Articles

α-(Fluoromethyl)dehydroornithine and α-(Fluoromethyl)dehydroputrescine Analogues as Irreversible Inhibitors of Ornithine Decarboxylase

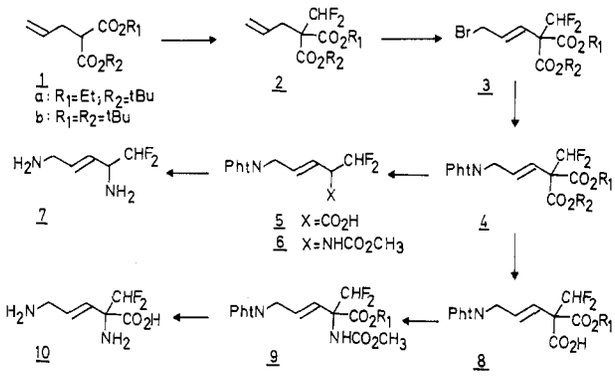
Philippe Bey,* F. Gerhart, V. Van Dorsselaer, and C. Danzin

Centre de Recherche Merrell International, 67084 Strasbourg Cedex, France. *Received January 24, 1983*

(*E*)-Dehydro analogues of α-(fluoromethyl)putrescine and -ornithine derivatives were synthesized and evaluated in vitro as irreversible inhibitors of a preparation of ornithine decarboxylase (ODC, EC 4.1.1.17) obtained from rat liver. The key step in the synthesis of (*E*)-α-(fluoromethyl)dehydroornithine (**17**) and -putrescine (**14**) was the addition of propenylmagnesium bromide to fluoroacetonitrile. The resulting unstable conjugated imine salt was reduced regioselectively in situ with $NaBH_4$ or was quenched with a solution of NaCN to give the corresponding unsaturated α-(fluoromethyl) amine and α-amino nitrile, respectively. These were transformed into **17** and **14** via a four-step sequence involving (a) phthaloyation of the amine function; (b) allylic bromination of the methyl group; (c) Gabriel reaction; and (d) hydrolytic cleavage of the protective groups. (*E*)-α-(Difluoromethyl)dehydroornithine (**10**) and -putrescine (**7**) were prepared from ethyl *tert*-butyl 2-(difluoromethyl)-2-(2-propenyl)malonate and di-*tert*-butyl 2-(difluoromethyl)-2-(2-propenyl)malonate, respectively, via a sequence similar to that reported previously for the synthesis of the saturated analogues. Compounds **17**, **14**, **10**, and **7** proved to be much more potent enzyme-activated irreversible inhibitors of ODC than the corresponding saturated analogues. The increase in potency is particularly marked in the α-fluoromethyl series. The apparent dissociation constants (K_I) and the times of half-inactivation of enzyme (τ_{50}) at infinite concentration of inhibitors are 2.7 μM and 2.6 min for **17** and 42 μM and 0.2 min for **14**. The K_I and τ_{50} of the corresponding saturated analogues are 75 μM and 1.6 min for the ornithine derivative and 56 μM and 4.4 min for the putrescine derivative.

A large body of experimental evidence has accumulated since 1960 to suggest that the polyamines putrescine, spermidine, and spermine play essential roles in cellular growth and/or differentiation.[1] As a consequence, the last decade has seen an intense research effort aimed at the design and synthesis of specific inhibitors of polyamine biosynthesis.[2] Along this line, a major breakthrough has been achieved with the development of enzyme-activated irreversible inhibitors of ornithine decarboxylase (EC 4.1.1.17, ODC), the pyridoxal phosphate dependent enzyme responsible for the synthesis of putrescine. Among these inhibitors, α-fluoromethyl analogues of ornithine[3] and putrescine[4] have proven to be particularly valuable.[5] Not only have they provided useful tools to study the physiological functions of putrescine and spermidine in eukaryotic[6] and prokaryotic cells,[7] but, in addition, one of them, 2-(difluoromethyl)ornithine, has potential for treating human and animal diseases characterized by rapid cell proliferation[8] or caused by parasitic protozoa.[9] We report now the synthesis of the (*E*)-dehydro analogues of α-(fluoromethyl)- and α-(difluoromethyl)ornithine (**10** and **17**) and -putrescine (**14** and **7**) and disclose that these dehydro derivatives are more potent enzyme-activated irreversible inhibitors of ODC than the corresponding saturated analogues. The increase in potency is particularly marked in the α-fluoromethyl series.

Scheme I. Synthesis of (*E*)-α-(Difluoromethyl)dehydroputrescine and -ornithine

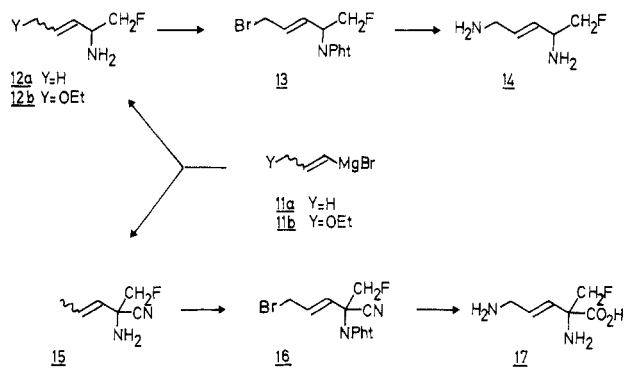


Chemistry. Our synthetic approach to (*E*)-α-(difluoromethyl)dehydroornithine (**10**) and -putrescine (**7**) is outlined in Scheme I. It is patterned after the reaction

(1) For a recent and comprehensive review, see O. Heby, *Differentiation*, *19*, 1 (1981).
(2) For reviews, see (a) L. Stevens and E. Stevens, "Polyamines in Biomedical Research", J. M. Gaugas, Ed., Wiley, Chichester, New York, Brisbane, and Toronto, 1980, p 167. (b) O. Heby and J. Jänne, In "Polyamines in Biology and Medicine", D. R. Morris and L. J. Marton, Eds., Marcel Dekker, New York, 1981, p 243.
(3) (a) B. W. Metcalf, P. Bey, C. Danzin, M. J. Jung, P. Casara, and J. P. Vevert, *J. Am. Chem. Soc.*, **100**, 2551 (1978). (b) J. Kollonitsch, A. A. Patchett, S. Marburg, A. L. Maycock, L. M. Perkins, G. A. Doldouras, D. E. Duggan, and A. S. Aster, *Nature (London)*, **274**, 906 (1978). (c) P. Bey, In "Enzyme-Activated Irreversible Inhibitors", N. Seiler, M. J. Jung, and J. Koch-Weser, Eds., Elsevier, Amsterdam, 1978, p 27.
(4) C. Danzin, P. Bey, D. Schirlin, and N. Claverie, *Biochem. Pharmacol.*, *31*, 3871 (1982).
(5) Enzyme-activated irreversible inhibitors, also referred to as suicide inhibitors, mechanism-based inhibitors, or k_{cat} inhibitors, are highly specific enzyme inhibitors by virtue of their mechanism of action. These inhibitors, which are chemically inert substrate or product analogues, incorporate in their structure latent reactive groupings that are transformed at the target enzyme's active site into an alkylating species as a result of the normal catalytic turnover. In this case, elimination of F^- after decarboxylation or α-hydrogen abstraction generated an electrophilic conjugated imine (see ref 3 and 4).
(6) P. S. Mamont, M. C. Duchesne, A. M. Joder-Ohlenbusch, and J. Grove, In ref 3c, p 43.
(7) A. Kallio, P. P. McCann, and P. Bey, *Biochem. J.*, **204**, 771 (1982).
(8) J. Koch-Weser, P. J. Schechter, P. Bey, C. Danzin, J. R. Fozard, M. J. Jung, P. S. Mamont, N. Seiler, N. J. Prakash, and A. Sjoerdsma, In ref 2b, p 437.
(9) C. J. Bacchi, H. C. Nathan, S. H. Hutner, P. P. McCann, A. Sjoerdsma, *Science*, **210**, 332 (1980).

0022-2623/83/1826-1551$01.50/0

Scheme II. Synthesis of
(*E*)-α-(Fluoromethyl)dehydroputrescine and -ornithine



sequence relying on a Curtius rearrangement of appropriately substituted α-(difluoromethyl)malonic acid hemiester and carboxylic acid derivatives that we developed for the preparation of the corresponding saturated analogues of ornithine and putrescine.[10] Thus, alkylation of the sodium salt of butenyl-substituted malonate (**1**) with chlorodifluoromethane provided the olefinic difluoromethyl malonate **2** in excellent yields. Elaboration of the allylamine chain of the target molecules was accomplished efficiently in two steps: allylic bromination of **2** with *N*-bromosuccinimide under light irradiation in benzene gave, after silica gel column chromatography, the rearranged bromides **3** as the major products, which upon treatment with potassium phthalimide in dimethylformamide were converted to the unsaturated phthalimides **4**. Selective cleavage of the *tert*-butyl ester from **4a** and **4b** with trifluoroacetic acid at 0 °C, followed in the case of **4b** by decarboxylation of the resulting diacid in acetic acid at 25 °C, cleanly provided the key α-(difluoromethyl)-malonic acid hemiester **8** and carboxylic acid **5**. As expected from our previous work,[10] when subjected to the standard Curtius rearrangement sequence, the hemiester **8** and the acid **5** led to the methyl carbamate derivatives **9** and **6**, respectively. Hydrolytic cleavage of the amine and carboxylic acid protecting groups of **9** and **6** under drastic acidic conditions afforded (*E*)-α-(difluoromethyl)dehydroornithine (**10**) and -putrescine (**7**), which were isolated as the hydrochloride and dihydrochloride salts, respectively.

In principle, the (*E*)-α-(fluoromethyl) dehydro analogues of ornithine (**17**) and putrescine (**14**) could be obtained through a similar sequence by replacing the difluoromethylating agent chlorodifluoromethane with a fluoromethylene halide, FCH_2X (X = Cl, Br, or I). However, the lack of convenient access to FCH_2X made this approach unattractive and led us to develop an alternate and more expeditious synthesis for **17** and **14**, which is depicted in Scheme II. Following the work of Bergman et al.,[11] who reported the successful addition of aromatic Grignard reagents to fluoroacetonitrile to give α-fluoroacetophenone derivatives after hydrolysis of the intermediate imines, we found that propenylmagnesium bromide (**11a**) adds to fluoroacetonitrile[12] in tetrahydrofuran at −30 °C. Most interestingly, we discovered that the resulting unstable conjugated imine salt could either be regioselectively reduced in situ to the unsaturated amine **12a** upon addition of a solution of sodium borohydride in water–methanol at low temperature ($t \leq -30$ °C) or would add cyanide in a 1,2 fashion to give directly the unsaturated (fluoromethyl) amino nitrile **15** upon quenching of the reaction mixture with an aqueous solution of sodium cyanide and ammonium chloride. Transformation of **15** and **12a** to (*E*)-α-(fluoromethyl)dehydroornithine (**17**) and (*E*)-α-(fluoromethyl)dehydroputrescine (**14**) was easily carried out via a four-step sequence involving (a) protection of the amine function as its phthalimide; (b) allylic bromination with *N*-bromosuccinimide under light irradiation in the presence of a catalytic amount of benzoyl peroxide; (c) displacement of the allylic bromide with potassium phthalimide; and (d) cleavage of the amine protecting groups and hydrolysis of the cyano group under strong acidic conditions. It is noteworthy that **15** and **12** were obtained as a mixture of *E* and *Z* isomers, the ratio of which appeared to be dependent upon the temperature and the concentration of the reactants. Separation of the geometric isomers was, however, not necessary, since isomerization of the double bond takes place during allylic bromination to give predominantly the (*E*)-allylic bromides **13** and **16**. The assignment of the *E* configuration to the double bond both in the fluoromethyl and difluoromethyl series was made from the analysis of the ^1H NMR spectra on the basis of the signal pattern and the values of the coupling constants of the vicinal vinylic protons. The geometric isomers of the phthalimido derivative of **12b** were separated by flash chromatography on silica gel. At 60 MHz, the *Z* and *E* isomers present clearly distinct signals for the ethylenic protons, with vicinal coupling constants of 11 and 15 Hz, respectively. In addition, the *E* isomer upon treatment with BBr_3 gave a bromide in all respects identical with **13**. At 60 MHz, the signals of the vinylic protons generally are complex overlapping multiplets not amenable to first-order analysis. At higher fields, however, the spectra are greatly simplified, and the measurement of the vicinal coupling constant can usually be achieved in a straightforward manner. For example, at 200 MHz, the ethylenic protons of **14** resonate at 6.12 and 5.98 ppm. Their signals appear as a doublet of doublets of doublets and a doublet of doublets, respectively, from which a vicinal coupling constant of 16 Hz can be measured. Similarly at 400 MHz, the resonance of the ethylenic protons of **17** occurs at 6.05 and 6.02 ppm. Their respective splittings are a doublet and a doublet of triplets, from which a vicinal coupling constant of 16.5 Hz can be measured (see Experimental Section). These values of the vicinal coupling constant are in agreement with a *E* configuration of the double bond.[13] Additional circumstantial evidence for the assignment of the *E* configuration to the double bond in **17** was provided by the stability of the corresponding methyl ester under basic conditions. No lactam formation could be detected. Under similar conditions, the methyl ester of the saturated analogues **18** underwent a rapid cyclization to the corresponding lactam.[14] Such a cyclization reaction would certainly have been favored in the *Z* series.

Enzyme Inhibition. The novel dehydroornithine and dehydroputrescine derivatives (**10**, **17**, **7**, and **14**) were

(10) P. Bey and D. Schirlin, D. *Tetrahedron Lett.*, 5225 (1978).
(11) E. D. Bergman, S. Cohen, E. Hoffman, and Z. Rand-Meir, *J. Chem. Soc.*, 3452 (1961).
(12) Fluoroacetonitrile (bp 79 °C) is conveniently prepared by dehydratation of fluoroacetamide over P_2O_5. Fluoroacetonitrile should be handled with great care under a well-ventilated hood. Its LD_{50} in mice (intraperitoneally injection) is 25 mg/kg. Accumulation of citric acid in heart and other organs is observed, which suggests a blockade of the Krebs cycle at the aconitase step similar to that caused by fluoroacetic acid.
(13) L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, Oxford, 1969, p 301.
(14) P. Bey, J. P. Vevert, V. Van Dorsselaer, and M. Kolb, *J. Org. Chem.*, 44, 2732 (1979).

Table I. Kinetic Constants for the Irreversible Inhibition of Rat Liver ODC by Ornithine and Putrescine Analogues

inhibitors	K_I, μM	τ_{50}, min	$t_{1/2}$ at 10 μM
Ornithine Analogues			
α-CH_3	40	no time-dependent inhibn	
α-CH_3-(*E*)-dehydro (**20**)	2.7	no time-dependent inhibn	
α-CH_2F (**18**)	75	1.6	14
α-CH_2F-(*E*)-dehydro (**17**)	2.7	2.6	3
α-CHF_2 (**19**)	39	3.1	15
α-CHF_2-(*E*)-dehydro (**10**)	30	2.6	10
Putrescine Analogues			
α-CH_2F	56	4.4	29
α-CH_2F-(*E*)-dehydro (**14**)	42	0.2	1
α-CHF_2	30	7.4	29
α-CHF_2-(*E*)-dehydro (**7**)	60	0.7	5

tested as time-dependent irreversible inhibitors of an ODC preparation obtained from livers of thioacetamide-treated rats following the protocol that was used for the corresponding saturated fluoromethyl analogues.[3a,4] For each compound, the minimum experimental kinetic criteria for an enzyme-activated irreversible inhibition[15] of ODC were met. Indeed, incubation of the ODC preparation with **10**, **17**, **7**, or **14** resulted in a rapid, total, and time-dependent loss of enzyme activity. The inactivation process followed pseudo-first-order kinetics for at least two half-lives. The rate of inactivation increased with the concentration of the inhibitor and decreased in the presence of the competitive inhibitor L-2-methylornithine or the substrate L-ornithine. Saturation kinetics were observed. The apparent dissociation constants (K_I) and the times of half-inactivation of ODC at infinite concentration of inhibitors (τ_{50}) listed in Table I were determined by plotting the half-lives of enzyme activity as a function of the reciprocal of the inhibitor concentration according to the method of Kitz and Wilson.[16] The inhibition was irreversible as demonstrated by the lack of any significant recovery of ODC activity after prolonged dialysis of the inactivated enzyme.

As clearly indicated by the half-lives of ODC activity in the presence of 10 μM inhibitor (Table I), the introduction of unsaturation in the chain increases the inhibitory potency of the α-(fluoromethyl)ornithine and -putrescine derivatives. In theory, the double bond in **10**, **17**, **14**, and **7** could replace the fluorine atom substituting the α-methyl group in the role of the latent reactive grouping in the inactivation process. There is precedent for such a mechanism of irreversible inhibition of a pyridoxal phosphate dependent enzyme in the described inactivation of glutamate decarboxylase by its unsaturated substrate analogue (*E*)-2-methyl-3,4-dehydroglutamic acid.[17] This possibility could, however, be ruled out on the basis that (*E*)-2-methyldehydroornithine (**20**) (Table I)[18] and (*E*)-dehydroornithine and (*E*)-dehydroputrescine as reported by Relya and Rando[19] display only a competitive inhibitory component toward ODC.

A comparison of the kinetic constants K_I and τ_{50} of the saturated and unsaturated inhibitors (Table I) shows that the beneficial effect of the double bond on the inactivation process is quite different in the putrescine and the ornithine series. The unsaturation in the putrescine derivatives dramatically increases the rate of inactivation but has little influence on the apparent affinity of the inhibitors. In the amino acid series, (*E*)-2-(fluoromethyl)-dehydroornithine (**17**) has a K_I 30 times lower than that of 2-(fluoromethyl)ornithine (**18**), while the τ_{50} of **17** and **18** remains within the same range. The increase of affinity provided by the unsaturation in **17** is in agreement with the findings that (*E*)-dehydroornithine[19] and (*E*)-2-methyldehydroornithine (Table I) bind ODC 60 and 15 times more tightly than the corresponding saturated analogues. It should be noted, however, that in the case of 2-(difluoromethyl)ornithine (**19**), the unsaturation only moderately increases the affinity for ODC.[20]

Conclusion

In summary, the introduction of an *E* double bond in the chain of α-(fluoromethyl)putrescine and α-(fluoromethyl)ornithine, two enzyme-activated irreversible inhibitors of ODC, considerably enhances their inhibitory potencies. These new ODC inhibitors and their derivatives obviously have high potential for depleting polyamines in biological systems. Moreover, the new and efficient process reported for the preparation of **14** and **17** from fluorinated acetonitrile clearly offers possibilities of extension to the synthesis of other fluorinated amines and amino acids of pharmacological and therapeutic interests.[3b]

Experimental Section

Melting points were determined on a Mettler FP 5 or a Kofler hot bank melting point apparatus and are uncorrected, as are boiling points. Microanalyses were conducted on a Perkin-Elmer 240 CHN analyzer. The IR, NMR, and UV data were consistent with the assigned structure. Unless otherwise stated, the ^1H NMR spectra were recorded at 60 MHz on a Varian Associates Model T60 spectrometer. The 200- and 400-MHz NMR spectra were recorded on a Brucker WP 200 and a Brucker WH 400 spectrometer, respectively. The NMR spectra are reported in parts per million from internal tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonate in the δ scale. Data are presented as follows: solvent, chemical shift, integration, multiplicity (s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet), interpretation, and coupling constants.

Caution: All experiments with fluoroacetonitrile should be carried out under a well-ventilated hood!

***tert*-Butyl 2-(Ethoxycarbonyl)-4-pentenoate (1a).** To a suspension of sodium hydride (0.16 mol, 6.98 g of a 55% dispersion in oil washed three times with tetrahydrofuran) in tetrahydrofuran

(15) R. H. Abeles and A. Maycock, *Acc. Chem. Res.*, **9**, 313 (1976).
(16) R. Kitz and I. B. Wilson, *J. Biol. Chem.*, **237**, 3245 (1962).
(17) E. Chrystal, P. Bey, and R. R. Rando, *J. Neurochem.*, **32**, 1501 (1979).
(18) (*E*)-2-Methyldehydroornithine (**20**) was synthesized from methyl 2-(benzylidenamino)-2-methyl-4-pentenoate (**21**) [P. Bey and J. P. Vevert, *Tetrahedron Lett.*, 1455 (1977)]. Hydrolytic cleavage of the Schiff base of **21**, followed by trifluoroacetylation of the resulting amino ester, afforded methyl 2-(trifluoroacetamido)-2-methyl-4-pentenoate, which was converted to **20** through a sequence similar to that of Scheme I.
(19) N. Relya and R. R. Rando, *Biochem. Biophys. Res. Commun.*, **67**, 392 (1975).
(20) The first steps in the enzymatic decarboxylation are the formation of a Schiff base between the α-amino group of ornithine and the aldehyde function of pyridoxal phosphate. It is therefore conceivable that the affinity of the inhibitors for ODC depends also upon the nucleophilicity of the α-amino group. The pK_a of the α-amino group decreases sharply as the number of fluorine atom substituting the α-methyl group increases. In the ornithine series, the pK_a drops by 1.3 units for the fluoromethyl derivatives and by 2.6 units for the difluoromethyl derivatives as compared with the value of 2-methylornithine. The β,γ-unsaturation decreases further the pK_a of the α-amino group by 0.5 unit.
(21) B. Miller and M. R. Saidi, *J. Am. Chem. Soc.*, **98**, 2227 (1976).
(22) P. Caubere, *Bull. Soc. Chim. Fr.*, 148 (1964).

(200 mL) was added *tert*-butyl ethyl malonate (30.08 g, 0.16 mol). After the mixture was stirred for 1 h at room temperature, a solution of allyl bromide (19.36 g, 0.16 mol) in tetrahydrofuran (120 mL) was rapidly added. Stirring was continued for 30 min. The mixture was then quenched with brine and extracted three times with diethyl ether (200 mL). The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was distilled to give 18.6 g of **1a** (50%), bp 50–52 °C (0.15 mm).

***tert*-Butyl 2-(*tert*-butyloxycarbonyl)-4-pentenoate (1b)** was obtained in 75% yield in a manner similar to that described for **1a**. Di-*tert*-butyl malonate was used in place of *tert*-butyl ethyl malonate, bp 46–47 °C (0.03 mm).

***tert*-Butyl 2-(Ethoxycarbonyl)-2-(difluoromethyl)-4-pentenoate (2a).** To a suspension of sodium hydride (0.08 mol, 3.49 g of a 55% dispersion in oil washed three times with tetrahydrofuran) in tetrahydrofuran (90 mL) was added, at room temperature under nitrogen, a solution of **1a** (18.4 g, 0.08 mol). After the mixture was stirred for 1 h, a stream of chlorodifluoromethane was rapidly bubbled through the carbanion solution maintained at 45 °C. Stirring was continued overnight at room temperature, and the mixture was quenched with brine and extracted with diether ether (3 × 150 mL). The organic layers were dried over $MgSO_4$ and concentrated in vacuo to give 20.78 g of crude **2a**, which was purified by chromatography on silica gel (eluant diethyl ether/petroleum ether, 5:95) (75%): bp 43–44 °C (0.06 mm); NMR (CCl_4) δ 6.16 (1 H, t, CHF_2, J = 53 Hz).

***tert*-Butyl 2-(*tert*-butyloxycarbonyl)-2-(difluoromethyl)-4-pentenoate (2b)** was prepared from **1b** in 77% yield in a manner similar to that described for **2a**: bp 48–49 °C (0.07 mm); NMR (CCl_4) 6.05 (1 H, t, CHF_2, J = 53 Hz).

***tert*-Butyl (*E*)-2-(Ethoxycarbonyl)-2-(difluoromethyl)-5-bromo-3-pentenoate (3a).** A solution of **2a** (0.0456 mol, 12.4 g), *N*-bromosuccinimide (0.0456 mol, 7.93 g), and benzoyl peroxide (30 mg) in benzene (240 mL) was heated under reflux temperature for 0.5 h. Then, 3 equiv of *N*-bromosuccinimide was added in three 1-equiv portions at 0.5-h intervals. Heating was continued for 0.5 h after the last addition, and the reaction mixture was concentrated under reduced pressure. The residue was taken up in CCl_4 (250 mL). The insoluble succinimide was filtered, and the filtrate was concentrated. The residue was purified by chromatography on silica gel. Diethyl ether/petroleum ether (2:98) eluted first 9 g of a mixture of starting material and bromo compound and then 5 g of pure **3a** (30%) as an oil: NMR (CCl_4) δ 1.3 (3 H, t, CH_3, J = 7 Hz), 1.5 [9 H, s, $C(CH_3)_3$], 3.9–4.1 (2 H, m, CH_2Br), 4.2 (2 H, q, OCH_2, J = 7 Hz), 5.86–6.15 (2 H, m, HC=CH), 6.12 (1 H, t, CHF_2, J = 54 Hz).

***tert*-Butyl (*E*)-2-(*tert*-butyloxycarbonyl)-2-(difluoromethyl)-5-bromo-3-pentenoate (3b)** was prepared from **2b** in 35% yield in a manner similar to that described for **3a**: mp 54 °C (from pentane at −78 °C); NMR ($CDCl_3$) δ 1.5 [18 H, s, $C(CH_3)_3$], 3.93–4.03 (2 H, m, CH_2Br), 5.93–6.06 (2 H, m, HC=CH), 6.16 (1 H, t, CHF_2, J = 54 Hz). Anal. ($C_{15}H_{23}BrF_2O_4$) C, H, N.

***tert*-Butyl (*E*)-2-(Ethoxycarbonyl)-2-(difluoromethyl)-5-phthalimido-3-pentenoate (4a).** A mixture of potassium phthalimide (3.88 g, 0.021 mol) and **3a** (5 g, 0.014 mol) in dimethylformamide (140 mL) was heated at 80 °C for 2 h. The reaction mixture was then concentrated in vacuo. The residue was extracted with CH_2Cl_2. The organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was taken up in benzene. The insoluble material was filtered. The solid obtained after concentration in vacuo of the filtrate was crystallized from diethyl ether/pentane to give 3.2 g of **4a** (50%): mp 79–80 °C; NMR ($CDCl_3$) δ 1.23 (3 H, t, CH_3, J = 7 Hz), 1.47 [9 H, s, $C(CH_3)_3$], 4.0–4.43 (4 H, m, CH_2N and CH_2O), 5.83–6.23 (2 H, m, HC=CH), 6.12 (1 H, t, CHF_2, J = 54 Hz), 7.5–8.0 (4 H, m, H aromatic). Anal. ($C_{21}H_{23}F_2NO_6$) C, H, N.

***tert*-Butyl (*E*)-2-(*tert*-butyloxycarbonyl)-2-(difluoromethyl)-5-phthalimido-3-pentenoate (4b)** was prepared from **3b** in 65% yield in a manner similar to that described for **4a**: mp 96 °C (diethyl ether/pentane); NMR ($CDCl_3$) 1.47 [18 H, s, $C(CH_3)_3$], 4.4 (2 H, d, CH_2N, J = 4.5 Hz), 5.8–6.3 (2 H, m, HC=CH), 6.16 (1 H, t, CHF_2, J = 54 Hz), 7.65–8.0 (4 H, m, H aromatic). Anal. ($C_{23}H_{27}F_2NO_6$) C, H, N.

Ethyl (*E*)-2-(Difluoromethyl)-2-[(methoxycarbonyl)amino]-5-phthalimido-3-pentenoate (9a). A solution of **4a** (2.73 g, 0.0065 mol) in trifluoroacetic acid (10 mL) was stirred at 25 °C for 2 h. Concentration in vacuo gave crude **8** (R_1 = Et) as an oil, which was dissolved in thionyl chloride (20 mL). The solution was heated at reflux temperature for 2 h. The excess thionyl chloride was evaporated in vacuo to give the corresponding acid chloride, which was dissolved in acetone (25 mL). The solution was cooled to 0 °C, and sodium azide (0.42 g, 0.00645 mol) in water (2.5 mL) was added dropwise. After stirring for 1 h at 20 °C, the reaction mixture was extracted with ether (3 × 30 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, and concentrated in vacuo at 20 °C. The crude acyl azide (1.6 g) was dissolved in anhydrous methanol (30 mL), and the solution was heated at reflux temperature for 12 h. The residue obtained after concentration in vacuo was purified by chromatography on silica gel. Elution with ethyl acetate/chloroform (4:96) afforded 1.24 g of **9a** (60%) as an oil: NMR ($CDCl_3$) δ 1.23 (3 H, t, CH_3, J = 7 Hz), 3.6 (3 H, s, OCH_3), 4.03–4.4 (4 H, m, CH_2N and CH_2O), 5.56 (1 H, br s, NH), 5.83–6.1 (2 H, m, HC=CH), 6.17 (1 H, t, CHF_2, J = 54 Hz), 7.46–7.83 (4 H, m, H aromatic).

(*E*)-2-(Difluoromethyl)dehydroornithine Hydrochloride (10). A mixture of **9a** (1.2 g), acetic acid (10 mL), and concentrated hydrochloric acid (30 mL) was heated at reflux temperature for 42 h. After concentration in vacuo, the residue was taken up with water (10 mL). The insoluble phthalic acid was filtered. The filtrate was treated with charcoal and concentrated in vacuo. The residue was dissolved in ethanol (10 mL). The solid which slowly precipitated upon addition of an excess of propylene oxide was crystallized from water–ethanol to afford 0.39 g (40%) of **10**: mp 151 °C; NMR (D_2O) δ 3.66–3.80 (2 H, m, CH_2N), 6.1–6.5 (2 H, m, HC=CH), 6.46 (1 H, t, CHF_2); 250-MHz NMR (D_2O) δ 6.11 (d, C=CHC, J_{HH} = 16.5 Hz). Anal. ($C_6H_{10}F_2N_2O_2$·HCl) C, H, N.

(*E*)-2-(Difluoromethyl)-5-phthalimido-3-pentenoic Acid (5). A solution of **4b** (1 g) in trifluoroacetic acid (10 mL) was stirred at 0 °C for 2 h and then at 20 °C for 15 min. The excess trifluoroacetic was evaporated under reduced pressure. The residue was dissolved in glacial acetic acid (30 mL). The solution was stirred at 20 °C for 12 h. The solid obtained after concentration in vacuo at room temperature was crystallized from chloroform/pentane to give 0.5 g (75%) of **5**: mp 154 °C; NMR ($CDCl_3$) δ 3.1–3.9 (1 H, m, CH), 4.2–4.5 (2 H, m, CH_2N), 5.7–6.1 (2 H, m, HC=CH), 6.0 (1 H, d of t, CHF_2, J_{HH} = 5 Hz, J_{HF} = 54 Hz), 7.6–8.0 (4 H, m, H aromatic). Anal. ($C_{14}H_{11}F_2NO_4$) C, H, N.

(*E*)-1,1-Difluoro-2-[(methoxycarbonyl)amino]-5-phthalimido-3-pentene (6) was prepared from **5** (in 32% yield) in a manner similar to that described for the synthesis of **9a** from **8**: mp 167 °C (CH_2Cl_2/pentane); NMR ($CDCl_3$) δ 3.66 (3 H, s, OCH_3), 4.2–4.4 (2 H, m, CH_2N), 4.4–6.7 (4 H, complex m, CHF_2, HC=CH and CHNH), 7.8–8.0 (4 H, m, H aromatic). Anal. ($C_{15}H_{14}F_2N_2O_4$) C, H, N.

(*E*)-2-(Difluoromethyl)dehydroputrescine Dihydrochloride (7). A mixture of **6** (0.38 g) and concentrated hydrochloric acid (20 mL) was heated at reflux temperature for 48 h. After concentration in vacuo, the residue was taken up in water (5 mL). The insoluble phthalic acid was filtered. The filtrate was treated with charcoal and concentrated in vacuo. The residue was crystallized 3 times in a mixture of ethanol, acetone, and diethyl ether to give 0.15 g (62%) of **7**: mp 186 °C; NMR (D_2O) δ 3.66 (2 H, d, CH_2N, J = 5 Hz), 5.9–6.4 (2 H, m, HC=CH), 6.14 (1 H, d of t, CHF_2, J_{HF} = 53 Hz, J_{HH} = 2.5 Hz). Anal. ($C_5H_{10}F_2N_2$·2HCl) C, H, N.

2-Amino-2-(fluoromethyl)-3-pentenenitrile (15). Propenylmagnesium bromide was prepared from magnesium turnings (9.72 g, 0.40 mol) and 1-bromo-1-propene (24.2 g, 0.20 mol) in anhydrous THF (200 mL) under an atmosphere of nitrogen. After removal of the magnesium in excess by filtration on glass wool, the Grignard solution was cooled to −40 °C. A solution of fluoroacetonitrile (10.62 g, 0.18 mol) in THF (50 mL) was then added slowly at such a rate as to maintain the temperature between −40 and −30 °C. Stirring was continued for 30 min at −30 °C, and then the reaction mixture was poured into a solution of sodium cyanide (19.6 g, 0.40 mol) and ammonium chloride (32 g, 0.60 mol) in water (200 mL) and ice (200 g). After stirring at room temperature for 1 h, the reaction mixture was saturated with sodium chloride. The THF layer was separated, and the aqueous phase

was extracted with diethyl ether. The organic phases were pooled and evaporated. The residue was dissolved in ether and extracted with 1 N HCl (250 mL). The aqueous phase was made alkaline with concentrated ammonia and reextracted with diethyl ether. The etheral layer was dried over $MgSO_4$ and concentrated in vacuo to give **15** as an oil (14.2 g) (64%).

2-(Fluoromethyl)-2-phthalimido-3-pentenenitrile. To a solution of **15** (14.34 g, 0.116 mol) and triethylamine (23.2 g, 0.232 mol) in anhydrous dichloromethane (100 mL), cooled in an ice-bath, was added slowly a solution of phthaloyl dichloride (23.5 g, 0.116 mol) in CH_2Cl_2 (50 mL). After stirring at room temperature overnight, the mixture was heated under reflux temperature for 3 h and washed 3 times with 1 N HCl and then with water. The organic layer was dried over $MgSO_4$, treated with charcoal, and then filtered on Celite covered with a layer of silica gel. Evaporation of the slightly yellow filtrate gave the expected phthalimide derivative as an oil (24 g, 80%).

(*E*)-2-(Fluoromethyl)-2-phthalimido-5-bromo-3-pentenenitrile (16). A mixture of crude 2-(fluoromethyl)-2-phthalimido-3-pentenenitrile (18.7 g, 72 mmol), NBS (13 g, 73 mmol), and a catalytic amount of benzoyl peroxide in anhydrous carbon tetrachloride was irradiated and heated under reflux by means of a 375-W lamp for 2.5 h. The succinimide that precipitated upon cooling was removed by filtration. The filtrate was washed with water, dried over $MgSO_4$, and evaporated. On standing overnight, the residue crystallized. Digestion with ether gave **16** as white crystals (14 g, 56%): mp 90 °C; NMR ($CDCl_3$) δ 4.03 (2 H, d, CH_2Br, J_{HH} = 6.5 Hz), 4.98 (1 H, d of d, CHHF, J_{HH} = 9.5 Hz, J_{HF} = 46 Hz), 5.35 (1 H, d of d, CHHF, J_{HH} = 9.5 Hz, J_{HF} = 46 Hz), 6.16 (1 H, d, C=CHC, J_{HH} = 15 Hz), 6.54 (1 H, d of t, CH_2CH=C, J_{HH} = 15 Hz, J_{HH} = 6.5 Hz), 7.9 (4 H, m, H aromatic).

(*E*)-2-(Fluoromethyl)-2,5-diphthalimido-3-pentenenitrile. A mixture of **16** (14 g, 41.5 mmol), potassium phthalimide (7.7 g, 41.5 mmol), and anhydrous DMF (50 mL) was stirred at 80 °C for 5 h. The solvent was removed under vacuum, and the residue was taken up with chloroform. The insoluble salts were filtered, and the filtrate was washed with 1 N NaOH and then 1 N HCl, dried over $MgSO_4$, and treated with charcoal. Filtration on Celite and evaporation gave a residue that crystallized on standing. Digestion with ether afforded the expected diphthalimido derivative as white crystals (14.6 g, 87%): mp 175 °C; NMR (Me_2SO-d_6) δ 4.4 (2 H, d, CH_2N, J = 4 Hz), 5.08 (1 H, d of d, CHHF, J_{HF} = 46 Hz, J_{HH} = 9 Hz), 5.50 (1 H, d of d, CHHF, J_{HF} = 46 Hz, J_{HH} = 9 Hz), 6.14 (1 H, d, C=CHC, J_{HH} = 15 Hz), 6.33 (1 H, d of t, CH=C, J_{HH} = 15 Hz, J_{HH} = 4 Hz), 8.0 (8 H, m, H aromatic). Anal. ($C_{22}H_{14}FN_3O_4$) C, H, N.

(*E*)-2-(Fluoromethyl)dehydroornithine Hydrochloride (17). (*E*)-2-(Fluoromethyl)-2,5-diphthalimido-3-pentenenitrile (14 g, 35 mmol) was heated with concentrated HCl under reflux temperature for 42 h. After cooling, phthalic acid was removed by filtration, and the filtrate was evaporated. The residue was dissolved in water, and the insoluble material was removed by filtration. The filtrate was extracted twice with ether and then concentrated in vacuo. The residue was dissolved in ethanol. The insoluble ammonium chloride was filtered, and crude **6** was precipitated by the addition of propylene oxide (6 g). After 2 h at 5 °C, the precipitate (5.2 g) was collected and redissolved in water (10 mL). Upon addition of ethanol (30 mL), **17** crystallized (4.0 g) (58%): mp 184 °C; 400-MHz NMR (D_2O) δ 3.70 (2 H, d, CH_AH_BN, $J_{H_AH_F} = J_{H_BH_F}$ = 5 Hz), 4.73 (1 H, d of d, CH_CH_DF, $J_{H_CH_D}$ = 10 Hz, J_{H_CF} = 46.5 Hz), 4.89 (1 H, d of d, CH_CH_DF, $J_{H_CH_D}$ = 10 Hz, J_{H_DF} = 45 Hz), 6.02 (1 H, d, C=CH_EC, $J_{H_EH_F}$ = 16.5 Hz), 6.05 (1 H, d of t, $CH_AH_BCH_F$=C, $J_{H_FH_E}$ = 16.5 Hz, $J_{H_FH_B} = J_{H_FH_A}$ = 5 Hz). Anal. ($C_6H_{12}FN_2O_2$·HCl) C, H, N.

1-Fluoro-2-amino-3-pentene (12a). Under an atmosphere of nitrogen, propenylmagnesium bromide was prepared from magnesium turnings (9.72 g or 0.4 mol) and freshly distilled 1-bromo-1-propene (cis/trans mixture, 24.2 g, 0.20 mol) in anhydrous THF (180 mL). The colored solution was separated from the excess of magnesium and cooled to −30 °C. Fluoroacetonitrile (11.8 g, 0.20 mol) in THF (50 mL) was added dropwise over a period of 20 min. The reaction mixture was kept at −30 °C for an additional 20 min and then cooled to −50 °C, whereupon a suspension of sodium borohydride (7.6 g, 0.2 mol) in methanol (400 mL) and water (8 mL) cooled to −50 °C was added all at once. The temperature rose to −10 °C. After the mixture was cooled to −30 °C, the temperature was allowed to rise to 0 °C over a period of 1.5 h. The mixture was acidified with 6 N HCl and evaporated. The residue was taken up in water. The aqueous solution was extracted twice with ether and then made alkaline with 4 N NaOH and extracted again twice with ether. After drying over Na_2SO_4 and filtering, anhydrous HCl was bubbled through the etheral solution. The oily precipitate (12 g, 43%) was dissolved in water. The solution was filtered and the filtrate was saturated with sodium chloride, made alkaline with 4 N NaOH, and extracted twice with small portions of ether. The organic layers were pooled and concentrated under atmospheric pressure. The black oily residue was distilled under reduced pressure (15 mm) to give a colorless liquid (4.4 g, bp 60–100 °C), which was distilled again under atmospheric pressure to afford **12a** (cis/trans mixture, 2.8 g, 13%) as a colorless oil, bp 110–118 °C.

1-Fluoro-2-phthalimido-3-pentene was prepared from **12a** in 84% yield in a manner similar to that described for the synthesis of 2-(fluoromethyl)-2-phthalimido-3-pentenenitrile, except that *N*-carbethoxyphthalimide was used in place of phthaloyl dichloride: NMR ($CDCl_3$) (cis/trans mixture) δ 1.63 and 1.70 (3 H, 2 d, CH_3, J_{HH} = 6 Hz and J_{HH} = 7 Hz), 3.90 to 5.57 (3 H, complex m, CH and CH_2F), 5.70 (2 H, m, CH=CH), 7.57 (4 H, m, H aromatic).

(*E*)-1-Fluoro-2-phthalimido-5-bromo-3-pentene (13) was prepared from 1-fluoro-2-phthalimido-3-pentene in 90% yield in a manner similar to that described for the synthesis of **16**: mp 70–72 °C (CH_3OH); NMR ($CDCl_3$) δ 3.87 (2 H, m, CH_2Br), 4.07–5.5 (3 H, complex m, CH and CH_2F), 6.03 (2 H, m, CH=CH), 7.70 (4 H, m, H aromatic).

(*E*)-1-Fluoro-2,5-diphthalimido-3-pentene was prepared from **13** in 61% yield in a manner similar to that described for the synthesis of (*E*)-1-(fluoromethyl)-2,5-diphthalimido-3-pentenenitrile: mp 160 °C ($CHCl_3/Et_2O$); NMR ($CDCl_3$) δ 4.28 (2 H, m, CH_2N), 4.13–5.5 (3 H, complex m, CH and CH_2F), 6.00 (2 H, m, CH=CH), 7.72 (8 H, m, H aromatic). Anal. ($C_{21}H_{15}N_2O_4F$) C, H, N.

(*E*)-2-(Fluoromethyl)dehydroputrescine Dihydrochloride (14). A suspension of (*E*)-1-fluoro-2,5-diphthalimido-3-pentene (10.5 g, 27.7 mmol) in concentrated HCl (250 mL) and acetic acid (100 mL) was heated at reflux temperature for 24 h. The residue obtained after concentration in vacuo was taken up in water. The insoluble phthalic acid was filtered. The filtrate was treated with charcoal and concentrated and the residue was crystallized from methanol–acetone to afford **14** (4.2 g, 80%): mp 176 °C; 200-MHz NMR (D_2O) δ 3.71 (2 H, d, $CH_AH_BNH_2$, $J_{H_AH_C} = J_{H_BH_C}$ = 6 Hz), 4.35 (1 H, d of m, CH_CNH_2), 4.69 (1 H, d of d of d, CH_DH_EF, $J_{H_DH_C}$ = 6 Hz, $J_{H_DH_E}$ = 10.5 Hz, J_{H_DF} = 46.5 Hz), 4.79 (1 H, d of d of d, CH_DH_EF, $J_{H_EH_C}$ = 3.5 Hz, $J_{H_EH_D}$ = 10.5 Hz, J_{H_EF} = 45 Hz), 5.98 (1 H, d of d, C=$CH_F$$CH_C$N, $J_{H_FH_C}$ = 16 Hz, $J_{H_FH_C}$ = 7 Hz), 6.12 (1 H, d of t, CH_G=C, $J_{H_GH_F}$ = 16 Hz, $J_{H_GH_A} = J_{H_GH_B}$ = 6 Hz). Anal. ($C_5H_{11}N_2F$·2HCl) C, H, N.

1-Fluoro-2-amino-5-ethoxy-3-pentene (12b) was prepared from 1-bromo-3-ethoxy-1-propene (bp 144 °C)[21,22] in 17% yield in a manner similar to that described for the synthesis of **12a**.

(*E*)- and (*Z*)-1-Fluoro-2-phthalimido-5-ethoxy-3-pentene were prepared from **12b** in 45% overall yield in the same manner as the one described for the synthesis of 1-fluoro-2-phthalimido-3-pentene. The *Z* and *E* isomers were separated by flash chromatography on silica gel (ethyl acetate/petroleum ether, 15:85). The *Z* isomer was eluted first: NMR ($CDCl_3$) of the *Z* isomer δ 1.17 (3 H, t, J_{HH} = 7 Hz, OCH_2CH_3), 3.47 (2 H, q, J_{HH} = 7 Hz, OCH_2CH_3), 4.08 (2 H, m, CH_2O), 4.22–5.65 (3 H, complex m, CH and CH_2F), 5.83 (2 H, m, HC=CH, J_{HH} = 11 Hz), 7.62 (4 H, m, H aromatic); IR (film) absence of CH out-of-plane deformation near 970–960 cm^{-1}; NMR of the *E* isomer ($CDCl_3$) δ 1.16 (3 H, t, J_{HH} = 7 Hz, OCH_2CH_3), 3.43 (2 H, q, OCH_2CH_3, J_{HH} = 7 Hz), 3.92 (2 H, m, OCH_2), 4.22–5.52 (3 H, complex m, CH and CH_2F), 5.92 (2 H, m, CH=CH, J_{HH} = 15 Hz); IR (film) 975 cm^{-1}.

Preparation of Compound 13 from (*E*)-1-Fluoro-2-phthalimido-5-ethoxy-3-pentene. Boron tribromide (106 mg, 0.42 mmol) in anhydrous methylene chloride (5 mL) was added slowly to a solution of (*E*)-1-fluoro-2-phthalimido-5-ethoxy-3-pentene (320 mg, 1.15 mmol) in anhydrous methylene chloride (10 mL) cooled to −78 °C. The temperature was allowed to rise to room temperature overnight, and concentration under vacuum

afforded a solid (345 mg, 95%) whose analytical data were identical with that of **13**.

Ornithine Decarboxylase Preparation. Rat liver ODC prepared from the livers of rats that had been injected with thioacetamide (150 mg/kg of body weight) 18 h before sacrifice was purified about 10-fold by acid treatment at pH 4.6 as described by Ono et al.[23] The specific activity of the preparation was 0.2 nmol of CO_2 min^{-1} (mg of protein)$^{-1}$.

Assay of Time-Dependent Inhibition of Ornithine Decarboxylase. Assay and measurement of the kinetic constants of the inhibition were performed as described previously.[3a] Kinetic constants were calculated by the method of Kitz and Wilson[16] by using a least-squares fit of the data points with a Hewlett-Packard 9820 calculator.

Acknowledgment. We are indebted to K. Jund, A. Czermak, and M. C. Chanal for valuable technical assistance. We thank also Dr. J. Wagner and E. Wolf for the measurement of the pK_a values of the putrescine and ornithine derivatives.

(23) M. Ono, H. Inoue, F. Suzuki, and Y. Takeda, *Biochim. Biophys. Acta*, **284**, 285 (1972).

Registry No. **1a**, 82006-42-6; **1b**, 62031-25-8; **2a**, 82031-21-8; **2b**, 86921-74-6; **3a**, 82006-43-7; **3b**, 86921-75-7; **4a**, 82006-44-8; **4b**, 82006-77-7; **5**, 82006-78-8; **6**, 86921-71-3; **7**, 86921-79-1; **7**·2HCl, 82006-79-9; **8**, 82006-45-9; **9a**, 82006-48-2; **10**, 82134-42-7; **10**·HCl, 82006-86-8; (*Z*)-**12a**, 82006-49-3; (*E*)-**12a**, 82006-50-6; (*E*)-**12b**, 82006-62-0; (*Z*)-**12b**, 82006-61-9; **13**, 82006-54-0; **14**, 82006-58-4; **14**·2HCl, 82006-56-2; **15**, 86921-72-4; **16**, 86921-73-5; **17**, 82006-84-6; **17**·HCl, 82006-71-1; *tert*-butyl ethyl malonate, 32864-38-3; allyl bromide, 106-95-6; di-*tert*-butyl malonate, 541-16-2; chlorodifluoromethane, 75-45-6; *N*-bromosuccinimide, 2439-85-2; potassium phthalimide, 1074-82-4; (*E*)-2-(difluoromethyl)-2-(ethoxycarbonyl)-5-phthalimido-3-pentenoyl chloride, 82006-46-0; ethyl (*E*)-2-(difluoromethyl)-2-(azidocarbonyl)-5-phthalimido-3-pentenoate, 82006-47-1; propenylmagnesium bromide, 14092-04-7; fluoroacetonitrile, 503-20-8; 2-(fluoromethyl)-2-phthalimido-3-pentenenitrile, 86921-76-8; phthaloyl dichloride, 88-95-9; (*E*)-2-(fluoromethyl)-2,5-diphathalimido-3-pentenenitrile, 86921-77-9; (*Z*)-1-fluoro-2-phthalimido-3-pentene, 82006-52-8; (*E*)-1-fluoro-2-phthalimido-3-pentene, 82006-53-9; *N*-carbethoxyphthalimide, 22509-74-6; (*E*)-1-fluoro-2,5-diphthalimido-3-pentene, 82006-55-1; 1-bromo-3-ethoxy-1-propene, 86921-78-0; (*E*)-1-fluoro-2-phthalimido-5-ethoxy-3-pentene, 82006-66-4; (*Z*)-1-fluoro-2-phthalimido-5-ethoxy-3-pentene, 82006-65-3; ornithine decarboxylase, 9024-60-6.

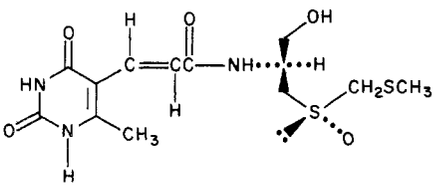
Synthesis and Biological Evaluation of Sparsomycin Analogues

Scherer S. Duke and Marvin R. Boots*

Department of Pharmaceutical Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298. *Received November 3, 1982*

Three series of sparsomycin analogues were prepared and examined for their ability to inhibit DNA or protein synthesis in bone marrow, P388 lymphocytic leukemia, and P815 mastocytoma cells. The compounds of series I and II, distinguished by the inclusion or exclusion of a hydroxymethyl functional group, were designed to elucidate the effect on activity of replacing the oxodithioacetal side chain of sparsomycin with 4-substituted benzyl groups. The series III analogues, which excluded the hydroxymethyl group and replaced the oxodithioacetal moiety of sparsomycin with a benzyl amide group, were designed to investigate the potential interaction of an amide oxygen in contrast to the sulfoxide oxygen of sparsomycin. Overall, the bromobenzyl-substituted analogues imparted the greatest inhibitory activity in the protein synthesis assay, while the methoxybenzyl-substituted analogues displayed the least. The methylbenzyl and the unsubstituted benzyl compounds were intermediate in inhibitory potential. The activity in the protein synthesis assay may correspond to the lipophilic and electronic characteristics of the substituents on the benzyl moiety of the analogues. All of the compounds were inactive in the DNA synthesis assay.

In 1962, Owen, Dietz, and Camiener reported the isolation of a novel antibiotic with antitumor, antifungal, and antibacterial activity from the culture filtrate of *Streptomyces sparsogenes*.[1] The structure of the crystalline antibiotic, named sparsomycin, remained elusive until 1970, when Wiley and MacKellar reported results of spectroscopic and degradation studies that elucidated the structure (**1**).[2,3] The molecule featured a trans olefin bond,



1 (sparsomycin)

a chiral carbon atom with an *S* configuration, and a chiral sulfur atom with a *R* configuration.[4] Due to the presence of the synthetically complex oxodithioacetal side chain, the first total synthesis of sparsomycin was not reported until 1979.[5,6] Since its discovery, the compound has attracted attention not only for its unique structural characteristics and challenging synthesis, but also for the antitumor, antibacterial, antifungal, and antiviral properties attributed to sparsomycin's ability to inhibit protein synthesis at the ribosomal level.[1,7–12] The most promising biological activity of sparsomycin was its antitumor ac-

(1) Owen, S. P.; Dietz, A.; Camiener, G. W. *Antimicrob. Agents Chemother.* **1962**, 772.
(2) Wiley, P. F.; MacKellar, F. A. *J. Org. Chem.* **1976**, *41*, 1858.
(3) Wiley, P. F.; MacKellar, F. A. *J. Am. Chem. Soc.* **1970**, *92*, 417.
(4) Ottenheijm, H. C. J.; Liskamp, R. M. J.; Helquist, P.; Lauher, J. W.; Shekhani, M. S. *J. Am. Chem. Soc.* **1981**, *103*, 1720.
(5) (a) Ottenheijm, H. C. J.; Liskamp, R. M. J.; Tijhuis, M. W. *Tetrahedron Lett.* **1979**, 387. (b) Ottenheijm, H. C. J.; Liskamp, R. M. J.; vanNispen, S. P. J. M.; Boots, H. A.; Tijhuis, M. W. *J. Org. Chem.* **1981**, *46*, 3273.
(6) Helquist, P. *J. Am. Chem. Soc.* **1979**, *101*, 1057.
(7) Argoudelis, A. D.; Herr, R. R. *Antimicrob. Agents Chemother.* **1962**, 780.
(8) Slechta, L. *Antimicrob. Agents Chemother.* **1965**, 326.
(9) Thiry, L. *J. Gen. Virol.* **1968**, *2*, 143.
(10) Bannister, E. R.; Hunt, D. E.; Pittillo, R. F. *Can. J. Microbiol.* **1966**, *12*, 595.
(11) Slechta, L. In "Antibiotics: Mechanism of Action"; Gottlieb, D.; Shaw, P. D., Eds.; Springer-Verlag: New York, 1967; Vol. I, p 410.
(12) Bhuyan, B. K.; Fraser, T. J. *Exp. Cell Res.* **1974**, *83*, 103.

0022-2623/83/1826-1556$01.50/0