suggest the importance of the hydrogen bonding of the 3-hydroxyl group.

As anticipated,[4] the statone-containing peptide **4d** is much less potent than the hydroxy analogue **4a**. The notion that substitution of fluorine atoms adjacent to the carbonyl group to increase its propensity toward addition of water and sp^3 hybridization is clearly illustrated in the increased inhibitory potency of the peptide **4e** over that of the nonfluorinated peptide **4d**.

A high degree of enzyme specificity is desirable for a potentially successful therapeutic agent. Pepstatin, for example, is a general aspartyl protease inhibitor and shows poor selectivity. A substrate analogue such a RIP[15] does not discriminate between renin and converting enzyme. The difluoro ketone **4f** does exhibit high renin specificity. It is a very poor inhibitor of converting enzyme and is 3–4 orders of magnitude less effective against pepsin and cathepsin D. Such target specificity is also important for a better understanding of a mechanistic based biological action.

The transition-state analogue concept remains a viable approach in the design of potent enzyme inhibitors as illustrated in aspartyl proteases.[16] The work presented in this report lends support to the value of an understanding of enzymatic mechanism as an aid to create effective inhibitors of therapeutically important enzymes.

Registry No. **1a**, 96056-65-4; 1b, 97920-08-6; **2a**, 97920-09-7; **2b**, 97995-50-1; **3a**, 97920-10-0; **3b**, 97995-51-2; **4a**, 97920-11-1; **4b**, 97920-12-2; **4c**, 97995-52-3; **4d**, 97920-13-3; **4e** (isomer 1), 97920-14-4; **4e** (isomer 2), 97995-53-4; **4e** tosyl deriv. (isomer 1), 97920-15-5; **4e** tosyl deriv. (isomer 2), 97995-54-5; BrF_2CCO_2Et, 667-27-6; Boc-His(Ts), 35899-43-5; Boc-Phe, 13734-34-4; ACE, 9015-82-1; Boc-L-leucinal, 58521-45-2; L-isoleucyl-2-pyridylmethylamide, 97920-16-6; renin, 9015-94-5; pepsin, 9001-75-6; cathepsin, 9004-08-4.

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Articles

N-(Phosphonoacetyl)amino Phosphonates. Phosphonate Analogues of *N*-(Phosphonoacetyl)-L-aspartic Acid (PALA)

Paweł Kafarski,[†] Barbara Lejczak,[†] Przemysław Mastalerz,*[†] Danuta Duś,[‡] and Czesław Radzikowski[‡]

Institute of Organic and Physical Chemistry, Technical University of Wrocław 50-370 Wrocław, and Tumor Immunology Department, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 53-114 Wrocław, Poland.
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Michaelis–Arbuzov reaction of *N*-(chloroacetyl)amino phosphonic acids or their esters, followed by acidolysis, gives moderate yields of *N*-(phosphonoacetyl) derivatives of a variety of (aminoalkyl)phosphonic acids, including analogues of the cytostatic agent PALA, in which the α- or β-carboxylic groups in the aspartate moiety are replaced by a PO_3H_2 function. Assay of cytostatic activity with human KB cell lines indicates that the substitution of any of the COOH groups in PALA with PO_3H_2 results in total loss of cytostatic activity. No activity was observed also in the case of other [*N*-(phosphonoacetyl)amino]alkylphosphonic acids described in this report.

N-(Phosphonoacetyl)-L-aspartic acid (PALA (1)) is a rationally designed transition-state analogue of aspartate transcarbamylase,[1-3] the second enzyme in the de novo pyrimidine biosynthesis. It also exhibits tumor-inhibitory activity against a number of transplantable solid murine tumors.[4-7] However, in comparison with other antimetabolites, the spectrum of activity of PALA is unique in that leukemias,[6-8] bladder cancer,[9] and hypernoma[9] are relatively or completely insensitive to the drug whereas Lewis lung carcinoma[10,11] and melanomas[4,12,13] are sensitive. The possibility of using PALA in polychemotherapy is also very promising since it was found to be synergistic with several popular anticancer drugs including fluorouracil, alanosine, acivicin, methotrexate, and pyrofurazin.[11,14-20]

Although PALA is a promising anticancer agent, its usefulness in human therapy seems to be limited. It fails to inhibit pyrimidine biosynthesis in vivo as strongly as expected from its effectiveness in vitro, despite an apparently effective concentration of the drug in tissues.[17]

[†] Technical University of Wrocław.
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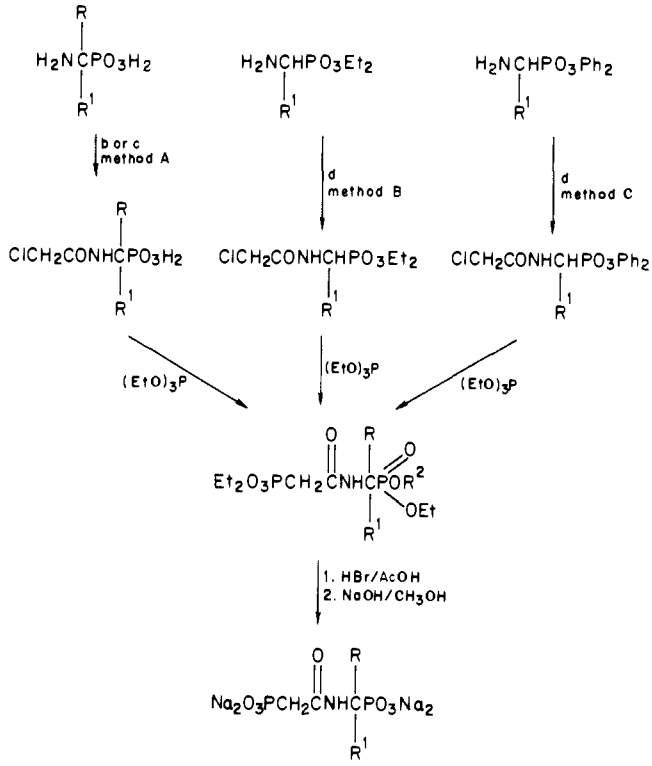
Scheme I[a]

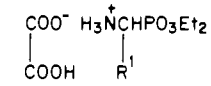


[a] R = H, CH_3; R^1 = CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH_2CH_2CH_3$, $C(CH_3)_3$, CH_2COO-*n*-Pr, $CH_2CH_2COOCH_3$, $CH_2CH_2CH_2COOEt$, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl; RR^1 = $-(CH_2)_4-$; R^2 = Et, Ph; R^3 the same as R^1 with exception for $-(CH_2)_nCOOH$ derivatives for which R^3 = $-(CH_2)_nCOONa$ while n = 1–3. [b] $ClCH_2COCl/NaOH(aq)$. [c] $(ClCH_2CO)_2O$/dioxane. [d] $ClCH_2COCl/Et_3N/CHCl_3$.

This is probably due to the difficult transportation of PALA (as the tetraanion only) to its sites of action.[21]

Over 25 years several groups, including ours, have been interested in the chemical and biological consequences of the replacement of the carboxyl groups of amino acids by phosphonic groups. New compounds obtained in this manner (the phosphonic analogues of amino acids) were frequently found to have interesting biological and biochemical activities.[22] Moreover, the N-acylated aminophosphonates are usually well transported through biological membranes.[23]

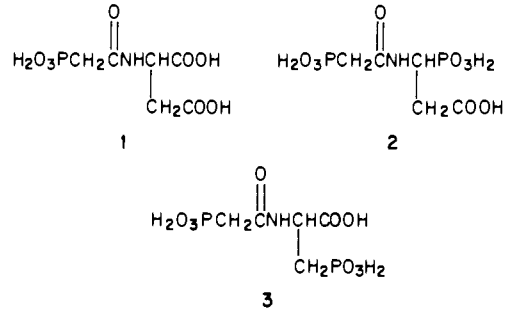
Table I. Diethyl (1-Aminoalkyl)phosphonate Oxalates[a]



R^1	mp, °C	yield,[b] %	formula	anal.[c]
$C(CH_3)_3$	69–72	22.5	$C_{11}H_{24}NO_7P$	N, P
$CH_2CH_2COOCH_3$	semisolid	7	$C_{11}H_{22}NO_9P$	N, P
$CH_2CH_2CH_2COOCH_2$-CH_3	semisolid	7	$C_{13}H_{26}NO_9P$	N, P
cyclopropyl	72–74	36	$C_{10}H_{20}NO_7P$	N, P
cyclobutyl	112–114	21	$C_{11}H_{22}NO_7P$	N, P
cyclopentyl	131–135	63	$C_{12}H_{24}NO_7P$	N, P
cyclohexyl	143–146	27.5	$C_{13}H_{26}NO_7P$	N, P

[a] Prepared by the method of Kowalik et al.[29] [b] In relation to starting acid chloride. The reaction scheme is as follows: $R^1COCl + (EtO)_3P \rightarrow R^1C(O)PO_3Et_2 \rightarrow R^1C(NOH)PO_3Et_2 \rightarrow R^1CH(NH_2)PO_3Et_2$. [c] Analyses within ±0.4% for indicated elements.

In this work we describe the preparation of phosphonic analogue (**2** and **3**) of PALA, as well as the synthesis of other *N*-(phosphonoacetyl)amino phosphonic acids for evaluation of their anticancer activity in human tumor cell lines.



Chemistry. The methods for the preparation of [[*N*-(phosphonoacetyl)amino]alkyl]phosphonic acids (sodium salts) are outlined in Scheme I. The syntheses involved chloroacetylation of (aminoalkyl)phosphonic acids (method A) or their diethyl (method B) or diphenyl (method C) esters followed by Michaelis–Arbuzov reaction and acidolysis of ester bonds.

Unexpectedly, we found the acylation of (aminoalkyl)-phosphonic acids (method A) difficult to achieve. This is probably due to the fact that the phosphonic group forms a mixed anhydride with the acylating agent, thus accelerating its hydrolysis.[24,25]

Chloroacetylation of the phosphonic analogues of aspartic acid was impaired also by the insolubility of the substrates and products in dioxane. This problem was solved by esterification of the carboxyl moieties with propanol prior to the acylation step.

The procedure starting from diethyl (aminoalkyl)-phosphonates (method B) is limited by two factors: the availability of starting esters (the obtained esters are presented in Table I) and the thermal instability of diethyl [[*N*-(chloroacetyl)amino]alkyl]phosphonates that decompose partially during Arbuzov reaction. The overall re-

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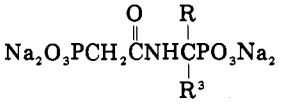
Table II. Sodium Salts of [[*N*-(Phosphonoacetyl)amino]alkyl]phosphonates



no.	R	R^3	method	yield,[a] %	formula	anal.[b]
13	H	CH_2COONa	A	42	$C_4H_5NNa_5O_9P_2$	N, P
14	$Na_2O_3PCH_2CONHCHCOONa$	$CH_2PO_3Na_2$	A	7.5	$C_4H_5NNa_5O_9P_2$	N, P
15	H	CH_3	A	22	$C_4H_7NNa_4O_7P_2$	N, P
			C	63.5		
16	CH_3	CH_3	A	29	$C_5H_9NNa_4O_7P_2$	N, P
17	H	$CH(CH_3)_2$	A	28	$C_6H_{11}NNa_4O_7P_2$	N, P
			C	63		
18	H	$CH_2CH_2CH_3$	A	16	$C_6H_{11}NNa_4O_7P_2$	N, P
			C	72		
19	H	$CH_2CH_2CH_3$	C	71	$C_7H_{13}NNa_4O_7P_2$	N, P
20	H	$C(CH_3)_3$	B	14.5	$C_7H_{13}NNa_4O_7P_2$	N, P
21	$(CH_2)_4$		A	22	$C_7H_{11}NNa_4O_7P_2$	N, P
22	H	CH_2CH_2COONa	B	25	$C_6H_8NNa_5O_9P_2$	N, P
23	H	$CH_2CH_2CH_2COONa$	B	15	$C_7H_{10}NNa_5O_9P_2$	N, P
24	H	cyclopropyl	B	26.5	$C_6H_9NNa_4O_7P_2$	N, P
25	H	cyclobutyl	B	11	$C_7H_{11}NNa_4O_7P_2$	N, P
26	H	cyclopentyl	B	21	$C_8H_{13}NNa_4O_7P_2$	N, P
27	H	cyclohexyl	B	21	$C_9H_{15}NNa_4O_7P_2$	N, P
28	$Na_2O_3PCH_2CONHCH_2CH_2CH_2PO_3Na_2$		B	20	$C_5H_9NNa_4O_7P_2$	N, P
29	$Na_2O_3PCH_2CONHCHPO_2Na_2$	C_6H_5	A	55	$C_9H_9NNa_4O_6P_2$	N, P
30	$Na_2O_3PCH_2CONHCH_2PC_2H_5$	ONa	A	61.5	$C_5H_{10}NNa_3O_6P_2$	N, P

[a] In relation to starting (1-aminoalkyl)phosphonic acid (method A) or its diethyl (method B) or diphenyl (method C) ester. [b] Within ±0.4% for indicated elements.

action yields were comparable with those obtained by method A.

The best preparative results were obtained starting from diphenyl (1-aminoalkyl)phosphonates (method C). In addition, this method proved to be interesting from the chemical point of view because, quite unexpectedly, the Arbuzov reaction of diphenyl [[*N*-(chloroacetyl)amino]-alkyl]phosphonates with triethyl phosphite yielded mixed ethyl phenyl instead of the diphenyl esters. Also unexpectedly, the deprotection of the mixed ester function proceeded readily with hydrogen bromide in glacial acetic acid. It should be noted that the diphenyl phosphonates are not cleaved by HBr.

Yields and physicochemical data for the [[*N*-(phosphonoacetyl)amino]alkyl]phosphonic acid sodium salts obtained by the methods described above are presented in Table II.

Cytostatic Activity. Cytostatic activity was tested on a stable human KB cell line, originated from oral carcinoma, in tissue monolayer culture. Since the determined ID_{50} for the model compound, PALA, was 240 $\mu g\ mL^{-1}$, all the compounds were tested at 1–400 $\mu g\ mL^{-1}$ doses.

The results are disappointing—even at the highest dose we did not observe any cytostatic activity. Thus, the replacement of any of the carboxyl moieties in PALA by a phosphonic function resulted in a total abolition of antitumor activity.

Experimental Section

Melting points (uncorrected) were determined on a Koeffler apparatus. [[*N*-(Phosphonoacetyl)amino]alkyl]phosphonic acid sodium salts did not melt up to 350 °C. The structures of all compounds were supported by their IR (Perkin-Elmer 621) and NMR (Tesla BS 467) spectra.

(Aminoalkyl)phosphonic acids were gifts from Dr. J. Oleksyszyn and Dr. M. Soroka. Diphenyl (1-aminoalkyl)phosphonates were prepared according to Oleksyszyn et al.[26] Tetrasodium *N*-(phosphonoacetyl)-L-aspartate was prepared as described earlier.[27]

***n*-Propyl 3-amino-3-phosphonopropionate** (4) was prepared as described earlier,[28] starting from 5.0 g (0.03 mol) of 3-amino-3-phosphonopropionic acid: yield 5.3 g (83.5%); mp 234–235 °C dec. Anal. ($C_6H_{14}NO_5P$) N, P.

***n*-Propyl 2-amino-3-phosphonopropionate** (5) was prepared by a previously reported method,[28] starting from 5.0 g (0.03 mol) of 2-amino-3-phosphonopropionic acid: yield 5.4 g (85%); mp 174–177 °C dec. Anal. ($C_6H_{14}NO_5P$) N, P.

Diethyl (1-aminoalkyl)phosphonate oxalates (6–12) were prepared by the method of Kowalik et al.[29] Their physicochemical data are presented in Table I.

Tetrasodium [1-[*N*-(Phosphonoacetyl)amino]cyclopentyl]phosphonate (21). **Typical Example of Method A.** (1-Aminocyclopentyl)phosphonic acid (3.3 g, 0.02 mol) and chloroacetic anhydride (13.7 g, 0.08 mol) were suspended in 100 mL of dioxane and refluxed 15 h. The unreacted aminophosphonic acid was removed by filtration, and the dioxane was removed on a rotary evaporator. The residue was dissolved in the mixture obtained by dissolving 10 mL of thionyl chloride in 75 mL of methanol and left overnight. This step was carried out to convert the unreacted chloroacetic anhydride and chloroacetic acid produced into methyl acetate. The volatile components were then removed under reduced pressure, and to the oily product was added 25 mL of triethyl phosphite. This mixture was heated at 160–180 °C for 1.5 h and cooled to room temperature, and the insoluble material was removed by filtration. Evaporation of the excess of triethyl phosphite yielded tetraethyl [1-[*N*-(phosphonoacetyl)amino]cyclopentyl]phosphonate: yield 2.4 g (30%); ^1H NMR ($CDCl_3$, HMDS) δ 1.25 (t, J = 7 Hz, 4 × CH_3), 1.25–2.65 (m, cyclopentyl CH_2), 2.82 (d, J = 21 Hz, CH_2P), 4.03 (qq, J =

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7 Hz, J_{PH} = 7 Hz, 4 × OCH_2), 7.32 (s, NH). Anal. ($C_{15}H_{31}NO_7P_2$) N, P.

This product was dissolved in 50 mL of 40% hydrogen bromide in glacial acetic acid solution and the resultant mixture left overnight. Evaporation of solvents and hydrogen bromide yielded an oily residue that was washed twice with dry diethyl ether and dissolved in methanol. The methanolic solution of [1-[*N*-(phosphonoacetyl)amino]cyclopentyl]phosphonic acid was titrated with 1 M sodium hydroxide solution in methyl alcohol to adjust to pH 7. This mixture was warmed to boiling and cooled to room temperature. The precipitated, hygroscopic product was collected by filtration: yield 1.65 g (22%); IR (KBr) ν 3700–2000, 3305 (NH), 1635 (CO), 1570 (NH), 1050 (PO_3^{2-}); ^{1}H NMR (D_2O, HMDS) δ 1.6–3.0 (m, cyclopentyl CH_2), 3.07 (d, J = 18.5 Hz, CH_2P).

Pentasodium 4-[*N*-(Phosphonoacetyl)amino]-4-phosphonobutyrate (22). Typical Example of Method B. Methyl 4-amino-4-(diethoxyphosphinoyl)butyrate (6.2 g, 0.0181 mol) was dissolved in 100 mL of dry chloroform containing 8.2 mL (0.0543 mol) of triethylamine, cooled to −5 °C, and acylated with 1.45 mL (0.019 mol) of chloroacetyl chloride. The resulting solution was washed successively with water, 5% hydrochloric acid solution, water, saturated solution of sodium bicarbonate, water, and brine and dried over anhydrous sodium sulfate. Evaporation of the chloroform yielded *C*-methyl *P,P*-diethyl 4-[*N*-(chloroacetyl)amino]-4-phosphonobutyrate, which was dissolved in 30 mL of triethyl phosphite and heated at 160–180 °C for 1.5 h. The insoluble products were removed by filtration, and the volatile components were removed under reduced pressure, yielding *C*-methyl *P,P,P,P*-tetraethyl 4-[*N*-(phosphonoacetyl)amino]-4-phosphonobutyrate: yield 4.4 g (56%); ^{1}H NMR ($CDCl_3$, HMDS) δ 1.33 (brt, J = 7 Hz, 4 × OCH_2CH_3), 1.6–2.8 (m, CH_2CH_2CO), 3.00 (d, J = 21.5 Hz, CH_2P), 3.66 (s, OCH_3), 3.95–4.9 (m, J = 7.5 Hz, 4 × OCH_2, NCHP), 8.01 (brd, J = 9.5 Hz, NH). Anal. ($C_{15}H_{31}NO_9P_2$) N, P.

This compound was acidolyzed with 70 mL of 40% hydrogen bromide in glacial acetic acid solution, and the resulting free acid was converted to the hygroscopic salt **22** as in method A: yield 1.85 g (25%); IR (KBr) ν 3700–2000, 1715 (CO), 1625 (CO), 1540 (NH), 1170, 1050 (PO_3^{2-}); ^{1}H NMR (D_2O, HMDS) δ 2.2–2.45 (m, CH_2CH_2CO), 3.49 (d, J = 19.5 Hz, CH_2P), 4.3–5.0 (m, NCHP).

Tetrasodium [1-[*N*-(Phosphonoacetyl)amino]-2-methylpropyl]phosphonate (17). Typical Procedure for Method C. Diphenyl 1-amino-2-methylpropylphosphonate (12.3 g, 0.04 mol) was acylated analogously as in method B, yielding [1-[*N*-(chloroacetyl)amino]-2-methylpropyl]phosphonate, which was reacted with 60 mL of triethyl phosphite at 160–180 °C for 15 h. Evaporation of the volatile components gave *P*-phenyl *P,P,P*-triethyl [1-[*N*-(phosphonoacetyl)amino]-2-methylpropyl]phosphonate: yield 12.5 g (72%); ^{1}H NMR ($CDCl_3$, HMDS) δ 1.02 and 1.08 (d, J = 7 Hz, 2 × $CHCH_3$), 2.0–2.55 (m, $CHCH_3$), 2.88 (d, J = 21.5 Hz, CH_2P), 3.4–4.2 (m, J = 7 Hz, J_{PH} = 7 Hz, 3 × OCH_2, NCHP), 6.82 (s, aromatic protons), 7.55 (d, J = 10 Hz, NH). Anal. ($C_{18}H_{31}NO_7P_2$) N, P.

Acidolysis of this product with 100 mL of hydrogen bromide in glacial acetic acid and neutralization with methanolic sodium hydroxide yielded the tetrasodium [1-[*N*-(phosphonoacetyl)-amino]-2-methylpropyl]phosphonate: yield 9.2 g (63%); IR (KBr) ν 3650–2700, 3300 (NH), 1630 (CO), 1545 (NH), 1095 (PO_3^{2-}); ^{1}H NMR (D_2O, HMDS) δ 1.38 (brd, 2 × CH_3), 1.36–2.0 (m, $CHCH_3$), 3.03 (d, J = 20.5 Hz, CH_2P), 4.22 (brd of brd, J = 7 Hz, J_{PH} = 17.5 Hz, CHP).

Biological Assays. Cells were grown in a modified minimal essential medium eagle with Earle's salts (EMEM) supplemented with 10% fetal calf serum (both Flow). Experiments were carried out according to the standard Geran's protocol,[30] using [[*N*-(phosphonoacetyl)amino]alkyl]phosphonic acid salts in the concentration range 1–400 μg mL^{-1}. The growth of cells in cultures was determined by measuring the total cell protein according to Lowry.[31] Tetrasodium *N*-(phosphonoacetyl)-L-aspartate was used as a positive control.

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Synthesis and Structure–Activity Relationships of Novel Arylfluoroquinolone Antibacterial Agents[1]

Daniel T. W. Chu,* Prabhavathi B. Fernandes, Akiyo K. Claiborne, Eva Pihuleac, Carl W. Nordeen, Robert E. Maleczka, Jr., and Andre G. Pernet

Antiinfective Research Division, Abbott Laboratories, North Chicago, Illinois 60064. *Received April 1, 1985*

A series of novel arylfluoroquinolones has been prepared. These derivatives are characterized by having a fluorine atom at the 6-position, substituted amino groups at the 7-position, and substituted phenyl groups at the 1-position. Structure–activity relationship (SAR) studies indicate that the in vitro antibacterial potency is greatest when the 1-substituent is either *p*-fluorophenyl or *p*-hydroxyphenyl and the 7-substituent is either 1-piperazinyl, 4-methyl-1-piperazinyl, or 3-amino-1-pyrrolidinyl. The electronic and spatial properties of the 1-substituent, as well as the steric bulk, play important roles in the antimicrobial potency in this class of antibacterials. As a result of this study, compounds **45** and **41** were found to possess excellent in vitro potency and in vivo efficacy.

Since the introduction of nalidixic acid (**1**)[2] in 1963 for the treatment of urinary tract infections, a large number of related derivatives have been synthesized. The earlier derivatives[3] such as oxolinic acid (**2**), rosoxacin (**3**), and pipemidic acid (**4**) have been used as gram-negative antibacterial agents. Recently, several new analogues containing fluorine atoms have been made; these compounds are potent broad-spectrum antibacterial agents. Included in this group are pefloxacin (**5**),[4] norfloxacin (**6**),[5] enoxacin (**7**),[6] ofloxacin (**8**),[7] and ciprofloxacin (**9**).[8]

(1) This work was presented in part at the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, Oct 8–10, 1984, Abstract 72.

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