7-(2-AMINOMETHYLPHENYLACETAMIDO)-3-(1-CARBOXYMETHYLTETRAZOL-5-YLTHIOMETHYL)-3-CEPHEM-4-CARBOXYLIC ACID*

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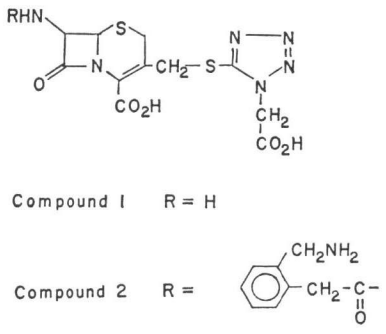
The synthesis of 7-(2-aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (BL–S786) is described and the antimicrobial activities are compared with cefazolin and cefamandole. The compound exhibits broad antimicrobial spectrum, produces high intramuscular blood levels in mice and demonstrates a high degree of therapeutic efficacy in experimental bacterial infections in rodents.

Subsequent to the work of COCKER *et al.*[1] describing the displacement of the acetoxy group of certain cephalosporanic acids with various nucleophiles, a large number of highly active cephalosporins have been prepared. It is the purpose of this paper to describe the synthesis of a new cephalosporin which possesses a broad antibacterial spectrum, exhibits high mouse blood levels and demonstrates impressive protective doses in mice. The heterocyclic thiol used for the preparation of this compound in the displacement of the acetoxy group from 7-aminocephalosporanic acid (7–ACA) is 1-carboxymethyl-5-mercaptotetrazole. The resulting 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (Compound **1**, Fig. 1) was acylated with 2-aminomethylphenylacetic acid to give compound **2** (Fig. 1). Although compound **2** is zwitterionic, it possesses high water solubility at pH 7.0 affording suitable concentrations for parenteral use and avoids the liability of crystalluria present with many zwitterionic cephalosporins. The high solubility is due to the added carboxylic acid group in the tetrazole moiety.

Chemistry

1-Methyl-5-mercaptotetrazole was prepared by the method of STOLLE and FR-HENKE-STARK[2]. Compound **2** was synthesized by displacing 7–ACA with 5-mercaptotetrazole acetic acid. This intermediate was then coupled with potassium 2-N-(1-carbethoxypropen-2-yl) aminomethylphenyl acetate[3,4] *via* a mixed carboxylic-carbonic anhydride reaction.

Fig. 1. Structure of cephalosporin compounds.



Microbiology

The *in vitro* minimum inhibitory concentrations (MICs) of **2** are compared with cefazolin and cefamandole in Table 1. The intramuscular blood levels are compared in Table 2 and the PD_{50}'s are

* This compound has also been referred to as BL–S786.

tabulated in Table 3. The data indicates that **2** has excellent antimicrobial activity, produces high blood levels after injection and demonstrates low protective doses in mice.

Table 1. Minimum inhibitory concentrations (MICs)*

Organism	Bristol No.	Compound **2**	Cefazolin	Cefamandole
Streptococcus pneumoniae	A-9585	0.13	0.063	0.063
Streptococcus pyogenes	A-20203	0.13	0.063	0.032
Staphylococcus aureus	A-9537	1.0	0.25	0.13
S. aureus	A-9606	2.0	0.25	0.5
Salmonella enteritidis	A-9531	0.13	1.0	0.13
Escherichia coli	A-15119	0.5	1.0	1.0
Escherichia coli	A-20110	4.0	8.0	32.0
Klebsiella pneumoniae	A-20579	0.25	1.0	0.25
K. pneumoniae	A-15130	0.25	1.0	1.0
Proteus mirabilis	A-9900	0.5	4.0	0.5
Proteus vulgaris	A-9717	0.5	8.0	2.0
Pseudomonas aeruginosa	A-9843A	>125.0	>125.0	>125.0
Serratia marcescens	A-20019	>125.0	>125.0	125.0

* The MIC values are in μg/ml and were determined by the 2-fold agar dilution method described by F. LEITNER and coworkers[5]. In the present experiments, MUELLER-HINTON Medium (Difco) was used as the test medium for all organisms. For the fastidious strains, *i.e., Streptococcus pneumoniae* and *Streptococcus pyogenes,* the medium was supplemented with 4% defibrinated sheep blood.

Table 2. Mouse blood levels*

Minutes after administration	Compound **2**	Cefazolin	Cefamandole
15	18.5	14.8	8.7
30	13.8	10.4	4.6
60	6.7	4.6	0.8
90	3.5	>3.0	>0.5

* A dose of 10 mg/kg was administered intramuscularly. The results are in μg/ml and are the average of 2~6 experiments using 8 mice per experiment.

Table 3. Efficacy of intramuscular treatment of mice*

Organism	Challenge number of organisms	PD_{50}/treatment (mg/kg)		
		Compound **2**	Cefazolin	Cefamandole
E. coli A 15119	2×10^5	0.4	1.7	0.8
P. mirabilis A 9900	4×10^5	2	16	18
K. pneumoniae A 15130	1×10^8	25	125	>330
S. aureus A 9606	2×10^9	9	14	8

* A total of 5 mice were used per dose level.

Experimental Section

Melting points were taken on a Fisher Johns melting point apparatus, i.r. spectra were taken on a Beckman 4240 Spectrophotometer and pmr spectra were taken on a P.E. R/28 Spectrometer.

1-Carboxymethyl-5-mercaptotetrazole

To a stirred solution of 5 g (0.043 mol) of 1-methyl-5-mercaptotetrazole in 150 ml of ether at −7°C under nitrogen was added, over a 5-minute period, 60 ml (0.086 mol) of 1.6 M *n*-butyllithium in *n*-hexane. The mixture was stirred at −5°C for 1/2 hour and warmed to 20°C over a 2-hour period. A total of 25 g of dry ice was added and the mixture was stirred for an additional 2 hours. The resultant white precipitate was removed by filtration, washed with 100 ml of ether, and dissolved in 50 ml of water. The solution was acidified to pH 2 with 6 N hydrochloric acid and extracted with 100 ml ethyl acetate. The organic phase was washed with water and evaporated at 40°C (15 mm) to a white solid. The product was slurried with 50 ml of boiling chloroform and collected to yield after drying *in vacuo* over phosphorous pentoxide, 3.7 g of white crystals; mp 167~170°C dec.

pmr spectrum (ppm δ in $DMSO_{d6}$): s, 5.10.

Anal. Calcd. for $C_3H_4N_4O_2S$: C, 22.50; H, 2.52; N, 34.98.
Found: C, 22.69; H, 2.69; N, 36.66.
IR spectrum (KBr): 1735, 1510, 1490 cm^{-1}.

7-Amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, **1**

To a stirred suspension of 1.0 g (0.0625 mol) of 1-carboxymethyl-5-mercaptotetrazole and 1.7 g (0.0625 mol) of 7-ACA in 50 ml of pH 6.4 (0.1 M) phosphate buffer at 55°C under nitrogen, was added in small portions 1.1 g of sodium bicarbonate. The solution was stirred for 4 hours, cooled to 5°C and acidified to pH 2.0 with 1:1 phosphoric acid. The resultant precipitate was collected by filtration, washed with 50 ml of water, 50 ml of acetone and dried *in vacuo* over phosphorous pentoxide yielding 2.3 g of solid. The solid was then slurried in 25 ml of tetrahydrofuran and concentrated hydrochloric acid was added dropwise until the mixture became clear. The solution was then treated with 100 mg of Darco KB carbon, filtered, and evaporated at 30°C (15 mm) to a volume of 10 ml. The crystalline product was collected by filtration and air-dried overnight to yield 660 mg; mp>170°C (dec.).

pmr spectrum (ppm δ in $DMSO_{d6}$+DCL−D_2O): s, 3.8, 2H; 4.4, DOH;
q, 5.1, 2H; s, 5.35, 2H.

Anal. Calcd. for $C_{11}H_{12}N_6O_5S_2 \cdot 1/2H_2O$: C, 34.63; H, 3.43; N, 22.04.
Found : C, 34.79; H, 3.82; N, 21.67.
IR spectrum (KBr) : 1800, 1730, 1620 cm^{-1}.

7-(2-Aminomethylphenylacetamido)-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, **2**

A mixture of 1.7 g (0.006 mol) of potassium 2-N-(1-carbethoxypropen-2-yl) aminomethylphenyl acetate[4] and 5 drops of N,N-dimethylbenzylamine in 24 ml of tetrahydrofuran at −40°C was stirred vigorously with 835 mg (0.006 mol) of isobutylchloroformate. The mixture was stirred for 5 minutes and added to a solution of 1.5 g (0.004 mol) of 7-amino-3-(1-carboxymethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid and 820 mg of N-methylmorpholine in 14 ml of water at 0°C. The solution was stirred for 2 hours and the tetrahydrofuran was evaporated at 30°C (15 mm). The solution was layered with 40 ml of ethyl acetate and adjusted to pH 2 dropwise with concentrated hydrochloric acid. The product was collected and washed with 20 ml of water to yield after air drying to constant weight, 150 mg; mp>150°C (dec.).

pmr spectrum (ppm δ in $DMSO_{d6}$): m, 3.5~4.0, 4H; m, 4.0~4.5, 4H; d, 5.0, 1H;
s, 5.35, 2H; d, 5.65, 1H; s, 7.45, 4H.

Anal. Calcd. for $C_{20}H_{21}N_7O_6S_2 \cdot H_2O$: C, 44.68; H, 4.31; N, 18.23.
Found : C, 45.07; H, 4.30; N, 18.00.
IR spectrum (KBr) : 1780, 1700, 1655 cm^{-1}.

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