# Synthesis and Biological Activity of Novel Cephalosporins Containing a (*Z*)-Vinyl Dimethylphosphonate Group

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A series of cephalosporins containing a novel 7-[2-(*Z*)-(2-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino] group were prepared and their antibacterial activity measured against a range of pathogens. In general the compounds displayed a broad spectrum of activity against both Gram positive and Gram negative organisms, except *Pseudomonas aeruginosa*. Activity against the latter could be achieved by introducing a catechol moiety at the 3 position of the cephalosporin. The methyl phosphonates in general were stable to a wide range of β-lactamases, including the TEM enzymes and the *Enterobacter cloacae* P99 chromosomal enzyme. In addition, they showed the advantage of being highly water soluble.

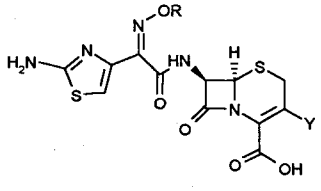
The emerging resistance to so-called third generation cephalosporins is largely due to the expression of the new, extended spectrum TEM derived β-lactamases. This is a dynamic problem and new agents are needed to moderate this threat. As part of our programme towards the development of novel broad spectrum cephalosporins with improved β-lactamase stability we embarked on the synthesis of a series of analogues derived from third generation aminothiazolyl oxime cephalosporins in which the oxime moiety **1** was replaced with a (*Z*)-substituted olefin **2**. At the commencement of this work it was well established in the literature that olefinic replacements for the oxime could lead to useful antibacterial activity. The Meiji group had described the synthesis of carboxyvinyl analogues **2a** which displayed good Gram-negative activity,[1] the orally active ceftibuten **2b** was known to display excellent Gram-negative activity and β-lactamase stability[2] and (*Z*)-vinyl sulphones **2c** had been prepared by the Bayer group.[3~4] To extend the range of olefin substituents we have prepared a series of (*Z*)-vinyl dimethylphosphonates **2d** and herein report their synthesis and biological properties.

## Results

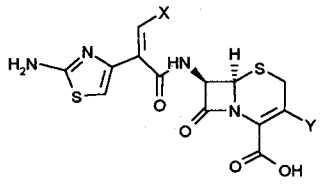
### Chemistry

Olefination of the glyoxalate **3a**[5] with the bis-phosphonate **4** afforded the vinylphosphonate ethyl ester **5**, as a 1 : 1 mixture of (*E*) and (*Z*) isomers which were

Illustration 1.

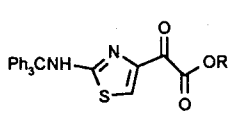


**1**

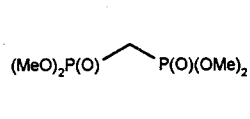


**2a** X=$CO_2R$
**b** X=$CH_2CO_2H$, Y=H
**c** X=$SO_2$-Alkyl
**d** X=P(O)$(OMe)_2$

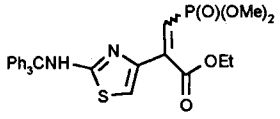
Illustration 2.



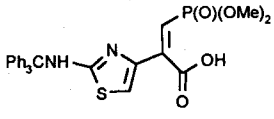
**3 a:** R = Et
**b:** R = Na
**c:** R = pentafluorophenyl



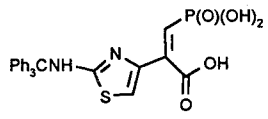
**4**



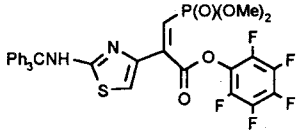
**5**



**6**



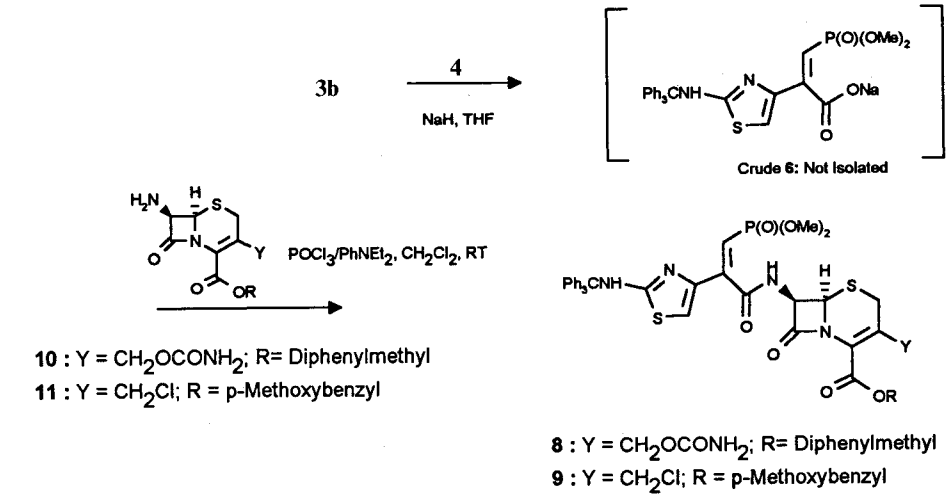
**7**



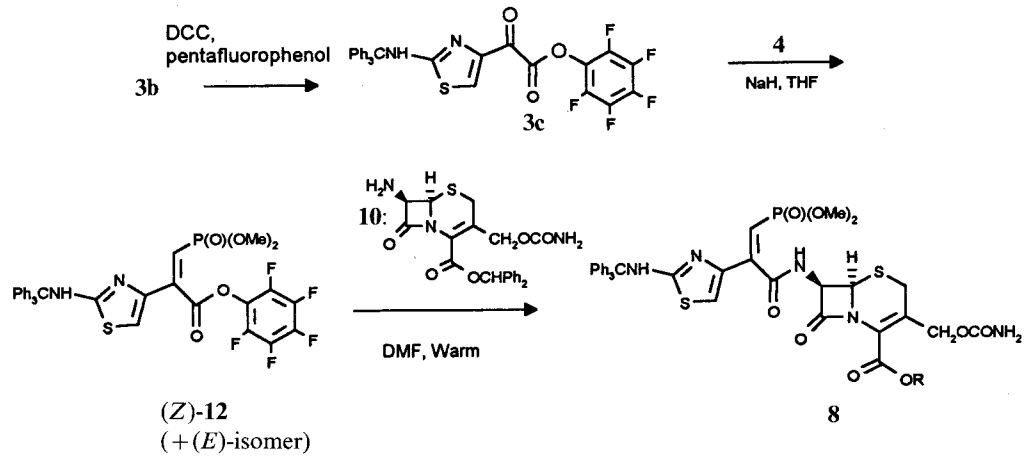
**12**

Scheme 1.

Method A



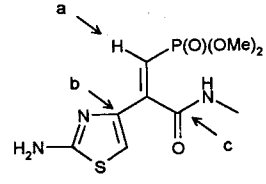

Method B




separated using conventional chromatography.

Attempts to prepare the dimethylphosphonate-carboxylic acid **6** by saponification of (*Z*)-**5** were unsuccessful due to the extreme instability of **6**. It appears that in compound **6** the methyl phosphonate esters are highly susceptible to hydrolysis catalysed by the neighbouring carboxylate group, and the fully hydrolysed compound **7** is rapidly formed. This intramolecular assisted hydrolysis of phosphonate esters has been observed in related systems.[6~8] To circumvent this problem the olefination reaction was carried out on the sodium salt **3b** (which is freely soluble in THF) (Scheme 1: Method A). The unstable **6** generated *in situ* in this reaction was then coupled directly with the cephalosporin amines **10** or **11** producing **8** or **9** respectively in a one pot process. Interestingly, only the (*Z*)-isomers **8** and **9** were produced under these conditions in moderate overall yields. The (*Z*)-stereochemistry of the vinyl phosphonates was inferred from the $^{13}C$-$^{1}H$ coupling constants between the olefinic proton and neighbouring carbon atoms using an inverse heteronuclear multiple

Fig. 1.



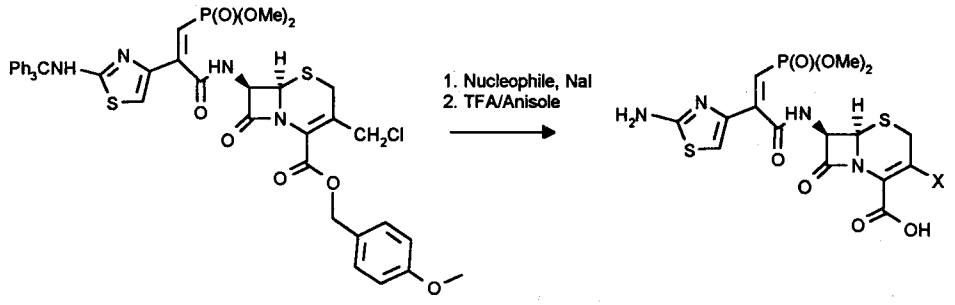

bond correlation (HMBC) experiment.[9] A 15 Hz coupling was observed between the olefinic proton and the carbonyl carbon, indicating a trans relationship, whereas a smaller 9 Hz coupling was observed between the olefinic proton and the carbon within the thiazole heterocycle (cis relationship, Fig. 1).

We attempted a variety of coupling conditions in order to improve the yields for this 'one pot' process. Phosphorus oxychloride ($POCl_3$) was found to be the most effective reagent for the reaction. Lower yields of coupled products **8** and **9** were obtained when bis (2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) was used as the coupling reagent. With other coupling reagents little or none of the desired products were

formed. In all experiments, compound **7** was formed as a major biproduct. Compound **7** did not undergo any coupling to cephalosporin amines **10** and **11** under any of the conditions employed.

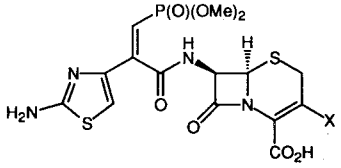
In an alternative approach, (Scheme 1: Method B), the pentafluorophenyl ester **12** was prepared from the sodium salt **3b** in two stages. Firstly **3b** underwent a dicyclohexyl carbodiimide (DCC) coupling with pentafluorophenol, and the crude, unstable product from this reaction **3c** was then treated with salts of bis-phosphonate **4** to generate the active ester **12**. A highly (*Z*)-selective olefination was observed when $KN(TMS)_2$ was used as base (potassium salt of **4**), but when sodium hydride was used to deprotonate **4**, a separable 1:1 mixture of

Scheme 2.




**9** **13~22**

Table 1-1. Yields for final stages and spectral data for **13~24**.



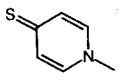
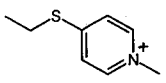
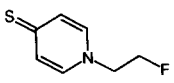
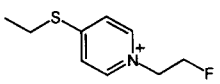
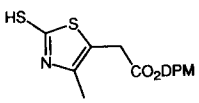
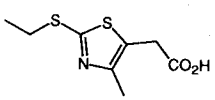
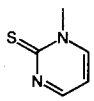
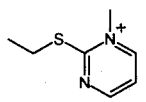
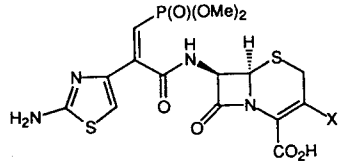

| Nucleophile | Yield Step 1 | Yield Step 2 | Product No. | X | $^1$H NMR (DMSO unless stated) | IR (nujol) $\beta$ lactam $cm^{-1}$ |
|---|---|---|---|---|---|---|
|  | 100 | 94 | **13** |  | 9.5 (1H, d, NH, *J* 7.5 Hz), 8.7, 8.0 (4H, 2d, pyridinium H, *J* 7.5 Hz), 7.3 (2H, br s, $NH_2$), 6.67 (1H, s, thiazole H), 6.18 (1H, d, olefin H, $J_{PH}$ 15 Hz), 5.78 (1H, dd, H-7), 5.22 (1H, d, H-6), 4.38 (2H, br s, $CH_2S$), 3.7 (2H, ABq, H-4), 3.65, 3.6 (6H, d, MeOP, $J_{PH}$ 15 Hz), 4.12 (3H, s, NMe) | 1779 |
|  | 88 | 59 | **14** |  | 9.6 (1H, d, NH, *J* 8 Hz), 8.8, 8.08 (4H, 2×d, pyridyl H, *J* 6 Hz), 6.92 (1H, s, thiazole H), 6.33 (1H, d, olefin H, $J_{PH}$ 12 Hz), 5.83 (1H, dd, H-7, *J* 5, 8 Hz), 5.29 (1H, d, H-6, *J* 5 Hz), 5.02, 4.91 (2H, 2m, $CH_2F$), 4.8 (2H, m, $NCH_2$), 4.45 (2H, ABq, $CH_2S$), 3.7 (2H, ABq, H-4), 3.75, 3.65 (6H, d, MeOP, $J_{PH}$ 15 Hz). | 1779 |
|  | 87 | 85 | **15** |  | 9.55 (1H, d, NH, *J* 8 Hz), 6.85 (1H, s, thiazole H), 6.25 (1H, d, olefin H, $J_{PH}$ 15 Hz, 5.75 (1H, dd, H-7, *J* 5, 8 Hz), 5.10 (1H, d, H-6, *J* 5 Hz), 4.52, 4.10 (2H, ABq, $CH_2S$), 3.75 (2H, s, $CH_2CO_2$), 3.7, 3.65 (6H, d, MeOP, $J_{PH}$ 15 Hz), 3.7 (2H, ABq, H-4), 2.25 (3H, s, MeAr). | 1782 |
|  | 70 | 99 | **16** |  | 9.39 (1H, d, NH, *J* 8 Hz), 9.12 (dd), 9.00 (dd), 7.76 (dd) (3H, pyrimidinyl H), 6.64 (1H, s, thiazole H), 6.10 (1H, d, olefin H, $J_{PH}$ 13.7 Hz), 5.65 (1H, dd, H-7, *J* 5, 8 Hz), 5.00 (1H, d, H-6, *J* 5 Hz), 4.72, 4.20 (2H, ABq, $CH_2S$), 3.92 (3H, s, NMe), 3.7, 3.4 (2H, ABq, H-4), 3.55, 3.49 (6H, d, MeOP, $J_{PH}$ 15 Hz). | 1784 |

Table 1-2. Yields for final stages and spectral data for **13**~**24**.



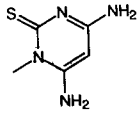
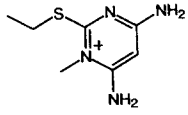
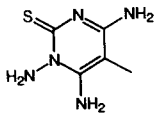
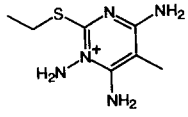
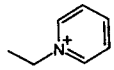
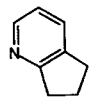
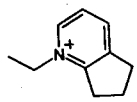
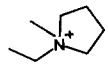
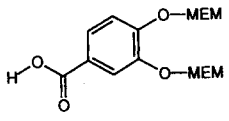
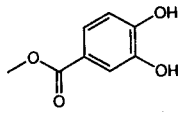
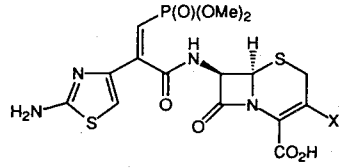
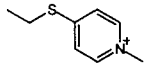
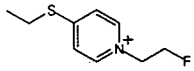
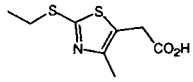
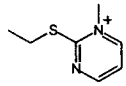
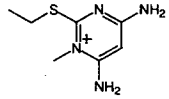
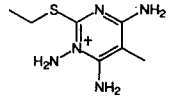
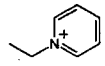
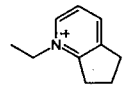
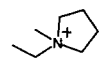
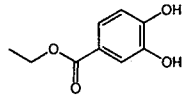
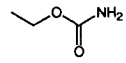

| Nucleophile | Yield Step 1 | Yield Step 2 | Product No. | X | $^1$H NMR (DMSO unless stated) | IR (nujol) $\beta$ lactam $cm^{-1}$ |
|---|---|---|---|---|---|---|
|  | 84 | 56 | **17** |  | 9.56 (1H, d, NH, *J* 8 Hz), 7.95 (2H, br s, $NH_2$), 6.85 (1H, s, thiazole H), 6.29 (1H, d, olefinic H, $J_{PH}$ 12.5 Hz), 5.80 (1H, dd, H-7, *J* 5, 8 Hz), 5.6 (1H, s, pyrimidinyl H), 5.22 (1H, d, H-6, *J* 5 Hz), 4.51, 4.20 (2H, ABq, $CH_2S$), 3.7, 3.65 (6H, d, MeOP, $J_{PH}$ 15 Hz), 3.45 (3H, s, NMe), 3.6 (2H, ABq, H-4) | 1778 |
|  | 100 | 61 | **18** |  | 9.45 (1H, d, NH, *J* 7.5 Hz), 7.8, 7.4, 6.0 (6H, 3 br s, 3 × $NH_2$), 6.68 (1H, s, thiazole H), 6.20 (1H, d, olefin H, $J_{PH}$ 15 Hz), 5.73 (1H, dd, H-7, *J* 5, 7.5 Hz), 5.18 (1H, d, H-6, *J* 5 Hz), 4.3, 3.95 (2H, ABq, $CH_2S$), 3.6 (2H, ABq, H-4), 3.65, 3.55 (6H, d, MeOP, $J_{PH}$ 15 Hz), 1.88 (3H, s, MeAr). | 1780 |
|  | 83 | 63 | **19** |  | 9.48 (1H, d, NH, *J* 7.5 Hz), 9.02 (2H, d), 8.63 (1H, t), 8.2 (2H, t, pyridyl H), 7.25 (2H, br s, $NH_2$), 6.62 (1H, s, thiazole H), 6.18 (1H, d, olefin H, $J_{PH}$ 15 Hz), 5.88 (1H, dd, H-7, *J* 5, 7.5 Hz), 5.55 (2H, ABq, $CH_2N$), 5.23 (1H, d, H-6), 3.6, 3.55 (6H, d, MeOP, $J_{PH}$ 15 Hz), 3.45 (2H, ABq, H-4) | 1780 |
|  | 49 | 90 | **20** |  | 9.5 (1H, d, NH, *J* 7.5 Hz), 8.65 (d), 8.4 (d), 7.95 (t) (3H, pyridyl H), 7.3 (2H, br s, $NH_2$), 6.62 (1H, s, thiazole H), 6.2 (1H, d, olefin H, $J_{PH}$ 15 Hz), 5.85 (1H, dd, H-7, *J* 5, 7.5 Hz), 5.5 (2H, m, $CH_2N$), 5.2 (1H, d, H-6), 3.65, 3.6 (6H, d, MeOP, $J_{PH}$ 15 Hz), 3.45 (2H, m, H-4), 3.3, 3.15 (4H, m), 2.2 (2H, m, cyclopentyl H). | 1778 |
|  | 68 | 90 | **21** |  product contains 20% of $\Delta$-2 isomer | 9.5 (1H, d, NH, *J* 7.5 Hz), 7.2 (2H, br s, $NH_2$), 6.65 (1H, s, thiazole H), 6.2 (1H, d, olefin H, $J_{PH}$ 15 Hz), 5.85 (1H, dd, H-7), 5.3 (1H, d, H-3), 4.5, 4.15 (2H, ABq, $CH_2$py), 3.9 (2H, ABq, H-4), 3.7~3.4 (4H, m, ring $CH_2N$), 3.6, 3.55 (6H, d, MeOP, $J_{PH}$ 15 Hz), 2.8 (3H, s, NMe), 2.2~2.0 (4H, m, $CH_2CH_2N$). | 1783 |
|  | 21 | 41 | **22** |  | 9.5 (1H, d, NH, *J* 7.5 Hz), 7.36 (1H, d, *J* 2.5 Hz), 7.34 (1H, dd, *J* 2.5, 8 Hz), 6.82 (1H, d, *J* 8 Hz, catechol H), 6.69 (1H, s, thiazole H), 6.19 (1H, d, olefin H, $J_{PH}$ 15 Hz), 5.8 (1H, dd, H-7, *J* 4, 7.5 Hz), 5.23 (1H, d, H-6, *J* 4 Hz), 5.3 (2H, ABq, $CH_2OCO$, *J* 13 Hz), 3.7 (6H, d, MeOP, $J_{PH}$ 15 Hz), 3.68 (2H, ABq, H-4, *J* 18 Hz). | 1776 |
| — | — | 94 | **23** | H | ($D_2O$): 7.15 (1H, s, thiazole H), 6.75 (1H, dd, H-3), 6.39 (1H, d, olefin H, $J_{PH}$ 12.5 Hz), 5.96 (1H, d, H-7, *J* 5 Hz), 5.25 (1H, d, H-6, *J* 5 Hz), 3.85, 3.80 (6H, d, MeOP, $J_{PH}$ 15 Hz), 3.68 (2H, m, H-4) | 1779 |
| — | — | 71 | **24** | $CH_2$–O–$CONH_2$ | ($D_2O$): 7.1 (1H, s, thiazole H), 6.4 (1H, d, olefinic H, $J_{PH}$ 12.5 Hz), 5.9 (1H, d, H-7, *J* 5 Hz), 5.25 (1H, d, H-6, *J* 5 Hz), 5.0, 4.8 (2H, ABq, $CH_2OCO$), 3.8, 3.75 (6H, d, MeOP, $J_{PH}$ 15 Hz), 3.62 (2H, ABq, H-4). | 1781 |

Table 2-1. Antimicrobial activities of **13**~**24** (MIC, $\mu$g/ml).



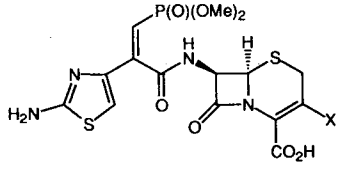

| | X | *S. aureus* 853E | *S. aureus* 663 | *E. coli* 851E | *E. coli* DC0 | *E. coli* DC2 | *E. coli* TEM3 | *E. coli* TEM9 | *E. coli* tonB | *E. cloacae* P99 |
|---|---|---|---|---|---|---|---|---|---|---|
| **13** |  | 4 | 2 | 0.5 | 0.5 | 0.25 | 0.25 | 4 | 1 | 2 |
| **14** |  | 4 | 2 | 0.25 | 1 | 0.25 | 1 | 1 | 1 | 2 |
| **15** |  | 31 | 16 | 2 | 4 | 4 | 8 | 16 | 2 | 16 |
| **16** |  | 31 | 16 | 2 | 4 | 2 | 8 | 8 | 4 | 16 |
| **17** |  | 2 | 1 | 0.25 | 0.5 | 0.13 | 0.5 | 0.5 | 0.5 | 1 |
| **18** |  | 1 | 1 | 0.13 | 0.13 | 0.06 | 0.25 | 0.5 | 0.25 | 1 |
| **19** |  | 8 | 8 | 0.5 | 1 | 1 | 1 | 8 | 1 | 4 |
| **20** |  | 8 | 8 | 2 | 4 | 4 | 8 | 31 | 4 | 16 |
| **21** |  | 16 | 16 | 1 | 4 | 2 | 8 | 16 | 2 | 8 |
| **22** |  | 4 | 4 | <0.03 | 0.06 | <0.03 | 0.06 | 0.13 | 8 | 16 |
| **23** | H | 31 | 31 | 0.5 | 0.5 | 0.06 | 2 | 4 | 1 | 8 |
| **24** |  | 8 | 8 | 0.5 | 0.5 | 0.06 | 4 | 2 | 0.5 | 16 |
| | Ceftazidime | 2 | 2 | <0.06 | 0.13 | <0.06 | 31 | >62 | 0.13 | 8 |

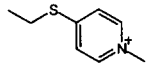
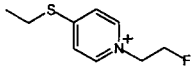
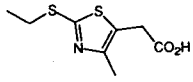
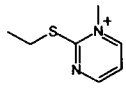
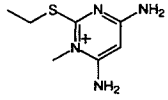
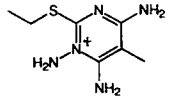
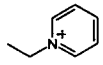
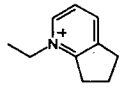
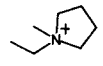
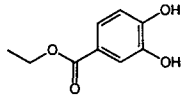
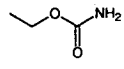
(*E*) and (*Z*) isomers was obtained. The activated ester (*Z*)-**12** generated by this method could be purified by chromatography, and underwent slow coupling to the cephalosporin amine **10** at 50°C in DMF. This achieved an overall improved yield of **8** based on the cephalosporin when compared to method A.

Utilising the coupling procedures outlined above we then used standard methods to elaborate the intermediates **8** and **9** and prepared a diverse range of cephalosporin derivatives **13**~**24**. Nucleophilic displacements of the chloride in **9** were accomplished under standard conditions with a selection of nucleophiles.[10,11] Subsequent deprotection with TFA/anisole afforded the target cephalosporins **13**~**22** (Scheme 2). Compound **23** was prepared by method A starting from (6*R*,7*R*)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *t*-butyl ester and utilising BOP-Cl as the coupling reagent. The intermediate **25** formed in this reaction was deprotected with TFA/anisole. Similarly compound **24** was prepared by direct TFA/anisole treatment of **8**. In all the above cases, the dimethylphosphonate group was completely stable to the TFA/anisole conditions used for removal of the protecting groups.

Yields for the nucleophilic displacement reactions are given in Table 1 together with spectroscopic data for **13**~**24**.

Table 2-2. Antimicrobial activities of **13**~**24** (MIC, μg/ml).




| | X | *K. aerogenes* K1 | *P. vulgaris* 1805E | *M. morganii* 1375E | *S. marcescens* | *A. calcoaceticus* 1955E | *P. aeruginosa* 150E | *P. aeruginosa* 1912E | sc $ED_{50}$ *S. aureus* |
|---|---|---|---|---|---|---|---|---|---|
| **13** |  | 0.5 | 2 | 0.5 | 1 | 31 | >62 | 62 | 6.2 |
| **14** |  | 0.25 | 2 | 0.5 | 2 | 31 | >62 | 62 | 3.6 |
| **15** |  | 2 | 2 | 1 | 4 | 62 | >62 | >62 | 50 |
| **16** |  | 2 | 4 | 1 | 8 | 16 | >62 | 62 | |
| **17** |  | 0.25 | 1 | 0.5 | 1 | 16 | >62 | 62 | 2.7 |
| **18** |  | 0.13 | 1 | 0.25 | 0.5 | 16 | >62 | >62 | 1.5 |
| **19** |  | 1 | 4 | 1 | 2 | 16 | >62 | 31 | |
| **20** |  | 2 | 8 | 4 | 8 | 31 | >62 | >62 | |
| **21** |  | 2 | 4 | 2 | 8 | 31 | >62 | >62 | |
| **22** |  | 0.06 | 0.5 | 0.5 | 0.13 | 0.06 | 1 | 1 | |
| **23** | H | 0.25 | 0.5 | 0.13 | 1 | 31 | 62 | 31 | |
| **24** |  | 0.25 | 0.25 | 0.13 | 1 | 16 | >62 | 62 | 6.3 |
| | Ceftazidime | 0.13 | <0.06 | <0.06 | <0.06 | 0.5 | 0.25 | 1 | 25 |

## Antimicrobial Activity

Compounds **13**~**24** were evaluated *in vitro* against a screen of commonly encountered bacteria comprising both Gram positive and Gram negative organisms (Table 2). Strains expressing plasmid- and chromosomally-mediated β-lactamases were also included.[12,13] The MIC values for ceftazidime against the same strains are shown for comparison. MICs were determined by a two-fold agar dilution method using Mueller-Hinton media (Becton Dickinson Ltd., U.S.A.). Exponentially growing cultures were inoculated onto the surface of agar plates using a multipoint inoculator (Denley, UK) to produce an inoculum of approximately $10^5$ cfu $ml^{-1}$. The plates were incubated at 37°C for 18 hours and the MIC value interpreted as the lowest antibiotic concentration which completely inhibited bacterial growth.

The more active derivatives were tested in an *in vivo* mouse model of *S. aureus* infection. The organism was administered by intraperitoneal inoculation into groups of five female CRH (Charles River Harefield) mice. The animals were dosed subcutaneously with antibiotic at four dose levels. The median effective dose ($ED_{50}$ $mgkg^{-1}$ $dose^{-1}$) was calculated using the method described by LITCHFIELD and WILCOXON.[14] The observed *in vivo* anti-Staphylococcal activity correlated well with the observed *in vitro* activity.

## Discussion

In general the dimethylphosphonates displayed useful

antibacterial activity against the Gram positive organism *S. aureus* (especially compounds **17** and **18** which possess a thiopyrimidyl 3-substituent of the type described by the Lucky group[15]) and good activity against *E. coli* and other Gram negative organisms except *Pseudomonas aeruginosa*. They were invariably more effective against organisms expressing the extended spectrum β-lactamases than was ceftazidime. This included significantly improved activity against organisms harbouring plasmids expressing TEM-3 (cefotaximase-type)[16] and TEM-9 (ceftazidimase-type)[17] enzymes. The compounds were also resistant to hydrolysis by chromosomally mediated β-lactamases (eg P99 and K1) and often had activity equivalent to or better than that of ceftazidime.

Compound **22** was exceptionally stable to most of the β-lactamase producing organisms and was the only phosphonate derivative active against *Pseudomonas aeruginosa*. The overall enhanced activity against Gram negative bacteria observed may be due to the catechol group present in the molecule, which could facilitate transfer of the compound across the outer membrane of Gram-negative bacteria *via* a component of the bacterial iron transport mechanism. A similar improvement in Gram negative activity on introducing a 3-catechol moiety is observed in other series of cephalosporins.[18,19] Lack of penetration of the outer membrane may be a limiting factor in activity against bacterial species such as *Pseudomonas*, which are usually resistant to β-lactam antibiotics. In *E. coli*, the overall activity of the iron transport system is thought to be under the control of the *tonB* gene.[20] The use of a mutant *E. coli* strain lacking this *tonB* gene, yields an increased MIC value when tested against an antibiotic which utilises the iron transport mechanism as an additional means of gaining entry into the organism. Such an increase was observed with compound **22** and may indicate active uptake *via* the iron transport system.

In conclusion, it appears that as a class the dimethyl phosphonates are somewhat less active antibacterial compounds than oximes but are significantly more stable to β-lactamases. Thus it seems that the additional steric bulk around the tetrahedral phosphorus atom reduces the ability of the β-lactam nucleus to acylate both penicillin binding proteins and β-lactamases. A final point of note is that compounds **13**~**24** are all highly water soluble thus making them ideal for use as injectable antibiotics.

## Experimental

FTIR spectra were recorded using a Nicolet 20SXB or a Bio-Rad FTS-7. $^1$H NMR spectra were recorded either at 250 MHz using a Bruker AC or AM 250 or at 400 MHz with a Varian VXR 400. Mass spectra were measured on a HP Engine (Thermospray positive) or VG Autospec Q (LSIMS). Routine microanalyses were performed on a Leco CHNS-932 or Carlo-Erba instrument. Fluorine analyses were carried out with a Phillips PW9415 ion selective meter and water analyses using a Mitsubishi CA-05. Flash chromatography was carried out using Merck Kieselgel 9385.

### i) Vinyl Phosphonate Formation

#### Method A

#### Bis (2-oxo-3-oxazolidinyl) Phosphinic Chloride (BOP-Cl) Coupling Procedure

a) (6*R*,7*R*)-7-[2-(*Z*)-(2-trityl-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-3-carbamoyl-oxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid diphenyl methyl ester (**8**)

The phosphonate **4** (0.695 g, 3 mmol) was dissolved in THF (5 ml) and cautiously added to a suspension of sodium hydride (60% dispersion, 0.12 g, 3 mmol) in THF (5 ml) under nitrogen at 0°C. The resulting clear solution was added dropwise at 0°C to a solution of the glyoxalate **3b** (1.31 g, 3 mmol) in THF (25 ml), producing a deep red solution. After 20 mins BOP-Chloride (0.73 g, 3 mmol) was added and the solution diluted with dichloromethane (100 ml). The solution was stored at 0°C for 1 hour. At this stage a clear yellow solution was obtained. To this was added a solution of the protected cephalosporin **10** (tosylate salt) (1.38 g, 2 mmol) neutralised with *N*,*N* diethylaniline (0.622 ml, 4 mmol) in dichloromethane (20 ml) and the resulting suspension allowed to warm to room temperature over 12 hours. Ethyl acetate (200 ml) and water (200 ml) were then added and the organic fraction washed with aqueous hydrochloric acid (2 M, 200 ml) then aqueous sodium bicarbonate solution (saturated, 200 ml). A yellow precipitate was produced at this stage which was shown to be (*Z*)-3-phosphono-2-[2-(trityl-amino)-thiazol-4-yl]-acrylic acid **7**. Compound **7**: $^1$H NMR (DMSO): 8.75 (1H, s, trityl NH), 7.4~7.1 (15H, m, 3×Ph), 6.5 (1H, s, thiazole H), 5.9 (1H, d, olefin H, $J_{PH}$ 15 Hz). FD-MS *m/z* 493 ($MH^+$), 243. $C_{25}H_{21}N_2O_5PS \cdot 2H_2O$ Requires C 56.8, H 4.7, N 5.3, S 6.1, $H_2O$ 6.8. Found C 56.7, H 5.0, N 5.4, S 6.0, $H_2O$ 6.4.

Filtration of the organic residue gave a brown solution which was dried (anhydrous magnesium sulphate), evaporated *in vacuo* and the residue purified by flash chromatography (eluted with ethyl acetate) to afford the title compound **8** (0.314 g, 17%) as a yellow foam. $^1$H NMR ($CDCl_3$) 7.45 (1H, d, NH), 7.4~7.2 (25H, m, 5×Ph), 6.92 (1H, s, C*H*$Ph_2$), 6.75 (1H, s, thiazole H), 6.65 (1H, br s, trityl NH), 6.58 (1H, d, olefin H, $J_{PH}$ 15 Hz), 5.95 (1H, dd, H-7), 5.04 (1H, d, H-6), 5.08, 4.7 (2H, ABq, $CH_2OCO$), 4.55 (2H, br s, $NH_2$), 3.8~3.7 (6H, 2×d, MeOP, $J_{PH}$ 15 Hz), 3.48 (2H, ABq, H-4). IR ($CHBr_3$): 1788, 1728, 1681, 1580, 1525. FD-MS *m/z* 942 ($MH^+$).

b) Similarly prepared from (6*R*,7*R*)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *t*-butyl ester was (6*R*,7*R*)-7-[2-(*Z*)-(2-trityl-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *t*-

butyl ester (**25**) in 25% yield. $^1$H NMR ($CDCl_3$): 7.35~7.25 (15H, m, 3×Ph), 7.2 (1H, d, NH amide, *J* 7.5 Hz), 6.72 (1H, s, trityl NH), 6.65 (1H, s, thiazole H), 6.55 (1H, d, olefin, $J_{PH}$ 15 Hz), 6.42 (1H, dd, H-3), 5.96 (1H, dd, H-7), 5.05 (1H, d, H-6), 3.7 (6H, 2×d, MeOP, $J_{PH}$ 15 Hz), 3.6, 3.4, (2H, 2m, H-4), 1.5 (9H, s, tBu). IR ($CHBr_3$): 3398, 1787, 1718, 1669, 1525. $C_{38}H_{39}N_4O_7PS_2$ Requires C 60.1, H 5.1, N 7.4. found C 59.3, H 5.2, N 7.0. FD-MS *m/z* 758 ($M^+$).

$POCl_3$ Coupling Procedure

(6*R*,7*R*)-7-[2-(*Z*)-(2-trityl-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-3-chloromethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *p*-methoxybenzyl ester (**9**)

The bis-phosphonate **4** (Lancaster Synthesis, 1.16 g, 5 mmol) was dissolved in dry THF (5 ml) and added cautiously at 0°C under nitrogen to a stirred suspension of sodium hydride (60% dispersion in oil, 200 mg, 5 mmol) in THF (5 ml). When all effervescence had ceased, the resulting clear solution was added in one portion to a solution of the sodium salt **3b** (2.18 g, 5 mmol) in dry THF (50 ml) at 0°C producing a deep red solution. After 30 minutes the mixture was concentrated to a small volume, diluted with dichloromethane (100 ml) and cooled to −30°C. *N,N* diethylaniline (1.6 ml, 10 mmol) was added, followed by phosphorus oxychloride (0.56 ml, 6 mmol) and then the hydrochloride salt of the cephalosporin **11** (2.03 g, 5 mmol). The resulting suspension was allowed to warm to room temperature over 30 minutes. Ethyl acetate (200 ml) was added and the organic solution washed sequentially with aqueous hydrochloric acid, water and saturated aqueous sodium bicarbonate solution. The organic layer was then dried (anhydrous $MgSO_4$), evaporated under vacuum and purified by flash chromatography (eluant ethyl acetate: petroleum ether, 3:1) affording the title compound **9** (1.64 g, 38%) as a yellow foam. $^1$H NMR ($CDCl_3$): 7.35~7.2 (18H, m, 3×Ph, NH, 2 Ar), 6.9 (2H, d, 2 Ar), 6.72 (1H, s, trityl NH), 6.65 (1H, s, thiazole H), 6.58 (1H, d, olefin H, $J_{PH}$ 13 Hz), 5.92 (1H, dd, H-7), 5.22 (2H, ABq, $CO_2CH_2$), 5.05 (1H, d, H-6), 4.5 (2H, ABq, $CH_2Cl$), 3.8 (3H, s, MeOAr), 3.78~3.70 (6H, 2×d, MeOP, $J_{PH}$ 15 Hz), 3.55 (2H, ABq, H-4). IR (nujol): 1786, 1724, 1677, 1515. $C_{43}H_{40}ClN_4O_8PS_2 \cdot 0.5H_2O$ Requires C 58.7, H 4.7, N 6.4, S 7.3. Found C 58.7, H 5.1, N 6.4, S 7.1. FD-MS *m/z* 871 ($MH^+$).

Method B

a) Preparation of (*Z*)-(dimethoxy-phosphoryl)-2-[2-(trityl-amino)-thiazol-4-yl]-acrylic acid 2,3,4,5,6-pentafluoro-phenyl ester (**12**)

The sodium salt **3b** was converted to the free acid by partitioning between ethyl acetate and aqueous hydrochloric acid. A mixture of the acid (1.04 g, 2.5 mmol) and pentafluorophenol (0.46 g, 2.5 mmol) were dissolved in dichloromethane (70 ml) and cooled to −40°C under nitrogen. To this was added dropwise a solution of dicyclohexylcarbodiimide (DCC) (0.52 g, 2.5 mmol) in dichloromethane (10 ml) maintaining the temperature below −30°C. The resulting slurry was allowed to warm to 0°C, then filtered, dried and evaporated to afford a bright orange foam containing the pentafluorophenyl glyoxalate **3c**. This compound was unstable and was used directly without further purification. The orange foam was dissolved in dry THF (30 ml) and treated at −30°C under nitrogen with a solution of the sodium salt of the bis phosphonate **4** (Lancaster Synthesis, 1.74 g, 7.5 mmol) in THF (10 ml) prepared as described in method A. Upon addition of the phosphonate the orange solution turned deep purple. Initially the colour was transient and rapidly dispersed, but at the end of the addition it persisted. The temperature of the reaction was allowed to rise to 0°C over 1 hour, and after 10 minutes at 0°C the reaction mixture was partitioned between ethyl acetate (200 ml) and water (200 ml). The organic layer was dried (anhydrous $MgSO_4$) and evaporated to a brown foam which was purified by flash chromatography (ethyl acetate: petroleum ether 1:2). The required compound (*Z*)-**12** was eluted first (223 mg) as a yellow foam, followed by the (*E*)-isomer (255 mg) (orange foam) together with a mixed fraction of (*E*) and (*Z*) isomers (208 mg). The combined yield of isomers from **3b** was 41%.

(*Z*)-isomer **12**: $^1$H NMR ($CDCl_3$): 7.4 (15H, m, 3×Ph), 6.7 (1H, d, olefin H), 6.71 (1H, s, trityl NH), 6.70 (1H, s, thiazole H), 3.7 (6H, d, MeOP, $J_{PH}$ 15 Hz). IR ($CHBr_3$): 1774, 1725, 1654, 1598, 1519. (*E*)-isomer $^1$H NMR ($CDCl_3$): 7.4 (15H, m, 3×Ph), 7.05 (1H, s, thiazole H), 6.95 (1H, d, olefin H), 6.75 (1H, br s, trityl NH), 3.6 (6H, d, MeOP, $J_{PH}$ 15 Hz). IR ($CHBr_3$): 1764, 1716, 1521. $C_{33}H_{24}F_5N_2O_5PS \cdot 0.5H_2O$ requires C 57.0, H 3.6, N 4.0, S 4.6, F 13.65. Found: (*Z*)-isomer C 56.8, H 3.7, N 4.1, S 4.3, F 13.2. (*E*)-isomer C 56.9, H 3.6, N 4.1, S 4.4, F 12.8.

The above experiment replacing sodium hydride with $KN(TMS)_2$ (1 M in toluene) to generate the anion of **4** afforded exclusively the required (*Z*)-isomer **12**.

b) Coupling of *Z*-pentafluorophenyl ester.

Coupling of (*Z*)-(dimethoxy-phosphoryl)-2-[2-(trityl-amino)-thiazol-4-yl]-acrylic acid 2,3,4,5,6-pentafluoro-phenyl ester **12** with **10**: Alternative preparation of (6*R*,7*R*)-7-[2-(*Z*)-(2-trityl-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-3-carbamoyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid diphenyl methyl ester (**8**).

(*Z*)-Pentafluorophenyl ester **12** (180 mg, 0.267 mmol) and protected cephalosporin amine **10** (tosylate salt) (185 mg, 0.267 mmol) were mixed in DMF (5 ml) at room temperature with *N,N* diethylaniline (82 μl, 0.53 mmol). A slow reaction took place at room temperature, so the solution was warmed to 50°C. After 48 hours all the initial cephalosporin had reacted and the solution was partitioned between ethyl acetate (50 ml) and water (50 ml). The organic layer was dried (anhydrous $MgSO_4$) evaporated and the residue purified by flash chromato-

graphy (eluant: ethyl acetate) affording **8** (113 mg, 46%) as a pale yellow foam identical with the material prepared by method A.

Coupling of (*Z*)-(dimethoxy-phosphoryl)-2-[2-(trityl-amino)-thiazol-4-yl]-acrylic acid 2,3,4,5,6-pentafluoro-phenyl ester **12** with **11**: Alternative preparation of (6*R*,7*R*)-7-[2-(*Z*)-(2-trityl-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-3-chloromethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid *p*-methoxybenzyl ester (**9**).

In a similar experiment to the one described above, (*Z*)-**12** (0.85 g, 1.24 mmol) with the hydrochloride salt of **11** (0.502 g, 1.24 mmol) produced **9** (0.435 g, 40%) identical to material prepared by method A.

ii) Elaboration of **8** and **9** to Target Compounds: Illustrative Procedures

a) Nucleophilic Displacement of **9**.

4-{(6*R*,7*R*)-7-[2-(*Z*)-(2-trityl-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-2-*p*-methoxy-benyloxycarbonyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl-methyl}-thio-1-(2-fluoroethyl)-pyridinium hydriodide (**14a**)

A solution of compound **9** (0.5 g, 0.58 mmol) and sodium iodide (101 mg, 0.638 mmol) in dry DMF (10 ml) was stirred under nitrogen for 10 mins when 1-(2-fluoroethyl)-4-pyridinethione (92 mg, 0.638 mmol) was added. The mixture was stirred at room temperature for a further 4 hours and the solvent was evaporated under high vacuum. The residue was partitioned between chloroform (100 ml) and water (100 ml). The aqueous layer was further washed with ethyl acetate (3 × 100 ml) and the combined organic layers washed with brine (50 ml), dried ($MgSO_4$) and evaporated to afford the fully protected cephalosporin **14a** as a brown foam (0.568 g, 88%). $^1$H NMR (DMSO): 9.43 (1H, d, NH, *J* 7.5 Hz), 8.82 (1H, s, trityl NH), 8.75, 8.0 (4H, 2 × d, pyridyl H), 7.3~7.1 (17H, m, 3 × Ph, 2 Ar), 6.85 (2H, d, 2 Ar), 6.6 (1H, s, thiazole H), 5.6 (2H, m, H-7, olefin H), 5.2 (3H, m, H-6, C$H_2$Ar), 5.0, 4.9 (2H, br m, C$H_2$F), 4.8 (2H, m, C$H_2$S), 4.35 (2H, m, C$H_2$N), 3.72 (3H, s, MeOAr), 3.65 (2H, ABq, H-4), 3.55, 3.5 (6H, d, MeOP, $J_{PH}$ 15 Hz). IR ($CHBr_3$): 3402, 2950, 1787, 1719, 1668, 1630. $C_{50}H_{48}FIN_5O_8PS_3$ Requires C 53.6, H 4.3, N 6.3, S 8.6. Found C 53.8, H 5.0, N 6.5, S 8.3.

b) Deprotections

4-{(6*R*,7*R*)-7-[2-(*Z*)-(2-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl-methyl}-thio-1-(2-fluoroethyl)-pyridinium hydriodide (**14**)

The fully protected cephalosporin **14a** (0.498 g, 0.44 mmol) was dissolved in anisole (2 ml) and TFA (5 ml) was added. The mixture was stirred for 10 minutes at room temperature then diluted with water (0.5 ml) and diisopropyl ether (30 ml). The resulting reddish-brown precipitate was treated with more water to give, after ultrasound treatment, a brown precipitate which was filtered and dried *in vacuo* at 40°C to give compound **14** (25 mg) .The filtrate was diluted with water and then concentrated, freeze dried and dried *in vacuo* at 35°C to give **14** (204 mg, total yield 59%) as a light brown solid still containing trifluoroacetic acid. $C_{23}H_{26}FIN_5O_7PS_3 \cdot 0.25\ CF_3CO_2H$ Requires C 35.9, H 3.4, N 8.7, S 12.2. Found C 36.1, H 3.5, N 8.5, S 11.9.

(6*R*,7*R*)-7-[2-(*Z*)-(2-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-3-carbamoyloxy-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetate (**24**)

Compound **8** (0.28 g, 0.298 mmol) was dissolved in anisole (1.5 ml) and treated with trifluoroacetic acid (5 ml) at room temperature for 20 minutes. The solution was then diluted with water (1 ml) and washed with diisopropyl ether (10 ml). The aqueous layer was freeze dried to afford the title compound as a pale yellow solid still containing trifluoroacetic acid and water. $C_{17}H_{20}N_5O_9PS_2 \cdot 1.35\ H_2O \cdot 1.9\ CF_3CO_2H$ Requires C 33.5, H 3.33, N 9.4, $H_2O$ 3.26. Found C 33.0, H 3.2, N 9.8, $H_2O$ 3.0.

Similarly prepared from compound **25** was (6*R*,7*R*)-7-[2-(*Z*)-(2-amino-thiazol-4-yl)-3-(dimethoxy-phosphoryl)-acryloylamino]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid trifluoroacetate (**23**). $C_{15}H_{17}N_4O_7PS_2 \cdot 1.1H_2O \cdot 1.3\ CF_3CO_2H$. Requires C 33.6, H 3.3, N 8.9, F 11.8, $H_2O$ 3.15. Found C 33.3, H 3.15, N 8.8, F 11.6 and $H_2O$ 3.0.

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