SEMISYNTHETIC CEPHALOSPORINS. IV SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF PARENTERALLY ACTIVE 7-[4-(SUBSTITUTED METHYL)PHENYL]-ACETAMIDO-3-CEPHEM-4-CARBOXYLIC ACIDS

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A group of novel 4-substituted phenylacetic acids were prepared and coupled with several 7-amino-Δ-3-cephems to afford a family of parenterally active cephalosporins. A compound designated **13l** had the broadest spectrum of activity and the highest potency of the group against both Gram-positive and Gram-negative bacteria. The activity of **13l** included high potency against penicillinase-producing staphylococci and activity against anaerobes, including *Bacteroides fragilis*.

In a previous publication[1] we have shown how a variety of novel substituted-aryl amino acids **1** were prepared by nucleophilic displacement of a selectively chloromethylated α-amino-4-hydroxyphenylacetic acid (**1**, R^1=Cl). Moreover once these amino acids were coupled with 7-aminocephems, a family of orally active cephalosporins **2** was obtained. In this paper we present the synthesis of a series of 4-substituted phenylacetic acids **3** and the group of parenterally active cephalosporins **4** derived from them (Scheme 1).

Chemistry

A new process for the preparation of 4-chloromethylphenylacetic acid[2] (**3a**, R^1=Cl in **3**) has been developed. Treatment of **5** with sodium acetate in acetic acid gave a mixture of **6**, unreacted **5** and a small amount of diester **7**, from which **6** was isolated and treated with sodium cyanide to give **8**. The cyanoester **8** when hydrolyzed with concentrated hydrochloric acid gave the desired **3a** in a 54% overall yield (Scheme 2).

The acid **3a** was converted to the acylchloride **9** which was then coupled by standard procedures[3] with 3-substituted-7-aminocephems **12**[4]. Subsequent displacement of the chloride group with a variety

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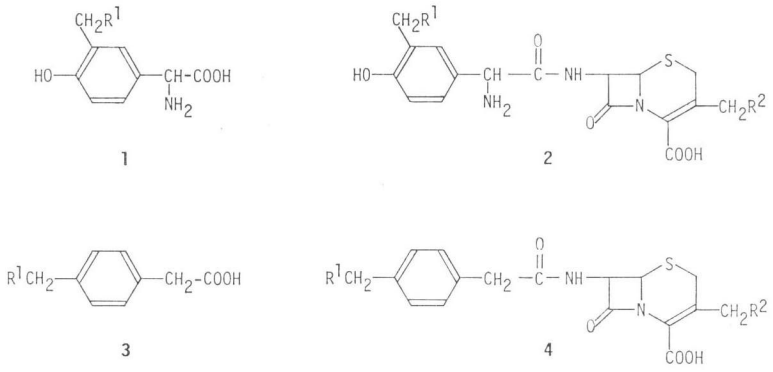
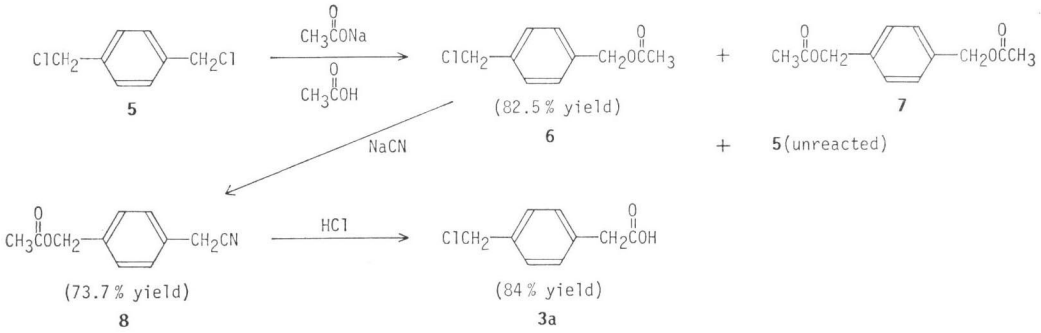
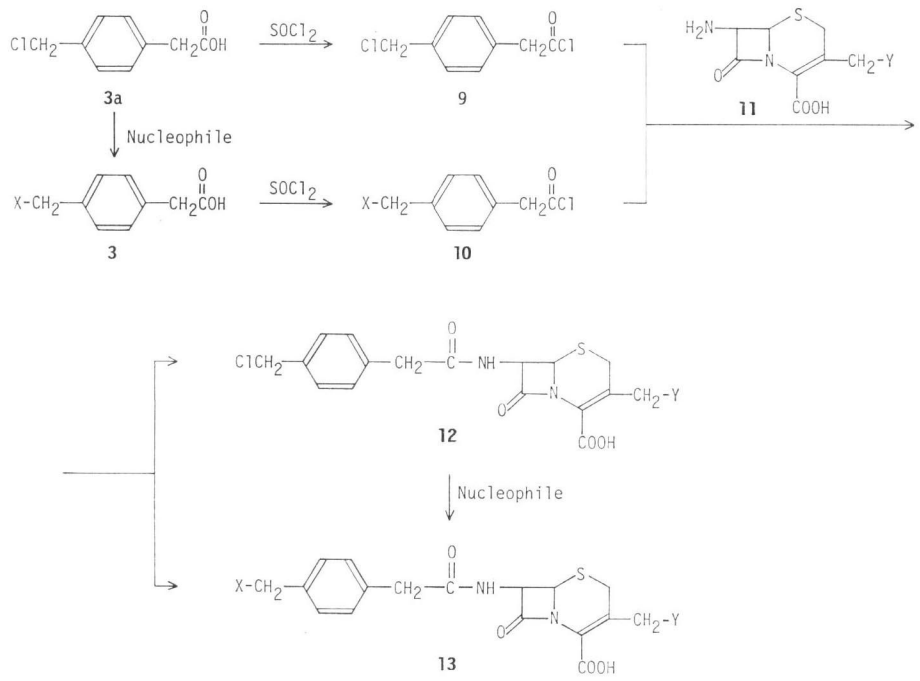
Scheme 1.



Scheme 2.



Scheme 3.



of nucleophiles afforded the novel parenterally active cephalosporins **13** (Scheme 3, Table 2). An alternative synthetic approach involved the prior convertion of **3a** to other *p*-substituted-methylphenylacetic acids **3** (Table 1), followed by direct coupling of the corresponding acyl halides **10** with the desired cephems **11**.

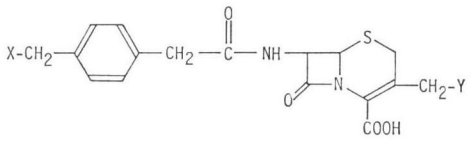
In Table 1 are listed some of the novel 4-substituted-methylphenylacetic acids **3** which have been prepared. In Table 2 are listed the cephalosporanic acid derivatives obtained on

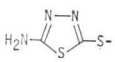
Table 1. 4-Substituted-methylphenylacetic acids (**3**)

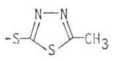
3(a∿e)

3a : X = Cl-
3b : X = NC-
3c : X = N_3-
3d : X = $CH_3\overset{O}{\overset{\|}{C}}-O$-
3e : X = $H_2N\overset{HN}{\diagdown}C-S$-

Table 2. 7-[4-Substituted-methylphenyl]acetamido cephems **12** and **13**.



#	X	Y	#	X	Y
12a **12b** **12c** **12d**	Cl–	DCA ACA CT CTD	**13p**		ACA
13a **13b**	NC–	DCA ACA	**13q**		ACA
13c **13d**	N_3–	DCA ACA	**13r** **13s**		ACA CT
13e **13f**		DCA ACA	**13t** **13u**		ACA CT
13g **13h**	$CH_3\overset{O}{\overset{\|}{C}}O$–	ACA DCA	**13v**	$Et_2N\overset{S}{\overset{\|}{C}}-S$–	ACA
13i		ACA	**13w**	$H_2N-\overset{S}{\overset{\|}{C}}-S$–	ACA
13j **13k** **13l** **13m**	$NH_2-\overset{NH}{\overset{\|}{C}}-S$–	DCA ACA CT CTD	**13x**	$H-\overset{O}{\overset{\|}{C}}-NH-N=\overset{NH_2}{\overset{\|}{C}}-S$–	CT
13n	NCS–	CT	**13y** **13z** **13AA**	HO_3S–	ACA CT CTD
13o	$H_2N-\overset{NH}{\overset{\|}{C}}-N=\overset{NH_2}{\overset{\|}{C}}-S$–	CT	**13BB** **13CC**	$H_2N-N=\overset{NH_2}{\overset{\|}{C}}-S$–	ACA CTD

	DCA	ACA	CT	CTD
Y=	–H	$-CH_2O\overset{O}{\overset{\|}{C}}CH_3$		

coupling the acids **3** with cephems **11** as well as those compounds obtained by nucleophilic displacement of chloride from the 7-[4-chloromethylphenyl]acetamido cephems **12**.

Bacteriology

Materials and Methods

Antibiotics

Cephalothin was kindly supplied by The Eli Lilly and Company and cefazolin by The Smith Kline & French Laboratories.

Antibiotic Spectrum

Minimal inhibitory concentrations (MICs) were determined using serial twofold dilutions of compounds in a growth medium followed by its inoculation. To obtain inocula for MIC determinations, all cultures except anaerobes were grown 18~24 hours at 37°C in Trypticase soy broth (BBL). The Trypticase soy broth tubes containing the antibiotics were inoculated so that the final concentration of cells represented a culture dilution of 1×10^{-3} for *Streptococcus pneumoniae* and *Streptococcus pyogenes* and 1×10^{-5} for all other cultures. Anaerobes were grown for 48 hours at 37°C in thioglycolate broth (BBL) and then diluted 1×10^{-1} in MUELLER-HINTON broth (BBL). Using the multiple inoculator of STEERS *et al.*,[5] the diluted cultures (0.003 ml) were applied to the surface of MUELLER-HINTON agar containing 1% Supplement C (Difco) and the antibiotics. Tubes were incubated at 37°C in air for 18 hours. Agar plates were incubated at 37°C for at least 24 hours in the atmosphere created by using the Gas Pak (BBL) hydrogen+carbon dioxide generating system in a Gas Pak Anaerobic Jar. After incubation, the lowest concentration of antibiotic causing inhibition of visible growth was considered to be the MIC.

Treatment of Experimental Infections in Mice

Albino CD-1 male mice weighing 20(±1) g were infected by intraperitoneal injection of a bacterial suspension containing a sufficient number of organisms to produce uniformly lethal infections. The suspensions were in brain heart infusion broth and were 0.1 to 1.0 ml in volume, depending upon the organism. Suspensions of *S. aureus* M238 and *Escherichia coli* Es59 contained 2.5% mucin. Groups of ten mice each were treated subcutaneously with appropriate concentrations of antibiotic at 1 and 4 hours after infection. The number of mice in each group surviving the challenge for 4 days was recorded and the ED_{50} (the dose in mg/kg required to protect 50% of the infected mice) determined by the method of REED and MUENCH[6].

Results and Discussion

Activity *In Vitro*

The *in vitro* spectra of activity and potencies of the more biologically interesting compounds against common pathogens are shown in Table 3. Of all of the compounds tested having the 7-aminocephalosporanic acid (7-ACA) nucleus, compound **13k** possessed the broadest spectrum was generally the most potent, and compared favorably to cephalothin, which also has the 7-ACA nucleus. The congener of **13k**, **13l**, which has a methyltetrazolylthio group in place of the acetoxy of **13k**, had the broadest spectrum and was the most potent of all of the compounds prepared. It was more potent than cephalothin or cefazolin (which also bears a methylthioheterocycle at C-3) against the Gram-positive bacteria and was equipotent to or more potent than the two reference compounds against all of the Gram-negative bacteria. Additional testing of these compounds found that **13k** and **13l** were more potent than their respective reference compounds, cephalothin and cefazolin, against penicillinase-producing staphylococci (Table 4), organisms against which the most recent generation of cephalosporins and cephalo-

Table 3. Activity *in vitro* of **13k**, **13l**, **13m**, **13o**, **13u**, **13x**, cephalothin and cefazolin.

Organism	MIC (μg/ml)							
	13k	Cephalothin	**13l**	**13m**	**13o**	**13u**	**13x**	Cefazolin
Gram-positive								
Staphylococcus aureus M240[a]	0.4	0.2	0.1	0.2	0.2	0.2	0.2	0.2
Staphylococcus aureus M238[b]	0.4	0.4	0.2	0.4	NT[c]	NT	NT	0.8
Streptococcus faecium St316	50	50	25	25	100	>100	>100	100~>100
Streptococcus pneumoniae D137	0.1	0.2	0.03	0.8	0.05	≤0.03	≤0.03	0.03~0.1
Streptococcus pyogenes St139	≤0.03	0.1	≤0.01	≤0.05	0.05	0.2	≤0.03	0.1
Gram-negative								
Escherichia coli Es59	25	12.5	1.6	12.5	25	6.2	25	1.6
Escherichia coli Es62	100	50	12.5	>50	NT	NT	NT	25
Klebsiella pneumoniae K39	12.5	6.2	3.1	6.2	25	3.1	12.5	1.6~3.1
Proteus mirabilis P5	12.5	12.5	6.2	50	NT	NT	NT	25
Proteus mirabilis P6	25	25	12.5	25	25	25	50	6.2~12.5
Salmonella shottmuelleri Sa27	6.2	0.8	0.4	3.1	3.1	≤1.6	≤3.1	1.6

[a] Benzylpenicillin-sensitive strain.
[b] Penicillinase-producing strain.
[c] NT, not tested.

Table 4. Activity *in vitro* of **13k**, **13l**, cephalothin and cefazolin against penicillinase-producing staphylococci.

Antibiotic	Organism (No. of strains)	Cumulative percent inhibited at MIC (μg/ml)					
		≤0.025	0.05	0.1	0.2	0.4	0.8
13k	*S. aureus* (16)				75	100	
Cephalothin					31	100	
13l					100		
Cefazolin						31	100
13k	*S. epidermidis* (5)	40		60	100		
Cephalothin				40		100	
13l		20	40	60	100		
Cefazolin					40	60	100

sporin-like molecules show considerably reduced potency as compared to cephalothin and cefazolin. Further studies *in vitro* revealed that **13l** was more potent than cefoxitin against representative strains of anaerobes, including *Bacteroides fragilis*; **13k** was similar to cefoxitin in potency (Table 5). None of the compounds showed noteworthy activity against *Enterobacter* sp., indole-positive *Proteus* sp., *Serratia marcescens, Pseudomonas aeruginosa, Neisseria gonorrhoeae,* or *Haemophilus influenzae*.

Table 5. Activity *in vitro* of **13k**, **13l** and cefoxitin against anaerobes.

Organism	MIC (μg/ml)		
	13k	**13l**	Cefoxitin
Bacteroides fragilis subsp. *fragilis*	3.1	3.1	6.2
Bacteroides fragilis subsp. *fragilis*	6.1	3.1	3.1
Bacteroides fragilis subsp. *thetaiotamicron*	12.5	6.2	12.5
Clostridium perfringens	3.1	≤1.6	1.6
Fusobacterium varium	1.6	≤1.6	1.6

Table 6. Activity *in vivo* of **13k**, **13l**, cephalothin and cefazolin.

Organism		Challenge (CFU)[b]	ED_{50} (mg/kg)[a]			
			13k	Cephalothin	**13l**	Cefazolin
Staphylococcus aureus	M240[c]	3.5×10^8	0.13	0.2	0.04	0.04
Staphylococcus aureus	M238[d]	6×10^7	NT[e]	NT	5.8	22.4
Streptococcus pneumoniae	D137	1.5×10^2	0.9	22	0.2	0.6
Streptococcus pyogenes	St139	2×10^4	0.1	1.4	0.1	0.5
Escherichia coli	Es59	1×10^3	22	41	3.0	3.7
Klebsiella pneumoniae	K39	4.5×10^3	34	112	10	17
Proteus mirabilis	P6	1×10^8	50	250	24	36
Salmonella schottmuelleri	Sa27	1×10^7	28	33	0.6	5.0

[a] Mice were treated 1 and 4 hours after infection.
[b] CFU, colony-forming units.
[c] Benzylpenicillin-sensitive strain.
[d] Penicillinase-producing strain.
[e] NT, not tested.

Activity *In Vivo*

All of the compounds were effective to varying degrees against certain Gram-positive and Gram-negative bacterial mouse infections when administered subcutaneously; none was active orally. The most potent compound was **13l**, being more potent than cephalothin and equipotent to or more potent than cefazolin, depending upon the mouse infection (Table 6).

Summary

In summary, **13l** was the most biologically interesting compound of the series described. It possessed a medium broad spectrum of activity, was highly potent against penicillinase-producing staphylococci, and it was active against anaerobes, including *Bacteroides fragilis*.

Experimental

The NMR spectra of all cephalosporin derivatives **12**, **13** were consistent with their proposed structures. Only the coupling of **9** with one 7-amino cephem **11** is presented as well as one general procedure for the nucleophilic displacement of the chloride. All other compounds listed in Table 2 were prepared by analogous methods.

α-Acetoxy-α′-chloro-*p*-xylene (**6**)

A mixture of α,α′-dichloro-*p*-xylene (**5**, 175 g, 1 mole) and 300 ml of glacial acetic acid was heated to 90°C with continuous stirring. At this temperature sodium acetate anhydrous (102.6 g, 1.25 mole) was added in 2~3 portions. The mixture was further heated at reflux temperature for 90 minutes, it was then cooled and filtered. The solid contained unreacted **5**, and sodium chloride and was further worked up to recover **5**. The filtrate was flash concentrated and filtered to give a solid consisting of a second batch of unreacted **5** and sodium acetate. The filtrate was then distilled. Three fractions were collected b.p. 158°C, 160~170°C and 188°C at 20 mmHg, consisting respectively of **5**, **6** and **7**. The second fraction weighed 75 g (85.2% yield) and was used without further purification. The crude **5** was purified by crystallization from ethanol. Recovered 100 g.

α-Acetoxy-α′-cyano-*p*-xylene (**8**)

A solution of sodium cyanide (39.4 g, 0.8 mole) in 130 ml of ethanol and 80 ml of water was heated to 60°C. At this temperature **6** (80 g, 0.4 mole) was added over a period of 90 minutes. At the end of

the addition the mixture was further heated at 70~80°C for 3 hours. The mixture was cooled and was extracted with five portions of 150 ml chloroform. The combined organic phase was washed twice with 150 ml of water, dried, treated with charcoal and evaporated to dryness to give 56.2 g (73.7% yield) of crude **8** which was used in the next step without further purification.

4-Chloromethylphenylacetic Acid (**3a**)

A mixture of **8** (54.6 g, 0.29 mole) and concentrated hydrochloric acid (550 ml) was heated to 70~80°C for 2 hours. Upon cooling to 10°C the crude acid **3a** which precipitated weighed 55.5 g, it was recrystallyzed from hot toluene (2,200 ml) to give a total of 44.5 g of **3a** (86% yield), m.p. 156~157°C (Ref.[2], 152~153°C) in two crops.

NMR (Me_2CO-d_6) δ 3.62 (s, 2), 4.68 (s, 2), 7.37 (s, 4).

4-Chloromethylphenylacetyl Chloride (**9**)

A solution of **3a** (10 g, 0.057 mole) in 60 ml of thionyl chloride was stirred at room temperature for 24 hours. The excess reagent was removed under high vacuum to give 10.5 g (95% yield) of crude crystalline **9** which was used without further purification.

NMR ($CDCl_3$) δ 4.17 (s, 2), 4.58 (s, 2), 7.48 (m, 4).

4-Cyanomethylphenylacetic Acid (**3b**)

A solution of **3a** (1 g, 5 mmole) and sodium cyanide (2 g, 40 mmole) in 30 ml of methanol was refluxed for 4 hours. The solvent was flash evaporated and the residue was dissolved in a mixture of dichloromethane and water which was acidified to pH 3 with 10% hydrochloric acid. The organic phase was separated, dried and concentrated to give a residue which was chromatographed on silica gel using (9: 1) benzene - acetone as eluent. A total of 0.69 g (75% yield) of **3b** was obtained, m.p. 123~125°C.

NMR (Me_2CO-d_6) δ 3.67 (s, 2), 3.93 (s, 2), 7.35 (s, 4).

Anal. Calcd. for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00.
Found: C, 68.38; H, 5.23; N, 8.20.

3-[(1-Methyltetrazol-5-ylthio)methyl]-7-[[2-[4-(isothioureamethyl)phenyl]acetyl]amino]-8-oxo-5-thia-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Hydrochloride (**13l**)

To a solution of sodium bicarbonate (1.5 g, 0.018 mole) and **11** (Y = 1-methyltetrazol-5-ylthio) (3.28 g, 0.01 mole), in 30 ml of water and 20 ml of acetone was added **9** (2.03 g, 0.01 mole) in 10 ml of acetone. The mixture was stirred for 45 minutes at room temperature and was then treated with charcoal and filtered. The filtrate was flash concentrated at 25°C, to remove the acetone. Thiourea (0.76 g, 0.01 mole) was added to the aqueous residue. After a short period of vigorous stirring a heavy precipitate formed. Stirring was continued for 30 minutes and the product isolated by filtration was washed repeatedly with water and dried, giving 4.2 g (78.5% yield), m.p. 142~144°C.

NMR (Me_2SO-d_6) δ 3.73 (m, 4), 3.90 (s, 3), 4.25 (s, 2), 4.40 (s, 2), 4.95 (d, 1), 5.55 (q, 1), 7.28 (m, 4), 9.20 (m, 4).

Anal. Calcd. for $C_{20}H_{23}ClN_8O_4S_3 \cdot 1.5H_2O$: C, 40.17; H, 4.40; N, 18.70.
Found: C, 40.20; H, 4.70; N, 18.00.

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