin C, cefoxitin and cefuroxime, was specifically designed for its predicted stability to human serum esterases.[12] Actually FCE 22101, *in vitro* roughly equivalent to the acetate 3c, proved superior when tested *in vivo*,[13] even beyond our expectations. In order to investigate the effects of *N*-substitution on antimicrobial activity, several congeners were synthesized (Scheme II).
A first set of products (29~32) arose in connection with a study aimed at an alternative to trichloroacetyl isocyanate for the pilot-plant carbamoylation of 1a. Addition of chlorosulphonyl isocyanate (1 mol equiv., $CaCO_3$, CH_2Cl_2, -60°C) to 1a gave 27a (not isolable), which could be hydrolyzed *in situ* smoothly, with concomitant loss of SO_3, by quenching the reaction mixture with aqueous dioxane and warming up to room temperature under vigorous stirring (2 h). Conventional deblocking of obtained 28a afforded FCE 22101 in 80% overall yield. By varying the hydrolytic conditions of 27a, the sodium *N*-sulphonate 30a (4% aq. $NaHCO_3$; 78%), the ethyl *N*-sulphonate 31a (EtOH, 1 h; 72%), and the *N*-sulphonamide 32a (30% aq. NH_4OH; 45%) were isolated. The allophanate 29a'' was obtained by iterative carbamoylation of trichloroethyl bis-protected penem carbinol 1a''. After CSI addition and hydrolysis to 28a'', the second carbamoyl unit was introduced by reaction with trichloroacetyl isocyanate (CH_2Cl_2, 10 min, 0°C) followed by silica gel catalyzed methanolysis (4 h, 25°C; 83%).
Synthesis of the *N*-methyl, *N*-cyclohexyl, and *N*-phenyl carbamates 33~35a, requiring DMAP catalysis for isocyanate addition, has already been reported.[14] It was interesting to ascertain whether the loss of activity against most *Enterobacteriacee* observed with the last two compounds could be related to their high lipophilicity. Introduction of a charged substituent (pyridiniomethyl; compound 39) was therefore sought.[15] Curtius degradation of *p*-(*tert*-butyldiphenylsilyloxymethyl)benzoyl azide (refluxing benzene, 4 h) afforded a solution of the corresponding isocyanate, which was directly added to penem carbinol 1a (DMAP 0.1 mol equiv., refluxing

Scheme II

2-(Carbamoyloxymethyl)penems



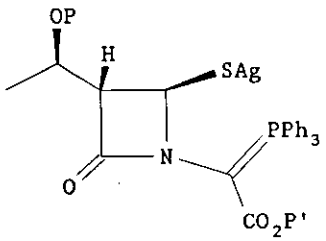
$CHCl_3$, 2 h) to produce 36a (75%). Selective desilylation and introduction of the pyridinium moiety (36a→37a→38a; 26%)strictly paralleled the previously described sequence leading to 22a. Tertiary carbamates were obtained by acylating 1a with chlorocarbonyl derivatives of secondary amines. Thus *N*-chlorocarbonylmorpholine reacted with 1a (*N*,*N*-diethylisopropylamine, CH_2Cl_2) within one night to give 40a (54%), while immediate acylation to 41a (51%) occurred with the more reactive 1-chlorocarbonyl-4-ethyl-2,3-dioxopiperazine.
Removal of protecting groups was carried out as usual. Both trichloroethyl esters in 29a'' were cleaved with Zn/HOAc (THF, 4 h), releasing 29c in 25% unoptimized yield after $NaHCO_3$ treatment and reverse-phase chromatography. Sequential desilylation and deallylation were performed on 30~35a, 40a, 41a to prepare the sodium salts 30~35c, 40c, 41c (60~80% overall). Similarly, zwitterion 39 (66%) was obtained from 38a, save that the Pd-mediated deallylation was carried out in the presence of excess HOAc in place of sodium ethylhexanoate. Unexpected by-products from these reactions included 28b (from 31a), denouncing cleavage of the N-S bond by the fluoride reagent, and FCE 22101 (27c), which formed in increasing amounts while recording the nmr spectrum of disodium salt 30c (DMSO-d_6, 45 °C). Following this observation, FCE 22101 allyl ester (28b) was quantitatively obtained from the sodium *N*-sulphonate 30b by thermolysis in DMSO-MeCN (1:9, 80 °C, 6 h).

2-(Alkoxymethyl)- and 2-(Aryloxymethyl)penems. Very recently, 3-alkoxymethylcephalosporins have received attention for their interesting pharmacokinetic properties, in particular improved oral bioavailability.[16] A brief investigation in the corresponding class of penems was therefore undertaken (Scheme III). Instability of 2-halomethylpenems to solvolytic conditions advised for a different approach, involving the versatile silver azetidinyl mercaptide 42b.[17] Acylation of the latter with the appropriate alkoxyacetyl chloride (CH_2Cl_2, -10 °C, 20 min) followed by intramolecular Wittig reaction (refluxing toluene, 2 h) proceeded smoothly, affording penems 43~48b in 50~70% overall yields. The 2-hydroxymethylpenem intermediate 1a could displace in acceptable yields (45~55%) the most electrophilic halides (allyl and benzyl bromides) in the presence of silver triflate (2,6-lutidine, CH_2Cl_2, 20 min), providing an alternative access to penem ethers 46a and 47a.
The presence of zinc at the active site of enzymes implicated in the degradation of β-lactam antibiotics (mammalian dehydropeptidases and class B bacterial β-lactamases) conferred potential interest to polyethers, such as MEM-ether 50a, THP-ether 51a, and orthoformate 52a. These products were uneventfully obtained from carbinol 1a under customary conditions (50a: MEM-chloride, Hünig base, CH_2Cl_2, overnight, 76%; 51a: 3,4-dihydro-2*H*-pyran, PTSA catalysis, CH_2Cl_2, 1h quantitative; 52a: neat trimethyl orthoformate, 60 °C, 7 h, 70%).
Aryloxymethylpenems present a substitution pattern unusual for hemisynthetic cephalosporins. For the synthesis of a few representatives (53c, 57c, 58c, 60c, 65, 66) the Mitsunobu-Volante procedure was again selected as the basic strategy. Not unexpectedly, yields of condensation impressively varied according to the pK_A of the reagent. Thus, 1a reacted with unsubstituted phenol (THF, 1 h) to give a very modest yield of 53a, *m*-nitrophenol gave a mixture of penem 55a and 2-exomethylenepenam 67a[18] (30:70; 36%), while the more acidic *o*- and *p*-isomers reacted within a few seconds at 0°C to give the anticipated products 54a (83%) and 56a (74%). Consistently, access to the *p*-hydroxymethyl derivative 62a by this procedure was unconvenient, and in fact *p*-(*tert*-butyldiphenylsilyloxymethyl)phenol condensed with 1a in low and capricious yields (up to 23%). Condensations targettet at the quaternary ammonium compounds 65, 66, and at carbamate 60c, were best run with *p*-hydroxybenzaldehyde, here selected as an electron-poor equivalent of *p*-(hydroxymethyl)phenol. Use of this reagent led to the formyl derivative 59a in yields

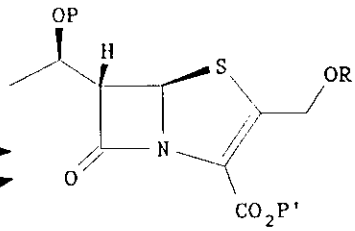
Scheme III

2-(Alkoxymethyl and Aryloxymethyl)penems

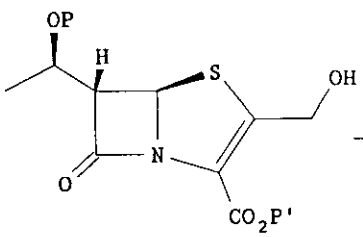
a	P= $SiMe_2t$-Bu	P'= allyl
b	P= H	P'= allyl
c	P= H	P'= Na



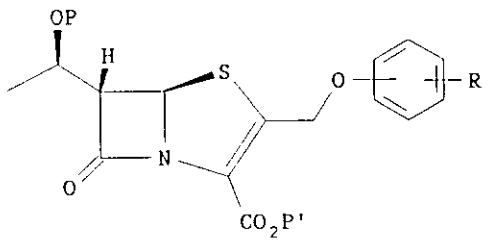
42 b



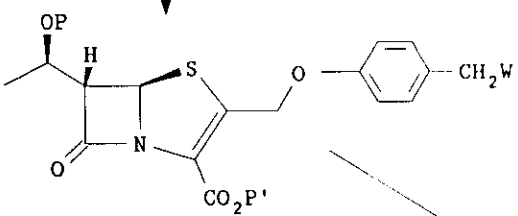
43 b,c	R= Me	48 b	R= $CH_2CO_2CH_2CH{=}CH_2$
44 b,c	R= Et	49 c	R= CH_2CO_2Na
45 b,c	R= n-Pr	50 a,b,c	R= $CH_2OCH_2CH_2OMe$
46 a,b,c	R= $CH_2CH{=}CH_2$		
47 a,b,c	R= CH_2Ph	51 a,b,c	R= 
		52 a	R= $CH(OMe)_2$

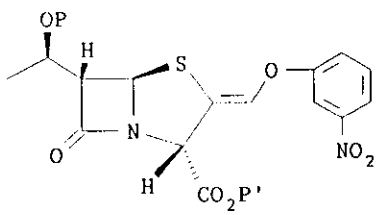


1 a



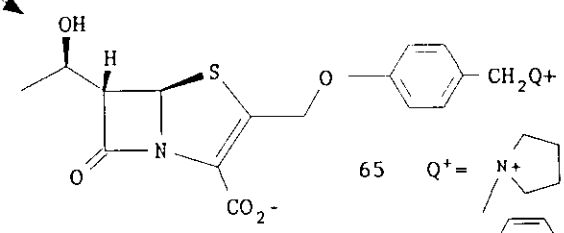
53 a,b,c	R= H	57 b,c	R= o-NH_2
54 a,b	R= o-NO_2	58 b,c	R= p-NH_2
55 a,b	R= m-NO_2	59 a	R= p-CHO
56 a,b	R= p-NO_2	60 a,b,c	R= p-CH_2OCONH_2





67 a,b

61 a	W= $OSiPh_2t$-Bu
62 a	W= OH
63 a,b	W=  $CF_3SO_3^-$
64 a,b	W=  $CF_3SO_3^-$



65	Q^+=
66	Q^+=

exceeding 80%; subsequent reduction, either by excess sodium cyanoborohydride (HOAc, THF, r.t., 2 h, 88%) or stoichiometric K-selectride (THF, -60°C, 30 min, 91%), afforded 62a in a reproducible sequence, setting the stage for carbamoylation to 60b ($Cl_3CCONCO$, CH_2Cl_2, -20°C, then MeOH/SiO_2, 4 h; 55%) or for quaternarization to 63a, 64a (isolated crude; quantitative) under conditions identical to those described for the synthesis of 21a, 22a.

Desilylation of 46a, 47a, 50a, 51a, 53a, 54a, 56a, 60a, 63a, 64a ($Bu_4NF \cdot 3H_2O$, HOAc, THF, overnight) proceeded uneventfully, save that the mixed orthoformate 52a released the hydroxymethylpenem 1b instead of 52b (quantitative conversion). The 30:70 mixture of *m*-nitrophenoxy endo/exo isomers afforded an unvaried proportion of desilylated compounds 55b, 67b (not processed further). The *o*- and *p*-nitro compounds 54b, 56b were subjected to dissolving metal reduction (Zn/HOAc, CH_2Cl_2, 30 min) to obtain the aminophenoxy derivatives 57b (68%) and 58b (48%). Finally, Pd-catalyzed transallylation with sodium 2-ethylhexanoate or excess acetic acid gave penem monosodium salts 43~47c, 50c, 51c, 53c, 57c, 58c, 60c (40~85%), the disodium salt 49c (82%), and zwitterions 65, 66 (30% overall from 62a) required for microbiological evaluation.

Table I - Spectral data of key intermediates

Compd	ir (ν_{max}, cm^{-1}) [a]	^1H nmr (δ, ppm) [b]
8a	1775,1735,1705	3.74(1H,dd,J=1.8 and 4.2Hz), 4.04(3H,s), 5.54(2H,ABq, J=15.4Hz), 5.74(1H,d,J=1.8Hz), 6.80(1H,s)
9a	1785,1740-1695	3.72(1H,dd,J=1.8 and 4.2Hz), 4.12(3H,s), 5.48(2H,ABq, J=15.1Hz), 5.61(1H,d,J=1.8Hz), 7.48(1H,s)
15a	1785,1750,1710	3.71(1H,dd,J=1.9 and 4.5Hz), 3.82(3H,s), 5.10 and 5.56 (2H,two d,J=15.0Hz), 5.60(1H,d,J=1.9Hz) [200MHz]
19a	1790,1750,1705	2.7(1H,br,exch.D_2O), 3.71(1H,dd,J=1.6 and 4.4Hz),4.20 (2H,s), 5.18 and 5.59(2H,two d,J=15.0Hz), 5.60(1H,d, 1.6Hz)
20a	1785,1715	2.34(1H,br,exch.D_2O), 3.65(1H,dd,J=2 and 4.4 Hz), 4.70 (2H,s), 5.45(2H,ABq,J=15.5Hz), 5.57(1H,d,J=2Hz)
28a''	3480,3320,1770, 1730,1700 (KBr)	3.93(1H,dd,J=2 and 8Hz), 4.73(2H,s), 4.81(2H,s), 5.26 (2H,ABq,J=15.5Hz), 5.62(1H,d,J=2Hz)
29a''	3500,3405,1805 1755,1725	4.02(1H,dd,J<2 and 7Hz), 4.77(2H,s), 4.87(2H,s), 5.38 (2H,ABq,J=15Hz), 5.70(1H,ABq,J<2Hz)
52a	1775,1700 (KBr)	3.35(6H,s), 3.67(1H,dd,J=1.6 and 4.8Hz), 4.67 and 4.89 (2H,two d,J=15.5Hz), 5.08(1H,s), 5.56(1H,d,J=1.6Hz)
54b	1790.1690 (KBr)	2.50(1H,br,exch.D_2O), 3.78(1H,dd,J=1.8 and 6.0Hz), 5.40(2H,ABq,J=16Hz), 5.65(1H,d,J=1.8Hz), 6.9-8.0(4H,m)
55b	1785,1705	3.75(1H,dd,J=2 and 4.3Hz), 5.38(2H,ABq,J=15Hz), 5.63 (1H,d,J=2Hz), 7.3-7.9(4H,m)
56b	1790,1695 (KBr)	3.76(1H,dd,J=1.8 and 6.1Hz), 5.40(2H,ABq,J=15.5Hz), 5.63(1H,d,J=1.8Hz), 6.95(2H,d,J=8.5Hz), 8.18(2H,d, J=8.5Hz)
59a	1790,1695	3.72(1H,dd,J=1.6 and 4.4Hz), 5.20 and 5.51(2H,two d, J=15.1Hz), 5.61(1H,d,J=1.6Hz), 7.03(2H,d,J=8.8Hz), 7.84(2H,d,J=8.8Hz), 9.90(1H,s) [200MHz]
62a	1790,1715	2.4(1H,br,exch.D_2O), 3.65(1H,dd,J=1.9 and 4.1Hz), 4.82 (2H,s), 5.47(2H,ABq,J=15.6Hz), 5.67(1H,d,J=1.9Hz), 7.49(2H,d,J=8Hz), 8.08(2H,d,J=8Hz)

([a]) In $CHCl_3$ unless otherwise stated.
([b]) In $CDCl_3$ at 60 MHz unless otherwise stated (salient data).

Table II - Spectral data of 2-(oxygen-substituted)methylpenem-3-carboxylic acids (sodium or internal salts)

Compd	ir (KBr) $\nu_{max}(cm)^{-1}$	uv (H_2O) $\lambda_{max}(nm)$	^1H nmr (D_2O)[1] δ (ppm)
2c	1765,1720, 1610,1585	258(ε=5358), 308(ε=7587)	1.30(3H,d,J=6.7Hz), 3.95(1H,dd,J=1.4 and 6.0Hz), 4.26(1H,dq,J=6.0 and 6.7Hz), 5.18 and 5.56(2H,two d,J=13.9Hz), 5.69(1H,d, J=1.4Hz), 8.19(1H,s)
3c	1770,1750, 1610	258(ε=4630), 308(ε=6870)	1.31(3H,d,J=6.5Hz), 2.14(3H,s), 3.93(1H,dd, J=1.4 and 5.8Hz), 4.26(1H,m), 5.10 and 5.46 (2H,two d,J=14.4Hz), 5.68(1H,d,J=1.4Hz)
4c	1760,1740, 1610,1590	214(ε=2688), 259(ε=2197), 307(ε=3708)	1.19(3H,t,J=7.5Hz), 1.56(3H,d,J=6.3Hz), 1.89(2H,m), 2.66(2H,t,J=7.1Hz), 4.16(1H,dd, J=1.6 and 6.0Hz), 4.50(1H,m), 5.38 and 5.72 (2H,two d,J=14.2Hz), 5.91(1H,d,J=1.6Hz) [taken at 45°C]
5c	1760,1605, 1580	306(ε=6267)	1.29(3H,d,J=6.5Hz), 3.90(1H,br.d,J=5.9Hz), 4.23(1H,dq,J=5.9 and 6.5Hz), 5.62(1H,br.s), 5.32 and 5.66(2H,two d,J=13.9Hz), 7.53(2H, m), 7.68(1H,m), 8.02(2H,d,J=8.2Hz)
6c		260(ε=2630), 305(ε=4879)	1.29(3H,d,J=6.5Hz), 2.32(3H,s), 3.92(1H,dd, J=1.5 and 5.7Hz), 4.24(1H,m), 5.15 and 5.53 (2H,each d,J=14.0Hz), 5.65(1H,d,J=1.5Hz)
8c	1760,1740, 1605,1580	304	1.32(3H,d,J=6.3Hz), 3.91(1H,dd,J=1.5 and 6.0Hz), 4.03(3H,s), 4.27(1H,m), 5.60(2H, ABq,J=14.0Hz), 5.66(1H,d,J=1.5Hz), 6.99 (1H,s) [60MHz]
11c	1755(br), 1595	258(ε=3370),	1.30(3H,d,J=6.4Hz), 3.17(1H,dd,J=2.5 and 15.2Hz), 3.41(1H,dd,J=1.5 and 15.2Hz), 3.93(1H,dd,J=1.5 and 5.9Hz), 4.25(1H,dq, J=5.9 and 6.4Hz), 4.41(1H,dd,J=2.4 and 5.6Hz), 5.19 and 5.60(2H,two d,J=14.0Hz), 5.67(1H,d,J=1.5Hz)
13c	1750(br), 1660,1610	258(ε=3121), 308(ε=4824)	
14c	1750(br), 1610-1580	255(ε=3058), 307(ε=3760)	1.35(3H,t,J=7.0Hz), 1.36(3H,d,J=6.4Hz), 3.95 (1H,dd,J=1.7 and 5.8Hz), 4.27(1H,m), 4.29 (2H,q,J=7.0Hz), 5.37(2H,ABq,J=14.0Hz), 5.68(1H,d,J=1.7Hz) [60MHz]
23	1750, 1620-1580	259(ε=2204), 308(ε=3020)	1.30(3H,d,J=6.5Hz), 3.94(1H,dd,J=1.6 and 5.8Hz), 3.98(2H,s), 4.25(1H,m), 5.19 and 5.61(2H,two d,J=13.8Hz), 5.69(1H,d,J=1.6Hz)
24	1775-1745, 1605,1585	258(ε=3258), 308(ε=4919)	1.29(3H,d,J=6.4Hz), 2.24(4H,m), 3.27(3H,s), 3.6-3.9(4H,m), 3.93(1H,dd,J=1.4 and 5.9Hz), 4.24(1H,dq,J=5.9 and 6.4Hz), 4.45(2H,s), 5.22 and 5.63(2H,two d,J=14.0Hz), 5.68(1H, d,J=1.4Hz)
25	1765,1720, 1605,1575	236(ε=18923), 307(ε=5600)	1.27(3H,d,J=6.3Hz), 3.79(1H,dd,J=1.6 and 5.8Hz), 4.20(1H,m), 5.12 and 5.42(2H,two d, J=14.8Hz), 5.51(1H,d,J=1.6Hz), 5.92(2H,s), 7.50 and 7.94(4H,two d,J=8.3Hz), 8.15(2H, m), 8.63(1H,m), 8.99(2H,m)

Table II - Continued

28c	1755, 1730, 1600-1570	258(ε=4150), 306(ε=6030)	1.31(3H,d,J=6.5Hz), 3.91(1H,dd,J=1.5 and 6.0Hz), 4.25(1H,dq,J=6.0 and 6.5Hz), 5.02 and 5.36(2H,two d,J=14.5Hz), 5.66(1H,J=1.5Hz)
29c	1770,1710, 1660,1600	257(ε=3548), 306(ε=4246)	1.29(3H,d,J=6.5Hz), 3.93(1H,dd,J=1.6 and 6.0Hz), 4.24(1H,m), 5.14 and 5.55(2H,two d, J=14.2Hz), 5.67(1H,d,J=1.6Hz)
30c	1775(br), 1605	255(ε=2349), 307(ε=3130)	1.30(3H,d,J=6.4Hz), 3.96(1H,dd,J=1.4 and 5.9Hz), 4.25(1H,m), 5.12 and 5.52(2H,two d,J=14.5Hz), 5.67(1H,d,J=1.4Hz)
31c	1760, 1650-1580	258(ε=4434), 307(ε=6632)	1.29(3H,d,J=6.3Hz), 1.33(3H,t,J=7.0Hz),3.91 (1H,dd,J=1.6 and 6.0Hz), 4.18(2H,q,J=7.0Hz), 4.24(1H,m), 5.03 and 5.38(2H,two d,J=14.9Hz), 5.64(1H,d,J=1.6Hz)
32c	1750, 1620(br)	256(ε=2326), 304(ε=2804)	1.31(3H,d,J=6.4Hz), 3.93(1H,dd,J=1.6 and 5.8Hz), 4.25(1H,m), 5.03 and 5.37(2H,two d, J=14.9Hz), 5.66(1H,d,J=1.6Hz)
33c	1770,1720, 1590 [Nujol]	304(ε=5670) [EtOH]	1.30(3H,d,J=6.5Hz), 2.73(3H,s), 3.90(1H,dd, J=1.6 and 6.0Hz), 4.24(1H,dq,J=6.0 and 6.5 Hz), 5.07 and 5.36(2H,two d, J=14.6Hz), 5.65 (1H,d,J=1.6Hz)
34c	1765,1705, 1590 [Nujol]	306(ε=7600) [EtOH]	1.31(3H,d,J=6.4Hz), 1.2-1.9(10H,m), 3.3-3.4 (1H,m), 3.90(1H,dd,J=1.3 and 6.2Hz), 4.24 (1H,dq,J=6.2 and 6.4Hz), 5.09 and 5.42(2H, two d,J=14.8Hz), 5.65(1H,d,J=1.3Hz)
35c	1760-1710, 1595	306(ε=5229) [EtOH]	1.29(3H,d,J=6.5Hz), 3.90(1H,dd,J=1.4 and 6.0Hz), 4.23(1H,dq,J=6.0 and 6.5Hz), 5.14 and 5.51(2H,two d,J=13.6Hz), 5.64(1H,d,J=1.4Hz), 7.2-7.4(5H,m)
39	1760,1720, 1590	307	1.24(3H,d,J=6.4Hz), 3.82(1H,dd,J=1.6 and 5.8Hz), 4.18(1H,dq,J=5.8 and 6.4Hz), 5.05 and 5.42(2H,two d,J=14.6Hz), 5.55(1H,d,J=1.6Hz), 5.75(2H,m), 7.43(4H,m), 8.05(2H,m), 8.53(1H,m), 8.89(2H,m)
40c	1755,1720, 1590	305(ε=5795)	1.31(3H,d,J=6.4Hz), 3.53(4H.m), 3.75(4H,m), 3.93(1H,dd,J=1.4 and 6.0Hz), 4.26(1H,dq,J=6.0 and 6.4Hz), 5.13 and 5.49(2H,two d,J=14.6Hz), 5.67(1H,d,J=1.4Hz)
41c	1755,1720, 1675,1600	224,306	1.20(3H,t,J=7.3Hz), 1.30(3H,d,J=6.4Hz), 3.51(2H,q,J=7.3Hz), 3.73(2H,m), 3.95(1H, dd,J=1.7 and 5.9Hz), 4.12(2H,m), 4.25(1H, m), 5.65(1H,d,J=1.7Hz), 5.26 and 5.66(2H, two d,J=14.2Hz)
43c	1755,1650, 1600,1580	258(ε=4044), 306(ε=6076)	1.30(3H,d,J=6.3Hz), 3.38(3H,s), 3.91(1H,dd, J=1.7 and 6.1Hz), 4.25(1H,dq,J=6.1 and 6.3 Hz), 4.50 and 4.80(2H,two d,J=14.0Hz), 5.66(1H,d,J=1.7Hz)
44c	1760,1605, 1580	306(ε=5613)	1.19(3H,t,J=7.1Hz), 1.30(3H,d,J=6.4Hz), 3.5-3.7(2H,m), 3.90(1H,dd,J=1.6 and 6.0Hz), 4.48(1H,dq,J=6.0 and 6.4Hz), 4.52 and 4.85 (2H,two d,J=14.2Hz), 5.65(1H,d,J=1.6Hz)

Table II - Continued

45c	1760, 1600-1560	258(ε=4100), 306(ε=6050)	0.92(3H,t,J=7.5Hz), 1.32(3H,d,J=6.5Hz), 1.61 (2H,m), 3.5-3.6(2H,m), 3.91(1H,dd,J=1.6 and 6.0Hz), 4.26(1H,dq,J=6.0 and 6.5Hz), 4.55 and 4.68(2H,two d, J=14.9Hz), 5.66(1H,d, J=1.6Hz)
46c	1760,1585	258(ε=3764), 307(ε=5637)	1.31(3H,d,J=6.5Hz), 3.92(1H,dd,J=1.6 and 5.9Hz), 4.0-4.2(2H,m), 4.26(1H,dq,J=5.9 and 6.5Hz), 4.55 and 4.90(2H,two d,J=14.1Hz), 5.2-5.4(2H,m), 5.67(1H,d,J=1.6Hz), 5.9-6.1 (1H,m)
47c	1760, 1590-1570	258(ε=3870), 307(ε=5474)	1.34(3H,d,J=6.5Hz), 3.88(1H,dd,J=1.4 and 4.7Hz), 4.24(1H,dq,J=4.7 and 6.5Hz), 4.61 (2H,m), 4.62 and 4.93(2H,two d,J=14.4Hz), 5.62(1H,d,J=1.4Hz), 7.44(5H,m)
49c	1765, 1590(br)	258(ε=3187), 307(ε=4864)	1.32(3H,d,J=6.5Hz), 3.94(1H,dd,J=1.6 and 6.0Hz), 3.93 and 4.01(2H,two d,J=15.5Hz), 4.26(1H,dq,J=6.0 and 6.5Hz), 4.59 and 4.89 (2H,two d,J=14.4Hz), 5.67(1H,d,J=1.6Hz9
50c	1760, 1600-1580	259(ε=3350), 307(ε=5412)	1.21(3H,d,J=6.4Hz), 3.32(2H,s), 3.54(2H,m), 3.68(3H,m), 4.22(1H,m), 4.71(2H,m), 4.80 and 4.93(2H,two d,J=13.8Hz), 5.66(1H, br s)
51c	1760,1580	260(ε=2678) 306(ε=5025)	1.32(3H,d,J=6.4Hz), 1.4-1.9(6H,m), 3.60 (1H,m), 3.90(2H,m), 4.25(1H,m), 4.58, 4.73, 4.94 and 5.05(2H,each d,J=14Hz), 4.78(1H, m), 5.67(1H,d,J=1.4Hz) [taken at 45°C]
53c	1760,1590	262(ε=4484) 308(ε=5513)	1.31(3H,d,J=6.5Hz), 3.89(1H,dd,J=1.6 and 6.0Hz), 4.25(1H,dq,J=6.0 and 6.5Hz), 5.20 and 5.53(2H,two d,J=14.6Hz), 5.62(1H,d,J= 1.6Hz), 7.06-7.46(5H,m)
57c	1765,1600	306(ε=5273)	1.27(3H,d,J=6.4Hz), 3.85(1H,dd,J=1.2 and 6.0Hz), 4.21(1H,m), 5.15 and 5.8(2H,two d, J=14.5Hz), 5.57(1H,d,J=1.2Hz), 6.75-7.3 (4H,m)
58c	1765, 1600-1580	305(ε=5466)	1.27(3H,d,J=6.4Hz), 3.86(1H,dd,J=1.6 and 6.0Hz), 4.21(1H,m), 5.07 and 5.41(2H,two d, J=14.5Hz), 5.58(1H,d,J=1.6Hz), 6.86(4H,m)
60c	1760,1705, 1610,1585	308(ε=5387)	1.26(3H,d,J=6.3Hz), 3.79(1H,dd,J=1.6 and 5.8Hz), 4.19(1H,dq,J=5.8 and 6.3Hz), 4.98 (2H,s), 5.12 and 5.44(2H,two d,J=14.6Hz), 5.52(1H,d,J=1.6Hz), 6.97(2H,d,J=8.5Hz), 7.31(2H,d,J=8.5Hz)
65	1765,1600	228,260sh, 308(ε=4780)	1.27(3H,d,J=6.3Hz), 2.22(4H,m), 2.93(3H,s), 3.30-3.65(4H,m), 3.84(1H,d,J=6.0Hz), 4.23 (1H,m), 4.45(2H,s), 5.19 and 5.51(2H,two d, J=14Hz), 5.58(1H,s), 6.95-7.55(4H,m)
66	1760,1605, 1580	258(ε=8935) 306(ε=5647)	1.22(3H,d,J=6.3Hz), 3.72(1H,dd,J=1.5 and 6.0Hz), 4.14(1H,dq,J=6.0 and 6.3Hz), 5.02 and 5.34(2H,two d,J=14.4Hz), 5.43(1H,d, J=1.5Hz), 5.70(2H,s), 6.95 and 7.39(4H,two d,J=(8.6Hz), 8.01(2H,dd,J=5.6 and 7.0Hz), 8.50(1H,d,J=7.0Hz), 8.87(2H,d,J=5.6Hz)

([a]) At 200 MHz unless otherwise stated.

Table III - *In vitro* antibacterial activity of penems

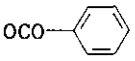
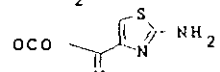
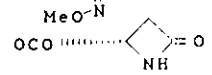
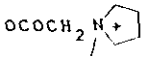
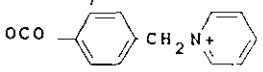
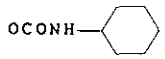
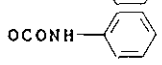
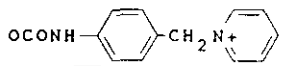
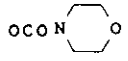
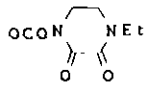
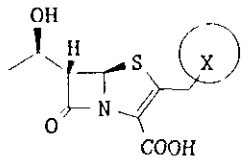
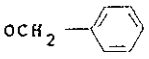
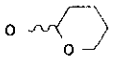
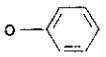
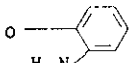
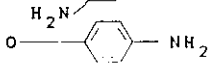
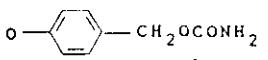
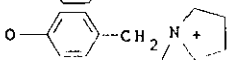
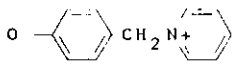
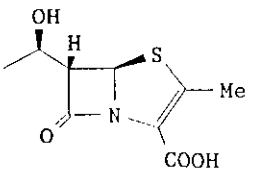
Compd		MIC([a])					
		S.a.	*S.f.*	*E.c.*	*E.c.*(+)	*S.t.*	*C.f.*(+)
				Acyloxymethyl compounds			
2c	OCHO	0.011	6.25	0.78	0.78	0.78	nd([b])
3c	OCOMe	0.022	3.12	0.39	0.39	0.39	nd
4c	OCOPr-*n*	0.045	6.25	0.39	0.78	12.5	>50
5c		0.022	3.12	0.19	0.78	50	>50
6c	$OCOCH_2COMe$	<0.09	nd	0.78	1.56	nd	nd
8c		0.09	12.5	nd	nd	>25	>25
11c		0.19	3.12	0.78	0.78	3.12	6.25
13c	$OCOCO_2Na$	0.09	12.5	1.56	6.25	0.78	nd
14c	OCOOEt	0.045	12.5	nd	nd	25	>25
23	$OCOCH_2NH_2$	nd	12.5	nd	nd	3.12	nd
24		0.045	12.5	3.12	3.12	3.12	nd
25		0.011	0.78	0.39	1.56	0.39	6.25
				Carbamoyloxymethyl compounds			
28c	$OCONH_2$	0.045	3.12	0.78	0.78	0.78	1.56
29c	$OCONHCONH_2$	0.011	6.25	0.78	3.12	1.56	nd
30c	$OCONHSO_3Na$	0.19	25	1.56	25	0.78	nd
31c	$OCONHSO_3Et$	0.78	50	6.25	25	3.12	>25
32c	$OCONHSO_2NH_2$	0.39	100	6.25	25	6.25	>25
33c	OCONHMe	0.22	3.12	0.78	0.78	0.78	nd
34c		0.045	50	0.78	0.78	100	>25
35c		0.022	1.56	0.19	0.39	6.25	>50
39		0.045	0.39	0.78	1.56	0.78	3.12
40c		0.09	6.25	0.78	6.25	25	>25
41c		0.39	12.5	0.78	25	0.78	nd

Table III - Continued

Compd		MIC([a])					
		S.a.	*S.f.*	*E.c.*	*E.c.*(+)	*S.t.*	*C.f.*(+)
		Hydroxy-, alkoxy-, aryloxymethyl compounds					
1c	OH	0.19	nd	3.12	6.25	3.12	≥25
43c	OMe	0.045	3.12	0.39	0.78	0.39	6.25
44c	OEt	0.09	12.5	0.78	0.78	1.56	25
45c	OPr-*n*	0.09	12.5	0.78	0.78	6.25	>50
46c	$OCH_2CH{=}CH_2$	0.045	12.5	0.39	0.39	1.56	>50
47c		0.045	12.5	0.78	0.78	>50	>50
49c	OCH_2COONa	1.56	>50	1.56	1.56	1.56	6.25
50c	$OCH_2OCH_2CH_2OMe$	0.09	12.5	0.39	0.39	0.78	>50
51c		0.09	12.5	0.78	0.78	12.5	>50
53c		0.011	1.56	0.78	1.56	100	>25
57c		0.022	1.56	1.56	3.12	25	>25
58c		0.022	nd	1.56	3.12	25	>25
60c		0.011	1.56	0.39	0.78	25	>25
65		0.022	3.12	0.78	1.56	1.56	nd
66		0.011	1.56	0.78	0.78	0.78	1.56
		Reference					
68		0.38	12.5	3.12	3.12	1.56	>25

([a]) MICs (mcg/ml) were determined by the standard two-fold agar dilution method in Bacto Antibiotic Medium 1 (Difco); inoculum size 10^4 cfu. Organisms included in this table are: *S.a.*, *Staphylococcus aureus* Smith; *S.f.*, *Streptococcus faecium* ATCC 8043; *E.c.*, *Escherichia coli* B; *E.c.*(+), *E. coli* B β-lactamase producer; *S.t.*, *Salmonella typhi* ATCC 14028; *C.f.*(+), *Citrobacter freundii* ATCC 4051 cephalosporin-resistant. ([b]) nd = Not determined.

Table III shows the *in vitro* activity of the title compounds against six representative bacterial strains, in comparison with the unsubstituted 2-methylpenem reference 68. In particular, *Staphylococcus aureus* Smith and *Salmonella typhi* ATCC 14028 were selected as common Gram-positive and Gram-negative organism, respectively; data on *Escherichia coli* B, characterized by a permeable outer membrane, reflect more closely the *intrinsic* activity of each compound. With the exception of *Pseudomonas aeruginosa*, which proved resistant to all of the tested compounds, and of some "difficult" opportunistic pathogens, here exemplified by *Citrobacter freundii* ATCC 4051, the vast majority of compounds showed good levels of activity, usually superior to that of the unsubstituted 2-methyl reference. It is hard to identify general features proper of this family of "2-CH_2X" penems, either in comparison with others[1,3,4] characterized by a different X hetero atom, or according to a division into chemical subclasses. Within each of these (esters, carbamates, ethers), simple substituents proved often the best for imparting a wide spectrum of activity (3c, 28c, 43c). Homologation of the carbon chain of the acyl (4c, 5c), *N*-substituted carbamate (34c, 35c), or ether residue (44c, 45c, 47c) almost immediately led to a decrease in activity against Gram-negative rods, as a result of impaired outer membrane penetration. Introduction of negatively ionizable groups (13c, 30~32c, 49c) restored good penetration properties but depressed the intrinsic activity (compare MIC values on *E. coli* and *S. typhi*).

On the contrary, introduction of a quaternary ammonium overcame the permeability problem without affecting intrinsic activity on both Gram-positive and -negative bacteria (25, 39, 65, 66). The 2-aryloxymethyl compounds, reported by Ciba scientist as devoid of activity against enterobacteria,[17] are particularly interesting in this respect. Compound 66 (FCE 24386) was selected for its good *in vitro* activity and unusually extended plasma half-life;[15] unfortunately, it also displayed unacceptable levels of CNS toxicity (anoxic convulsions in mice), which prevented further development.

Other sidechain modifications met with little success. Introduction of the aminothiazolyl moiety (8c), or basic groups (23, 57c, 58c), or potential metal ligands (49c, 50c, 51c) did not result in improved antibacterial potency and anti-pseudomonal activity, nor in increased stability towards renal degrading enzymes (dehydropeptidases, data not shown). Further works confirmed 28c (FCE 22101) and 43c (FCE 24964)[19] as the most interesting compounds in this penem family. An oral prodrug formulation of the latter, FCE 25199,[20] now undergoing toxicological studies in animals, in a short time will hopefully join the exiguous maniple of clinically evaluated penems.

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