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Synthesis and Biological Activity of Some Broad-Spectrum *N*-Acylphenylglycine Cephalosporins[1]

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The synthesis and the in vitro and in vivo antibacterial activities of a series of N-acylated phenylglycine cephalosporins are described. These compounds exhibit activity against a broad spectrum of gram-positive and gram-negative bacteria including some strains of *Pseudomonas aeruginosa*, a bacterial species normally insensitive to the cephalosporin antibiotics. The cephalosporins were prepared by acylation of cephaloglycin or its 3-tetrazolylthiomethyl analogue. In several cases, the acylations produced mixtures of diastereomeric cephalosporins, the components of which, when separated, showed different levels of antibiotic activity. Optimum activity was obtained when the acyl moiety on the phenylglycine nitrogen contained an oxygen atom centrally located between the amide carbonyl and a carboxyl substituent, preferably in a three- or five-membered ring. Replacement of acetoxymethyl by (1-methyl-1*H*-tetrazol-5-yl)thiomethyl at the 3 position resulted in overall improvement in activity both in vitro and in vivo. Against a group of *P. aeruginosa* strains, the best compounds of this series showed activity on the order of carbenicillin.

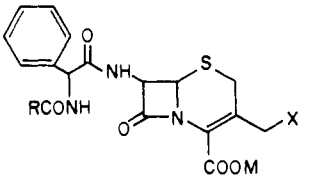
Chemical modifications of the cephalosporin structure have produced numerous derivatives which are active against a broad spectrum of gram-positive and gram-negative bacteria. In spite of intensive synthetic efforts, the overwhelming majority of these derivatives show no significant antibacterial activity against *Pseudomonas aeruginosa*. Recently several laboratories have described three types of semisynthetic cephalosporins of diverse structure which are active against this species: 3-(substituted)vinyl cephalosporins;[2] 7-(α-sulfocephalosporins);[3] and 7-(α-ureidophenylacetyl) cephalosporins.[4] This report describes the synthesis and the in vitro and in vivo activities of a series of N-acylated phenylglycine cephalosporins which are active against a broad spectrum of gram-positive and gram-negative bacteria including some strains of *P. aeruginosa* which are normally insensitive to most cephalosporin antibiotics.

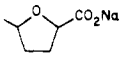
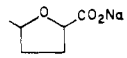
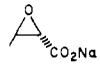
Chemistry. The cephalosporins listed in Table I were prepared by acylation of cephaloglycin **1**[5] or its 3-tetrazolylthiomethyl analogue **2**[6] (Scheme I). These zwitterionic cephalosporins (**1** and **2**) were relatively insoluble in most organic solvents but they could be acylated under very mild conditions by stirring a suspension of them in an inert solvent such as acetone with a suitably activated carboxylic acid. Cephalosporins **12–14** and **20–22** were obtained using commercially available anhydrides **3–5**. Cephalosporins **15–18** and **23–26** were prepared using cyclic anhydrides **6–8** of the corresponding dicarboxylic acids which were obtained by known procedures.[7-10]

Biology. In this series, substitution of 3-tetrazolylthiomethyl for acetoxymethyl exerts no significant influence on the level of gram-positive activity. The MIC's against a penicillin-G resistant strain of *Staphylococcus aureus* [*S.a.*(R)] for the tetrazole-containing analogues (Table III) are all within one twofold dilution of those obtained for the corresponding acetoxy analogues (Table II). On the other hand, this change results in an overall improvement of the MIC's against the gram-negative bacteria for the majority of compounds tested. These results are consistent with other structure–activity relationship studies which have shown the same general trends reported here.[12]

Significant changes in MIC's are observed with variation of the *N*-acyl group attached to the phenylglycine portion of the molecule within each series. Compounds **12–14** (Table II) and **20–22** (Table III) show the effects of altering

Table I. Structures of Cephalosporin Analogues



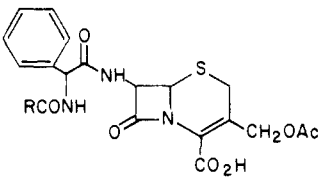
Compd	R	M	X	Formula	Analyses
12	$(CH_2)_3CO_2Na$	Na	OAc	$C_{23}H_{23}N_3O_9SNa_2 \cdot H_2O$	C, H, N
13	$CH_2SCH_2CO_2H$	H	OAc	$C_{22}H_{21}N_3O_9S_2 \cdot 0.25H_2O$	C, H, N
14	$CH_2OCH_2CO_2Na$	Na	OAc	$C_{22}H_{21}N_3O_{10}SNa_2 \cdot 2H_2O$	C, H, N
15		Na	OAc	$C_{23}H_{21}N_3O_9SNa_2 \cdot 2.5H_2O$	C, H, N
16		Na	OAc	$C_{22}H_{19}N_3O_{10}SNa_2 \cdot 2.5H_2O$	C, H, N
17		Na	OAc	$C_{22}H_{19}N_3O_{10}SNa_2 \cdot 2.5H_2O$	C, H, N
18		Na	OAc	$C_{24}H_{23}N_3O_{10}SNa_2$	C, H, N
19		Na	OAc	$C_{21}H_{20}N_3O_8SNa \cdot 1.75H_2O$	C, H, N
20	$(CH_2)_3CO_2Na$	Na	SMTZ[a]	$C_{23}H_{23}N_7O_7S_2Na_2 \cdot 0.75H_2O$	C, H, N
21	$CH_2SCH_2CO_2Na$	Na	SMTZ	$C_{22}H_{21}N_7O_7S_3Na_2 \cdot 1.5H_2O$	C, H, N
22	$CH_2OCH_2CO_2Na$	Na	SMTZ	$C_{22}H_{21}N_7O_8S_2Na_2 \cdot 2.75H_2O$	C, H, N
23		Na	SMTZ	$C_{23}H_{21}N_7O_7S_2Na_2 \cdot 1.5H_2O$	C, H, N
24		Na	SMTZ	$C_{22}H_{19}N_7O_8S_2Na_2 \cdot 3H_2O$	C, H; N[b]
25		Na	SMTZ	$C_{22}H_{19}N_7O_8S_2Na_1 \cdot 1.5H_2O$	C, H; N[c]
26		Na	SMTZ	$C_{24}H_{23}N_7O_8Na_2 \cdot 2.25H_2O$	C, H, N
27		Na	SMTZ	$C_{21}H_{20}N_7O_6S_2Na \cdot 1.75H_2O$	C, H, N
28		Na	SMTZ	$C_{22}H_{19}N_7O_8S_2Na_2 \cdot 2.5H_2O$	C, H, N

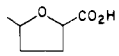
[a] SMTZ =  . [b] N: calcd, 14.56; found, 14.04. [c] N: calcd, 15.06; found, 12.99.

the central portion of the chain from CH_2 to S to O. While the levels of gram-positive activity remain essentially unchanged, there is a stepwise improvement among the gram-negative activities with the oxygen-containing cephalosporins showing the best MIC's in both series. If the central methylene is constrained within a cyclopropane ring with a cis configuration for the two carboxyls, the cephalosporins (**15** and **23**) have better MIC's than the corresponding straight-chain analogues (**12** and **20**). This effect may be somewhat greater than the MIC's indicate since the cyclopropyl cephalosporins are mixtures of diastereomers of which one component should be more potent than the mixture (vide infra). While the data for the corresponding thiiranes are not available to compare with those of the open-chain sulfur analogues (**13** and **21**), the results for the oxiranes (**16**, **17**, **24**, and **25**; cf. **14** and **22**) do fit well into the developing SAR. The components of diastereomeric pairs **16**, **17** and **24**, **25** were separated and tested. While the absolute configurations of these side chains are unknown, the data show that the more active components (**16** and **24**) are two to eight times more potent than the less active components (**17** and **25**). The antibacterial spectrum of **24** is particularly noteworthy. Its gram-positive activity is not outstanding but it is highly active against those gram-negative organisms normally susceptible to the cephalosporins (*E.c.*, *K.p.*, and *Sal.p.*) as well as some of the strains generally insensitive to these antibiotics (*E.a.*, *Pr.m.*(+), *Ps.aer.*). Compound **24** is more active against gram-negative organisms than the straight chain oxygen analogue **22**, the cyclopropyl derivative **23**, or the two cephalosporin standards. Its activity against indole-positive *Proteus* sp. and *P. aeruginosa* is about equal to that of carbenicillin.

Enlarging the ring size of the acyl moiety by two carbons to the tetrahydrofuranyl derivatives **18** and **26** does not result in a major difference in the potency or spectrum of either of these compounds relative to that of the epoxide. Since these compounds are mixtures of diastereomers, the components should differ in levels of activity in a way similar to the diastereomeric epoxides discussed above. Alteration of the ring size produces only minor changes in the spectrum, but removal of the second carboxyl alters the biological profile of these compounds (**19** and **27**). For these two analogues the gram-positive activity has improved but the gram-negative activity is lessened. An even more pronounced change is encountered when the stereochemistry of the second carboxyl of the epoxide is altered from cis to trans as in compound **28**, which has

Table II. In Vitro and in Vivo Activities of 7-(2-Acylamino-2-phenyl)acetamidocephalosporanic Acids



Compd	R	Minimum inhibitory concentration, μg/mL[a]							Mouse PD_{50} vs. *E. coli*, mg/kg sc[c]
		S.a.(R)[b]	*E.c.*	*K.p.*	*Sal.p.*	*E.a.*	*Pr.m.*(+)	*Ps.aer.*	
12	$CH_2CH_2CH_2CO_2H$	6.3	50	25	25	100	>200	>200	100
13	$CH_2SCH_2CO_2H$	3.1	12.5	6.3	6.3	25	12.5	>200	64
14	$CH_2OCH_2CO_2H$	3.1	6.3	3.1	12.5	12.5	12.5	>200	21.5
15[d]		6.3	12.5	3.1	6.3	12.5	12.5	>200	25
16[e]		6.3	1.6	0.8	1.6	3.1	3.1	100	12.5
17[e]		12.5	12.5	6.3	6.3	25	200	>200	72
18[d]		6.3	12.5	6.3	12.5	25	50	200	140
19[d]		1.6	25	12.5	12.5	25	25	>200	100
	Cephaloglycin	3.1	3.1	3.1	3.1	50	>200	>200	
	Cephalothin	0.2	3.1	1.6	0.8	12.5	>200	>200	50
	Carbenicillin	3.1	6.3	6.3	6.3	12.5	0.8	50	25

[a] The in vitro antibacterial activities are reported as minimum inhibitory concentrations (MIC) in μg/mL. The MIC's were determined by the twofold agar dilution method [T. Jen, B. Dienel, J. Frazee, and J. Weisbach, *J. Med. Chem.*, 15, 1172 (1972)] on Trypticase soy agar buffered to pH 6.0. Organisms selected for inclusion in this table are *S.a.*(R), *Staphylococcus aureus* HH 127 (penicillin G resistant); *E.c.*, *Escherichia coli* 12140; *K.p.*, *Klebsiella pneumoniae* 4200; *Sal.p.*, *Salmonella paratyphi* ATCC 12176; *Sh.p.*, *Shigella paradysenteriae* HH 117; *E.a.*, *Enterobacter aerogenes* ATCC 13048; *Pr.m.*(+), *Proteus morgani* 179 (indole positive); *Ps.aer.*, *Pseudomonas aeruginosa* HH 63. [b] MIC's were also determined vs. *Staphylococcus aureus* 23390 (Smith) and in all cases the MIC values were within one twofold dilution of those reported for *Staphylococcus aureus* HH 127. [c] The PD_{50} values are expressed as the total dose of compound in mg/kg which afforded protection to 50% of the mice challenged intraperitoneally with approximately 2×10^3 organisms per mouse of an *E. coli* 12140 culture diluted in 5% hog gastric mucin to produce uniformly lethal infections. Fourfold dilutions of each compound were injected subcutaneously in equally divided portions at 1 and 5 h postinfection to five groups of ten mice each. Survivors were observed for 3 days and the mean protective dose (PD_{50}) was calculated by the method of Litchfield and Wilcoxon, *J. Pharmacol. Exp. Ther.*, 96, 99–113 (1949). [d] Mixture of diastereomers. [e] Compounds 16 and 17 are diastereomers. The absolute configurations are unknown.

significantly lower activity than **24**.

The protective effectiveness of these compounds in mice infected with *E. coli* (PD_{50}, Tables II and III) reflects the trends observed in vitro. In vivo protection increases as the central portion of the acyl moiety changes from CH_2 to S to O (**12–14**, Table II; **20–22**, Table III). It is further increased when the CH_2 or O is part of a three- or five-membered ring (**15–18**, Table II; **23–26**, Table III). Additionally, a second carboxyl substituent cis to the amide group is required for optimum activity (**19**, **27**, and **28**). The differences in the MIC values for the diastereomers are reflected in the observed PD_{50} values also. Cephalosporins **16** and **24** (PD_{50} 12.5, 7.0 mg/kg) are more active in vivo than their diastereomers **17** and **25** (PD_{50} 72, 35 mg/kg). Of all the compounds tested, compound **24** provided the best protection to mice infected with *E. coli*, in agreement with the observed in vitro test results.

The in vitro activities against eight strains of *P. aeruginosa* for those compounds having a 3-tetrazolylthiomethyl substituent are shown in Table IV. Against this group of selected clinical isolates which are generally resistant to cephalosporins, **24** and **26** show activity on the order of carbenicillin.

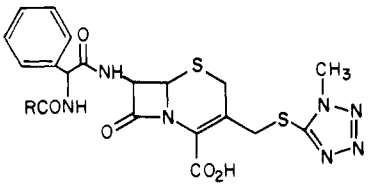
Experimental Section

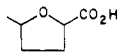
Melting points were determined in open capillary tubes using a Thomas-Hoover Uni-Melt apparatus. Unless indicated otherwise, IR spectra were obtained in Nujol mull using a Perkin-Elmer Infracord; NMR spectra were obtained in Me_2SO-d_6 or Me_2SO-d_6–D_2O on a Varian T-60 spectrometer using Me_4Si as an internal standard. The IR and NMR data of all compounds are consistent with structure. Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. All solvents were evaporated under vacuum; $MgSO_4$ was used as the drying agent for organic extracts.

Tetrahydrofuran-*cis*-2,5-dicarboxylic Acid Anhydride (8). A solution of 4.96 g (31 mmol) of tetrahydrofuran-*cis*-2,5-dicarboxylic acid[9] in 50 mL of THF was treated with 6.38 g (31 mmol) of DCC in 50 mL of THF. The reaction was stirred at room temperature for 2 h, filtered to remove precipitated urea, and evaporated to give a white solid which was crystallized from CCl_4 to give 3.0 g (68%) of white crystals: mp 125–126 °C (lit.[13] 128–129 °C; lit.[14] 134–135 °C); NMR δ 4.95 (m, 2), 2.25 ppm (m, 4); IR 1825, 1775 cm^{-1}. Anal. Calcd for $C_6H_6O_4$: C, 50.71; H, 4.26. Found: C, 50.37; H, 4.34.

7-[2-(*N*-Glycidamido)-2-phenyl]acetamido-3-cephem-4-carboxylic Acids (19 and 27). A suspension of 0.63 g (5 mmol) of potassium glycidate[11] in 50 mL of THF containing 2 drops of *N*-methylmorpholine was cooled to −10 °C. To this was added 0.68 g (5 mmol) of isobutyl chloroformate and the mixture stirred at −10 °C for 15 min. A cold solution of 2.31 g (5 mmol) of cephalosporin **2** in 40 mL of 50% aqueous THF containing 0.5 g (5 mmol) of Et_3N was added dropwise at a rate slow enough to maintain the temperature below −5 °C. The resulting mixture was stirred in the cold for 1 h and then at room temperature for 2 h. The THF was evaporated; the mixture was diluted with 50 mL of water, adjusted to pH 7.5 with Et_3N, and extracted twice

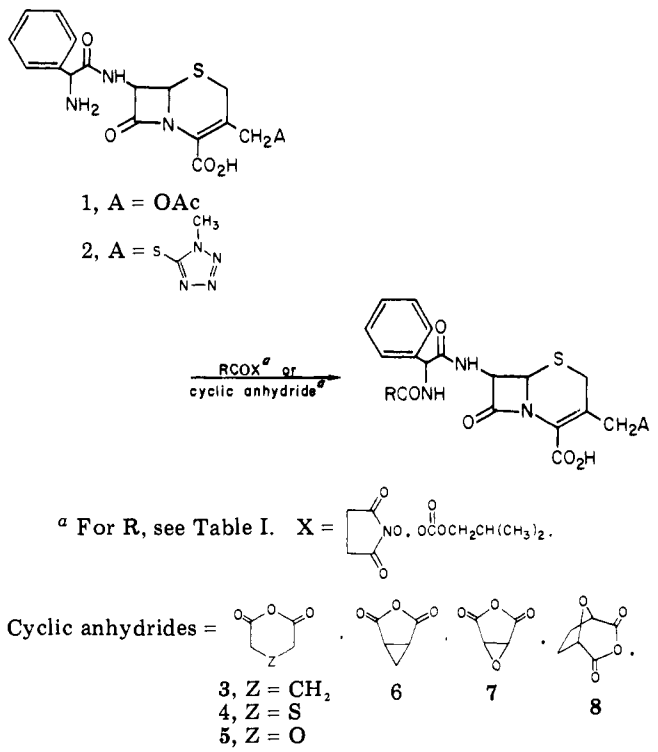
Table III. In Vitro and in Vivo Activities of 7-(2-Acylamino-2-phenyl)acetamido-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acids



Compd	R	Minimum inhibitory concentrations, μg/mL[a]							Mouse PD_{50} vs. *E. coli*, mg/kg sc[c]
		S.a.(R)[b]	*E.c.*	*K.p.*	*Sal.p.*	*E.a.*	*Pr.m.*(+)	*Ps.aer.*	
20	$CH_2CH_2CH_2CO_2H$	3.1	12.5	6.3	6.3	25	12.5	>200	100
21	$CH_2SCH_2CO_2H$	3.1	6.3	3.1	3.1	3.1	1.6	>200	50
22	$CH_2OCH_2CO_2H$	3.1	1.6	0.8	1.6	3.1	0.4	200	8.7
23[d]		6.3	6.3	3.1	3.1	6.3	6.3	200	25
24[e]		6.3	0.8	0.8	1.6	1.6	0.4	50	7
25[e]		12.5	6.3	3.1	6.3	12.5	3.1	200	35
26[d]		3.1	3.1	1.6	3.1	3.1	1.6	100	25
27[d]		1.6	12.5	6.3	6.3	12.5	3.1	200	45
28[d]		1.6	25	12.5	12.5	25	25	>200	150
	RCO replaced by H	3.1	3.1	1.6	1.6	1.6	6.3	>200	1.4
	Cephalothin	0.2	3.1	1.6	0.8	12.5	>200	>200	50
	Carbenicillin	3.1	6.3	6.3	6.3	12.5	0.8	50	25

[a-d] See corresponding footnotes to Table II. [e] Compounds **24** and **25** are diastereomers. The absolute configurations are unknown.

Scheme I



[a] For R, see Table I. X =

Cyclic anhydrides =

3, Z = CH_2; 4, Z = S; 5, Z = O; 6; 7; 8

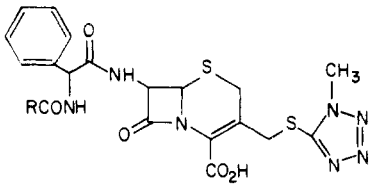
with EtOAc. The solution was adjusted to pH 2.5 with 3 N HCl and extracted with three 75-mL portions of EtOAc and the extracts were combined, dried, and evaporated to a gum. This was dissolved in 20 mL of MeOH, cooled in an ice bath, and adjusted to pH 6.9 with 5% $NaOCH_3$ in MeOH. The product was fractionally precipitated by the dropwise addition of Et_2O, collected by filtration, and dried under vacuum to give **27**.

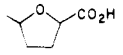
Substitution of cephalosporin **1** in the above procedure gave the corresponding 3-acetoxymethyl analogue **19**.

General Procedure for Acylation of Cephalosporins 1 or 2 with Cyclic Anhydrides. Synthesis of Cephalosporins 12–18 and 20–26. A suspension of 10 mmol of cephalosporin **1** or **2** in 50 mL of dry acetone was stirred at room temperature and 10 mmol of the appropriate anhydride was added in one portion. If the anhydride was not sufficiently soluble in acetone to effect acylation (5 min to 2 h as judged by complete solution of the suspended cephalosporin) 20 mL of dry DMF was added as a co-solvent. When the reaction was complete the solvents were evaporated and the residue was triturated with Et_2O giving a crude solid. The crude products were dissolved in MeOH (~20 mL) and the solution was adjusted to pH 7.0 with 5% $NaOCH_3$ in MeOH. In most cases the products were fractionally precipitated by the dropwise addition of Et_2O to give diastereomeric mixtures of sodium salts. For compounds **16**, **17** and **24**, **25**, cooling and scratching of the methanolic solution of sodium salts caused selective crystallization of one of the diastereomers in both cases. The crystalline products were collected by filtration and the remaining diastereomer precipitated from the filtrate by the dropwise addition of Et_2O. TLC on silica gel using $CHCl_3$–MeOH–HCOOH (60:40:3) showed that the crystalline diastereomers (**16** or **24**) were pure and were the slower running of the pair, while the diastereomers **17** or **25** which were precipitated from the filtrates were contaminated with a minor amount of the slower moving isomer.

***trans*-2,3-Epoxysuccinic Acid *N*-Hydroxysuccinimidyl Ester (11) (Scheme II).** A solution of 10.2 g (77.4 mmol) of *trans*-2,3-epoxysuccinic acid[7] in 150 mL of THF was treated dropwise with a solution of 15.5 g (77.4 mmol) of *O*-*tert*-butyl-*N*,*N*-diisopropylpseudourea in 75 mL of THF. After addition was complete, the reaction was stirred at room temperature overnight. The mixture was filtered and the filtrate was evaporated to a residue which was partitioned between 150 mL of H_2O and 150 mL of Et_2O. The Et_2O was dried and evaporated to leave a suspension of a solid in an oil. This was triturated with CH_2Cl_2

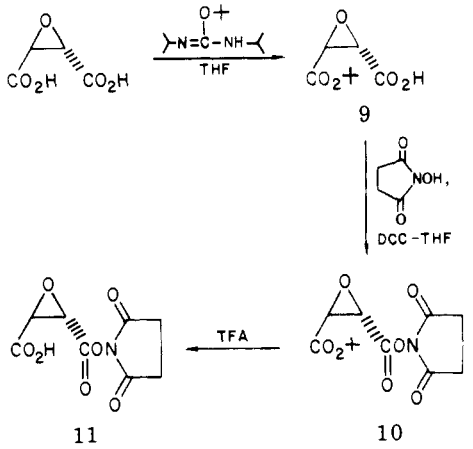
Table IV. In Vitro Activities of 7-(2-Acylamino-2-phenyl)acetamido-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid Analogues against *Pseudomonas aeruginosa*



Compd	R	Minimum inhibitory concentration, μg/mL[a]							
		1504	1042	219	647	864	1062	1063	1106
20	$(CH_2)_3CO_2H$	>500	>500	>500	>500	>500	>500	>500	>500
21	$CH_2SCH_2CO_2H$	500	>500	500	>500	>500	500	>500	>500
22	$CH_2OCH_2CO_2H$	125	250	125	125	500	250	500	500
23[b]		125	250	125	125	500	250	500	>500
24		63	63	125	63	500	63	500	500
25		500	>500	500	>500	500	>500	>500	>500
26[b]		63	125	125	125	500	125	250	500
27[b]		500	>500	>500	>500	>500	>500	>500	>500
	Carbenicillin	63	63	63	63	500	63	63	125

[a] Assays were carried out in unbuffered Mueller-Hinton broth using the standard microtiter technique (T. L. Gavin and D. A. Butler, "Current Techniques for Antibiotic Susceptibility Testing", A. Ballows, Ed., Charles C Thomas, Springfield, Ill., 1974, Chapter IX. [b] Mixture of diastereomers.

Scheme II



and filtered, and the filtrate was evaporated to give 10.7 g (74%) of the mono-*tert*-butyl ester **9** as a colorless oil: NMR (acetone-d_6) δ 3.6 (m, 2), 1.5 ppm (s, 9). A solution of 9.0 g of ester **9** in 100 mL of THF containing 5.52 g (48 mmol) of *N*-hydroxysuccinimide was treated dropwise with 9.9 g (48 mmol) of DCC in 50 mL of THF. After stirring overnight at room temperature the solution was filtered and evaporated to give a white, foamy solid. This was stirred with Et_2O and filtered, and the filtrate was dried and evaporated to give 5.6 g (41%) of the activated ester **10** as a white solid: NMR (acetone-d_6) δ 4.18 (d, 1, J = 2 Hz), 3.80 (d, 1, J = 2 Hz), 2.95 (s, 4), 1.55 ppm (s, 9). This was added to 40 mL of cold TFA and the resulting solution stirred in an ice bath for 1.5 h. The TFA was evaporated and the residue was triturated with Et_2O to give 1.9 g (43%) of **11** as a white solid: NMR (acetone-d_6) δ 4.2 (d, 1, J = 2 Hz), 3.85 (d, 1, J = 2 Hz), 2.95 ppm (s, 4).

7-D-α-(*trans*-Epoxysuccinamido)phenylacetamido-3-(1-methyltetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (28). A mixture of 2.3 g (5 mmol) of cephalosporin **2** and 1.15 g (5 mmol) of activated ester **11** was stirred in 50 mL of DMF for 3 h. A small amount of insoluble material was removed by filtration and the filtrate was evaporated to a gum. This was partitioned between H_2O and EtOAc at pH 7.5 and the organic phase discarded. The aqueous phase was adjusted to pH 2.5 with 3 N HCl and extracted with EtOAc, and the dried extract was evaporated to a foamy solid. This was dissolved in ~20 mL of cold MeOH, the solution adjusted to pH 7.0 with 5% $NaOCH_3$ in MeOH, and the product isolated by fractional precipitation with Et_2O.

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Synthesis and Some Pharmacological Properties of [4-Homoserine]oxytocin

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The incorporation of homoserine [Hse] into synthetic peptides has proved extremely difficult due to the ease with which homoserine derivatives form a γ-lactone. We now wish to report the synthesis of [4-homoserine]oxytocin. We wished to obtain this peptide to test our earlier hypothesis as to why threonine substitution in the 4 position in oxytocin enhanced both oxytocic activity (O) and oxytocic–antidiuretic (O/A) selectivity relative to oxytocin. The key to this synthesis was the preparation of N^{α}-*tert*-butyloxycarbonyl-*O*-benzyl-L-homoserine [Boc-Hse(Bzl)] by a modification of a recently published method used for the benzylation of N^{α}-*tert*-butyloxycarbonylserine (Boc-Ser). Boc-Hse(Bzl) was incorporated into the protected nonapeptide Z-Cys(Bzl)-Tyr(Bzl)-Ile-Hse(Bzl)-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 using the solid-phase method by procedures previously described from these laboratories. Following deblocking, cyclization, and purification in the usual manner the desired peptide, [4-homoserine]oxytocin, was obtained. An examination of its pharmacological properties provided the following potencies (units/mg): rat oxytocic (O) 125 ± 13; rat antidiuretic (A) 0.24 ± 0.03. Its oxytocic–antidiuretic ratio is thus 521. These data indicate that threonine and homoserine when substituted at position 4 in oxytocin exert similar effects on O/A selectivity (cf. [4-threonine]oxytocin has an O/A ratio of 512) but that threonine exerts a unique effect in leading to enhanced oxytocic activity (cf. [4-threonine]oxytocin has an oxytocic potency of 923 ± 96 units/mg). This successful synthesis of Boc-Hse(Bzl) and its incorporation into [4-homoserine]oxytocin now make possible the synthesis of homoserine analogues of other peptides.

The substitution of threonine for glutamine in oxytocin gave a compound [Thr^4]oxytocin which possesses very unusual properties.[1] Its oxytocic potency is almost twice that of oxytocin whereas its antidiuretic potency is less than one-third that of oxytocin. In attempting to explain why threonine had such unusual effects, we proposed that examination of the properties of [Hse^4]oxytocin might shed some light on this intriguing question.[2] Thus in 1970 we undertook the synthesis of this compound. This synthesis proved extremely difficult and has only recently been finally accomplished. The difficulty in incorporating homoserine into synthetic peptides stems from the well-known tendency of γ-hydroxyamino acids and their derivatives to form a γ-lactone.[3-15] Thus to date to our knowledge there is only one reported instance in which homoserine was incorporated into a synthetic peptide.[11] The method employed the coupling of homoserine as the C-terminal lactone of a dipeptide to give an analogue of tetragastrin in a yield of only 20%. We have tried unsuccessfully to adapt this approach to the synthesis of [Hse^4]oxytocin. After many unsuccessful attempts to synthesize [Hse^4]oxytocin, we now report its successful synthesis by an approach which should be widely applicable for the synthesis of homoserine analogues of other peptides.

The key to this synthesis was the preparation of N^{α}-*tert*-butyloxycarbonyl-*O*-benzyl-L-homoserine [Boc-Hse(Bzl)] using an adaptation of a recently published procedure for the benzylation of N^{α}-*tert*-butyloxycarbonyl-L-serine (Boc-Ser).[16] Boc-Hse(Bzl) was incorporated into the required protected nonapeptide intermediate by the solid-phase method[17] as described for the synthesis of [Thr^4]oxytocin.[1] Deblocking with sodium in liquid ammonia,[18] cyclization, and purification by gel filtration on Sephadex G-15 by the two-step procedure involving 50% AcOH and 0.2 N AcOH as eluents, respectively, as previously described[19,20] afforded the desired free peptide. The peptide was assayed for oxytocic activity on the isolated rat uterus suspended in solutions containing no Mg^{2+} or 0.5 mM Mg^{2+}.[21] Milk-ejection activity was measured by intravenous injection of the peptide into lactating rats anesthetized with pentobarbital.[22] Antidiuretic activity was estimated by intravenous injection into hydrated rats anesthetized with ethanol.[23] Vasopressor assays were performed on phenoxybenzamine-treated rats under urethane anesthesia.[24] The USP posterior pituitary reference standard was used in all assays.

Results and Discussion

Synthetic Aspects. Attempts to incorporate homoserine into synthetic peptides have to date met with little success. Morley and Smith coupled Z-Trp-DL-Hse lactone and Asp-Phe-NH_2 to obtain Z-Trp-DL-Hse-Asp-Phe-NH_2 in low yield.[11] When these authors attempted to couple Z-Trp-DL-Hse azide (obtained from the above lactone), the end product contained mainly lactone. We attempted unsuccessfully to couple *tert*-butyloxycarbonylhomoserine lactone to Asn-Cys(Bzl)-Pro-Leu-Gly-resin. From these results it was clear that N-protected homoserine lactone or N-protected peptides ending with homoserine lactone are not active enough to give satisfactory yields in the peptide bond forming step unless promoted by other driving forces. Such forces have been very elegantly demonstrated in the synthesis of [52-homoserine] basic pancreatic trypsin inhibitor (BPTI) by cyanagen bromide cleavage of the native molecule at the methionine residue in position 52, followed at neutral pH by a conforma-