DIASTEREOMERIC 7-UREIDOACETYL CEPHALOSPORINS. II

7β-[[[(AMINOCARBONYL) AMINO]-2-THIENYLACETYL] AMINO]-7-METHOXY-3-[[(1-METHYL-1H-TETRAZOL-5-YL) THIO] METHYL]-8-OXO-5-THIA-1-AZABICYCLO [4. 2. 0] OCT-2-ENE-2-CARBOXYLIC ACID[1]

H. E. APPLEGATE, C. M. CIMARUSTI, J. E. DOLFINI, W. H. KOSTER, M. A. ONDETTI, W. A. SLUSARCHYK and M. G. YOUNG
The Squibb Institute for Medical Research, Princeton, New Jersey 08540, U.S.A.

H. BREUER and U. D. TREUNER
Squibb International Research Center, Regensburg, Germany

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The synthesis and antibacterial activity *in vitro* of 7-methoxylated cephalosporins having a thienylureidoacetyl or a thienylglycyl C-7 side-chain are described. Acylation of 7β-amino-7-methoxycephems with a novel 2-aminooxazolone hydrochloride under neutral conditions gave the thienylureidoacetyl derivatives in good yield with retention of configuration. 7β-[[D-[(Aminocarbonyl) amino]-2-thienylacetyl] amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl) thio] methyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid sodium salt (SQ 14,359) was found to have a broad-spectrum of antibacterial activity *in vitro*, particularly against β-lactamase-producing organisms.

The discovery in our laboratories that the thienylureidoacetyl cephalosporin **1**, having the L-configuration in the C–7 side-chain, was more active against resistant Gram-negative organisms than the corresponding D-isomer[2] initiated an investigation of the synthesis and biological properties of analogous 7α-methoxy cephalosporin diastereomer **2**. It was anticipated that 7α-methoxylation would increase the stability of the cephalosporins to hydrolysis by β-lactamases[3~4], thereby complementing the broad-spectrum activity.

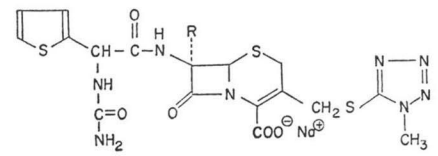
Chemistry

We have utilized two routes for the preparation of **2**: (1) direct introduction of the 7α-methoxy substituent on an acylated cephalosporin[5~7], and (2) acylation of a pre-formed 7β-amino-7-methoxycephalosporin with a suitably activated side-chain. In the first approach, protected thienylglycine derivative **3** [D(S) or L(R)] was coupled to amine **4** (DCC method) to give **5**[2]. Oxidation of **5** with lithium methoxide-*t*-butyl hypochlorite[5~7] gave 7α-methoxycephalosporin **6** in 30~40% yield. Deprotection of **6** yielded the trifluoroacetate salt **7**, which was treated with potassium cyanate to convert the amino group to the ureido moiety. A single diastereomer, **2** (D)* or **2** (L)*, was obtained in each case, indicating that the side-chain configuration was maintained throughout the reaction sequence. On a large scale, the methoxylation step was capricious, and difficulties were encountered in the purification of product **6**.

To circumvent this problem, acylation of methoxyamine **12**[8] was examined. Thus, coupling of

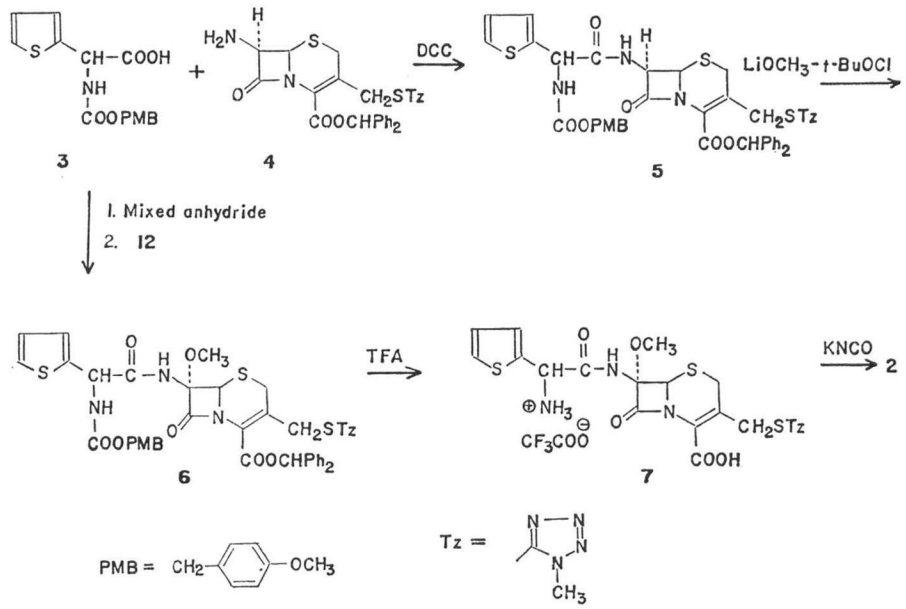
* The configuration of the asymmetric center in the side-chain is shown in parenthesis after the structure number. Throughout this paper D and L correspond to S and R absolute configurations, *respectively, of all* thienylglycyl derivatives.

amine **12** with thienylglycine derivative **3** (DL), *via* a mixed anhydride method, provided **6** (DL). However, under the same conditions, coupling of amine **12** with **3** (L) resulted in epimerization yielding **6** (DL) again. A similar mixture of the diastereomers **6** (DL) was isolated when **12** was treated with the symmetrical anhydride obtained from either **3** (L) or **3** (D) and 0.5 equivalent of N, N'-dicyclohexylcarbodiimide. Using the procedure already described, the product **6** (DL) obtained *via* mixed anhydride coupling was converted to **2** (DL). Analysis of **2** (DL) by reverse phase high performance liquid chromatography indicated that the mixture consisted of the **2** (D) and **2** (L) isomers in a ratio of 60: 40, respectively, and small quantities of the corresponding D- and L-double bond isomers[9].



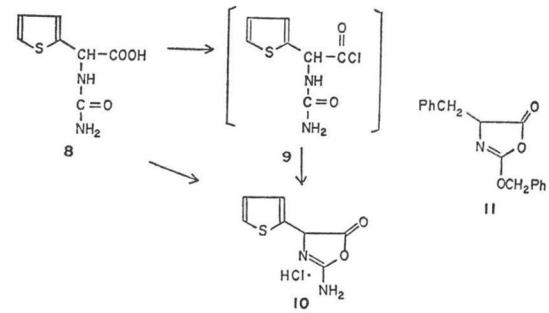
1 R = H
2 R = OCH_3

Scheme 1.

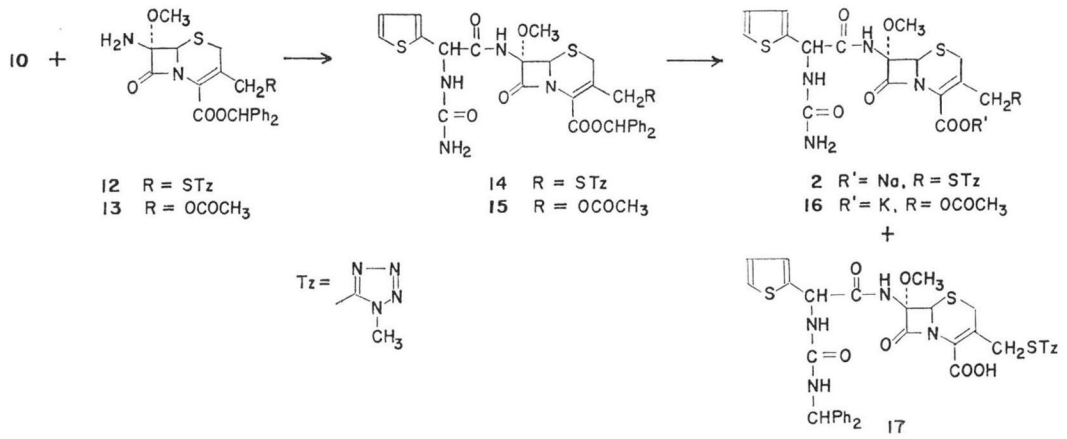


Although the mixed anhydride method was suitable for the preparation of large quantities of **2** (DL), an alternative method was needed for the synthesis of the pure diastereomers. Therefore, we attempted to synthesize acid chloride **9** for coupling with cephalosporin **12**. When 2-thienylureidoacetic acid (**8**) was reacted with phosphorus pentachloride or thionyl chloride in acetonitrile, an insoluble solid formed. A high frequency carbonyl absorption at 1870 cm^{-1} precluded assignment of acid chloride structure **9**.

Scheme 2.



Scheme 3.



Spectral and chemical properties of the solid were consistent with structure **10**, supported by analogy with oxazolone **11** (carbonyl absorption at 1840 cm^{-1} [10]). The difference in the carbonyl frequencies (30 cm^{-1}) of **10** and **11** was ascribed to protonation of the amidino moiety in **10**.

Acylation of 7β-amino-7-methoxycephalosporins **12** and **13**[11] with oxazolone **10** proceeded in good yield when propylene oxide, bis-trimethylsilylacetamide, potassium acetate, or N, N-diethylaniline was employed as acid scavenger. The configuration of the side-chain was retained in all instances except when N, N-diethylaniline was employed, where up to 20% epimerization was observed. However, no coupling occurred when a stronger organic base (N, N-diisopropylethylamine) was added, since deprotonation of **10** probably occurs, decreasing the electrophilicity of the oxazolone and allowing the free amine to polymerize.

The benzhydryl esters of the pure diastereomers **14** (D) and **14** (L) were cleaved using trifluoroacetic acid in the presence of anisole to yield the acids of **2** (D) and **2** (L), respectively, which were converted to sodium salts. Similarly, the 3-acetoxymethyl derivative **15** (DL) was converted to **16** (DL). Formation of small quantities of **17** during de-esterification of **14** indicated that the terminal ureido amino group was capable of competing with anisole for the benzhydryl carbonium ion. When the DL-oxazolone hydrochloride **10** was used in this route, asymmetric induction occurred providing a mixture (60: 40) of D: L diastereomers **2** (DL). In summary, the 2-aminooxazolone hydrochloride **10** provided the best method for acylating weakly nucleophilic 7β-amino-7-methoxycephalosporins while preserving the configuration of the starting amino acid.

Microbiology

In Table 1, the activity *in vitro* of the non-methoxylated and methoxylated thienylureido cephalosporins **1**, **2**, and **16** are compared. Minimum inhibitory concentrations (MIC) were determined by the conventional twofold dilution method with brain heart infusion broth[18]. The phenomenon of the L-thienylureidoacetyl cephalosporin **1** (L) having a higher degree of antimicrobial activity than the D diastereomer **1** (D) against resistant organisms is reversed in the corresponding methoxylated derivatives (cf. **2** (D) and **2** (L)). The 60 : 40 mixture of D : L isomers **2** (DL) possesses a spectrum of activity similar to that of the D diastereomer **2** (D) in most cases. An acetoxymethyl group at the C-3 position

Table 1. Microbiological evaluations of the thienylureidoacetyl cephalosporins[a]. Antibacterial activity (MIC in μg/ml).

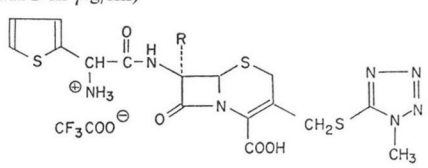
Organism		1(D)	1(L)	2(D)	2(L)	2(DL)	16(DL)	Cefoxitin	Cefamandole nafate
Sensitive strains:									
Staphylococcus aureus	SC 2399	0.78	4.7	0.8	3.1	0.8	4.7	1.2	0.13
Escherichia coli	SC 8294	1.4	0.8	0.8	3.1	0.8	6.3	0.8	0.4
Klebsiella pneumoniae	SC 8340	1.8	0.6	1.6	1.6	0.4	3.1	1.2	0.4
Salmonella schottmuelleri	SC 3850	0.13	0.6	0.4	3.1	0.4	6.3	1.2	0.03
Resistant strains:									
Staphylococcus aureus	SC 2400	1.6	3.1	0.8	6.3	1.6	9.4	1.6	2.4
Escherichia coli	SC 3552	3.1	1.6	1.6	6.3	1.6	25	1.6	0.8
Enterobacter cloacae	SC 8415	>100	1.2	3.1	12.5	6.3	12.5	>50	1.6
Proteus rettgeri	SC 8217	18.7	3.1	4.7	12.5	1.6	12.5	12.5	4.7
Pseudomonas aeruginosa	SC 8329	>50	75	50	>50	>50	>50	>50	>50
Serratia marcescens	SC 9782	>100	6.3	0.8	3.1	0.8	6.3	4.7	12.5

a) side-chain configuration is shown in parenthesis.

significantly decreases the activity of the methoxylated derivatives across the entire spectrum of organisms (cf. **2** (DL) and **16** (DL)). Against sensitive strains, **2** (D) and **2** (DL) compare favorably with cefoxitin and cefamandole. However, in our preliminary screen, **2** (D) is more active than cefoxitin and equivalent to cefamandole nafate against resistant β-lactamase producing organisms, with the exception of *Serratia* where **2** (D) displays significantly greater activity than either antibiotic.

The antimicrobial activity of the intermediate trifluoroacetate salt **7** (D) is compared with that of the non-methoxylated analog **18** (D)[12] in Table 2. The high MIC's of **7** (D) indicate that methoxylation of the 7-position lowers activity in this particular instance. The stability of **7** (D) and **18** (D) was monitored by following the disappearance of the U.V. chromophore at 270 nm in 0.1 M phosphate buffer (pH 7) at ambient temperature. A three to ninefold increase in the rate of decomposition of the 7-methoxy analog **7** (D) was noted relative to that

Table 2. Microbiological evaluation of thienylglycyl cephalosporins[a]. Antibacterial activity (MIC in μg/ml)



18 R = H
7 R = OCH_3

Organism		**18** (D)	**7** (D)
Staphylococcus aureus	SC 2399	3.13	37.5
Escherichia coli	SC 8294	2.4	>50
Klebsiella pneumoniae	SC 8340	1.6	50
Salmonella schottmuelleri	SC 3850	0.6	18.7
Staphylococcus aureus	SC 2400	6.3	>50
Escherichia coli	SC 3552	9.4	>50
Enterobacter cloacae	SC 8415	12.5	>50
Proteus rettgeri	SC 8217	25	>50
Pseudomonas aeruginosa	SC 8329	>50	50
Serratia marcescens	SC 9782	>50	>50

(a) side-chain configuration is shown in parenthesis.

of the non-methoxylated cephalosporin **18** (D), which may account for the low antimicrobial activity of **7** (D).

In conclusion, we have demonstrated exceptionally good activity for the thienylureidoacetyl cephamycin derivatives, SQ 14,359 (**2** (D)) and SQ 13,426 (**2** (DL)) in our preliminary studies *in vitro*. This activity has been supported in extended studies *in vitro* and *in vivo* that will be the subject of subsequent publications[13].

Experimental Section

The NMR spectra were determined on Varian nuclear magnetic resonance spectrometers (models T-60 and XL-100–15) using tetramethylsilane as an internal standard, and chemical shifts are reported on the δ scale. Infrared spectra were recorded on Perkin-Elmer spectrometers (model 257 and 621). Melting points are uncorrected.

DL-2-[[[(4-Methoxyphenyl)methoxy]carbonyl]amino]-2-thiopheneacetic acid [**3**(DL)]

DL-2-Thienylglycine was used to prepared **3**(DL) according to the procedure described for the synthesis of **3** (D) [and the synthesis of **3**(L) is also described][3]. The product, after trituration with pentane, was a crystalline solid, mp 108~109.5°C; PMR ($CDCl_3$) δ 3.77 (s, 3H, OCH_3), 5.03 (s, 2H, OCH_2), 5.63 (m, 2H, NH and CH), 6.7~7.4 (m, 7H, aromatic).

7β-[[L-[[[(4-Methoxyphenyl) methoxy] carbonyl] amino] - 2-thienylacetyl] amino] - 3 - [[(1 - methyl-1H-tetrazol-5-yl)thio]-methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, diphenylmethyl ester [**5**(L)]

Compound **5**(L) was prepared by the method described by BREUER, *et al.*[2] and was purified by preparative TLC (silica gel) using benzene - EtOAc (4: 1). It was obtained in 59% yield as an amorphous residue; PMR ($CDCl_3$) δ 3.65 (broad s, 2H, H_2), 3.77 and 3.80 (two s's 6H, OCH_3 and NCH_3), 4.30 (ABq, J=13 Hz, 2H, CH_2S), 4.93 (d, $J_{H_6-H_7}$=5 Hz, 1H, H_6), 5.00 (s, 2H, OCH_2), 5.73 (m, 2H, H_7 and CHCO), 5.98 (d, J_{NH-CH}=7 Hz, 1H, NH), and 6.7~7.7 (m, 19H, aromatic, OCH and NH).

5 (D):

Isomer **5** (D), prepared by the same method[2], crystallized on concentration of the organic extract to give 10.2 g (73%), mp 128~132°C; PMR ($CDCl_3$) δ 3.65 (broad d, 2H, H_2), 3.80 (s, 6H, OCH_3 and NCH_3), 4.28 (broad d, 2H, CH_2S), 4.97 (d, $J_{6,7}$=4.5 Hz, 1H, H_6), 5.07 (broad s, 2H, OCH_2), 5.83 (m, 2H, H_7 and CHCO), 6.08 (d, J_{NH-CH}=7 Hz, NH), 6.8~7.6 (m, 19H, aromatic, OCH and NH).

7α-Methoxy-7-[[D-[[[(4-methoxyphenyl) methoxy] carbonyl] amino] - 2-thienylacetyl] amino] - 3 - [[(1-methyl-1H-tetrazol-5-yl) - thio] methyl] -8-oxo-5-thia-1-azabicyclo [4.2.0] oct - 2 - ene - 2 - carboxylic acid, diphenylmethyl ester [**6** (D)]

To a stirred solution of commercial lithium methoxide (132 mg, 3.45 mmol) in dry CH_3OH (4 ml) under nitrogen at room temperature was added anhydrous tetrahydrofuran (25 ml). The solution was cooled to −74°C, and cephem **5** (D) (797 mg, 1 mmol) in dry tetrahydrofuran (2.5 ml) was added over 15 seconds, immediately followed by dropwise addition of *tert*-butyl hypochlorite (0.16 ml, 1.33 mmol) with vigorous stirring. The mixture was stirred for 15 minutes at −74°C, and then glacial HOAc (1 ml) was added. After pouring into $CHCl_3$-water, the organic layer was washed successively with water, water adjusted to pH 7.5, and water, dried (Na_2SO_4), and evaporated *in vacuo* to a residue (825 mg). Purification by preparative TLC on silica gel using benzene - EtOAc (4: 1) gave **6** (D) (290 mg, 35%); PMR ($CDCl_3$) δ 3.47 (broad s, 2H, H_2), 3.52 (s, 3H, C_7-OCH_3), 3.78 (s, 6H, OCH_3 and NCH_3), 4.35, (ABq, J=14 Hz, 2H, CH_2S), 5.07 (s, 3H, H_6 and OCH_2), 5.82 (d, J_{CH-NH}=7 Hz, 1H, CHCO), 6.12 (d, J_{CH-NH}=7 Hz, 1 H, NH), 6.7~7.8 (m, 19H, aromatic, OCH and NH).

6 (L):

Diastereomer **6** (L) was prepared as described above for **6** (D). After chromatography, **6** (L) was obtained in 40% yield as a mixture containing 20% of **5** (L) and it was used without further purification; PMR ($CDCl_3$) δ 3.37 (s, 3H, C_7-OCH_3), 3.50 (broad s, 2H, H_2), 3.77 and 3.80 (two s's, 6H, OCH_3 and

NCH_3), 4.35 (ABq, J=14 Hz, 2H, CH_2S), 5.03 (broad s, 3H, H_6 and OCH_2), 5.80 (d, J_{CH-NH}=7 Hz, 1H, CHCO), 6.08 (d, J_{CH-NH}=7 Hz, 1H, NH).

6 (DL):

To a solution of the racemic thienylglycine derivative **3** (DL) (10.35 g, 32.2 mmol) in dry CH_2Cl_2 (200 ml) and diisopropylethylamine (4.06 g, 31.4 mmol) at 0°C was added isobutyl chloroformate (4.29 g, 31.4 mmol). The solution was stirred for 5 minutes and 7β-amino-7-methoxycephem **12** (13.76 g, 26.2 mmol) was added; the mixture was stirred at room temperature under nitrogen for 3 hours, and a second portion of the mixed anhydride, prepared as described above, was added to the reaction mixture. Stirring was continued for an additional 3.5 hours, and finally a one-half portion of the mixed anhydride was added. The mixture was stirred overnight, and the resulting solution was washed successively with water, 3% aqueous $NaHSO_4$ solution, water, saturated aqueous $NaHCO_3$ solution, and water, dried (Na_2SO_4), and evaporated *in vacuo* to a crude oil. Subsequent chromatography on silica gel using CH_2Cl_2-EtOAc provided 12.14 g (46%) of **6** (DL) as an amorphous powder after precipitation (CH_2Cl_2-Et_2O); PMR ($CDCl_3$) δ 3.27~3.58 (m, 5H, OCH_3 and H_2), 3.78 (m, 6H, OCH_3 and NCH_3), 4.35 (ABq, J=14 Hz, 2H, CH_2S), 5.08 (s, 3H, H_6 and OCH_2), 5.82 (d, J_{CH-NH}=7.5 Hz, 1H, CHCO), 6.08 (d, J_{CH-NH}=7.5 Hz, 1H, NH), 6.78~7.6 (m, 19H, aromatic, OCH and NH); ir ($CHCl_3$) 1785 (β-lactam C=O), 1710 cm^{-1} (broad, ester and amide C=O).

7β-[[D-Amino-(2-thienyl)acetyl]amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, trifluoroacetic acid salt [**7** (D)]

To a stirred solution of **6** (D) (262 mg, 0.316 mmol) in anisole (0.8 ml) under nitrogen at 0~5°C was added trifluoroacetic acid (4 ml). The mixture was stirred at 0~5°C for 10 minutes, and then the trifluoroacetic acid was removed *in vacuo*. The residue was then taken up in acetone, and addition of Et_2O gave a precipitate, which was collected, washed with Et_2O, and dried *in vacuo* to afford **7** (D) as a powder (153 mg, 79%), mp 133°C (dec.); PMR (CD_3OD) δ 3.45 (broad s, 2H, H_2), 3.53 (s, 3H, OCH_3), 3.97 (s, 3H, NCH_3), 4.32 (broad s, 2H, CH_2S), 5.07 (s, 1H, H_6), 5.47 (s, 1H, CHCO), 6.9~7.7 (m, 3H, aromatic); ir (KBr) 1775 (β-lactam C=O), 1705 (acid C=O), 1680 (amide C=O), 1625 cm^{-1} ($CF_3COO^{\ominus}$).

Anal. Calc'd. for $C_{19}H_{20}F_3N_7O_7S_3$: C, 37.32; H, 3.30; N, 16.04.
Found: C, 37.60; H, 3.42; N, 16.04.

7 (L):

Crude **6** (L) was converted to **7** (L) using the procedure described for the preparation of **7** (D). Although the product contained some of the corresponding non-methoxylated derivative, it was used in the subsequent reaction without further purification.

7 (DL):

A stirred solution of **6** (DL) (11.48 g, 13.9 mmol) and anisole (3.8 ml, 34.8 mmol) in dry CH_2Cl_2 (120 ml) at 0°C under nitrogen was treated with trifluoroacetic acid (30 ml) for 1.5 hours. The solvent was removed *in vacuo*, and the residue was precipitated from acetone-Et_2O to give **7** (DL) as a tan powder (7.8 g), mp 122~125°C (dec.); NMR (DMSO-d_6) δ 3.22 and 3.45 (two s's, OCH_3), 3.90 and 3.95 (two s's, NCH_3), 4.26 (broad m, CH_2S), 5.19 (s, H_6), 5.42 (broad s, CHCO), 6.8~7.7 (m, aromatic); ir (KBr) 1780 (β-lactam C=O), 1705 (acid C=O), 1680 (amide C=O), 1630 cm^{-1} ($CF_3COO^{\ominus}$).

Anal. Calc'd. for $C_{19}H_{20}F_3N_7O_7S_3 \cdot 1/4\ C_4H_{10}O$: C, 38.12; H, 3.60; N, 15.56.
Found: C, 37.88; H, 3.40; N, 15.46.

D-2-Amino-4-(2-thienyl)-5(4H)-oxazolone hydrochloride [**10** (D)]

To a stirred suspension of **8** (D)[2] (5.20 g, 26 mmol) in dry CH_3CN (150 ml) at 0~5°C under nitrogen was added thionyl chloride (4.64 ml). After 5 minutes, dry Et_2O (250 ml) was added. The resulting slurry was stirred for 10 minutes, filtered rapidly under nitrogen, washed with Et_2O, and dried *in vacuo* to give the oxazolone hydrochloride **10** (D) as a powder (4.89 g, 86%); ir (Nujol) 1870, 1720 cm^{-1} (broad).

Anal. Calc'd. for $C_7H_7ClN_2O_2S$: C, 38.46; H, 3.23; N, 12.82; S, 14.64.
Found: C, 38.00; H, 3.23; N, 12.66; S, 14.42.

10 (L) and **10** (DL):

2-Amino-4-(2-thienyl)-5(4H)-oxazolone hydrochlorides, **10** (L) and **10** (DL), were prepared from L- and DL-thienylglycine, respectively, using the procedure above. Both compounds exhibited strong infrared absorptions at 1870 and 1720 cm^{-1}.

7β-[[D-[(Aminocarbonyl)amino]-2-thienylacetyl]amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, diphenylmethyl ester [**14** (D)]

A solution of 7β-amino-7-methoxycephem **12** (4.85 g, 9.25 mmol) and propylene oxide (8 ml) in anhydrous dimethylformamide (50 ml) and dry CH_2Cl_2 (50 ml) at $-60° \pm 10°C$ under nitrogen was treated with 2-aminooxazolone hydrochloride **10** (D) (4.89 g, 22.3 mmol) and stirred for 3 hours. After diluting the mixture with EtOAc-water, the aqueous layer was extracted with EtOAc and the combined organic extracts were washed successively with dilute aqueous $NaHCO_3$ solution, water, dilute HCl, and water. Evaporation of the dried (Na_2SO_4) extract *in vacuo* provided a residue, which was crystallized from CH_2Cl_2-Et_2O and then recrystallized from CH_2Cl_2-EtOAc to give **14** (D) (3.12 g, 48%), mp 135°C (dec.); PMR (CD_3COCD_3) δ 3.53 (s, 3H, OCH_3), 3.63 (ABq, J=18 Hz, 2H, H_2), 3.93 (s, 3H, NCH_3), 4.38 (ABq, J=13 Hz, 2H, CH_2S), 5.13 (s, 1H, H_6), 5.58 (broad s, 2H, NH_2), 6.15 (d, J_{CH-NH}=8 Hz, 1H, CHCO), 6.65 (d, J_{CH-NH}=8 Hz, 1H, NH), 6.9~7.9 (m, 14H, OCH and aromatic); ir (KBr) 1770 (β-lactam C=O), 1712 (ester C=O), 1650 cm^{-1} (amide C=O).

Anal. Calc'd. for $C_{31}H_{30}N_8O_6S_3$: C, 52.69; H, 4.28; N, 15.86.
Found: C, 52.26; H, 4.13; N, 15.46.

14 (L):

The crude product obtained from 7β-amino-7-methoxycephem **12** (6.12 g, 11.7 mmol) and 2-aminooxazolone hydrochloride **10** (L) (6.39 g, 29.2 mmol) was chromatographed on silica gel eluting with EtOAc - CH_2Cl_2 (3: 2). Trituration of the purified product with Et_2O provided 3.79 g (46%) of **14** (L); NMR (CD_3COCD_3) δ 3.37 (s, 3H, OCH_3), 3.63 (m, 2H, H_2), 3.93 (s, 3H, NCH_3), 4.37 (ABq, J=13.5 Hz, 2H, CH_2S), 5.08 (s, 1H, H_6), 5.5 (broad s, 2H, NH_2), 6.25 (d, J_{CH-NH}=8 Hz, 1H, CH), 6.77 (d, J_{CH-NH}=8 Hz, 1H, NH), 6.85~7.72 (m, 14H, OCH and aromatic).

14 (D, L):

The crude product obtained from acylation of **12** with **10** (D, L) was precipitated from CH_2Cl_2-Et_2O, and then chromatographed on silic gel using CH_2Cl_2-EtOAc. Subsequent precipitation (CH_2Cl_2-Et_2O) gave **14** (D, L) as a colorless powder (52% yield).

3-[(Acetyloxy)methyl]-7β-[[DL-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-7-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, diphenylmethyl ester [**15** (DL)]

Acylation of **13** with **10** (DL) gave a crude product, which was purified by TLC on silica gel using EtOAc - MeOH (9: 1); **15** (D, L) was obtained as a foam (35% yield); PMR ($CDCl_3$) δ 1.98 and 2.03 (two s's, 3H, CH_3CO), 2.9~3.6 (m, 5H, OCH_3 and H_2), 4.6~5.6 (m, 5H, CH_2O, H_6 and NH_2); ir ($CHCl_3$) 1780 (β-lactam C=O), 1740 (acetate C=O), 1725 (sh, ester C=O), 1660 (amide and ureido C=O's).

7β-[[D-[(Aminocarbonyl)amino]-2-thienylacetyl]amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, sodium salt [**2** (D)]

Method A: From trifluoroacetate salt **7** (D)

To a stirred suspension of **7** (D) (122 mg, 0.20 mmol) in water was added potassium cyanate (32 mg, 0.40 mmol). After stirring at 25°C for 3 hours, the pH was adjusted to 2.0 with 1 N HCl in the presence of EtOAc and the organic layer was separated. Repeated extraction of the aqueous layer with EtOAc gave a combined extract which was dried (Na_2SO_4) and evaporated *in vacuo* to give the free acid of **2** (D).

Method B: *Via* cleavage of benzhydryl ester **14** (D)

To a cold solution of ester **14** (D) (3.00 g, 4.24 mmol) and anisole (2 ml) in dry CH_2Cl_2 under nitrogen was added trifluoroacetic acid (8 ml). The mixture was stirred at 0°C for 1 hour and then concentrated *in vacuo*. The residue was treated with EtOAc-dilute aqueous $NaHCO_3$ solution, and the aqueous layer (pH 7.5) was adjusted to pH 2.0 (1 N HCl) in the presence of fresh EtOAc. Repeated extraction with EtOAc gave a combined extract, which was dried (Na_2SO_4) and evaporated to a pink powder.

Trituration with acetone-Et_2O gave the free acid of **2** (D) as a solid (2.0 g, 87%). The acid crystallized from CH_3OH-CH_2Cl_2, mp 204~205°C (dec.); PMR ($CDCl_3$-CD_3OD) δ 3.43 (ABq, J=18 Hz, 2H, H_2), 3.53 (s, 3H, OCH_3), 3.93 (s, 3H, NCH_3), 4.37 (broad s, 2H, CH_2S), 5.03 (s, 1H, H_6), 5.83 (s, 1H, CHCO), 6.87~7.40 (m, 3H, thienyl); ir (KBr) 1765 (β-lactam C=O), 1690 (acid C=O), 1645 cm^{-1} (amide and ureido C=O's).

Anal. Calc'd. for $C_{18}H_{20}O_6N_8S_3$: C, 39.99; H, 3.73; N, 20.73.
Found: C, 39.65; H, 3.27; N, 19.87.

The free acid (1.87 g, 3.46 mmol) was dissolved in dry dimethylformamide (5 ml) and acetone (20 ml). Addition of 2 M sodium 2-ethylhexanoate in *n*-BuOH (2.16 ml, 4.32 mmol) and then Et_2O (50 ml) gave a precipitate, which was collected by centrifugation, washed with Et_2O, and dried *in vacuo* at 25°C to give **2** (D) as a white powder (1.84 g, 92%), mp 163°C (dec.); PMR (D_2O) δ 3.44 (ABq, J=18 Hz, 2H, H_2), 3.58 (s, 3H, OCH_3), 4.03 (s, 3H, NCH_3), 4.16 (ABq, J=14 Hz, 2H, CH_2S), 5.10 (s, 1H, H_6), 5.65 (s, 1H, CHCO), 7.0~7.55 (m, 3H, thienyl); ir (KBr) 1770 (β-lactam C=O), 1680 (amide and ureido C=O), 1615 cm^{-1} ($COO^{\ominus}$).

Anal. Calc'd. for $C_{18}H_{19}N_8O_6S_3Na \cdot H_2O$; C, 37.25; H, 3.65; N, 19.30; S, 16.54.
Found: C, 37.83; H, 3.40; N, 18.78; S, 16.67.

2 (L):

Diastereomer **2** (L) was obtained by either of the two methods used to prepare **2** (D). Chromatography [silica gel TLC, acetone - HOAc (16: 1)] of the acid obtained by Method A removed non-methoxylated material resulting from the impure precursor **7** (L). From both procedures, the acid was obtained as an amorphous powder and converted to sodium salt **2** (L); PMR (D_2O) δ 3.40 (s, 3H, OCH_3), 3.51 (ABq, J=17 Hz, 2H, H_2), 4.03 (s, 3H, NCH_3), 4.17 (ABq, J=16 Hz, 2H, CH_2S), 5.07 (s, 1H, H_6), 5.67 (s, 1H, CHCO), 7.01~7.54 (m, 3H, thienyl); ir (KBr) 1760 (β-lactam C=O), 1665 (amide C=O), 1605 cm^{-1} ($COO^{\ominus}$).

Anal. Calc'd. for $C_{18}H_{19}N_8O_6S_3Na$: C, 38.43; H, 3.40; N, 19.92; S, 17.10.
Found: C, 38.71; H, 3.46; N, 18.59; S, 16.20.

2 (DL):

From either **7** (DL) or **14** (DL) the crude mixture of diastereomeric sodium salts **2** (DL) was obtained. Precipitation from acetone - CH_3OH - Et_2O gave **2** (DL) as a powder; ir (KBr) 1775 (β-lactam C=O), 1680 (amide and ureido C=O), 1620 cm^{-1} ($COO^{\ominus}$).

Anal. Calc'd. for $C_{18}H_{19}N_8O_6S_3Na$: C, 38.43; H, 3.40; N, 19.92; S, 17.10.
Found: C, 38.72; H, 3.32; N, 19.85; S, 16.90.

Analysis by reverse phase HPLC of **2** (DL) on a C_{18} μ-Bondapak column eluted with 10% methanol - 90% 0.01 M aqueous $(NH_4)_2HPO_4$ at 0.5 ml/min using a u. v. detector (254 nm) indicated the salt was a 60: 40 mixture of D: L diastereomers[9].

7β-[[D-[(N-Benzhydrylaminocarbonyl)amino]-2-thienylacetyl]-amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [**17** (D)]

During the cleavage of the benzhydryl ester **14** (D), the acid **17** (D) formed as a by-product in 1~6% yield. Chromatography of the crude reaction mixture on silica gel plates using *n*-BuOH - HOAc - H_2O (3: 1: 1) gave the pure acid of **2** (D) Rf (0.4) and benzhydrylureido acid **17** (D) (Rf 0.6). The latter had PMR (CD_3COCD_3) δ 3.47 (s, 3H, OCH_3), 3.58 (ABq, J=18 Hz, 2H, H_2), 3.99 (s, 3H, NCH_3), 4.42 (ABq, J=14 Hz, 2H, CH_2S), 5.05 (s, 1H, H_6), 5.94 and 6.11 (two d's, $J_{CH\text{-}NH}$=8 Hz, 2H, two CH's), 6.54 and 6.80 (two d's, $J_{CH\text{-}NH}$=8 Hz, 2H, two NH's), 6.9~7.4 (m, 13H, aromatic). Treatment of acid **17** with sodium 2-ethylhexanoate gave the sodium salt, mp 155~157°C; ir (KBr) 1755 (β-lactam C=O), 1765 (sh, amide and ureido C=O's), 1600 cm^{-1} ($COO^{\ominus}$).

3-[(Acetyloxy)methyl]-7β-[[DL-[(aminocarbonyl)amino]-2-thienylacetyl]amino]-7-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, potassium salt [**16** (DL)]

Treatment of the benzhydryl ester **15** (DL) with trifluoroacetic acid-anisole followed by potassium 2-ethylhexanoate gave crude potassium salt **16** (DL) as a precipitate. Reprecipitation from CH_3OH

with Et_2O, after charcoal treatment gave **16** (DL) as a colorless powder; PMR (D_2O) 2.04 (s, $COCH_3$), 3.30 and 3.48 (two s's, OCH_3), 5.06 (s, H_6), 5.58 and 5.61 (two s's, CHCO); ir (KBr) 1770 (β-lactam C=O), 1740 (sh, acetate C=O), 1675 (amide and ureido C=O's), 1615 cm^{-1} ($COO^{\ominus}$).

Anal. Calc'd. for $C_{18}H_{16}N_4O_8S_2K$: C, 41.61; H, 3.10; N, 10.78; S, 12.34.
Found: C, 41.80; H, 3.15; N, 10.78; S, 12.00.

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