2-(HETEROATOM-SUBSTITUTED)METHYL PENEMS. III[1]. NITROGEN DERIVATIVES

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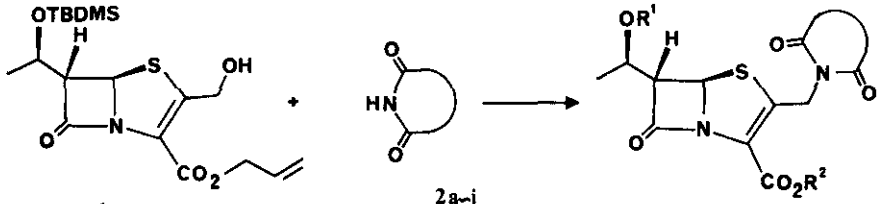
Abstract - The synthesis of "2-CH_2X" penems wherein X is N-imidoyl, N-heterocyclyl, amino or quaternary ammonium is described. The observed in vitro antibacterial activity marginally correlates with the electronic activation induced by the X group on the ß-lactam ring.

In the attractive and varied scenario of ß-lactam compounds, the penem nucleus stands as an artificial entry[2], yet endowed with remarkable biological properties. Since Woodward's pioneering synthesis[3] of the 6-acylamino derivatives, improvements have been sought through the introduction of different C_2 and C_6 side chains. While an optimal C-6 substituent was recognized in the thienamycin α-hydroxyethyl group, many efforts are still being devoted to C_2 functionalization. The observation that a major determinant of the ß-lactam activation of cephalosporins is the presence of a heteroatom at the C_3' position prompted us to synthesise penems carrying heteroatom-linked substituents at their electronically equivalent C_2-methylene[1]. Here we report the series of the nitrogen derivatives, arbitrarily divided according to whether the nitrogen atom is part of an imide, a heterocycle, a (substituted) amine or a quaternary ammonium salt.

Introduction of an imido group as the ß-lactam carbonyl electronic activator was firstly conceived, in spite of the scanty attention received by this kind of substituent in the cephalosporin field[4]. To this end, an array of five membered cyclic imides were found to react smoothly with 2-hydroxymethylpenem 1[5] by exploitation of the Mitsunobu[6] condensation procedure (Scheme I). Typically, the addition of a slight excess of preformed triphenylphosphine - diethyl azodicarboxylate complex to a solution of alcohol 1 and imides 2a-j[7] in dry THF at r.t. led to a virtually instantaneous reaction. The protected 2-imidomethylpenems 3a-j[8] were isolated after purification by flash chromatography (silica gel, cyclohexane-ethyl acetate), with the exception of 3e, which crystallised directly from the reaction mixture. Yields ranged from good to excellent (81-98%), apart from 3d (48%), 3h (59%), 3j (57%). The low solubility of hydantoin in THF and the large excess ($\geq$ 5 mol equiv.) of TPP-DEAD complex needed in the case of saccharin might account for the observed decrements; in both cases, a parasite reaction[9] leading to the hydrazino derivative 6a (separated and characterised only after desilylation at C_8) could not be suppressed. On the other hand, parabanic acid (2h), possessing two identical imidic protons, gave a substantial amount of dimer 7 (25%), even when a stoichiometric quantity of the Mitsunobu reagent was used.

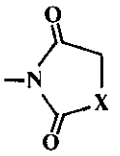
SCHEME I



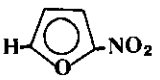
1 2a–j

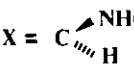
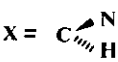
(TBDMS = $SiMe_2Bu^t$)

3a–j ; R^1 = TBDMS ; R^2 = $CH_2CH{=}CH_2$
4a–j ; R^1 = H ; R^2 = $CH_2CH{=}CH_2$
5a–j ; R^1 = H ; R^2 = Na

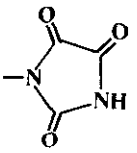


a ; X = CH_2
b ; X = O
c ; X = S
d ; X = NH

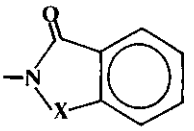
e ; X = 

f ; X =  (compds. 2,3,4f)
X =  (compd. 5f)

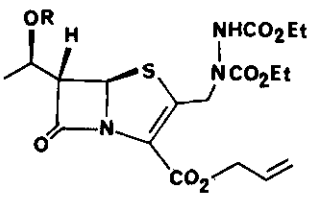
g ; X =  (compds. 2,3,4g)
X =  (compd. 5g)



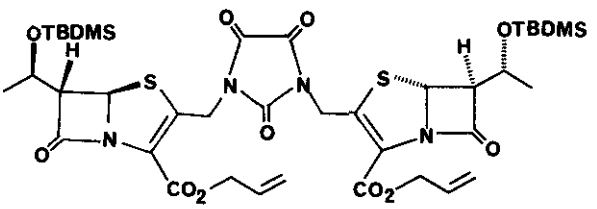
h



i ; X = CO
j ; X = SO_2



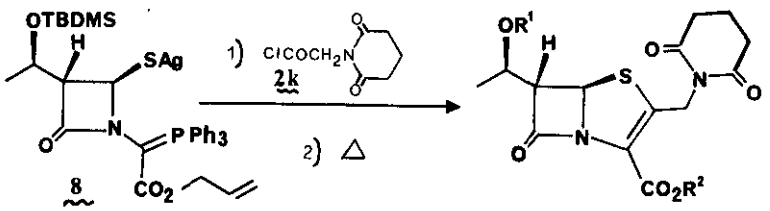
6a ; R = TBDMS
6b ; R = H



7

By contrast, saturated[10] 6-membered cyclic imides, owing to their lower pK_a values, under the above conditions proved inadequate to act as acidic components in the Mitsunobu reaction. Thus glutarimidomethylpenem 3k, a representative of this homologous series, was prepared by a two-step procedure entailing acylation of the silver thiolate 8[11] with the required chloride (CH_2Cl_2, r.t.) and Wittig condensation of the resulting thioester-phosphorane (Scheme II).

SCHEME II

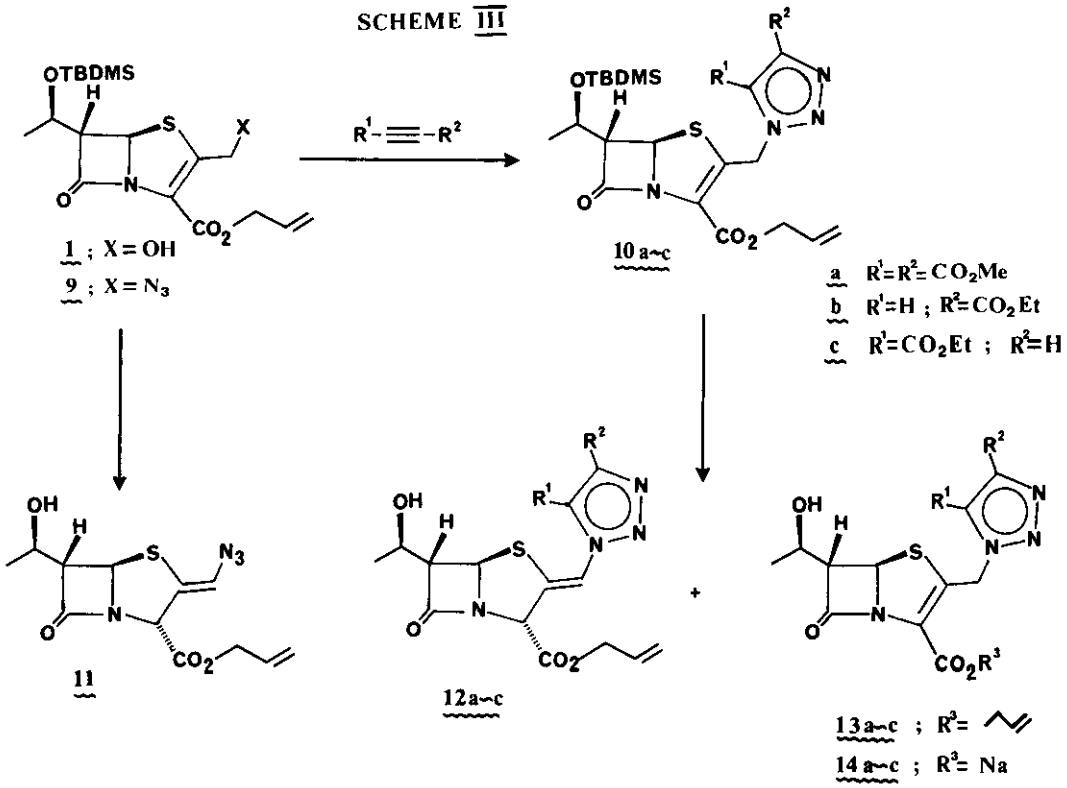


3k ; R^1=TBDMS ; R^2= 
4k ; R^1=H ; R^2= 
5k ; R^1=H ; R^2= Na

When exposed to tetrabutylammonium fluoride (buffered with acetic acid, THF, overnight)[12], silyl ethers 3a-k were smoothly deblocked to hydroxy derivatives 4a-k[8]. Palladium mediated transallylation[13] ($Pd(PPh_3)_4$, PPh_3, CH_2Cl_2-THF, 10-40 min) of 4a-e,h-k in the presence of sodium 2-ethylhexanoate (1 mol equiv.) afforded the corresponding sodium salts 5a-e,h-k, while the presence of excess acetic acid and longer reaction times (3-5 h) were required to achieve zwitterions 5f,g.

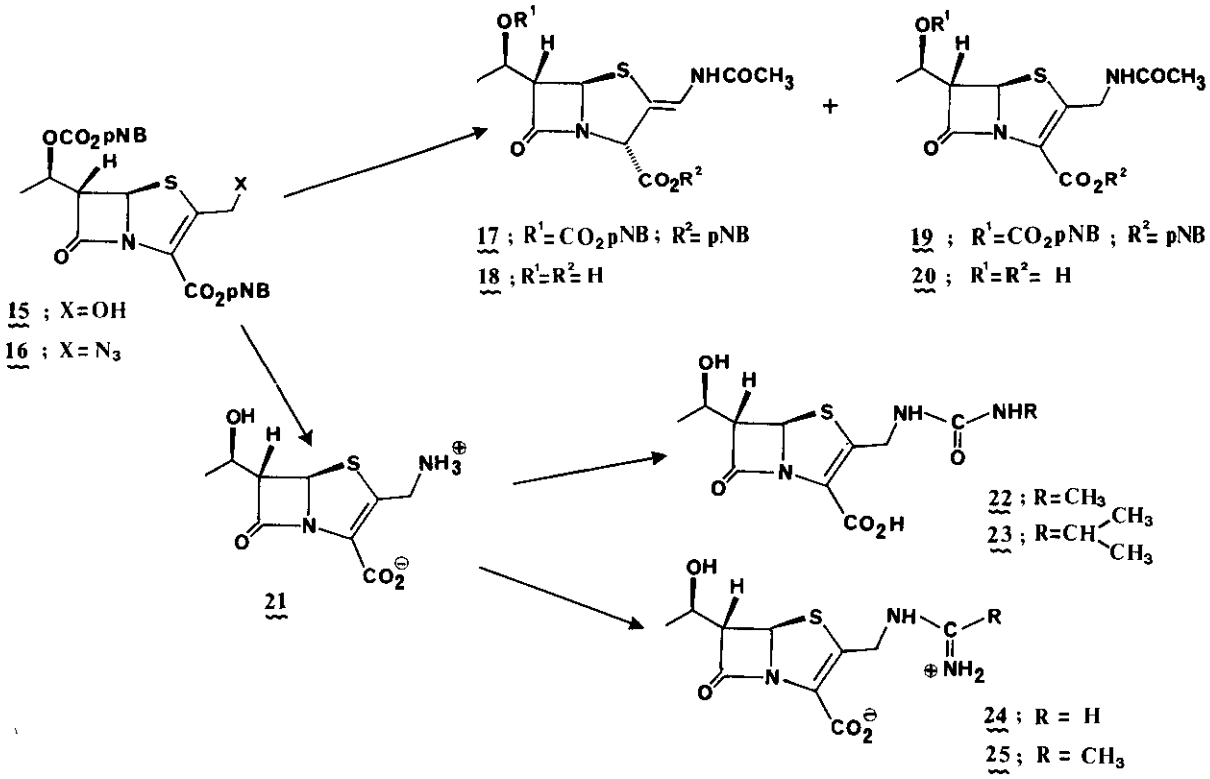
Next, we turned our attention to the 2-(triazol-1-yl)methylpenems 14a-c (Scheme III), which we considered representative[14] and accessible[15] testing samples of the vast class of the heterocyclic nitrogen derivatives. Our initial attempts to obtain the required 2-azidomethyl precursor by nucleophilic substitution of 2-chloro[9] or 2-mesyloxymethylpenem[16] with sodium azide in polar aprotic solvents were thwarted by low yields and lack of reproducibility. An excellent alternative was found in the reaction of 2-hydroxymethylpenem 1 with hydrazoic acid under Mitsunobu-Volante conditions, whereupon the crystalline 2-azidomethylpenem 9 was isolated in 95% yield. Thermal addition of acetylenedicarboxylate (THF, 60°C) to azide 9 produced 10a uneventfully (70%), while use of ethyl propiolate (refluxing toluene) led to a mixture of isomers (10b,10c, ratio 2.5:1, 64%) whose regiochemistry, predicted on the basis of electronic factors governing dipolar additions[17], was confirmed by spectral evidence. Stepwise removal of protecting groups afforded the target penems 14a-c in acceptable yields (35-42%).

SCHEME III



Endo-exo double bond shift, which had plagued the synthesis of 2-thiomethylpenems[16], was not detected at any extent in the 2-imidomethyl derivatives. However, small percentages of triazolylmethylenepenams 12a-c were formed during desilylation of 10a-c, and upon exposure of 9 to the same conditions only the vinyl azide 11 was isolated. The disappointing antibacterial profile exhibited by the triazolyl penems 14a-c diverted our efforts from synthesising further analogues. Instead, the azido intermediates 9 and 16 (Scheme IV) were used for preparing the 2-aminomethylpenem 21[18], a valuable target both per se and as a direct source of attractive derivatives.

SCHEME IV

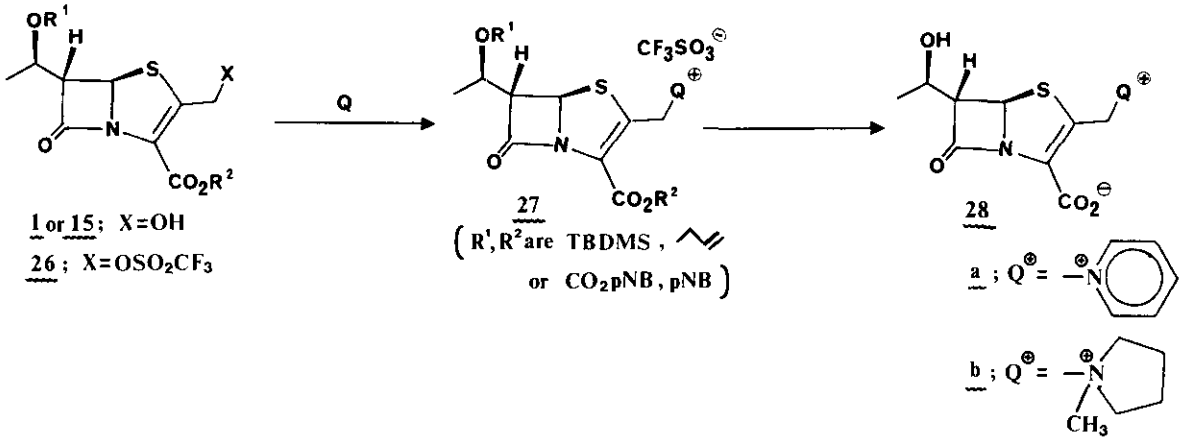


Preliminary attempts were carried out through the Staudinger reaction[19]: upon addition of PPh_3 (1 mol equiv.) to azido derivative 16 immediate nitrogen evolution occurred. The supposed imino-phosporane intermediate could be acetylated (AcCl, CH_2Cl_2, 0°C, 3h) to a 30:70 equilibrium[20] mixture of exo-endo acetamido derivatives 17, 19 in moderate yield (52%), but not hydrolyzed to free amino[21] under neutral or slightly acidic conditions, nor converted into a protected amino function by treatment with chloroformates. Therefore, 2-aminomethylpenem 21 was obtained from the azide 16 by catalytic hydrogenation (H_2 6 atm, 10% Pd/C, DME-Et_2O-H_2O, 3h, 40%), while reductive deblocking with Fe-NH_4Cl(THF-H_2O, 90 min, 14%) was preferred for the acetamido compounds 18,20. After completion of our work, 21 gained popularity as the Ciba-Geigy clinical penem candidate (CGP 31608)[22].

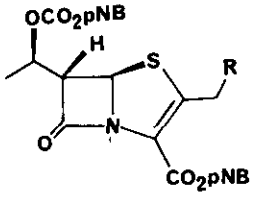
As expected, compound 21 evinced remarkable potential for further synthetic transformations: ureido-penems 22 and 23 were obtained by treatment of silylated 21 (8 mol equiv. BSA, THF, 1h) with methyl isocyanate (16 mol equiv., 1h, 63%) or isopropyl isocyanate (3.5 mol equiv., 24h, 41%), while amidino penems 24 and 25 became accessible by reaction of zwitterionic 21 with ethyl formimidate or ethyl acetimidate (1 mol equiv., $NaHCO_3$, H_2O, 2h, 43% and 61% respectively).

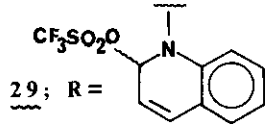
Nonetheless, the most remarkable products were found within the class of the quaternary ammoniomethyl derivatives 28 (Q^+ = pyridinium, trialkylammonium, dialkylanilinium, cycloalkylammonium, quinuclidinium), whose antibacterial activity has been recently anticipated by us[23].

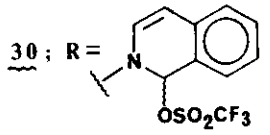
SCHEME V



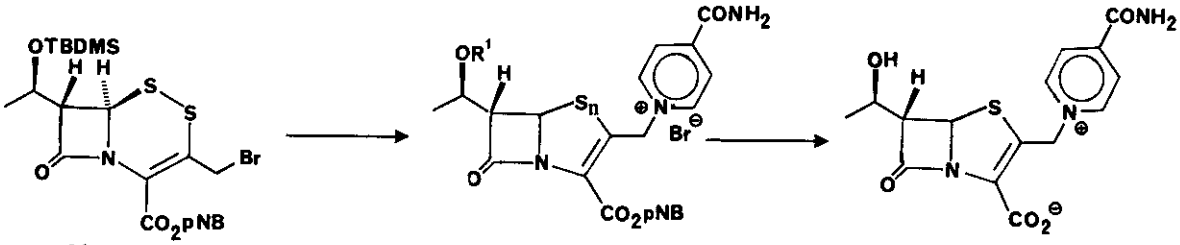
The procedure of choice for the synthesis of penems 28 is outlined in Scheme V for Q^+ = pyridinium. The in situ prepared triflates 26 (1.5 mol equiv. of triflic anhydride, 3 mol equiv. of pyridine, CH_2Cl_2, -40°C) in the presence of excess pyridine underwent smooth nucleophilic displacement affording crude 27, which were subjected to routine deprotection (TBAF-HOAc-THF overnight, 45%, followed by $Pd(PPh_3)_4$-PPh_3-HOAc-CH_2Cl_2, 80%; or Fe powder-NH_4Cl-H_2O-THF, 40 min, 30%) yielding zwitterion 28a. Failures to reproduce this sequence were occasionally encountered: for example, quinoline and isoquinoline with triflate 26 (R^1=CO_2pNB, R^2=pNB) afforded diastereoisomeric mixtures of non-ionic products, which were assigned the dihydro structures 29 and 30 on the basis of their ^1H NMR and FD mass spectra (Table I). Steric hindrance, low nucleophilicity or the presence on the amine of functional groups sensitive to triflic anhydride were other reasons for failure. Thus, untractable tars were obtained from reaction of 26 with 2,6-lutidine or isonicotinamide. The isonicotinioamido derivative 35a was therefore obtained by substituting 3-bromomethyl-2-thiacephem 31 for the triflate 26 as the electrophilic partner (Scheme VI).







SCHEME VI



31

32 ; R=TBDMS ; n = 2 ; 6R epimer

33 ; R=H ; n=2 ; 6R epimer

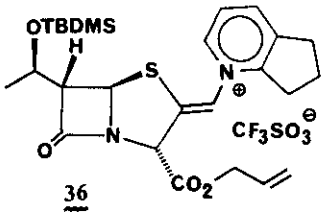
34a ; R=H ; n=1 ; 5R epimer

34b ; R=H ; n=1 ; 5S epimer

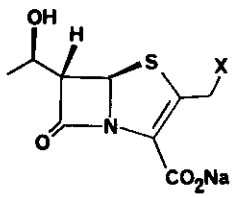
35a ; 5R epimer

35b ; 5S epimer

Following nucleophilic displacement of 31[24] by isonicotinamide (DMF, 20h, 66%), desilylation ($BF_3 \cdot Et_2O$, MeCN, 30 min, 0°C) and desulphurative ring contraction (PPh_3, acetone)[24] afforded a separable C_5-diastereoisomeric pair of penems, 34a,b (3:2, 41% overall from 32), which were individually deprotected (Fe-NH_4Cl, THF-H_2O, 30%) to give the target product, 35a, and its biologically inactive 5S-epimer 35b. Isotopic exchange of the C-2' methylene protons of 34 could be accomplished under neutral conditions (D_2O, acetone; few minutes), witnessing their acidity increase imparted by the quaternary ammonium substituent and showing that in this case the absence of any detectable endo-exo double bond equilibration is the result of thermodynamic control. This preference for the ammoniomethylpenem structure was experimentally ascertained to be a common event, the 2-(cyclopentenopyridinium)methylenepenam 36 being the only enammonium salt isolated in appreciable amount throughout our work.

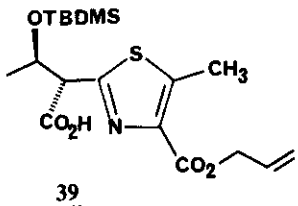


36

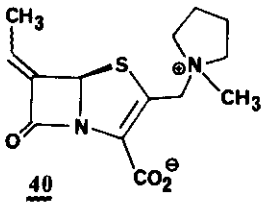


37 X=$OCONH_2$

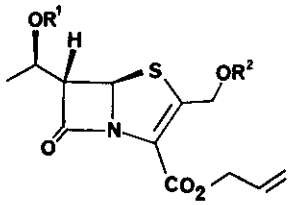
38 X=H



39



40



41 ; $R^1=R^2=H$

42 ; $R^1=H$; $R^2=SiMe_3$

43 ; $R^1=R^2=SiMe_3$

44 ; $R^1=SiMe_3$; $R^2=H$

Modulation of the ß-lactam reactivity and of the C_2' substituent nucleofugality[25] was at the basis of our research in 2-(heteroatom-substituted)methylpenems. The quaternary ammonium compounds marked a strong increment of both parameters as compared with the carbamate 37 (our current clinical candidate, FCE 22101[5]) and with the 2-unsubstituted methylpenem reference 38, sometimes accompained by an impressive increase of the *in vitro* antimicrobial activity (compound 28b, Table III). Thus, the pseudo-first order rate constant of ß-lactam cleavage in alkaline solution (pH 9, 37°C, HPLC determination) was 0.702 h^{-1} for 28b, 0.146 h^{-1} for 37 and 0.030 h^{-1} for 38. The leaving group ability of the quaternary ammonium moiety was testified by difficulties experienced in the removal of the tert-butyldimethylsilyl group under standard conditions (TBAF/HOAc/THF), which led to competitive ß-lactam cleavage, expulsion of the tertiary amine and aromatisation of the resulting exomethylenethiazoline intermediate to give the thiazole 39 and further degradation products[26]. On the other hand, 6,8-elimination interferred with the triflate-amine displacement step when a carbonate was used as the C_8 hydroxyl protector; for example, the 6-ethylidenepenem 40 (65:35 Z/E mixture) was isolated instead of 28b when the p-nitrobenzylcarbonate 15 was used as the starting hydroxymethylpenem reagent. To obviate these difficulties, the more labile trimethylsilyl ether 44 was used in place of 1 or 15 in gram-scale preparations of 28b. That entailed conventional desilylation of 1 (TBAF 3 mol equiv., HOAc-THF 1:3, overnight) and exhaustive silylation of the crude dicarbinol 41 to give 43 (excess BSA in CH_2Cl_2, 5 h; monosilylation gives the isolable primary ether 42), whose carefully controlled monodesilylation (HOAc-THF-H_2O 0.1:3:1, 15 min) yielded 44 (70% overall from 1 after $NaHCO_3$ quenching and flash-chromatography).

Table I - Spectral data of key intermediates and byproducts

Compd.	ir(νmax, cm^{-1})[1]	^1H nmr (δ, ppm)[2]
2f[3] 2g[3]	($CHCl_3$)1790,1735	(60MHz)2.70(1H,dd,J=17 and 7Hz),3.05(1H,dd,J=17 and 8Hz),4.35-4.65 (3H,m),5.0-5.4(2H,m),5.5-6.10(1H,m),6.30(1H,d,J=6.5Hz,exch.D_2O),9.7 (1H,s,exch.D_2O)
6b	($CHCl_3$)1785,1745 1715	(90MHz)1.06(6H,t,J=7Hz),3.74(1H,dd,J=1.9 and 6.4Hz),4.21(4H,d,J=7Hz), 4.90(2H,ABq,J=16.8Hz),5.61(1H,d,J=1.9Hz),6.82(1H,br s, exch. D_2O)
7[3]	1775,1745,1705	3.71(1H,dd,J=1.8 and 4.3Hz),4.81 and 5.38(2H,each d,J=16.8Hz),5.59 (1H,d,J=1.8Hz)
9[3]	2080,1765,1695	(60MHz)3.71(1H,dd,J=1.7 and 4.4Hz),4.52(2H,ABq,J=15.5Hz),5.56(1H,d, J=1.7Hz)
11[3]	($CHCl_3$)2105,1780 1745	(60MHz)3.37(1H,dd,J=1 and 6.2Hz),5.30(2H,br s),6.56(1H,d,J≲1Hz)
12a	(film)1785,1730	(60MHz)3.14(1H,dd,J=1 and 6.5Hz),4.02(6H,s),5.46(1H,d,J=1Hz),5.63 (1H,d,J=1Hz),7.90(1H,d,J=1Hz)
29	1790,1745,1710	(1:1 diaster.mixture)3.87 and 3.89(1H,each dd,J=1.7 and 7.9Hz,H_6), 4.60,5.12 and 4.81,4.93(2H,each d,J=16Hz,CH_2N),5.52 and 5.61(1H, each d,J=1.7Hz,H_5),5.77 and 5.80(1H,each d,J=4.0Hz,CHOS),6.1-6.2 (1H,two dd,J=4.0 and 9.5Hz, ),6.85(1H,d,J=9.5Hz, ),7.3-7.6(4H,m,Ar)
30[3]	1790,1745,1710	(3:5 diaster.mixture)3.88 and 3.90(1H,each dd,J=1.8 and 7.8Hz,H_6), 3.89,5.12 and 4.78,4.87(2H,each d,J=16Hz,CH_2N),5.55 and 5.61(1H, each d,J=1.8Hz,H_5),6.21(1H, br s, CHOS),6.38 and 6.39(1H,each d, J=7.5Hz, ),6.68(1H,br d, ),7.3-7.6(4H,m,Ar)

Table I - Continued

Compd.	ir(νmax, cm^{-1})[1]	^{1}H nmr (δ, ppm)[2]
32	1785,1720,1690	(CD_3COCD_3)0.05(6H,s),0.75(9H,s),1.27(3H,d,J=6.1Hz),3.68(1H,dd,J=2.6 and 3.3Hz),4.45(1H,dq,J=3.3 and 6.1Hz),4.97(1H,d,J=2.6Hz),5.49 and 5.70(2H,ABq,J=13.0Hz),6.29 and 6.47(2H,ABq,J=15.0Hz),7.82 and 8.20 each 2H,d,J=8.5Hz),7.92 and 9.6(2H,each br.s, exch. D_2O),8.99 and 9.72(each 2H,d,J=6.2Hz)
39	($CHCl_3$)1715	0.06(6H,s),0.85(9H,s),1.10(3H,d,J=6.2Hz),2.74(3H,s),4.27(1H,d,J=3.4 Hz),4.28(1H,dq,J=3.4 and 6.2Hz),4.67(2H,m),5.21-5.40(2H,m),5.94-5.99(1H,m)
44	n.d.	(60MHz)0.08(6H,s),1.23(3H,d,J=6.5Hz),3.66(1H,dd,J=1.5 and 5.5Hz), 4.18(1H,dq,J=5.5 and 6.5Hz),4.5-4.7(4H,m),5.15 and 5.37(2H,m),5.48 (1H,d,J=1.5Hz),5.65-6.20(1H,m)

[1] In KBr unless otherwise stated. [2] In $CDCl_3$,25°C, at 200MHz unless otherwise stated; except 2f,g, 32,39 and 41, signals relative to the hydroxyethyl side chain and to protecting groups have been omitted. [3] Additional data: 2f, $[\alpha]_D$ +32.0°(1% EtOH);2g; $[\alpha]_D$ -33.3°(1%EtOH);7,MS(FD)876m/z(M^+);9,mp 65°C; 10,uv λmax(EtOH)275nm(ε=10.950);30,MS(FD)820m/z(M^+).

Table II - Spectral data of 2-(nitrogen substituted)methylpenems and byproducts

Compd.	ir(KBr) νmax(cm^{-1})	uv(H_2O) λmax(nm)	^{1}H nmr(D_2O)[1] δ(ppm)
5a	1770,1700	260,306	(90MHz)1.30(3H,d,J=6.0Hz),2.85(4H,s),3.90(1H,dd,J=1.8 and 6.0 Hz),4.24(1H,m),4.86(2H,ABq,J=16.0Hz),5.60(1H,d,J=1.8Hz)
5b	1820,1745,1610, 1585	258,306	1.29(3H,d,J=6.5Hz),3.92(1H,dd,J=1.6 and 6.0Hz),4.23(1H,dq,J=6.0and 6.5Hz),4.64 and 5.21(2H,each d,J=16.2Hz),4.93(2H,s), 5.65 (1H,d,J=1.6Hz)
5c	1765,1675,1600, 1580	306(ε=4850)	1.33(3H,d,J=6.0Hz),3.96(1H,dd,J=5.0 and 1.8Hz),4.30(2H,s), 4.25(1H,m),4.80 and 5.33(2H,each d,J=17Hz),5.68(1H,d,J=1.8Hz)
5d	1770,1710,1600	259(ε=4518), 305(ε=5630)	1.33(3H,d,J=6.3Hz),3.95(1H,dd,J=1.3 and 6.0Hz),4.16(2H,s),4.68 and 5.15(2H,each d,J=16.8Hz),5.68(1H,d,J=1.3Hz)
5e	1765,1720,1690, 1670	266(ε=16544) 366(ε=18450)	1.28(3H,d,J=6.4Hz),3.92(1H,dd,J=1.5 and 6.0Hz),4.23(1H,dq,J=6.0 and 6.4Hz),4.54(2H,s),4.72 and 5.27(2H,each d,J=16.5Hz), 5.64(1H,d,J=1.5Hz),7.15(1H,d,J=3.9Hz),7.61(1H,d,J=3.9Hz),7.85 (1H,s)
5f	1770,1710,1590	255,305	1.27(3H,d,J=6.4Hz),2.72(1H,dd,J=18.1 and 5.5Hz),3.24(1H,dd,J=18.1 and 8.9hz),3.90(1H,dd,J=6.0 and 1.6Hz),4.22(1H,dq,J=6.4 and 6.0Hz),4.23(1H,dd,J=5.5 and 8.9Hz),4.60 and 5.15(2H,each d,J=16.2Hz),5.60(1H,d,J=1.6Hz)
5g	1770,1715,1585	270,307	1.29(3H,d,J=6.4Hz),2.90(1H,dd,J=5.7 and 18.3Hz),3.32(1H,dd,J=9.2 and 18.3Hz),3.92(1H,dd,J=1.6 and 6.0Hz),4.24(1H,dq,J=6.0 and 6.4Hz),4.48(1H,dd,J=5.7 and 9.2Hz),4.63 and 5.20(2H,each d,J=16.2Hz),5.62(1H,d,J=1.6Hz)
5h	1770,1750,1700	n.d.	n.d.
5i	1775,1720,1600, 1580	260(ε=6579), 304(ε=10240)	1.26(3H,d,J=6.4Hz),3.83(1H,dd,J=1.5 and 5.6Hz),4.20(1H,m)4.73 and 5.19(2H,each d,J=16.6Hz),5.53(1H,d,J=1.5Hz),7.82(4H,s)

Table II – Continued

Compd.	ir(KBr) νmax(cm^{-1})	uv(H_2O) λmax(nm)	^{1}H nmr(D_2O)[1] δ(ppm)
5j	1770,1730,1610, 1580	256(ε=6707), 307(ε=5312)	1.31(3H,d,J=6.6Hz),3.92(1H,dd,J=1.6 and 5.9Hz),4.26(1H,dq,J= 6.6 and 5.9Hz),5.02 and 5.50(2H,each d,J=16.7Hz),5.65(1H,d,J= 1.6Hz),8.0-8.2(4H,m,Ar)
5k	1770,1675,1605, 1580	256,305	(35°C)1.28(3H,d,J=6.5Hz),1.98(2H,q,J=6.2Hz),2.50(4H,t,J=6.2Hz), 3.86 (1H,dd,J=1.6 and 6.0Hz),4.22(1H,dq,J=6.0 and 6.5Hz),4.88 and 5.26(2H,each d,J=16.3Hz),5.58(1H,d,J=1.6Hz)
14a	1770-1730,1610, 1585	310(ε=5038)	(60MHz)1.33(3H,d,J=6.5Hz),3.90(1H,dd,J=1.8 and 6.0Hz),4.03(3H,s), 4.08(3H,s),4.25(1H,m),5.67(1H,d,J=1.8Hz),6.11(2H,ABq,J=16Hz)
14b	1770,1725,1610, 1585	213(ε=16352), 293(ε=5864)	(60MHz)1.34(3H,d,J=6.5Hz),1.43(3H,t,J=7Hz),3.90(1H,dd,J=1.8 and 6.0 Hz),4.19-4.63(3H,m),5.67(1H,d,J=1.8Hz),5.93(2H,ABq,J= 16Hz),8.31(1H,s)
14c	1770,1725,1610	310(ε=6783)	1.28(3H,d,J=6.5Hz),1.34(3H,t,J=7.2Hz),3.85(1H,dd,J=1.5 and 5.9 Hz),4.19(1H,m),4.40(2H,q,J=7.2Hz),5.56(1H,d,J=1.5Hz),5.86 and 6.30(2H,each d,J=16.5Hz),8.30(1H,s)
18	1780,1735,1680	265	1.31(3H,d,J=6.4Hz),2.09(3H,s),3.50(1H,dd,J=1.3 and 6Hz),4.29 (1H,m),5.25(1H,d,J 0.5Hz),5.39(1H,d,J=1.3Hz),6.94(1H,d,J 0.5Hz)
21[2]	1770,1575	310(ε=4540)	1.29(3H,d,J=6.5Hz),3.98(1H,dd,J=1.4 and 6.0Hz),4.06(2H,ABq,J=15 Hz),4.25(1H,m),5.71(1H,d,J=1.5Hz)
22	1770,1620,1575	306(ε=4659)	(DMSO-d_6,50°C)1.13(3H,d,J=6.2Hz),2.54(3H,d,J=4.4Hz),3.57(1H,dd, J=1.6 and 6.3Hz),3.93(1H,dq,J=6.3 and 6.2Hz),3.96(2H,ABq,J=16 Hz),5.46(1H,d,J=1.6Hz),6.18(1H,br s,exch.D_2O)
23[2]	1775,1635,1565	306(ε=4505)	1.09(6H,d,J=6.6Hz),1.28(3H,d,J=6.1Hz),3.71(1H,m),3.92(1H,dd, J=1 and 6Hz),4.22(1H,m),4.40(2H,s),5.62(1H,d,J=1Hz)
24	1770,1710,1575	308(ε=4370)	1.28(3H,d,J=6.3Hz),3.91(1H,dd,J=1.4 and 6.0Hz),4.22(1H,m), 4.61(2H,ABq,J=15Hz),5.68(1H,d,J=1.4Hz),7.85(1H,s)
25	1775,1685 sh, 1630,1580	308(ε=4902)	1.31(3H,d,J=6.4Hz),2.24(3H,s),3.93(1H,dd,J=1.4 and 5.9Hz), 4.26(1H,dq,J=6.4 and 6.0Hz),4.59(2H,s),5.71(1H,d,J=1.4Hz)
28a	1770,1610	258,314	1.27(3H,d,J=6.5Hz),3.98(1H,dd,J=1.4 and 5.8Hz),4.24(1H,dq,J= 5.8 and 6.5Hz),5.69(1H,d,J=1.4Hz),5.68 and 6.20(2H,each d,J= 14.9Hz),8.10(2H,dd,J=6.1 and 7.7Hz),8.61(1H,t,J=7.7Hz),8.95 (2H,d,J=6.1Hz)
28b	1775,1615,1575	256(ε=2766), 316(ε=4739)	1.29(3H,d,J=6.4Hz),2.23(4H,m),3.11(3H,s),3.59-3.63(4H,m), 4.03(1H,dd,J=1.6 and 5.8Hz),4.26(1H,dq,J=5.8 and 6.4Hz),4.78 (2H,ABq,J=13.8Hz),5.75(1H,d,J=1.6Hz)
35a	1775,1690,1600	262,306	1.28(3H,d,J=6.4Hz),4.00(1H,dd,J=1.6 and 5.9Hz),4.24(1H,dq,J= 5.9 and 6.4Hz),5.71(1H,d,J=1.6Hz),5.75 and 6.26(2H,each d,J= 14.8Hz),8.40 and 9.13(each 2H,d,J=6.8Hz)
35b	1775,1700,1605	262,305	1.38(3H,d,J=6.5Hz),4.01(1H,d,J=4.0 and 9.0Hz),4.33(1H,dq,J=9.0 and 6.5Hz),5.78 and 6.28(2H,each d,J=14.8Hz),5.80(1H,d,J=4.0Hz), 8.38 and 9.13(each 2H,d,J=6.8Hz)
40	1765,1700,1610	292,334	(1:2 E:Z mixture)(80MHz)1.85 and 2.09(3H,each d,J=7.2Hz, CH_3CH of Z and E),2.0-2.5(4H,m),3.15(3H,s),3.4-3.8(4H,m), 4.45 and 5.12(2H,each d,J=13Hz),5.21(1H,br s,H_5),6.33 and 6.63(1H,each q,J=7.2Hz;CH_3CH of E and Z)

[1] At 200MHz, 25°C, unless otherwise stated. [2] Additional data: 21, MS(FD)254, 244(M^+),226 m/z; 23, MS(FD)330, 329(M^+) m/z.

Table III shows the in vitro activity of the title penems observed against seven representative bacterial strains, in comparison with the reference compounds 37 (FCE 22101) and 38. The most striking results are the extraordinary anti-pseudomonal activity of the aminomethylpenem 21 (disappointingly decreasing upon amidino derivatisation, in contrast with the improved activity of Imipenem over thienamycin[27]), and the impressive potency of the quaternary ammonium representative 28b. On the other hand, substantial or complete loss of activity was associated with the 6-ethylidene chain (40), the exomethylene tautomeric structure (18), and the 5S configuration (35b). No straightforward structure-activity relationship is apparent beyond the gross correlation observed along the sequence 38 < 37 < 28b between antibacterial potency and alkaline hydrolysis rate. Clearly, the individual antimicrobial profiles are heavily affected by less predictable variations in the molecular recognition by the cell wall penetration properties.

Table III - In vitro antibacterial activity[1,2] of penems

Compd.	S.a.	E.f.	E.c.$^+$	E.cl.$^+$	K.a.$^+$	C.f.	P.a.
5a	0.18	n.d.	0.78	6.25	1.56	1.56	>50
5b	0.09	25	0.78	1.56	0.78	0.78	>50
5c	0.18	n.d.	3.12	12.5	3.12	6.25	>50
5d	0.09	n.d.	0.78	6.25	1.56	0.78	>50
5e	0.39	n.d.	6.25	12.5	6.25	25	>50
5f	0.77	12.5	1.56	3.12	1.56	1.56	>100
5g	0.39	50	0.78	1.56	0.78	1.56	>100
5h	0.39	n.d.	1.56	12.5	1.56	1.56	>100
5i	<0.005	6.25	0.39	100	12.5	12.5	>100
5j	0.045	3.12	0.39	50	25	12.5	>100
5k	0.18	50	0.78	12.5	0.78	0.78	>100
14a	0.045	n.d.	25	⩾ 50	25	⩾ 25	>100
14b	0.09	n.d.	6.25	> 25	25	6.25	>100
14c	0.18	n.d.	25	> 50	> 50	25	>100
18	>25	>100	>100	>100	> 100	>100	>100
21	0.18	25	1.56	6.25	6.25	3.12	0.78
22	0.39	25	6.25	12.5	6.25	12.5	⩾ 50
23	0.18	6.25	6.25	6.25	12.5	6.25	>100
24	0.02	6.25	0.39	0.78	0.39	0.78	12.5
25	0.045	12.5	6.25	6.25	12.5	25	50
28a	0.005	12.5	0.39	0.78	0.78	1.56	6.25
28b	<0.005	6.25	0.045	0.045	0.045	0.09	25
35a	0.005	12.5	1.56	3.12	1.56	3.12	25
35b	12.5	>100	>100	100	100	100	>100
40	0.19	>100	25	6.25	12.5	50	25
FCE 22101	0.045	1.56	0.78	1.56	0.78	1.56	> 50
38	0.39	12.5	3.12	3.12	3.12	3.12	>100

1) Minimal inihibitory concentrations (MICs, μg/ml) were determined by the standard two-fold agar dilution method in Bacto Antibiotic Medium 1 (Difco). 2) Organisms included in this table are: S.a., Staphylococcus aureus Smith; E.f., Enterococcus faecium ATCC 8043; E.c.$^+$, Escherichia coli B ß-lactamase producer; E.cl.$^+$, Enterobacter cloacae P99 ß-lactamase producer; K.a.$^+$, Klebsiella aerogenes 1082 E ß-lactamase producer; C.f., Citrobacter freundi ATCC 8090; P.a., Pseudomonas aeruginosa ATCC 19660.

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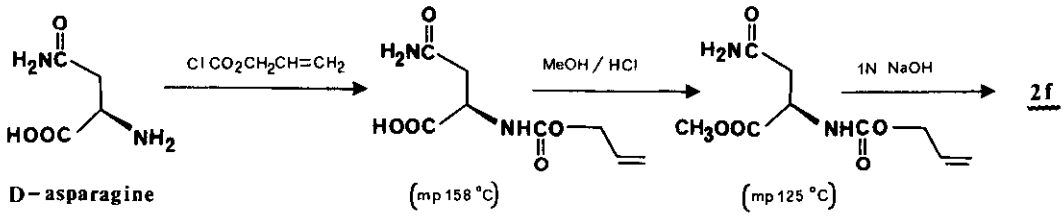
REFERENCES AND NOTES

1. Part II of this series: M. Alpegiani, E. Perrone, and G. Franceschi, Heterocycles, 1988,27,49.
2. I. Ernest in "Chemistry and Biology of ß-Lactam Antibiotics", R.B. Morin and M. Gorman, eds., Academic Press, London, 1982, Vol.2, pp. 315-360.
3. R.B. Woodward in "Recent Advances in the Chemistry of ß-Lactam Antibiotics", J. Elks, ed., Special Publication No. 28, Chem. Soc., London, 1976, pp. 167-180.
4. S. Horii, H. Fukase, and N. Mizokami, Jpn. Kokai Tokkyo Koho 78 119,885 (Chem. Abstr., 1979,90, 168628t), and ibidem, 79 92,986 (Chem.Abstr., 1980,93,2644w). For a saccharin precedent see: J.S. Skornicki and D.P. Strike, J. Antibiotics, 1986,39,380.
5. G. Franceschi, M. Foglio, M. Alpegiani, C. Battistini, A. Bedeschi, E. Perrone, F. Zarini, F. Arcamone, C. Della Bruna, and A. Sanfilippo, J. Antibiotics, 1984,37,685.
6. O. Mitsunobu, Synthesis, 1981, 1; R.P. Volante, Tetrahedron Lett., 1981,22,3119.
7. Imides 2 are commercial products, apart from 2b, which was prepared from glycolamide (V.H. Wallingford, M.A. Thorpe, and R.W. Stoughton, J. Am. Chem. Soc., 1945,67,522) and the new protected aminosuccinimides 2f, 2g, which were obtained from asparagine (54% overall) by analogy with a literature precedent (E. Sondheimer and R.W. Holley, J. Am. Chem. Soc., 1954,76,2647):



Elaboration of L- asparagine produced the enantiomeric imide 2g (Table I).
8. Melting point of crystalline compounds: 3b,132°C;3c,138°C;3e,>230°C;3h,174°C;3i,130°C;4b,130°C; 4c,135°C;4e,159-162°C.
9. M. Alpegiani, A. Bedeschi, and E. Perrone, Gazz. Chim. Ital., 1985,115,393.
10. Unsatured 6-membered analogs, such as uracil and its derivatives, gave problems of chemoselectivity (N-versus-O-alkylation) and regioselectivity. For a precedent see M.S. Manhas, W.H. Hoffman, B. Lal, and A.K. Bose, J. Chem. Soc., Perkin Trans I, 1975,461.
11. Compound 8 was prepared by modification of a patented procedure: M. Menard and A. Martel, U.S.Patent 4,272,437 (Chem. Abstr. 1981,95,203941s).
12. G. Just and T.J. Liak, Can. J. Chem., 1978,56,211.
13. P.D. Jeffrey and S.W. McCombie, J. Org. Chem., 1982,47,587.
14. Similar products were independently pursued at Ciba-Geigy by a different route: M. Lang, P. Schneider, W. Tosch, R. Scartazzini, and O. Zak, Program and Abstracts of the 25th Intersci. Conf. on Antimicrob. Agents Chemother., No 376, p.159, Minneapolis, 1986.

15. [3+2]Dipolar cycloaddition of acetylenes to azides constitutes a powerful synthetic tool for the late construction of thiazoles on sensitive molecules. For a recent application see R.G. Micetich, S.N. Maiti, P. Spevak, T.W. Hall, S. Yamabe, N. Ishida, M. Tanaka, T. Yamazaki, A. Nakai, and K. Ogawa, J. Med. Chem., 1987, 30, 1469.

16. M. Alpegiani, A. Bedeschi, E. Perrone, F. Zarini, and G. Franceschi, Heterocycles, 1985, 23, 2255.

17. H.G. Viehe, ed., "Chemistry of Acetylenes", Marcel Dekker, New York, 1969.

18. Racemic 21 had been previously patented by Bristol-Myers Co. (ref. 11).

19. H. Staudinger and J. Meyer, Helv. Chim. Acta, 1919, 2, 635.

20. Once separated, 17 (0.1g) gave the same 70:30 proportion of 17 and 18 when treated with triethylamine (0.1 ml) in dichloromethane (1 ml, 6 h).

21. M. Vaultier, N. Knouzi, and R. Carrié, Tetrahedron Lett., 1983, 24, 763.

22. M. Lang, E. Hungerbühler, P. Schneider, R. Scartazzini, W. Tosch, E.A. Konopka, and O. Zak, Helv. Chim. Acta, 1986, 69, 1576.

23. E. Perrone, M. Alpegiani, A. Bedeschi, F. Giudici, F. Zarini, G. Franceschi, C. Della Bruna, D. Jabes, and G. Meinardi, J. Antibiotics, 1986, 39, 1351.

24. E. Perrone, M. Alpegiani, A. Bedeschi, F. Giudici, M. Foglio, and G. Franceschi, Tetrahedron Lett., 1983, 24, 3283.

25. C.J.M. Stirling, Acc. Chem. Res., 1979, 12, 198.

26. A full account of the complex array of products resulting from hydrolytic cleavage of "2-CH_2X" penems will be given on a separate paper.

27. W.J. Leanza, K.J. Wildonger, T.W. Miller, and B.G. Christensen, J. Med. Chem., 1979, 22, 1435.

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