AS-924, a Novel Orally Active Bifunctional Prodrug of Ceftizoxime. Synthesis and Relationship between Physicochemical Properties and Oral Absorption

Masayasu Kasai, a, Satoru Hatano, a Meiko Kitagawa, a Akihisa Yoshimi, a Ken-ichi Nishimura, a Nobuhiro Mori, b Atsushi Sakai, b and Taisuke Sugihara b

Research Laboratories, Kyoto Pharmaceutical Industries, Ltd. a 38 Nishinokyo, Tsukinowa-cho, Nakakyo-ku, Kyoto 604-8444, Japan and Laboratory for Pharmacokinetic Research, Institute for Life Science Research, Asahi Chemical Industry Co., Ltd. b 2–1 Samejima, Fuji, Shizuoka 416–8501, Japan. Received February 18, 1999; accepted May 6, 1999

Ceftizoxime (CZX), a parenteral cephalosporin, has potent and broad antibacterial activity. To improve its oral absorption, we synthesized a series of monofunctional and bifunctional prodrugs of CZX. In rabbits, urinary recovery after oral administration of CZX was improved by esterification of the carboxyl group at the C-4 position with various lipophilic moieties (monofunctional prodrugs), and was further increased by introduction of a hydrophilic l-alamine to the amino group on the thiazole ring at the C-7 position (bifunctional prodrugs). Least-squares analysis showed good parabolic correlations between logP and urinary recovery for monofunctional and bifunctional prodrugs, respectively. AS-924, a bifunctional prodrug with a pivaloxymethyl and l-alanyl moiety had the best balance of lipophilicity and water-solubility for oral absorption among the prodrugs synthesized.

Key words AS-924; ceftizoxime alapivoxil; cephalosporin; bifunctional produrg; lipophilicity; water-solubility

The so-called third generation parenteral cephalosporins bearing an aminothiazole–methoxyimino moiety at the C-7 position of the cephem nucleus, such as ceftizoxime (CZX), cefotaxime and cefmenoxime, possess a broad antibacterial spectrum and potent activity against gram-positive and gram-negative bacteria. However, they are poorly absorbed from the gastrointestinal tract because of their low lipophilicity due to the low pK_s value of the carboxyl group at the C-4 position of the cephalosporin nucleus. In recent years, C-3 substituted cephalosporins bearing an aminothiazole–methoxyimino moiety at the C-7 position, including ceferam pivoxil (CFTM-PI) 1 and cefpodoxime proxetil (CPDX-PR), 2 have been developed as orally active prodrugs, in which the carboxyl group is esterified to increase lipophilicity. However, their antibacterial activities are still not satisfactory compared to the parenteral cephalosporins.

CZX, a parenteral cephalosporin with an aminothiazole–methoxyimino moiety, has a broad antibacterial spectrum against bacteria isolated from patients with respiratory or urinary tract infections, and more potent activity against gram-negative rods than oral cephalosporins used clinically. 3 It shows bactericidal activity at concentrations close to its minimal inhibitory concentrations (MIC) and is resistant to bacterial β-lactamas. 3 Moreover, CZX ester is assumed to be stable to isomerization from the Δ2 to the Δ2 ester because it has no substituent at the C-3 position. 4 Thus, CZX is considered to be a suitable parent compound for orally active prodrug of cephalosporin exhibiting both potent antibacterial activity and good oral absorption. The drug administered orally as a solid is first dissolved in the gastrointestinal fluid, and then absorbed across the lipoidal intestinal membrane. Therefore, the drug needs to possess a good balance of water-solubility and lipophilicity to ensure good oral absorption and bioavailability. CZX has a weakly basic amino-thiazole moiety on its side chain at the C-7 position (pK_s 2.95), and esterification alone may result in an increase of lipophilicity accompanied by reduction of water-solubility needed for dissolution into the gastrointestinal fluid. We previously resolved this problem by introduction of a hydrophilic moiety such as an amino acid into the ester of cephalosporin. 5–7

In the present study, to further increase the oral absorption of CZX esters (monofunctional prodrugs) by improvement of water-solubility, we introduced various amino acids as basic moieties onto the weakly basic amino group on the thiazole ring (bifunctional prodrugs). Then, we examined the correlation between oral absorption and physicochemical properties for monofunctional and bifunctional prodrugs. Among the bifunctional prodrugs examined, pivaloxymethyl 7β[(Z)-2-(2-(5)-alanylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate hydrochloride (ceftizoxime alapivoxil, AS-924) was demonstrated to have the best balance of lipophilicity and water-solubility for oral absorption (Fig.1).

Chemistry The synthetic routes of the monofunctional prodrug compounds 2a–2f, esterified with various lipophilic moieties at the C-4 carboxyl group of CZX, are shown in Chart 1. The free acid compound of CZX was treated with iodomethyl pivalate in N,N-dimethylacetamide (DMAc) in the presence of dicyclohexylamine (DCHA) to afford the ester compound 2a. The other monofunctional prodrugs 2b–2f were prepared by esterification of CZX with the corresponding halides under the same reaction conditions.

The synthetic routes of the bifunctional prodrugs 4g–4p, in which various amino acids were introduced into the monofunctional prodrugs, are shown in Chart 2. The ester compound 2a was treated with N-tert-butoxycarbonyl(N-Boc)-l-alamine in methylene chloride in the presence of 4-dimethylaminopyridine (DMAP), using 1-ethyl-3-(3-diethylaminopropyl)carbodiimide hydrochloride (EDC•HCl) as a condensing agent to afford compound 3a. Finally, the N-Boc group of compound 3a was removed by treatment with hydrochloride in formic acid to afford compound 4a (AS-924). The other bifunctional prodrugs 4g, 4i–4p were prepared by the same procedure from the corresponding monofunctional

* To whom correspondence should be addressed.
prodrugs. The synthetic routes for compounds 7a—7f, in which various amino acids were introduced onto the amino group of the thiazole ring of CZX, are shown in Chart 3. Compounds 7a—7f were prepared using the diphenylmethyl ester of CZX (5) as the starting material under reaction conditions similar to those shown in Chart 2.

**Biological Results**

**Oral Absorption and Physicochemical Properties of Monofunctional Prodrugs** A series of monofunctional prodrugs (2a—2f) was synthesized by esterification of the C-4 carboxyl group of CZX with various lipophilic moieties. Physicochemical properties and urinary recovery (UR) after oral administration of the prodrugs in rabbits were determined and are summarized in Table 1. The UR was markedly increased by esterification from 5.36% for CZX to 11.12—31.59%. Among the monofunctional prodrugs synthesized, the pivaloyloxymethyl (POM) ester of CZX (2a) showed the highest UR. Least-squares analysis showed a parabolic relationship between log $P$ and UR values for the monofunctional prodrugs with an optimum log $P$ value of 1.81 (Fig. 2).

UR = $-6.730 + 24.333 \log P + 10.469$ ($r^2=0.864$)

Water-solubility values for the synthesized compounds at pH 4.5 and 6.0, which are the virtual pH on the surface of small intestinal mucosa, were determined and are summarized in Table 1. Compounds 2a, 2d and 2e with relatively high log $P$ values were poorly soluble in water, indicating that oral absorption of these prodrugs might be further increased by improvement of the water-solubility.

**Oral Absorption and Physicochemical Properties of Bifunctional Prodrugs** To increase water-solubility, various amino acids were introduced as basic moieties onto the weakly basic amino group on the thiazole ring of the POM ester of CZX (2a), which showed the best oral absorption among the monofunctional prodrugs examined as described
above. Introduction of amino acids to 2a markedly increased the water-solubility at both pH 4.5 and 6.0. However, UR was increased only in the L-alanyl derivative 4h (AS-924), indicating that the L-alanyl moiety is the most effective amino acid residue for bifunctional prodrugs of CZX (Table 2).

In the next set of experiments, we introduced L-alanine into various esters of CZX. Their physicochemical properties and UR are shown in Table 3. The apparent log P values of bifunctional prodrugs 4m—4p were calculated from the relation between 2a and AS-924. Introduction of L-alanine into CZX esters increased the water-solubility and decreased the log P value to a similar extent. However, apparent log P values for bifunctional prodrugs 4m—4p were still higher than that of CZX. UR of the bifunctional prodrugs 4m—4p were also higher than those of corresponding monofunctional prodrugs 2b—2e. For the bifunctional prodrugs, a parabolic correlation between log P and UR was obtained with an optimum log P value of 1.22 (Fig. 2).

Figure 2 clearly shows that oral absorption of all the monofunctional prodrugs was increased by introduction of an L-alanyl moiety (bifunctional prodrugs).

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R¹</th>
<th>log P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Water-solubility&lt;sup&gt;b&lt;/sup&gt; (mg/ml)</th>
<th>UR&lt;sup&gt;c&lt;/sup&gt; (%)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH 4.5</td>
<td>pH 6.0</td>
<td></td>
</tr>
<tr>
<td>CZX</td>
<td>Na</td>
<td>3.29</td>
<td>&gt;25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>2a</td>
<td>CH₃</td>
<td>1.57</td>
<td>0.42</td>
<td>0.38</td>
</tr>
<tr>
<td>2b</td>
<td>CH₃</td>
<td>0.46</td>
<td>2.08</td>
<td>1.72</td>
</tr>
<tr>
<td>2c</td>
<td>CH₃</td>
<td>0.96</td>
<td>0.80</td>
<td>0.58</td>
</tr>
<tr>
<td>2d</td>
<td>CH₃</td>
<td>1.37</td>
<td>0.43</td>
<td>0.30</td>
</tr>
<tr>
<td>2e</td>
<td>CH₃</td>
<td>2.52</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>2f</td>
<td>CH₃</td>
<td>0.17</td>
<td>0.11</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Table 1. Physicochemical Properties and Oral Absorption in Rabbits of Monofunctional Prodrugs of CZX

---

<sup>a</sup> 1/15 M phosphate buffer (pH 6.5)/ n-octanol.  
<sup>b</sup> 1/15 M phosphate buffer.  
<sup>c</sup> Urine was collected for 6 h after oral administration.  
<sup>d</sup> Each value represents the mean±S.D. of three animals.

**Hydrolysis of CZX with Various Amino Acids**

Prodrugs are required to be stable in the gastrointestinal lumen and to be rapidly converted to the parent drugs during and/or after absorption. Esters of cephalosporins at the C-4 position are known to be rapidly hydrolyzed by intestinal esterase.
However, little is known about hydrolysis of the amide bond between amino acids and amino moieties on the thiazole ring at the C-7 position of cephalosporins. Thus, hydrolysis of CZX with various amino acid moieties and release of CZX were examined in rabbit intestinal homogenate (Fig. 3). Hydrolysis of the CZX amides varied according to the amino acid species introduced, with half-lives ranging from 5.90 to 79.47 min. The L-valine derivative (7c) was hydrolyzed most slowly, and more than 60% remained after incubation for 60 min at 37 °C, while the L-leucine (7d) and L-lysine derivatives (7f) were almost completely degraded within 30 min. The L-alanine (7b) derivative disappeared over 60 min. The disappearance of CZX derivatives with various amino acid moieties in the intestinal homogenate showed the same time course as the appearance of CZX.

**Oral Absorption of 4h (AS-924) and Other Cephalosporin Esters** In rabbits, oral absorption of AS-924 was compared with other orally active cephalosporin prodrugs used clinically. The UR of AS-924, CFTM-PI, CPDX-PR and cefotiam hexetil (CTM-HE) were 42.82 ± 2.63, 27.41 ± 6.06, 55.62 ± 8.20 and 28.76 ± 2.37% (mean ± S.D.), respectively, after oral administration at 20 mg/kg as an active compound. AS-924 was shown to be orally absorbed more efficiently than CFTM-PI and CTM-HE. The UR of CPDX-PR was higher than that of AS-924.

**Oral Absorption of a Monofunctional and Bifunctional Prodrug of CZX Administered Using a Capsule** In dogs, the values of $C_{\text{max}}$, $T_{\text{max}}$ and area under the plasma concentration ($AUC_{0-\infty}$) of CZX after oral administration of AS-924, a bifunctional prodrug and 2a, a monofunctional prodrug filled in a capsule was determined (Table 4). The $C_{\text{max}}$, $T_{\text{max}}$ and $AUC_{0-\infty}$ of CZX after the administration of AS-924 at a dose
of 10 mg/kg as an active compound were 3.41 ± 0.36 μg/ml, 1.71 ± 0.40 h and 10.38 ± 0.98 μg · h/ml, while those of 2a were 1.80 ± 0.68 μg/ml, 2.25 ± 1.89 h and 5.38 ± 2.57 μg · h/ml (mean ± S.D.). The C\textsubscript{max} and AUC\textsubscript{0–8} of CZX after oral administration of AS-924 was about two times higher than that of 2a.

**Discussion**

In general, β-lactam antibiotics are poorly absorbed from the gastrointestinal tract because of their low lipophilicity due to the low pK\textsubscript{a} value of the carboxyl group. There have been some reports of successful prodrug design by esterification of the carboxyl groups in penicillin and cephalosporin derivatives.\textsuperscript{2,3,9–14} In the present study, a series of monofunctional prodrugs of CZX was prepared by esterification with various lipophilic pro-moieties at the C-4 position. The lipophilicity of CZX determined as log\textit{P} (2.39) was markedly increased by esterification in monofunctional prodrugs (0.17–2.52). Compounds 2a, 2d and 2e with log\textit{P} values of 1.37–2.52 showed relatively high UR, and 2f with the lowest log\textit{P} value 0.17 showed the lowest UR. The UR values showed a parabolic relationship against log\textit{P} values as reported for other cephalosporins.\textsuperscript{15a,b} The POM ester of CZX (2a) showed the best UR among the monofunctional prodrugs examined and had a nearly optimum log\textit{P} value of 1.57 for oral absorption. A log\textit{P} value favorable for oral absorption is generally considered to be about 2.0,\textsuperscript{16} and has been reported to be 1.23–2.14 for cephalosporins with an esterified moiety at the C-7 position,\textsuperscript{15a} and 1.52–2.67 for POM esters of C-3 substituted cephalosporins.\textsuperscript{15b}

However, oral absorption of compounds with relatively high log\textit{P} values may be limited by low water-solubility insufficient for dissolution in intestinal fluid. In cephalosporins with a neutral or weakly basic moiety at the C-7 position, esterification at the C-4 position may not improve the oral absorption because of reduction of water-solubility. It has been reported that oral absorption of cephalosporin prodrugs is dependent on water-solubility, if the lipophilicity and hydrolysis rate are sufficiently high.\textsuperscript{8a} To improve oral absorption of esterified prodrugs of cephalosporins, we increased the water-solubility by introduction of a glycyl moiety onto the ester moiety at the C-4 position\textsuperscript{17} and an l-alanyl moiety onto the hydroxyl group of the side chain at the C-7 position.\textsuperscript{5–7} Among the monofunctional prodrugs synthesized, compound 2a showed the best log\textit{P} and UR but was hardly soluble in water, indicating that absorption can be further increased by improvement of its water-solubility. Therefore, various amino acids as basic moieties were introduced into the prodrug design.

**Table 4. Pharmacokinetic Parameters after Oral Administration of AS-924 and 2a in Capsules at a Dose of 10 mg/kg in Dogs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>C\textsubscript{max} (μg/ml)</th>
<th>T\textsubscript{max} (h)</th>
<th>AUC\textsubscript{0–8} (μg · h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS-924</td>
<td>3.41 ± 0.36</td>
<td>1.71 ± 0.40</td>
<td>10.38 ± 0.98</td>
</tr>
<tr>
<td>2a</td>
<td>1.80 ± 0.68</td>
<td>2.25 ± 1.89</td>
<td>5.38 ± 2.57</td>
</tr>
</tbody>
</table>

\(\text{a) Each value represents the mean±S.D. of six animals.}\)
onto the weakly basic amino group at the C-7 position in 2a, a POM ester of CZX. AS-924, a bifunctional prodrug of CZX with an \( \text{L}-\)alanyl and POM moiety, showed a good log \( P \) value for absorption and the highest oral absorption among the prodrugs with other amino acids. The log \( P \) value was too low in compounds with glyclyl and \( \text{L}-\)lysyl moieties (4g, 4i), and too high in compounds with \( \text{L}-\)leucyl and \( \text{L}-\)iso-leucyl moieties (4j, 4k). In the CZX ester with a \( \text{L}-\)valyl moiety (4l), oral absorption was not increased despite an appropriate log \( P \) value and water-solubility. Therefore, factors other than lipophilicity and water-solubility appeared to influence its absorption. Prodrugs are required to be converted to their parent compounds at the appropriate place and time during and/or after absorption. Compound 4i was resistant to hydrolysis in rabbit intestinal homogenate.

The UV values of the bifunctional prodrugs, in which \( \text{L}-\)alanine was introduced to the various esters of CZX showed a parabolic correlation to log \( P \). The log \( P \) value of AS-924 was closest to the calculated optimal values for absorption among the prodrugs examined. At all log \( P \) values, the UV values of bifunctional prodrugs were higher than those of the corresponding monofunctional prodrug, clearly indicating that introduction of an \( \text{L}-\)alanyl moiety improved the oral absorption by increasing the water-solubility (Fig. 2).

In rabbits, AS-924 showed a higher UV value after oral administration than CFTM-PI and CTM-HE, both orally active cephalosporins used clinically. Drugs given orally as a form of CZX were not absorbed immediately, washed several times with ice-cooled saline to expel the luminal contents, and homogenized with 3 volumes of 1/15s phosphate buffer (pH 7.0). An aliquot of the homogenate was centrifuged at 15,000 rpm for 20 min at 0°C, and the supernatant was diluted with 4 volumes of 1/15s phosphate buffer, and this solution was used as a 5% small intestine homogenate.

Several amino acid derivatives of CZX as substrates were dissolved in \( \text{N},\text{N}-\)dimethylformamide at a concentration equivalent to 2 mg/ml of CZX. The solution (5 µl) was rapidly added to the 5% small intestine homogenate (0.5 ml) preheated at 37°C so that the final concentration of the substrate was equivalent to 20 µg/ml of CZX. Sampling was carried out at 2.5, 5, 10, 20, 30 and 60 min of incubation at 37°C. The sample was added to the same volume of a mixture of 6% trichloroacetic acid–acetonitrile (1:1), and the precipitate was filtered, washed successively with acetic acid, \( \text{NaHCO}_3 \), and ethyl acetate. The residue was chromatographed on a silica gel column (Daisogel No.1001W, eluent; Benzene–EtOAc, 3 : 1) to give 2a (8.66 g) in 73% yield. IR (Nujol) cm\(^{-1}\): 1785, 1750, 1680. \( \text{H}-\)NMR (DMSO–d\(_6\) Δ): 1.16 (s, 9H, 1J, 5 = 5.0 Hz), 3.80—4.30 (m, 2H), 3.81 (s, 3H), 5.13 (d, 1H, J = 5.0 Hz), 5.70—6.10 (m, 3H), 6.50—6.70 (m, 1H), 6.73 (s, 1H), 7.12 (br, 2H), 9.67 (d, 1H, J = 8.0 Hz).

The ester compounds 2b—2f were prepared by a procedure similar to that described above. IR and \( \text{H}-\)NMR spectral data are listed in Table 6.

**Pivaloxyloxyethyl 7-(2-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (2a)** To a solution of the free acid (9.14 g) in DMSO (45 ml) was added DCHA (15.2 mmol) at 0°C under argon in vacuo. The resulting precipitate was collected by filtration. The solid was dissolved in \( \text{HCl} \) (9.15 N) and this solution was used as a 5% small intestine homogenate.

Various amino acid derivatives of CZX as substrates were dissolved in \( \text{N},\text{N}-\)dimethylformamide at a concentration equivalent to 2 mg/ml of CZX. The solution (5 µl) was rapidly added to the 5% small intestine homogenate (0.5 ml) preheated at 37°C so that the final concentration of the substrate was equivalent to 20 µg/ml of CZX. Sampling was carried out at 2.5, 5, 10, 20, 30 and 60 min of incubation at 37°C. The sample was added to the same volume of a mixture of 6% trichloroacetic acid–acetonitrile (1:1), and the precipitate was filtered, washed successively with acetic acid, \( \text{NaHCO}_3 \), and ethyl acetate. The residue was chromatographed on a silica gel column (Daisogel No.1001W, eluent; Benzene–EtOAc, 3 : 1) to give 2a (8.66 g) in 73% yield. IR (Nujol) cm\(^{-1}\): 1785, 1750, 1680. \( \text{H}-\)NMR (DMSO–d\(_6\) Δ): 1.16 (s, 9H, 1J, 5 = 5.0 Hz), 3.80—4.30 (m, 2H), 3.81 (s, 3H), 5.13 (d, 1H, J = 5.0 Hz), 5.70—6.10 (m, 3H), 6.50—6.70 (m, 1H), 6.73 (s, 1H), 7.12 (br, 2H), 9.67 (d, 1H, J = 8.0 Hz).

The ester compounds 2b—2f were prepared by a procedure similar to that described above. IR and \( \text{H}-\)NMR spectral data are listed in Table 6.

**Pivaloxyloxyethyl 7-(2-[2-(2-N-[2-(Ethoxy)benzoyl)carbonyl)]-(S)-alaninom nitroimidazo[4-yl]-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (3h)** To a solution of 2a (9.6 g) and \( \text{N}-\)Boc-\( \text{L}-\)alanine (7.8 g) in methyl ether (100 ml) was added EDC HCl (7.9 g) and DMAP (0.23 g) at room temperature. After stirring for 2 h, the reaction mixture was washed with 10% aq. citric acid, 5% aq. \( \text{NaHCO}_3 \), and ethyl acetate. The residue was chromatographed on a silica gel column (Daisogel No.1001W, eluent; Benzene–EtOAc, 3 : 1) to give 3h (6.45 g) in 50% yield. IR (Nujol) cm\(^{-1}\): 1780, 1755, 1680. \( \text{H}-\)NMR (DMSO–d\(_6\) Δ): 1.16 (s, 9H), 1.27 (d, 1H, J = 6.0 Hz), 1.36 (s, 9H), 3.50—3.80 (m, 2H), 3.89 (s, 3H), 3.90—4.40 (m, 1H), 5.14 (d, 1H, J = 5.0 Hz), 5.70—6.10 (m, 3H), 6.50—6.80 (m, 1H), 7.05 (d, 1H, J = 5.0 Hz), 7.30 (s, 1H), 9.17 (d, 1H, J = 8.0 Hz), 12.4 (brs, 1H).

Compounds 3g, 3i—3p were prepared by a procedure similar to that described above. IR and \( \text{H}-\)NMR spectral data are listed in Table 6.

**Pivaloxyloxyethyl 7-(2-[2-(S)-Alanylnitroimidazo[4-yl]-2-methoxyiminoacetamido]-3-cephem-4-carboxylate Hydrochloride (4h)** To a solution of 3h (4.9 g) in formic acid (25 ml) was added 9.15% HCl-isopropanol (3.2 ml) at 7°C with stirring. After stirring for 5 min at 0—5°C, the reaction mixture was poured into diethyl ether (100 ml). The resulting precipitate was collected by filtration. The solid was dissolved in methanol and poured into diisopropyl ether to give AS-924 (4.08 g) as a powder in 92% yield. IR (Nujol) cm\(^{-1}\): 1775, 1750, 1670. \( \text{H}-\)NMR (DMSO–d\(_6\) Δ): 1.20 (s, 9H), 1.52 (d, 3H, J = 6.0 Hz), 3.50—3.90 (m, 2H), 3.89 (s, 3H), 3.90—4.40 (m, 1H), 5.14 (d, 1H, J = 5.0 Hz), 5.70—6.10 (m, 3H), 6.50—6.80 (m, 1H), 7.05 (d, 1H, J = 5.0 Hz), 7.30 (s, 1H), 9.17 (d, 1H, J = 8.0 Hz), 12.4 (brs, 1H).
3.90 (s, 3H), 3.90—4.30 (m, 1H), 5.16 (d, 1H, J = 5.0 Hz), 5.65—6.10 (m, 3H), 6.50—6.80 (m, 1H), 7.44 (s, 1H), 8.40—8.90 (br, 3H), 9.68 (d, 1H, J = 8.0 Hz), 13.0 (br, 1H). Anal. Caled for C_{19}H_{21}ClN_{3}O_{2}S: C, 43.44; H, 4.86; Cl, 5.83; N, 13.82; S, 10.48. Found: C, 43.21; H, 4.82; Cl, 5.80; N, 13.82; S, 10.48.

Compounds 4g, 4i—4p were prepared by a procedure similar to that described above. IR and 1H-NMR spectral data are listed in Table 7.

**Diphenylmethyl 7b-[Z]-2-[[2-[[N-(tert-Butyloxycarbonyl)-(S)-alanaminothiazol-4-yl)]-2-methoxyiminoacetoamido]-3-cephem-4-carboxylate (6b)** To a solution of 5 (2.0 g) and N-Boc-D-alanine (1.51 g) in methylene chloride (10 mL) was added EDC·HCl (1.53 g) and DMAP (1.65 g) at room temperature.

For stirring for 2 h, the reaction mixture was washed with 10%aq. citric acid, 5%aq. NaHCO3 and brine. The organic layer was dried (Na2SO4) and concentrated in vacuo. The residue was chromatographed on a silica gel column (Daisogel No. 1001 W, eluent; Benzene-EtOAc: 4:1—3:1—2:1) to give 6b (1.6 g) in 61% yield. IR (Nujol) cm⁻¹: 3270, 1750, 1735, 1680. 1H-NMR (DMSO-d₆): δ: 1.27 (d, 3H, J = 3.70 Hz), 1.40 (s, 9H), 3.46—3.83 (m, 2H), 3.94 (s, 3H), 4.00—4.50 (m, 1H), 5.18 (d, 1H, J = 5.0 Hz), 6.01 (dd, 1H, J = 5.0, 9.0 Hz), 6.54—7.04 (m, 1H), 6.94 (s, 1H), 7.04—7.89 (m, 12H), 9.76 (d, 1H, J = 9.0 Hz), 12.76 (br, s, 1H).

Compounds 6a—6f were prepared by a procedure similar to that described above.

**7b-[[Z]-2-[[2-[[S]-Alanaminothiazol-4-yl)]-2-methoxyiminoacetoamido]-3-cephem-4-carboxylate Acid Hydrochloride (7b)** To a solution of 6b (1.2 g) in formic acid (7.2 mL) was added 10.1 g of HCl-isopropanol (1.2 mL) at 7°C with stirring. After stirring for 1 h at 0—5°C, the reaction mixture was poured into diethyl ether (400 ml). The resulting precipitate was collected by filtration. The solid was dissolved in methanol and poured into diisopropyl ether to give 7b (0.52 g) as a powder in 63% yield. IR (Nujol) cm⁻¹: 3400, 3250, 1785, 1700, 1660. 1H-NMR (DMSO-d₆): δ: 1.53 (d, 3H, J = 7.0 Hz), 3.66 (br, s, 2H), 3.94 (s, 3H), 3.90—4.40 (m, 1H), 5.14 (d, 1H, J = 5.0 Hz), 5.90 (dd, 1H, J = 5.0, 9.0 Hz), 6.20—6.70 (m, 1H), 7.54 (s, 1H), 8.00—14.00 (br, 5H), 9.75 (d, 1H, J = 8.0 Hz).

Compounds 7a—7f were prepared by a procedure similar to that described above. 1H-NMR spectral data are listed in Table 8.
References


