J. Med. Chem. 1991, 34, 1447–1454

Synthesis and in Vitro Biological Activity of New Deaza Analogues of Folic Acid, Aminopterin, and Methotrexate with an L-Ornithine Side Chain[1]

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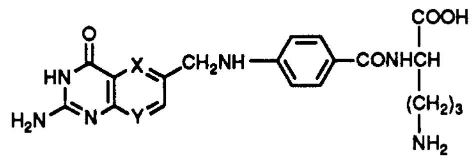
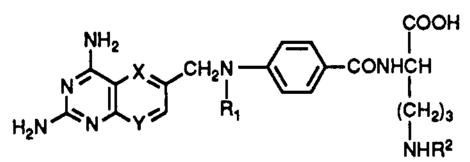
Received July 26, 1990

The 5-deaza and 5,8-dideaza analogues of N^{α}-pteroyl-L-ornithine (Pter-Orn), the 5-deaza, 8-deaza, and 5,8-dideaza analogues of N^{α}-(4-amino-4-deoxypteroyl)-L-ornithine (APA-Orn), and the N^{δ}-carboxymethyl derivative of N^{α}-(4-amino-4-deoxy-N^{10}-methylpteroyl)-L-ornithine (mAPA-Orn) were synthesized and tested as inhibitors of dihydrofolate reductase (DHFR) and as inhibitors of tumor cell growth in culture. Reductive amination of 2-acetamido-6-formylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one with methyl N^{α}-(4-aminobenzoyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate followed by removal of the blocking groups afforded the 5-deaza analogue of Pter-Orn, whereas N-alkylation of methyl N^{α}-(4-aminobenzoyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate with 2-amino-6-(bromomethyl)quinazolin-4(3*H*)-one and deprotection gave the corresponding 5,8-dideaza analogue. Reductive coupling of 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carbonitrile and 4-aminobenzoic acid followed by reaction with 95–97% formic acid yielded 4-amino-4-deoxy-5-deaza-N^{10}-formylpteroic acid, which on condensation with methyl N^{δ}-(benzyloxycarbonyl)-L-ornithinate and deprotection gave the 5-deaza analogue of APA-Orn. A similar sequence starting from 2,4-diaminoquinazoline-6-carbonitrile led to the corresponding 5,8-dideaza compound, whereas treatment of 2,4-diaminopyrido[3,2-*d*]pyrimidine-6-methanol with phosphorus tribromide followed by condensation with methyl N^{α}-(4-aminobenzoyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate and deprotection afforded the 8-deaza analogue. For the preparation of the N^{δ}-carboxymethyl derivative of mAPA-Orn, N^{α}-(benzyloxycarbonyl)-L-ornithine was subjected to N^{δ}-monoalkylation with glyoxylic acid and sodium cyanoborohydride, followed by N^{δ}-acylation with ethyl trifluoroacetate, N^{α}-deprotection by hydrogenolysis, condensation with 4-amino-4-deoxy-N^{10}-methylpteroic acid, and N^{δ}-deprotection by gentle treatment with ammonia. The 2,4-diamino derivatives all inhibited the growth of tumor cells in culture, with IC_{50} values of 0.2–2 μM, and inhibited purified DHFR with IC_{50} values of 0.02–0.08 μM. Deletion of ring nitrogens and N^{δ}-carboxymethylation both increased potency in the cell growth assay; however, the ornithine derivatives were less potent than aminopterin or methotrexate.

The folic acid analogue N^{α}-pteroyl-L-ornithine (1)[2,3] is a potent inhibitor of folylpolyglutamate synthetase (FPGS), an enzyme considered essential for cell growth,[2] whereas the aminopterin (AMT) analogue N^{α}-(4-amino-4-deoxypteroyl)-L-ornithine (APA-Orn, 2) has been found[4–9] to be an inhibitor of FPGS as well as dihydrofolate reductase (DHFR), another pivotal enzyme in folic acid metabolism.[10] Similarly, the 5-chloro-5,8-dideaza analogue of 1 inhibits FPGS, whereas the 5-chloro-5,8-dideaza analogue of 2 inhibits both DHFR and FPGS.[11] As part of a broader investigation of folic acid, AMT, and methotrexate (MTX) analogues,[12–15] we have synthesized the 5-deaza and 5,8-dideaza analogues of 1 (3 and 4, respectively) and the 5-deaza, 8-deaza, and 5,8-dideaza analogues of 2 (5, 6, and 7, respectively). Also prepared was the MTX analogue 8, a previously undescribed derivative of N^{α}-(4-amino-4-deoxy-N^{10}-methylpteroyl)-L-ornithine (mAPA-Orn, 9).[4,16] The synthesis of 2–8 and their activity as inhibitors of DHFR and cell growth in culture are the subject of this paper. The activity of these compounds as FPGS inhibitors will be reported separately as part of a larger study currently in progress, which includes analogues with side-chain amino acids other than ornithine.



1: X = Y = N
3: X = CH, Y = N
4: X = Y = CH



2: X = Y = N, R¹ = H
5: X = CH, Y = N, R¹ = R² = H
6: X = N, Y = CH, R¹ = R² = H
7: X = Y = CH, R¹ = R² = H
8: X = Y = N, R¹ = Me, R² = CH_2COOH
9: X = Y = N, R¹ = Me, R² = H

Chemistry

Our syntheses of N^{α}-(5-deazapteroyl)-L-ornithine (5-dPter-Orn, 3) and N^{α}-(5,8-dideazapteroyl)-L-ornithine (5,8-ddPter-Orn, 4) are depicted in Schemes I and II, respectively. Trisformylmethane, prepared via 2-[(*N*,*N*-dimethylamino)methylene]-1,3-bis(*N*,*N*-dimethylimmonio)propane bis(tetrafluoroborate) according to a recently described improved method,[17] was condensed directly with 2,6-diaminopyrimidin-4(3*H*)-one, and the product was acetylated to obtain 2-acetamido-6-formylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (10).[18] Reductive amination of 10 with methyl N^{α}-(4-aminobenzoyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate (11) in the presence of $Me_2NH{\cdot}BH_3$ afforded the protected coupling product 12, which on re-

† Dana-Farber Cancer Institute.
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(1) Paper 44 in this series. For previous paper, see: Rosowsky, A.; Bader, H.; Freisheim, J. H. *J. Med. Chem.* 1991, *34*, 574.
(2) George, S.; Cichowicz, D. J.; Shane, B. *Biochemistry* 1987, *26*, 522.
(3) Rosowsky, A.; Bader, H.; Forsch, R. A. *Pteridines* 1989, *1*, 91.
(4) Rosowsky, A.; Freisheim, J. H.; Moran, R. G.; Solan, V. C.; Bader, H.; Wright, J. E.; Radike-Smith, M. *J. Med. Chem.* 1986, *29*, 655.
(5) Clarke, L.; Rosowsky, A.; Waxman, D. J. *Mol. Pharmacol.* 1987, *31*, 122.
(6) Rosowsky, A.; Moran, R. G.; Freisheim, J. H.; Bader, H.; Forsch, R. A.; Solan, V. C. *NCI Monogr.* 1987, *5*, 145.
(7) McGuire, J. J.; Hsieh, P.; Franco, C. T.; Piper, J. R. *Biochem. Pharmacol.* 1986, *35*, 2607.
(8) McGuire, J. J.; Piper, J. R.; Coward, J. K.; Galivan, J. *NCI Monogr.* 1987, *5*, 139.
(9) McGuire, J. J.; Bolanowska, W. E.; Piper, J. A. *Biochem. Pharmacol.* 1988, *37*, 2931.

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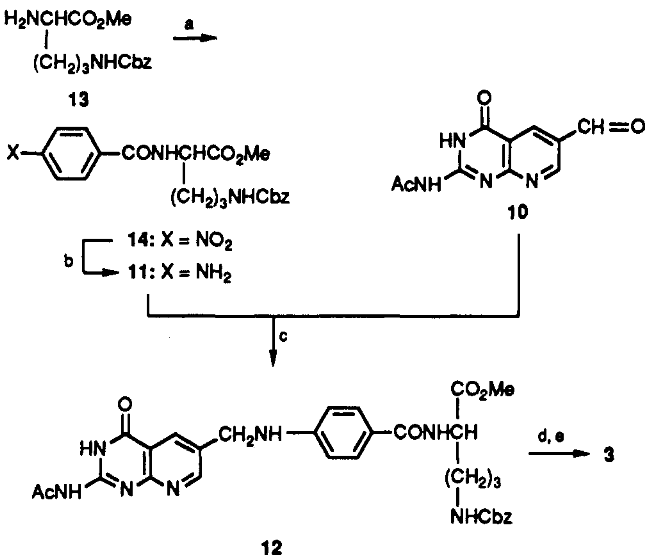
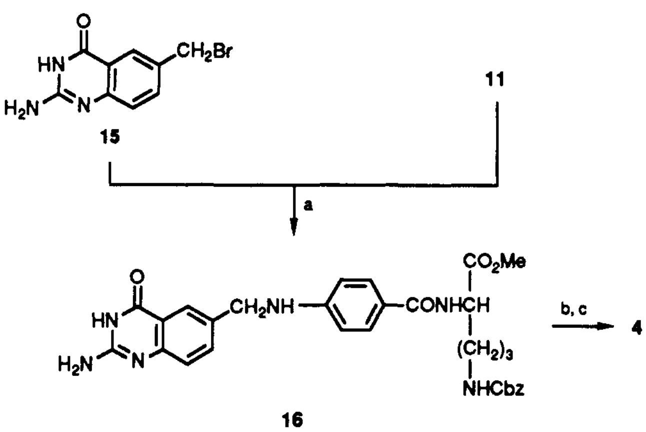
Scheme I[a]



[a](a) $4\text{-}O_2NC_6H_4COCl$; (b) $SnCl_2$; (c) $Me_2NH{\cdot}BH_3$; (d) CF_3CO_2H/PhSMe; (e) NaOH.

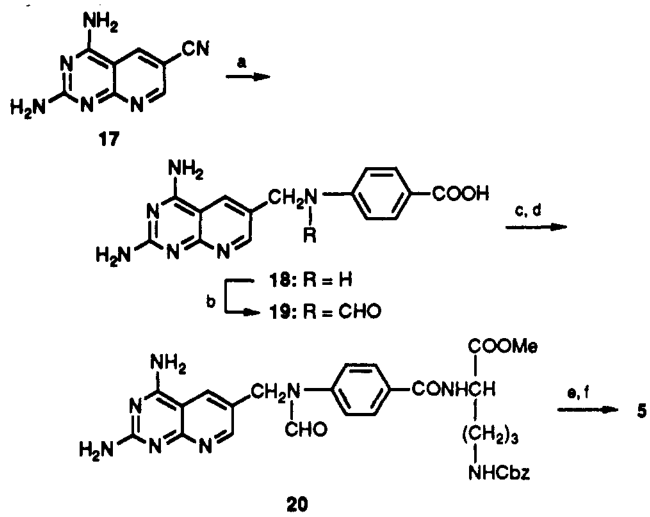
Scheme II[a]



[a](a) $i\text{-}Pr_2NEt$; (b) HBr/AcOH; (c) aqueous HBr.

moval of the benzyloxycarbonyl (Cbz) group with trifluoroacetic acid in thioanisole followed by cleavage of the methyl ester with NaOH gave the desired target compound **3**. Purification of **12** was easily carried out on a silica gel column, but purification of **3** was greatly complicated by

Scheme III[a]



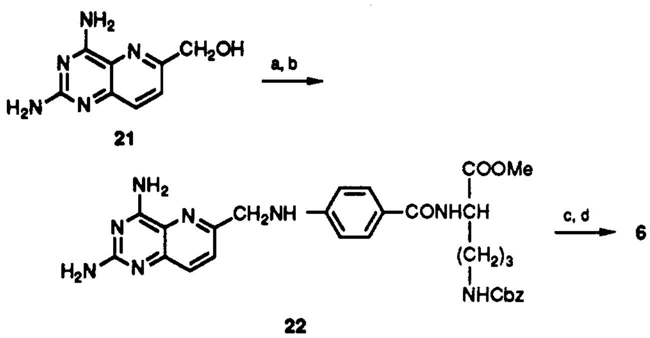
[a](a) $4\text{-}H_2NC_6H_4COOH/AcOH/H_2$/Raney Ni; (b) HCOOH; (c) $i\text{-}BuOCOCl/Et_3N$; (d) $H_2NCH(COOMe)(CH_2)_3NHCbz$ (**13**); (e) HBr/AcOH; (f) aqueous HBr.

the zwitterionic character of the ornithine moiety, which limited our ability to use ion exchange columns as a means of removing inorganic salts. Desalting was ultimately accomplished by preparative HPLC on C_{18} silica gel with aqueous 1% AcOH/5% EtOH as the eluent. Freeze drying gave **3** in the form of a hydrated acetate salt. The UV spectrum of **3** [λ_{max} (0.1 N NaOH) 243, 278, 285(infl), 331(infl) nm] was consistent with that reported for the corresponding glutamate analogue.[19] The previously undescribed side-chain fragment **11** was synthesized from methyl N^δ-(benzyloxycarbonyl)-L-ornithine (**13**) by reaction with 4-nitrobenzoyl chloride and reduction of the ensuing N^α-(4-nitrobenzoyl) derivative **14** with $SnCl_2$. The protected amino acid **13** was prepared as the HCl salt by treatment of N^δ-(benzyloxycarbonyl)-L-ornithine with $SOCl_2$ and MeOH. For the preparation of **4** (Scheme II), 2-amino-6-(bromomethyl)quinazolin-4(3*H*)-one (**15**) was prepared according to Acharya and Hynes[20] and used to alkylate **11**. Sequential treatment of the protected coupling product **16** with HBr/AcOH to cleave the Cbz groups and aqueous HBr to cleave the ester afforded the target compound **4**, which was purified by preparative HPLC (C_{18} silica gel, 1% AcOH/5% EtOH). The UV spectrum of **4** [λ_{max} (0.1 N NaOH) 226, 278, 288–291 (plateau) nm] was consistent with data reported previously for the glutamate analogue.[21]

The synthesis of N^α-(4-amino-4-deoxy-5-deazapteroyl)-L-ornithine (5-dAPA-Orn, **5**) is summarized in Scheme III. Reductive coupling of 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carbonitrile (**17**)[22] with 4-aminobenzoic acid (H_2/RaNi/AcOH) gave 4-amino-4-deoxy-5-deazapteroic acid (**18**), a sparingly soluble compound which was not characterized further but was treated directly with 95–97% HCOOH at 75 °C for 2 h to obtain the more soluble N^{10}-formyl derivative **19**. Condensation of **19** with **13** by the mixed carboxylic-carbonic anhydride method (i-BuOCOCl/Et_3N/DMF) afforded the ester **20**. The NMR spectrum of **20**, in DMSO-d_6 solution, showed the C_5 and C_7 protons as broad one proton singlets at δ 8.22

(10) For an overview of the enzyme pathways of folate metabolism, see: Kisliuk, R. L. In *Folate Antagonists as Therapeutic Agents*; Sirotnak, F. M., Burchall, J. J., Ensminger, W. D., Montgomery, J. A., Eds.; Academic Press: Orlando, FL, 1984; Vol. 1, pp 1–68.
(11) Patil, S. A.; Shane, B.; Freisheim, J. H.; Singh, S. K.; Hynes, J. B. *J. Med. Chem.* **1989**, *32*, 1559.
(12) Rosowsky, A.; Forsch, R. A.; Freisheim, J. H.; Moran, R. G.; Wick, M. *J. Med. Chem.* **1984**, *27*, 600.
(13) Rosowsky, A.; Moran, R. G.; Forsch, R. A.; Radike-Smith, M.; Colman, P. D.; Wick, M. M.; Freisheim, J. H. *Biochem. Pharmacol.* **1986**, *35*, 3327.
(14) Rosowsky, A.; Forsch, R. A.; Moran, R. G.; Kohler, W.; Freisheim, J. H. *J. Med. Chem.* **1988**, *31*, 1326.
(15) Rosowsky, A.; Bader, H.; Kohler, W.; Freisheim, J. H.; Moran, R. G. *J. Med. Chem.* **1988**, *31*, 1338.
(16) Piper, J. R.; McCaleb, G. S.; Montgomery, J. A.; Schmid, F. A.; Sirotnak, F. M. *J. Med. Chem.* **1985**, *28*, 1016.
(17) Keshavarz-K., M.; Cox, S. D.; Angus, R. O., Jr.; Wudl, F. *Synthesis* **1988**, 641.
(18) Taylor, E. C.; Palmer, D. C.; George, T. J.; Fletcher, S. R.; Tseng, C. P.; Harrington, P. J.; Beardsley, G. P. *J. Org. Chem.* **1983**, *48*, 4852.
(19) Temple, C., Jr.; Elliott, R. E.; Montgomery, J. *J. Org. Chem.* **1982**, *47*, 761.
(20) Acharya, S.; Hynes, J. *J. Heterocycl. Chem.* **1975**, *12*, 1283.
(21) Davoll, J.; Johnson, A. *J. Chem. Soc. (C)* **1970**, 997.
(22) Piper, J. R.; McCaleb, G. S.; Montgomery, J. A.; Kisliuk, R. L.; Gaumont, Y.; Sirotnak, F. M. *J. Med. Chem.* **1986**, *29*, 1080.

Scheme IV[a]



[a] (a) PBr_3/THF; (b) $H_2NC_6H_4CONHCH(COOMe)(CH_2)_3NHCbz$ (11); (c) HBr/AcOH; (d) aqueous HBr.

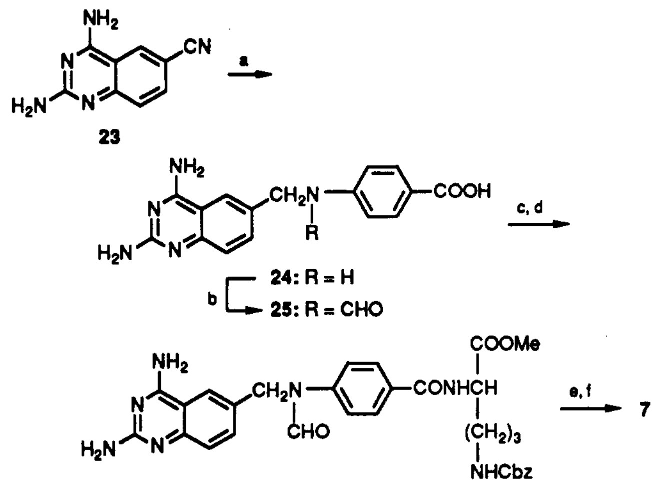
and 8.55, respectively. An interesting feature of the ^1H NMR spectrum of 20 was also the presence of two closely spaced singlets of unequal area at δ 8.70 and 8.77 for the N^{10}-formyl group. The total area for this pair of singlets corresponded to one proton. These results were consistent with a mixture of sterically hindered rotomers about the N^{10}-CHO bond, which has also been observed in recent work on 5-deazafolate derivatives by Taylor and coworkers.[23] Deprotection of **20** was accomplished as in the synthesis of **4** from **16**. The product obtained after neutralization with NaOH was partially purifiable by column chromatography on Dowex 50W-2X (H^+) and DEAE-cellulose (HCO_3^-), but could only be isolated in analytically pure state, free of NaBr, by preparative HPLC (C_{18} silica gel, 1% AcOH/5% EtOH).

Our synthesis of 4-amino-4-deoxy-8-deazapteroyl-L-ornithine (**6**, 8-dAPA-Orn) is summarized in Scheme IV and is patterned after that of 8-deazaaminopterin by Srinivasan and Broom.[24] Bromination of 2,4-diaminopyrido[3,2-*d*]pyrimidine-6-methanol (**21**) with PBr_3, followed directly by condensation with **11** gave the protected coupling product **22**. Deprotection of **22** was accomplished by sequential treatment with HBr/AcOH and aqueous HBr as described above (see deprotection of **20**). Analytically pure **6** was obtained as an acetate salt after chromatography on Dowex 50W-X2 (H^+) and DEAE-cellulose (HCO_3^-) columns, followed by preparative HPLC (C_{18} silica gel, 1% AcOH/5% EtOH) to remove residual salts.

The synthesis of N^{α}-(4-amino-4-deoxy-5,8-dideazapteroyl)-L-ornithine (**7**, 5,8-ddAPA-Orn) is depicted in Scheme V. Catalytic reduction of 2,4-diamino-6-nitroquinazoline followed by diazotization and reaction with $CuSO_4$ and KCN essentially as described by Davoll and Johnson[21] afforded 2,4-diaminoquinazoline-6-carbonitrile (**23**). Reductive coupling of **23** with 4-aminobenzoic acid in 50% AcOH in the presence of Raney nickel then gave 5,8-dideazapteroic acid (**24**), which was not purifiable and therefore was converted directly into its more soluble and previously uncharacterized N^{10}-formyl derivative **25** by treatment with 95–97% HCOOH at 70–75 °C for 1.5 h. Mixed anhydride (*i*-BuOCOCl/Et_3N) condensation of **25** and **13** in DMF gave **26**, which was easily purified on silica gel with $CHCl_3$/MeOH (9:1 to 6:1) as the eluent. Deprotection of **26** and purification of **7** were carried out as in the synthesis of **5** and **6** from **20** and **22**, respectively.

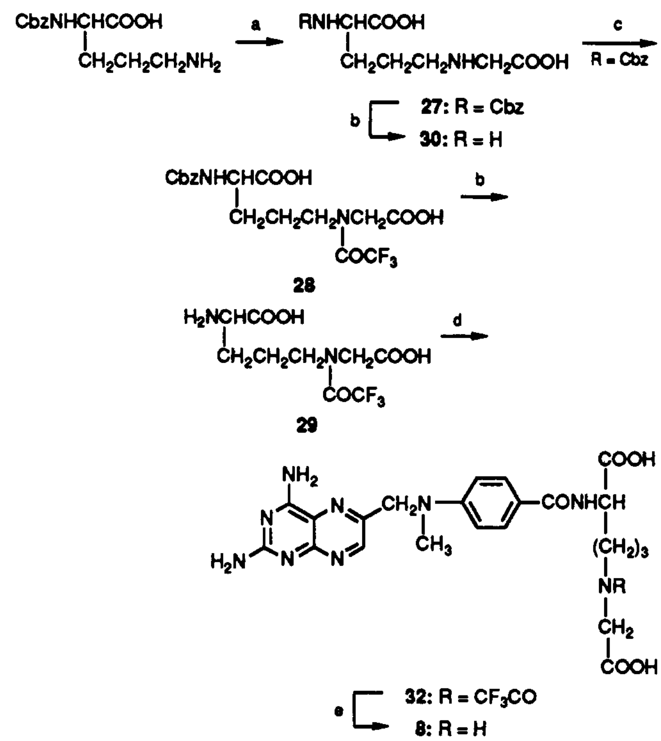
The route followed for the preparation of **8** is shown in Scheme VI. Reductive alkylation of N^{α}-(benzyloxycarbonyl)-L-ornithine with glyoxylic acid and sodium cyanoborohydride gave **27**, which on reaction with ethyl trifluoroacetate followed by catalytic hydrogenolysis (H_2/Pd–C) was converted to the diacids **28** and **29**. Catalytic hydrogenolysis of **27** afforded the heretofore undescribed diamino diacid **30** (91%). Compound **28** was isolated and characterized as a bis(*N*,*N*-dicyclohexylammonium) salt from which the free acid was obtained by acidification with dilute HCl. The deprotected amino diacid **29** was isolated as a hydrochloride from which the free base could be obtained on treatment with pyridine. Tris(trimethylsilylation) of **29** followed by condensation with 4-amino-4-deoxy-N^{10}-methylpteroic acid (**31**) by the diethyl phosphorocyanidate method[25] yielded the N^{δ}-tri-

Scheme V[a]



[a] (a) 4-$H_2NC_6H_4COOH$/AcOH/H_2/Raney Ni; (b) HCOOH; (c) *i*-BuOCOCl/Et_3N; (d) $H_2NCH(COOMe)(CH_2)_3NHCbz$ (13); (e) HBr/AcOH; (f) aqueous HBr.

Scheme VI[a]



[a] (a) $OHCCOOH{\cdot}H_2O/NaCNBH_3$; (b) H_2/Pd–C; (c) CF_3COOEt/Et_3N; (d) 4-amino-4-deoxy-N^{10}-methylpteroic acid (mAPA, 31); (e) NH_4OH.

(23) Taylor, E. C.; Hamby, J. M.; Shih, C.; Grindey, G. B.; Rinzel, S. M.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1989**, *32*, 1517.

(24) Srinivasan, A.; Broom, A. D. *J. Org. Chem.* **1981**, *46*, 1777.

Table I. DHFR Inhibition and Cell Growth Inhibition by Ornithine Analogues of Antifolates

compd	DHFR: IC_{50}, μM^a	cells: IC_{50}, μM L1210[b]	WI-L2[b]
2[c]	0.072	1.30	2.6
3	8.5	>5.0	ND
4	0.75	>5.0	ND
5	0.027	1.40	0.86
6	0.016	0.66	0.16
7	0.035	0.28	0.18
8	0.050	1.1	4.3
9[c]	0.16	1.30	ND

[a] DHFR activity was measured spectrophotometrically at 340 nm, with purified enzyme from human leukemic lymphoblasts (WI-L2/M4)[28] for compounds **2–5** and with enzyme from L1210/R71 murine leukemic cells[12] for compounds **2** and **9**. Results are given for a DHFR concentration of 0.05 μM in the cuvette. IC_{50} values obtained with the human and mouse enzyme generally do not differ by more than 2-fold. Thus, the IC_{50} of MTX is 0.035 μM against enzyme from L1210/R71 cells[4] and 0.02 μM against enzyme from WI-L2/M4 cells.[29] [b] Cell growth inhibition was determined by an adaptation[30] of the tetrazolium salt method[31] after a 48-h incubation in RPMI 1640 medium containing 10% fetal bovine serum and antibiotics. ND = not determined. [c] Data for compounds **2** and **9** are from ref 4.

fluoroacetyl derivative **32** (43%). Gentle treatment of **32** with ammonia then gave the target compound **8**.

Biological Activity

Compounds **2–8** were tested according to previously described assay procedures for the ability to inhibit purified DHFR from leukemic cells and for the ability to inhibit the growth of leukemic lymphoblasts in culture. The results (Table I) allow a comparison to be made between Pter-Orn (**1**) and its deaza analogues **3** and **4**, between APA-Orn (**2**) and its deaza analogues **5–7**, between the 2-amino-4-oxo derivatives **3** and **4** and the corresponding 2,4-diamines **5** and **6**, and between the N^δ-carboxymethyl analogue **8** and mAPA-Orn (**9**).

It has been observed previously that APA-Orn (**2**) and mAPA-Orn (**9**) are both less active as DHFR inhibitors than their glutamate counterparts, AMT and MTX,[4,6-9] and it was surmised that the presence of a polar, positively charged amino group on the end of the side chain interferes with binding to the active site. It was therefore of interest to determine whether part of the tight binding of AMT to DHFR lost on ornithine for glutamate substitution might be "recaptured" by replacing one or both pyrazine ring nitrogens by carbon. The results in Table I indicate that 8-dAPA-Orn (**6**) is a stronger inhibitor than APA-Orn (**2**), and that 5-dAPA-Orn (**5**) and 5,8-ddAPA-Orn (**7**) are less active than **6**. This suggests that carbon for nitrogen substitution at position 8 is favorable for DHFR binding. Carboxymethylation of the δ-amino group in mAPA-Orn (**9**) also appears to partially restore the binding activity lost on replacement of the glutamate side chain of MTX by ornithine. This is most likely due to decreased protonation of the nitrogen, as illustrated by the 6-fold difference in basicity of the amine nitrogen in sarcosine (pK_a = 10.0) versus methylamine (pK_a = 10.8).

The ability of antifolates to block cell replication in culture reflects not only their interaction with enzymes of the folate pathway, such as DHFR, but also their ability to be efficiently taken up and retained by cells. In the case of classical antifolates with a glutamate side chain, the efficiency with which they are converted to noneffluxing polyglutamate metabolites is a major determinant of their ability to accumulate in cells; indeed, where classical antifolates are concerned, differences in polyglutamylation probably contribute more in this regard than differences in transport.[26] With antifolates that cannot form polyglutamates, on the other hand, transport across the cell membrane is likely to be limiting. Such compounds are therefore of interest because they allow transport and polyglutamylation to be uncoupled. In this context we have compared the ability of compounds **2–9** to inhibit the growth of L1210 mouse leukemia cells and WI-L2 human leukemic lymphoblasts with their ability to inhibit DHFR. As shown in Table I, C for N replacement at position 8 gave a 4.6-fold increase in activity against L1210 cells, whereas C for N replacement at both positions 5 and 8 gave only a 2-fold increase and C for N replacement at position 5 alone led to no increase at all. In assays against WI-L2 cells, C for N replacement at position 8 as well at positions 5 and 8 gave a 15-fold increase in activity, whereas C for N replacement at position 5 gave an increase of only 3-fold. It thus appears that replacement of N^8 by carbon is a favorable change for WI-L2 cell growth inhibition, just as it is for DHFR inhibition, but that N^5 and N^8 both have to be replaced by carbon to produce the same favorable effect against L1210 cells.

It is of interest to note that **5** was less active than **6** against WI-L2 cells even though it was a better inhibitor of purified DHFR from the same cells, and that **7** was as active as **6** against the cells even though it was a weaker inhibitor of the enzyme. Since none of these compounds can be polyglutamylated, it seems likely that the lack of accord between cell growth inhibition and DHFR inhibition among these compounds reflects differences in transport. When the IC_{50} (growth inhibition)/IC_{50} (DHFR inhibition) ratios for **5–7** were normalized relative to the compound with the lowest IC_{50} for DHFR inhibition (**6**), the following values were obtained: **5**, 3.2; **6**, 1.0; **7**, 0.5. Although direct kinetic measurements would be needed to rigorously prove this point, our analysis suggests that transport of the ornithine analogues into WI-L2 cells obeys the order 5,8-dideaza > 8-deaza > 5-deaza.

The 2-amino-4-oxo compounds **3** and **4** were poor inhibitors of DHFR in comparison with the 2,4-diamines **5** and **6**. The IC_{50} of **3** was 315-fold higher than that of **5**, and the IC_{50} of **4** was 47-fold higher than that of **6**. The IC_{50} of both **3** and **4** as inhibitors of the growth of L1210 cells was >5 μM. These results are qualitatively in agreement with those reported previously[11] for other pteroyl-L-ornithine analogues with hetero atoms (O,S) at position 10.

The carboxymethyl derivative **8** was approximately as active against L1210 cells as the parent compound mAPA-Orn (**9**). Thus, decreasing the basicity of the terminal nitrogen does not appear to increase cell growth inhibition, just as it seems to have little effect on DHFR inhibition. It is of interest to note that the length of the side chain in **8** differs by only one atom from that of N^α-(4-amino-4-deoxy-N^{10}-methylpteroyl)-2-aminononanedioic acid, whose IC_{50} against L1210 cells was previously found to be only 0.0012 μM.[15] Since the 1000-fold differences between these two compounds is unlikely to be due to this small steric difference, it may be concluded that the introduction of a basic nitrogen in the side chain, even when the two carboxyl groups are

(25) Rosowsky, A.; Forsch, R.; Uren, J.; Wick, M. *J. Med. Chem.* 1981, *24*, 1450.

(26) For a comprehensive review of FPGS and its role in folate metabolism and antifolate chemotherapy, see: McGuire, J. J.; Coward, J. K. In *Folates and Pterins*; Blakley, R. L., Benkovic, S. J., Eds.; Wiley Interscience: New York, 1984; pp 135–190.

present, is very unfavorable for transport.

Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer; only peaks above 1200 cm^{-1} are reported. UV spectra were obtained on a Varian Model 210 instrument. ^1H NMR spectra were obtained on a Varian EM360L spectrometer with Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the reference. TLC analyses were done on fluorescent Baker Si250F silica gel plates, Eastman 13181 silica gel sheets, or Eastman 13254 cellulose sheets. Spots were visualized under 254-nm UV illumination or with the aid of ninhydrin. Column chromatography was carried out on Baker 3405 (60–200 mesh), Dowex 50W-X2, or Amberlite IR120 sulfonic acid resins, and Whatman DE-52 preswollen (*N,N*-diethylamino)ethyl cellulose (DEAE-cellulose). Solvents used in moisture-sensitive reactions were dried over Linde 4A molecular sieves (Fisher, Boston, MA). Analytical HPLC was done on Waters C_{18} cartridge column (5-μm particle size, 5 mm i.d. × 10 cm) connected to a Waters Model 400 instrument equipped with a Model 490 multiwavelength detector and Model 660 programmable solvent gradient system. Preparative HPLC was carried out on a Waters C_{18} cartridge column (15 μm particle size, 25 × 100 mm) connected to a Waters Delta-Prep 3000 system. 2,4-Diamino-6-nitroquinazoline was purchased from Fairfield Chemical, Blythewood, SC, and N^{α}-(benzyloxycarbonyl)-L-ornithine from Bachem, Torrance, CA. Other chemicals were from Aldrich, Milwaukee, WI. Known literature methods were used for the preparation of 2-acetamido-6-formylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**10**),[18] 2-amino-6-(bromomethyl)quinazolin-4-(3*H*)-one (**15**),[20] 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carbonitrile (**17**),[21] 2,4-diaminopyrido[3,2-*d*]-pyrimidine-6-methanol (**21**),[24] 2,4-diaminoquinazoline-6-carbonitrile (**23**),[21] and *N*-(4-amino-4-deoxy-N^{10}-methyl)pteroic acid (**31**).[25] In the synthesis of **22**, the method of Srinivasan and Broom[24] was modified slightly in that chlorination of the 2,4-dioxo compound with $POCl_3$ was performed in the presence of *N,N*-diethylaniline instead of Et_3N. Melting points were determined in Pyrex capillary tubes in a Mel-Temp apparatus (Cambridge Laboratory Devices, Cambridge, MA) and are not corrected. Microanalyses were by Robertson Laboratory, Madison, NJ.

Methyl N^{δ}-(Benzyloxycarbonyl)-L-ornithinate Hydrochloride (13·HCl). N^{δ}-(Benzyloxycarbonyl)-L-ornithine (2.66 g, 0.01 mmol) in MeOH (100 mL) was cooled in an ice bath and treated dropwise with $SOCl_2$ (6.55 g, 4.02 mL, 0.055 mol) while keeping the internal temperature below 10 °C. The bath was removed and the solution was left at room temperature for 18 h. The solvent was evaporated, and the solid was recrystallized from MeOH/EtOAc and dried in vacuo over P_2O_5 at 60 °C to obtain colorless flakes (2.95 g, 94% yield): mp 138–139 °C (lit.[27] mp 132–134 °C); IR (KBr) ν 1755 (ester C=O), 1700 (carbamate C=O) cm^{-1}; NMR (DMSO-d_6) δ 1.70 (m, CH_2CH_2), 3.00 (m, CH_2NCHbz), 3.70 (s, CH_3), 3.97 (m, α-CH), 4.98 (s, $OCH_2C_6H_5$), 7.28 (s, $OCH_2C_6H_5$), 8.63 (s, α-NH_3^+).

Methyl N^{α}-(4-Nitrobenzoyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate (14). A mixture of the blocked amino acid **13**·HCl (1.58 g, 0.005 mol) and 4-nitrobenzoyl chloride (0.93 g, 0.005 mol) in CH_2Cl_2 (25 mL) was treated with Et_3N (1.01 g, 1.39 mL, 0.01 mol), and after 5 min of stirring, the mixture was washed with H_2O and evaporated to dryness. Recrystallization from MeOH afforded white crystals (1.7 g, 79% yield): mp 141–142 °C; IR (KBr) ν 1750, 1700, 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.72 (m, CH_2CH_2), 3.25 (m, CH_2N), 3.78 (s, OCH_3), 4.85 (br m, α-CH, NH), 5.08 (s, $OCH_2C_6H_5$), 7.30 (s, $OCH_2C_6H_5$), 7.97 (d, J = 9 Hz, C_2-H and C_6-H), 8.27 (d, J = 8 Hz, C_3-H and C_5-H). Anal. ($C_{21}H_{23}N_3O_7$) C, H, N.

Methyl N^{α}-(4-Aminobenzoyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate (11). A mixture of **14** (21.5 g, 0.05 mol) and $SnCl_2 \cdot 2H_2O$ (56.4 g, 0.25 mol) in EtOAc (200 mL) was refluxed for 30 min, during which time it became homogeneous. The mixture was then stirred while 5% $NaHCO_3$ was added until the aqueous layer remained alkaline to pH paper. The salts were filtered (a large funnel should be used, as the filtration is slow), and the filter cake was washed with EtOAc. The combined filtrate and wash solution were evaporated to obtain a white solid (14 g, 70%): mp 136–137 °C; R_f 0.6 (silica gel, 19:1 $CHCl_3$/MeOH); IR (KBr) ν 1745, 1695, 1635 cm^{-1}; NMR ($CDCl_3$) δ 1.75 (m, CH_2CH_2), 3.20 (m, CH_2N), 3.72 (s, OCH_3), 3.98 (m, NH_2), 4.83 (m, α-CH, NH), 5.05 (s, $OCH_2C_6H_5$), 6.60 (d, J = 8 Hz, overlapping a multiplet, C_3-H, C_5-H, and NH), 7.27 (s, $OCH_2C_6H_5$), 7.60 (d, J = 8 Hz, C_2-H and C_6-H). In some runs, the product had to be additionally purified by column chromatography on silica gel with 19:1 $CHCl_3$/MeOH as the eluent. Anal. ($C_{21}H_{25}N_3O_5$) C, H, N.

Methyl N^{α}-(2-Acetamido-5-deazapteroyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate (12). A mixture of **10** (237 mg, 1.0 mmol) and **11** (399 mg, 1.0 mmol) in glacial AcOH (15 mL) was stirred at room temperature for 18 h, and $Me_2NH \cdot BH_3$ (24 mg, 0.4 mmol) was added. The precipitate which had formed overnight redissolved upon addition of the reducing agent. After 1.5 h at room temperature, the solution was warmed at 60 °C for 10 min [TLC R_f 0.4, 0.5, 0.6 (silica gel, 19:1 $CHCl_3$/MeOH)]. The reaction mixture was evaporated to dryness and the residue triturated with Et_2O to remove the spot with R_f 0.6. The remainder of the solid was chromatographed on a silica gel column (22 g, 2 × 24 cm) with 19:1 $CHCl_3$/MeOH as the eluent. Fractions containing the R_f 0.4 spot were pooled, concentrated to a small volume, and diluted with Et_2O. The precipitate was collected and dried in vacuo at 65 °C over P_2O_5 to obtain an off-white solid (195 mg, 32%): mp 128–135 °C; IR (KBr) ν 3430, 2970, 1710 sh, 1690, 1645, 1620, 1575, 1525, 1470, 1415, 1385, 1320, 1270, 1205 cm^{-1}; NMR ($CDCl_3$ + 4 drops DMSO-d_6) δ 1.80 (m, 4 H, CH_2CH_2), 2.30 (s, 3 H, CH_3CO), 3.22 (m, 2 H, CH_2NHCOO), 3.73 (s, 3 H, MeO), 4.50 (m, 3 H, benzylic CH_2N and α-CH), 5.10 (s, 2 H, benzylic CH_2O), 6.62 (d, J = 8 Hz, 2 H, 3′- and 5′-H), 7.35 (s, 5 H, C_6H_5), 7.50 (s, 1 H, NH), 7.72 (d, J = 8 Hz, 2 H, 2′- and 6′-H), 8.50 (br s, 1 H, C_5-H), 8.85 (m, 1 H, C_7-H). Anal. ($C_{31}H_{33}N_7O_7$) C, H, N.

N^{α}-(5-Deazapteroyl)-L-ornithine (5-dPter-Orn, 3). Compound **12** (144 mg, 0.23 mmol) was allowed to stand in a mixture of CF_3CO_2H (4 mL) and thioanisole (1.2 mL) for 4.5 h. The mixture was evaporated under reduced pressure and the residue triturated with Et_2O. After decantation of the Et_2O, 0.1 N NaOH (25 mL) was added and the mixture heated at 80 °C for 50 min. The solution was cooled, adjusted to pH 8–9 with HCl, and added onto a DEAE-cellulose column (HCO_3^- form, 1.5 × 25 cm) which was eluted first with H_2O, then 0.4 M NH_4HCO_3, and finally 3% Et_3N to remove the product. Freeze drying of pooled fractions containing the product gave a solid weighing 131 mg, indicating that inorganic salts had not been completely removed. Furthermore, HPLC (C_{18}, 6% MeCN in 0.1 M NH_4OAc, pH 7) showed two peaks with retention times of 7.5 and 22.5 min. Final desalting and product purification were accomplished by preparative HPLC (1% AcOH containing 5% EtOH). Appropriate fractions were pooled, concentrated by rotary evaporation, and subjected to prolonged freeze drying to obtain a white solid (26 mg, 22%): mp >300°C dec; HPLC 6.1 min (C_{18}, 1% aqueous AcOH containing 5% EtOH, flow rate 1.0 mL/min); IR (KBr) ν 3440, 2970, 1700 sh, 1615, 1580, 1520, 1410, 1340–1300 sh, 1270, 1200 cm^{-1}; UV λ_{max} (0.1 N NaOH) 243 nm (ϵ 23 600), 278 (24 000), 285 infl (23 600), 331 infl (10 000). Anal. ($C_{20}H_{23}N_7O_4 \cdot CH_3COOH \cdot 1.5H_2O$) C, H, N.

Methyl N^{δ}-(Benzyloxycarbonyl)-N^{α}-(5,8-dideazapteroyl)-L-ornithinate (16). Compound **15** (279 mg, 1.1 mmol) was added in small portions over 5 min to a stirred solution of **11** (440 mg, 1.1 mmol) and *i*-Pr_2NEt (192 μL, 142 mg, 1.1 mmol) in dry DMF (10 mL). The solution was left to stand at room temperature for 3 days, then concentrated to 3 mL under reduced pressure, and added dropwise with stirring to H_2O (50 mL). The solid was collected and taken up in a mixture of $CHCl_3$ and MeOH [TLC R_f 0.0, 0.3, 0.7 (silica gel, 9:1 $CHCl_3$/MeOH)]. The product was purified by column chromatography on silica gel (23 g, 2 × 25 cm). The column was eluted first with 9:1 $CHCl_3$/MeOH, then with 6:1 $CHCl_3$/MeOH. Fractions containing material with R_f 0.3 were pooled, concentrated to a small volume, and diluted with Et_2O. The solid was filtered and dried in vacuo at 60 °C over

(27) Synge, R. *Biochem. J.* **1948**, *42*, 99.
(28) Delcamp, T. J.; Susten, S. S.; Blankenship, D. T.; Freisheim, J. H. *Biochemistry* **1983**, *22*, 633.
(29) Rosowsky, A.; Forsch, R. A.; Freisheim, J. H.; Moran, R. G. *J. Med. Chem.* **1989**, *32*, 517.
(30) Rosowsky, A.; Bader, H.; Cucchi, C. A.; Moran, R. G.; Kohler, W.; Freisheim, J. H. *J. Med. Chem.* **1988**, *31*, 1332.
(31) Mosman, J. *J. Immunol. Meth.* **1983**, *65*, 55.

P_2O_5 to obtain a white solid (124 mg, 20%): mp 159–167 °C; IR (KBr) ν 3420, 2960, 2870, 1700, 1650, 1615, 1575, 1550, 1520, 1490, 1455, 1390, 1340, 1320 sh, 1270, 1220, 1200 cm^{-1}; NMR (DMSO-d_6 + D_2O) δ 1.65 (m, 4 H, CH_2CH_2), 3.02 (m, 2 H, CH_2NHCOO), 3.6 (large H_2O peak), 4.37 (m, 3 H, benzylic CH_2N and α-CH), 4.98 (s, 2 H, benzylic CH_2O), 6.58 (d, J = 8 Hz, 2 H, 3′- and 5′-H), 7.1–7.8 (m, 9 H, 2′-H, 6′-H, C_5-H, C_7-H, C_6H_5), 8.22 (d, J = 8 Hz, 1 H, C_8-H). Anal. ($C_{30}H_{32}N_6O_6{\cdot}H_2O$) C, H, N.

N^{α}-(5,8-Dideazapteroyl)-L-ornithine (5,8-ddPter-Orn, 4). Compound **16** (118 mg, 0.2 mmol) was dissolved in glacial AcOH (2.5 mL) with the aid of an ultrasonic bath, and 30% HBr in AcOH (2.5 mL) was added. A precipitate formed on addition of the HBr, but redissolved on continued ultrasonication (20 min). The solution was left to stand at room temperature for 30 min, and then treated with 48% HBr (5 mL). After another 40 min, the solution was evaporated under reduced pressure (caution: the lachrymator benzyl bromide is formed). The residue was dissolved in 28% NH_4OH by dropwise addition of 2 N NaOH and the solution partially desalted on Dowex 50W-X2 resin (H^+ form, 1.5 × 20 cm) by successive washing with H_2O and 3% Et_3N. Fractions containing the product according to HPLC were pooled and concentrated to dryness. The residue was suspended in 3% Et_3N, and just enough 2 N NaOH was added dropwise until a clear solution formed. The solution was applied onto a DEAE-cellulose column (HCO_3^- form, 1.5 × 24 cm). Elution with H_2O removed several impurities, but the product could not be eluted with H_2O, 0.2 M NH_4HCO_3, or 0.2 M NH_4HCO_3 adjusted to pH 10 with concentrated NH_4OH, but could be removed from the column with 3% Et_3N. Freeze drying of appropriate combined fractions afforded 43 mg of material which was still impure. Final purification was by preparative HPLC (C_{18}, 1% aqueous AcOH containing 5% EtOH). Pooled elutes containing the product were subjected to rotary evaporation and prolonged freeze drying to obtain a white powder (13 mg, 13%): mp >300 °C dec; HPLC: 11.7 min (C_{18}, 0.1 M NH_4OAc, pH 7.0, containing 5% MeCN, flow rate 1.0 mL/min); IR (KBr) ν 3440, 2970, 2940, 1715, 1615, 1575, 1520, 1455, 1410, 1340, 1285, 1200 cm^{-1}; UV λ_{max} (0.1 N NaOH) 226 nm (ε 46 700), 278 (23 900), 288–291 (plateau, 22 800). Anal. ($C_{21}H_{24}N_6O_4{\cdot}CH_3COOH{\cdot}2H_2O$) C, H, N.

Methyl N^{α}-(4-Amino-4-deoxy-5-deaza-N^{10}-formylpteroyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate (20). Raney nickel (0.2–0.3 g) was added to a solution of 4-aminobenzoic acid (3.71 g, 0.0271 mol) and **17** (5.04 g, 0.271 mol) in 67% AcOH (300 mL), and the mixture was shaken in a low-pressure Parr apparatus under 3 atm of H_2 for 18 h. The catalyst was removed by filtration, the solvents were evaporated, and the residue was suspended in H_2O (300 mL) to which 2 M NaOH was added to bring the pH to >12. Insoluble material was removed by filtration, and the filtrate was acidified with AcOH. The precipitate was collected, washed with H_2O, and dried in vacuo over P_2O_5 to obtain crude **18** as a bright orange powder (310 mg). The product was taken up directly in 95–97% formic acid (10 mL), and the solution was kept in an oil bath at 75 °C for 2 h before being evaporated to dryness under reduced pressure. The residue was taken up in 5% NH_4OH (30 mL), a small amount of insoluble material was removed by filtration, and the filtrate was acidified with 10% AcOH. The precipitate was collected and dried in vacuo at 90 °C over P_2O_5 to obtain **19** as a tan powder (239 mg): R_f 0.5 (silica gel, 5:4:1 $CHCl_3$/MeOH/28% NH_4OH); dec >220 °C; NMR (DMSO-d_6) δ 5.08 (s, CH_2N), 5.65 (br m, NH_2), 6.2–6.8 (m, NH_2), 7.43 (d, J = 8 Hz, $C_{3'}$- and $C_{5'}$-H), 7.92 (d, J = 8 Hz, $C_{2'}$- and $C_{6'}$-H, 8.20 (br s, C_5-H), 8.55 (br s, C_7-H), 8.80 (s, N^{10}-CHO). A similar procedure on a 10-fold larger scale resulted in a 48% yield.

A solution of nonpurified **19** from the preceding step in dry DMF (15 mL) in an ice bath was treated with Et_3N (303 mg, 417 μL, 3.0 mmol) and i-BuOCOCl (96 mg, 91 μL, 0.7 mmol). After 10 min of stirring, the blocked amino acid **13**·HCl (222 mg, 0.7 mmol) was added, followed by a second portion of i-BuOCOCl (27 mg, 26 μL, 0.2 mmol). After 10 min a second portion of the **13**·HCl (63 mg, 0.2 mmol) was added, and the cycle of additions (0.2 mmol of each reactant) was repeated one more time. The mixture was then concentrated to a volume of ca. 3 mL and added dropwise with stirring to 2% NH_4OH (50 mL) to obtain a gummy solid and cloudy liquid. The liquid was extracted with $CHCl_3$ and the gum was dissolved in the same $CHCl_3$ solution. The $CHCl_3$ solution was washed with H_2O and evaporated, and the residue was chromatographed on a silica gel column (20 g, 2 × 22 cm) with 9:1 $CHCl_3$/MeOH as the eluent. TLC homogeneous fractions (R_f 0.30, blue-fluorescent; silica gel, 9:1 $CHCl_3$/MeOH) were pooled, and hexane or Et_2O was added to precipitate a pale-yellow solid; yield 86 mg (20%) after drying in vacuo over P_2O_5 at 60 °C: mp 219–221 °C dec; IR (KBr) ν 3420, 2960, 1750, 1685, 1650, 1625, 1585, 1555, 1510, 1465, 1410, 1375, 1360, 1345, 1315, 1300 cm^{-1}; NMR (DMSO-d_6) δ 1.65 (m, CH_2CH_2), 3.02 (m, CH_2NHCbz), 3.62 (s, OCH_3), 4.40 (m, α-CH), 5.00 (s, $OCH_2C_6H_5$), 5.10 (s, CH_2NHAr), 6.22 (br s, NH_2), 7.43 (m, $C_{3'}$-H and $C_{5'}$-H, $OCH_2C_6H_5$, and NH_2), 7.88 (d, J = 8 Hz, $C_{2'}$-H and $C_{6'}$-H), 8.22 (br s, C_5-H), 8.55 (br s, C_7-H and NHCO), 8.70 (s), and 8.77 (s) (CHO rotomers). Anal. ($C_{30}H_{32}N_8O_6{\cdot}0.5H_2O$) C, H, N.

4-Amino-4-deoxy-5-deazapteroyl-L-ornithine (5-dAPA-Orn, 5). Compound **20** (619 mg, 1.03 mmol) was dissolved in 2 N HBr in AcOH (20 mL), the solution was allowed to stand at room temperature for 45 min, 48% HBr (20 mL) was added, and after another 20 h at room temperature, the hydrolysis mixture was evaporated under reduced pressure. The residue was taken up in a minimal volume of 1 N NaOH and the solution applied onto a Dowex 50W-X2 column (H^+ form, 2 × 22 cm) which was washed with a large volume of H_2O to remove salts and then with 3% NH_4OH to remove the product. The 3% NH_4OH eluates were pooled and freeze-dried, the residue was taken up in a small volume of 3% NH_4OH, a small amount of insoluble material was filtered off, the solution was applied onto a DEAE-cellulose column (HCO_3^- form, 1.5 × 24 cm), and the column was eluted with H_2O (300 mL). It should be noted that because the product was not retained on the DEAE-cellulose column, this step achieved partial purification but did not completely remove final traces of salt. Fractions were monitored by HPLC on C_{18} silica gel, using an 85:15 mixture of 0.1 M NH_4OAc, pH 7.0, and MeCN as the eluent, at a flow rate of 1.0 mL/min. The main product (HPLC, 4.3 min) was preceded by two minor impurities (HPLC, 6.3 min and 14.5 min). Pooled fractions that were >95% pure according to HPLC analysis were freeze-dried to obtain a pale-yellow powder (235 mg, 45% yield): dec >300 °C; IR (KBr) ν 3430, 2990 sh, 1620, 1590, 1565, 1520, 1475, 1415, 1340 cm^{-1}; NMR (1:1 DMSO-d_6/D_2O) δ 1.75 (m, CH_2CH_2), 2.83 (m, CH_2NH), 4.0–4.5 (H_2O), 6.63 (d, J = 8 Hz, $C_{3'}$-H and $C_{5'}$-H), 7.60 (d, J = 8 Hz, $C_{2'}$-H and $C_{6'}$-H), 8.28 (br s, C_5-H), 8.65 (br s, C_7-H); UV λ_{max} (0.1 N HCl) 219 nm (ε 40 900), 285 sh (17 000), 301 (18 700); λ_{max} (pH 7.4) 217 nm (ε 36 700), 248 (20 300), 281 (22 600), 294 sh (21 500), 338 infl (7600); λ_{max} (0.1 N NaOH) 248 nm (ε 22 600), 280 (23 600), 290 sh (22 500), 334–344 plateau (8000). The analytical sample was purified by preparative HPLC (C_{18} silica gel, 1% AcOH/5% EtOH; 162 mg recovered out of 201 mg). Anal. ($C_{20}H_{24}N_8O_3{\cdot}1.25CH_3COOH{\cdot}3H_2O$) C, H, N.

Methyl N^{α}-(4-Amino-4-deoxy-8-deazapteroyl)-N^{δ}-(benzyloxycarbonyl)-L-ornithinate (22). A suspension of **21** (233 mg, 1.22 mmol) in dry THF (3 mL) was stirred with PBr_3 (0.25 mL) for 8 h, and the solid was collected, washed with Et_2O, and added directly to a solution of the blocked amino acid **11** (499 mg, 1.25 mmol) in dry DMF (5 mL). Addition of i-Pr_2NEt (323 mg, 436 μL, 2.5 mmol) to the mixture produced a color change and a rise in temperature. The mixture was stirred at room temperature for 4 days and then added dropwise to H_2O (100 mL). The precipitate was filtered, dried, and triturated with 1:1 $CHCl_3$/MeOH (30 mL). Insoluble material was removed, the filtrate was evaporated, and the residue was chromatographed on a silica gel column (22 g, 2 × 22 cm), with TLC (silica gel, 9:1 $CHCl_3$/MeOH) used to monitor fractions. Elution with 9:1 $CHCl_3$/MeOH gave material with R_f 0.7, which proved to be unreacted **11** (290 mg, 58% recovery). Further elution with 6:1 $CHCl_3$/MeOH gave material with R_f 0.5. Fractions containing only the spot with R_f 0.5 were pooled, concentrated to a small volume, and treated with Et_2O to obtain a light-yellow solid (71 mg, 10% yield): mp 115–120 °C (softening above 110 °C); IR (KBr) ν 3420, 2970, 1735 sh, 1710, 1640 sh, 1620, 1580, 1525, 1465, 1425, 1395, 1320 br, 1270 cm^{-1}; NMR ($CDCl_3$) δ 3.68 (s, CH_3), 5.02 (s, CH_2NH), 7.23 (br s, C_7-H and C_8-H). Anal. ($C_{29}H_{32}N_8O_5{\cdot}0.5H_2O$) C, H, N.

N^{α}-(4-Amino-4-deoxy-8-deazapteroyl)-L-ornithine (8-dAPA-Orn, 6). Compound **22** (114 mg, 0.2 mmol) was slowly dissolved in 2 N HBr in AcOH (5 mL) over a 1-h period with the help of a sonication bath, and 48% HBr (5 mL) was added. The resulting solution was kept at room temperature for 1 h, the solvents were evaporated under reduced pressure, and the residue was dissolved in a small volume of H_2O made alkaline with 28% NH_4OH. The solution was placed onto a Dowex 50W-X2 column (H^+ form, 1.5 × 20 cm) which was eluted first with H_2O until the eluate was neutral and then with 3% Et_3N to remove **6** and several byproducts. HPLC analysis (0.1 M NH_4OAc, pH 7.5, with 12% MeCN, 1.0 mL/min) showed two major peaks at 3.5 min and 10.0 min, respectively, and several other peaks between 3.5 and 10.0 min. The crude mixture was freeze-dried and reapplied, in aqueous 3% Et_3N solution, to the top of a DEAE-cellulose column (HCO_3^- form, 1.5 × 24 cm). Elution with H_2O gave several fractions containing only impurities, and then a fraction containing only the product (HPLC 10.0 min). Pooled fractions containing the product were freeze-dried to obtain a light-yellow solid (32 mg, 30% yield); IR (KBr) ν 3390, 1660 sh, 1615, 1575, 1520, 1465 sh, 1460, 1410 sh, 1395, 1340, 1295, 1210 sh; NMR (DMSO-d_6 + D_2O) δ 1.72 (m, CH_2CH_2), 2.82 (m, CH_2NH_2), 4.47 (m, CH_2NH), 6.75 (d, J = 8 Hz, $C_{3'}$-H and $C_{5'}$-H), 7.57 (m, $C_{2'}$-H, C_6-H, C_7-H, C_8-H), UV (0.1 N NaOH) λ_{max} 222 (ϵ 29 100), 280 (21 000), 337 infl (5840); UV (pH 7.4) λ_{max} 221 nm (ϵ 37 700), 281 (25 300), 337 infl (7500); UV (0.1 N HCl) λ_{max} 221 nm (ϵ 44 400), 301 (15 000) with minor inflections at 233, 242, 282, and 330. The analytical sample was obtained by preparative HPLC on C_{18} silica gel with 1% AcOH/5% EtOH as the eluent. Anal. ($C_{20}H_{24}N_8O_3 \cdot 1.7CH_3COOH \cdot 3.3H_2O$) C, H, N.

4-Amino-4-deoxy-N^{10}-formyl-5,8-dideazapteroic Acid (25). A solution of **23** (1.26 g, 6.82 mmol) and 4-aminobenzoic acid (0.96 g, 7 mmol) in 50% AcOH (80 mL) was shaken with Raney nickel (ca. 0.2 g) in a Parr low-pressure apparatus under 2 atm of H_2 for 18 h. A heavy precipitate formed during the reaction. Solvents were evaporated, the residue (including the catalyst) was stirred with 10% NH_4OH (250 mL) for 15 min, and the insoluble material was filtered off. Concentration of the filtrate to 75 mL on a rotary evaporator caused acid **24** to precipitate. Filtration and drying in vacuo at 90 °C over P_2O_5 gave a pale-yellow solid (1.58 g, 75%). A larger run on a 2.7-fold scale gave a 53% yield.

A solution of crude **24** (1.24 g, 0.004 mol) in 95–97% HCO_2H (40 mL) was kept in an oil bath at 70–75 °C for 1.5 h, the HCO_2H was evaporated under reduced pressure, and the residue was stirred for 10 min with H_2O (130 mL) to which NH_4OH was added to make the solution strongly alkaline. The mixture was filtered and the filtrate acidifed by dropwise addition of glacial AcOH. The precipitate was filtered and dried in vacuo at 90 °C over P_2O_5: yield 0.91 g (68%); R_f 0.7 (silica gel, 5:4:1 $CHCl_3$/MeOH/28% NH_4OH). The analytical sample was prepared from a 0.3-g portion of the product by chromatography on a DEAE-cellulose column (HCO_3^- form, 1.5 × 20 cm). When a solution of the compound in NH_4OH was applied to the top of the column a dense precipitate formed. The column was washed with H_2O (200 mL), then with 0.2 M NH_4HCO_3 adjusted to pH 9.5 with NH_4OH. Colorless fractions containing minor impurities were discarded, and the eluent was changed to H_2O to which enough Et_3N was added to bring the pH to 11. The precipitate at the top of the column slowly dissolved, leaving dark immobile impurities on the column. Colorless fractions containing a single spot with R_f 0.7 (see above for TLC system) were pooled, concentrated to a volume of 40 mL, and acidified with glacial AcOH. The precipitate was filtered and dried in vacuo at 90 °C over P_2O_5 to obtain **25** as a white powder (0.16 g, starting from 0.3 g of crude material): IR (KBr) ν 3450, 1680 br, 1615, 1545-1510 br, 1395, 1335, 1275 1230 cm^{-1}; NMR (DMSO-d_6) δ 5.07 (br s, CH_2N), 7.13 (complex m, $C_{3'}$-H, $C_{5'}$-H, C_5-H, C_7-H, C_8-H, and NH_2), 7.83 (m, $C_{2'}$-H and $C_{6'}$-H), 8.77 (s, CHO). Another reaction conducted on a 2.7-fold larger scale and heated to reflux for 1 h gave a yield of 67%. Anal. ($C_{17}H_{15}N_5O_3 \cdot H_2O$) C, H, N.

Methyl N$^{\delta}$-(Benzyloxycarbonyl)-N^{α}-(4-amino-4-deoxy-N^{10}-formyl-5,8-dideazapteroyl)-L-ornithinate (26). A suspension of **25** (2.75 g, 7.75 mmol) in dry DMF (75 mL) was cooled in an ice bath while Et_3N (0.783 g, 1.078 mL, 7.75 mmol) followed by i-BuOCOCl (1.06 g, 1.01 mL, 7.75 mmol) was added with stirring. The solid did not completely dissolve. Another 10% each of Et_3N and i-BuOCOCl were therefore added, with trituration and sonication until nearly all the solid dissolved. To the mixture was then added **13**·HCl (2.45 g, 7.75 mol) followed again by Et_3N (0.783 g, 1.08 mL, 7.75 mmol). The solution was concentrated to ca. 10 mL and added dropwise with stirring to 2% NH_4OH (100 mL). The mixture was stirred in the ice bath for 5 min and filtered, and the solid was chromatographed on a silica gel column (50 g, 2.5 × 47 cm) with use of 9:1 $CHCl_3$/MeOH followed by 6:1 $CHCl_3$/MeOH as eluents. Pooled TLC-homogeneous fractions (R_f 0.3, blue-fluorescent spot, silica gel, 9:1 $CHCl_3$/MeOH) were concentrated to a small volume, and Et_2O was added. The precipitate was collected and dried in vacuo at 60 °C over P_2O_5 to obtain **26** as a pale-yellow powder (2.16 g, 46% yield): mp 113–119 °C; IR (KBr) ν 3400, 2970, 1730 sh, 1655, 1615, 1580–1570 br, 1520, 1465, 1420, 1365, 1340, 1295, 1270, 1230, 1210 cm^{-1}; NMR ($CDCl_3$) δ 1.67 (m, CH_2CH_2), 3.17 (m, CH_2NHCbz), 3.60 (s, CH_3O), 4.70 (br m, α-CH, OH, and NH_2), 4.98, s, OCH_2H_5), 5.50 (br m, CH_2NCHO), 6.65–7.90 (complex m, $OCH_2C_6H_5$, C_5-H, C_7-H, C_8-H, $C_{2'}$-H, $C_{3'}$-H, $C_{5'}$-H, $C_{6'}$-H, and NH_2), 8.37 (br s, CH). Anal. ($C_{31}H_{33}N_7O_6 \cdot 0.5H_2O$) C, H, N.

4-Amino-4-deoxy-5,8-dideazapteroyl-L-ornithine (5,8-ddA-PA-Orn, 7). Compound **26** (2.16 g, 3.61 mmol) was dissolved in 2 N HBr in AcOH (50 mL), the solution was left at room temperature for 30 min, 48% HBr (40 mL) was added, and the hydrolysis mixture was kept at room temperature for 17 h. The solvent was evaporated under reduced pressure, and H_2O (50 mL) was added to obtain a white precipitate (presumed to be a dihydrobromide salt), which was collected and dried on a lyophilizer. The residue (now soluble and presumed to be a monohydrobromide salt) was taken up in H_2O (50 mL) and the pH adjusted to 11 with NaOH. A trace of insoluble residue was removed by filtration, and the filtrate was passed through a Dowex 50W-X2 column (H^+ form, 1.5 × 28 cm), with use of H_2O first to remove salts and then 3% aqueous Et_3N to elute the product. Concentration of fractions containing the product according to HPLC analysis (C_{18}, 0.1 M NH_4OAc, pH 7.5, with 12% MeCN, 1.0 mL/min flow rate, detection at 292 nm) gave a solid, which was collected and dried on a lyophilizer to obtain a light-yellow powder (1.21 g, 79%): mp >250 °C dec; IR (KBr) ν 3440 br, 2980 sh, 1645, 1615, 1580, 1540 sh, 1520, 1460 sh, 1415, 1345, 1280 cm^{-1}; NMR (2:1 DMSO-d_6 + D_2O) δ 1.70 (m, CH_2CH_2), 2.87 (m, CH_2NH_2), 3.8–4.4 (br H_2O peak), 6.67 (d, J = 8 Hz, $C_{3'}$-H and $C_{5'}$-H), 7.35 (d, J = 8 Hz, C_7-H), 7.58 (m, $C_{2'}$-H, $C_{6'}$-H, and C_8-H), 8.07 (s, C_5-H); UV (0.1 N HCl) λ_{max} 232 nm (ϵ 51 500), 265 sh (12 000), 303 (12 000); UV (pH 7.4) λ_{max} 229 nm (ϵ 49 200), 296 (23 800; UV (0.1 N NaOH) λ_{max} 231 nm (ϵ 50 400), 279 (25 300), 290 infl (23 600), 340 infl (4700). HPLC analysis (see above) showed a single peak with an elution time of 12.1 min. Microanalytical data were consistent with a hydrated partial HBr salt. Anal. ($C_{21}H_{25}N_7O_3 \cdot 0.65HBr \cdot 2H_2O$) C, H, N, Br.

N^{α}-(Benzyloxycarbonyl)-N^{δ}-(carboxymethyl)-L-ornithine (27). N$^{\alpha}$-(Benzyloxycarbonyl)-L-ornithine (6.65 g, 25 mmol) was added to a stirred solution of glyoxylic acid monohydrate (2.64 g, 29 mmol) in MeOH (250 mL), and 1 N NaOH was added dropwise to the resulting slurry until the pH reached 8.0. The resulting solution was treated with $NaCNBH_3$ (1.57 g, 25 mmol), followed by enough 10% AcOH to bring the pH to 7.00 (meter). The pH was kept carefully at 7.00 ± 0.05 for 3 h by occasional dropwise addition of AcOH, and the solution was then left for 2 days with pH adjustment only every 10–15 h. After being concentrated to a small volume, the solution was applied onto a quaternary ammonium resin column (Amberlite CG-400, 160 g, 5 × 24 cm), which was eluted successively with a large volume of H_2O and then with 2–10% AcOH. Eluates were monitored by TLC, and fractions with R_f 0.24 (silica gel, 5:4:1 $CHCl_3$/MeOH/28% NH_4OH, ninhydrin positive) were combined and concentrated to a small volume. The solid which crystallized was collected, washed with Me_2CO, and dried on a lyophilizer; yield 3.91 g (48%): mp 185.5 °C; IR (KBr) ν 3390, 1695–1730, 1605 cm^{-1}; NMR (CF_3COOH) δ 1.93 (m, 4 H, CH_2CH_2), 3.40 (t, 2 H, CH_2NH), 4.18 (t, 2 H, $NHCH_2COOH$), 4.60 (m, 1 H, α-CH), 5.23 (s, 2 H, $C_6H_5CH_2O$), 7.33 (s, 5 H, $C_6H_5CH_2O$). Anal. ($C_{15}H_{20}N_2O_6 \cdot 0.13H_2O$) C, H, N.

N^{α}-(Benzyloxycarbonyl)-N^{δ}-(carboxymethyl)-N^{δ}-(trifluoroacetyl)-L-ornithine (28). A solution of **27** (1.47 g, 4.5 mmol), CF_3COOEt (1.28 g, 9 mmol), and Et_3N (1.06 g, 10.5 mmol) in MeOH (20 mL) was kept at 25 °C for 5 days while TLC (silica gel, 5:4:1 $CHCl_3$/MeOH/28% NH_4OH) was used to follow the disappearance of starting material (R_f 0.16) and formation of product (R_f 0.89). The reaction mixture was concentrated to dryness under reduced pressure, the residue was taken up in H_2O

(15 mL), and the solution was applied onto an Amberlite IR120 column (H^+ form, 2.0 × 21 cm). The column was eluted with H_2O, the eluates evaporated to a clear oil (1.76 g), and the oil taken up in Et_2O (100 mL). The solution was treated with *N,N*-dicyclohexylamine (1.79 g, 9.9 mmol) in Et_2O (25 mL) and stirred for 18 h until a granular solid formed. Recrystallization of the crude product (2.35 g) from *i*-PrOH to gave a *bis-(N,N-dicyclohexylammonium) salt* (2.2 g, 62% yield): mp 233–236 °C; TLC R_f 0.88 (silica gel, 5:4:1 $CHCl_3$/MeOH/28% NH_4OH); IR (KBr) ν 3450, 2940, 2880, 1700, 1635 cm^{-1}. Anal. ($C_{17}H_{19}F_3N_2O_7\cdot 2C_{12}H_{23}N$) C, H, N.

The free diacid was obtained by EtOAc extraction of a solution of the bis(*N,N*-dicyclohexylammonium) salt in dilute HCl. The extracts were washed with a small volume of H_2O, dried over $MgSO_4$, and evaporated to a thick oil which was used directly in the next step.

***N*$^\delta$-(Carboxymethyl)-*N*$^\delta$-(trifluoroacetyl)-L-ornithine (29).** A solution of the diacid **28**, obtained as described above from the bis(*N,N*-dicyclohexylammonium) salt (2.14 g, 1.74 mmol), was dissolved in a mixture of EtOH (150 mL) and AcOH (30 mL) and shaken under 2–3 atm of H_2 for 18 h in the presence of 10% Pd–C (150 mg) in a Parr low-pressure apparatus. The catalyst was removed by filtration, the solution was evaporated, and the residue (ninhydrin-positive) was dissolved in a mixture of MeOH and EtOH to which 6 N HCl (0.5 mL) had been added. The solution was evaporated to dryness, and residual traces of water were removed by azeotropic distillation, first with benzene and EtOH and then with EtOH alone. The resultant solidified foam was dried in vacuo over P_2O_5 at room temperature at 25 °C to obtain a hygroscopic *HCl salt* (0.94 g, 100% yield): mp 50–52 °C. Anal. ($C_9H_{13}F_3N_2O_5\cdot HCl\cdot 0.45C_2H_5OH$) C, H, Cl, N.

A portion of the HCl salt was dissolved in H_2O, and pyridine and EtOH were added. Concentration of the solution to a small volume and storage at 0 °C led to formation of a gelatinous solid. Filtration, washing with cold EtOH and Et_2O, and drying in vacuo over P_2O_5 at 35 °C gave the free amino diacid: mp 111 °C (sintering above 60 °C). Anal. ($C_9H_{13}F_3N_2O_5\cdot 0.25H_2O\cdot 0.25C_2H_5OH$) C, H, N.

***N*$^\delta$-(Carboxymethyl)-L-ornithine (30).** A mixture of compound **27** (0.5 g, 1.53 mmol) and 10% Pd–C (50 mg) in MeOH (25 mL), AcOH (10 mL), and H_2O (15 mL) was shaken overnight under 2–3 atm of H_2 in a Parr low-pressure apparatus. After filtration of the catalyst, the solvents were evaporated under reduced pressure, the residue was extracted with 10% NH_4OH, and the extracts were evaporated to dryness to obtain a colorless solid. The solid was digested with boiling H_2O (8.5 mL), a small amount of insoluble flocculent material was removed, and the filtrate was diluted with Me_2CO (40 mL) to obtain crystals. Drying in vacuo at 80 °C over P_2O_5 afforded colorless plates (0.27 g): mp 241–242 °C; IR (KBr) ν 3420, 3020, 1625 cm^{-1}; $[\alpha]^{20}_D$ +1.5° (H_2O). Anal. ($C_7H_{14}N_2O_4\cdot 0.25H_2O$) C, H, N.

***N*$^\alpha$-(4-Amino-4-deoxy-*N*^{10}-methylpteroyl)-*N*$^\delta$-(carboxymethyl)-*N*$^\delta$-(trifluoroacetyl)-L-ornithine (32).** A stirred suspension of **29**·HCl (0.652 g, 1.9 mmol) in CH_2Cl_2 (40 mL) was cooled in an ice bath and treated successively with Et_3N (1.16 mL, 0.845 g, 8.36 mmol) and Me_3SiCl (0.619 g, 5.7 mmol). A clear solution was obtained, and stirring was continued at 25 °C for 18 h. The solvent was evaporated under reduced pressure, the residue redissolved in dry DMF (35 mL), and the solution set aside for the coupling step (see below).

Diethyl phosphorocyanidate (1.00 g, 6.18 mmol) was added to a stirred suspension of **31** (0.678 g, 1.9 mmol, assumed to contain 1.75 mmol H_2O)[25] in dry DMF (60 mL) containing Et_3N (0.625 g, 6.18 mmol). After 4 h at 25 °C, the DMF solution of silylated **29** (from the HCl salt; see above) was added and stirring was continued for 48 h. The reaction was quenched with MeOH (10 mL), the solvents were removed by rotary evaporation, and the residue was dissolved in a minimum volume of DMF. Portions (1–2 mL) of the solution were diluted 2-fold with a 1:9 mixture of EtOH and 0.1 M NH_4OAc, pH 7, and subjected to low-pressure reversed-phase chromatography on C_{18} silica gel. Elution was performed with 0.1 M NH_4OAc, pH 7.0, containing increasing amounts of EtOH from 15 to 30%. Fractions that were pure by HPLC (C_{18}, 10% MeCN in 0.1 M NH_4OAc, pH 6, retention time 51 min) were pooled, evaporated, and freeze-dried to a yellow powder (0.53 g, 43% yield): mp 231 °C; TLC R_f 0.29 (silica gel, 5:4:1 $CHCl_3$/MeOH/28% NH_4OH); IR (KBr) ν 3400, 3170, 1685, 1640, 1610 cm^{-1}. Anal. ($C_{24}H_{26}F_3N_9O_6\cdot 3.3H_2O$) C, H, N.

***N*$^\alpha$-(4-Amino-4-deoxy-*N*^{10}-methylpteroyl)-N$^\delta$-(carboxymethyl)-L-ornithine (8).** A solution of **32** (0.205 g, 0.31 mmol) in 10% NH_4OH (15 mL) was kept at 25 °C for 24 h, concentrated to a small volume by rotary evaporation, and applied onto a DEAE-cellulose column (20 g, HCO_3^- form, 2.0 × 26 cm). The column was eluted with a large volume of H_2O to remove salts, and then with 1% NH_4HCO_3. Fractions showing a TLC spot with R_f 0.12 (silica gel, 5:4:1 $CHCl_3$/MeOH/28% NH_4OH) were combined, reduced in volume, filtered, and freeze-dried. Drying of the residue in vacuo over P_2O_5 at 100 °C gave a yellow powder (91 mg, 49% yield): mp >300 °C; IR (KBr) ν 3440, 1650–1610 cm^{-1}; UV λ_{max} (0.1 N HCl) 252 nm (ϵ 20 850), 305 (22 100), 335 infl (14 500), 350 infl (11 100); λ_{max} (0.1 N NaOH) 220 nm (ϵ 23 050), 259 (25 450), 301 (23 800), 372 (8300). Anal. ($C_{22}H_{27}N_9O_5\cdot 0.25CF_3COOH\cdot 3.5H_2O$) C, H, N.

Acknowledgment. This work was supported in part by grants CA39867 (to A.R.), CA25394 (to A.R.), and CA41461 (to J.H.F.) from the National Cancer Institute, DHHS.

Registry No. **3** (free base), 132343-84-1; **3**·HOAc, 132374-28-8; **4** (free base), 118675-83-5; **4**·HOAc, 132374-29-9; **5** (free base), 132343-85-2; **5**·*x*HOAc, 132374-30-2; **6** (free base), 132343-86-3; **6**·*x*HOAc, 132374-31-3; **7** (free base), 132374-27-7; **7**·*x*HBr, 132374-32-4; **8** (free base), 132344-00-4; **8**·$^1/_4$TFA, 132344-01-5; **10**, 87373-56-6; **11**, 132343-87-4; **12**, 132343-88-5; **13**·HCl, 5874-75-9; **14**, 132343-89-6; **15**, 58677-08-0; **16**, 132343-90-9; **17**, 101810-72-4; **19**, 132343-91-0; **20**, 132343-92-1; **21**, 76822-61-2; **22**, 132343-93-2; **23**, 18917-68-5; **25**, 69827-84-5; **26**, 132343-94-3; **27**, 132343-95-4; **28** (free acid), 132343-96-5; **28**·2DCHA, 132344-02-6; **29** (free base), 132343-97-6; **29**·HCl, 132344-03-7; **30**, 132343-98-7; **31**, 19741-14-1; **32**, 132343-99-8; FPGS, 63363-84-8; DHFP, 9002-03-3; 4-$H_2NC_6H_4COOH$, 150-13-0; $(HO)_2CHCOOH$, 298-12-4; 4-$O_2NC_6H_4COCl$, 122-04-3; H-Orn(Cbz)-OH, 3304-51-6; Cbz-Orn-OH, 2640-58-6.