Synthesis and In Vitro Antitumor Activity of New Deaza Analogues of the Nonpolyglutamatable Antifolate N^{α}-(4-Amino-4-deoxypteroyl)-N^{δ}-hemiphthaloyl-L-ornithine (PT523)

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Received November 8, 2001

Details are disclosed for the synthesis of N^{α}-[4-[2-(2,4-diaminoquinazolin-6-yl)ethyl]benzoyl]-N^{δ}-hemiphthaloyl-L-ornithine (**2**) and N^{α}-[4-[5-(2,4-diaminoteridin-6-yl)pent-1-yn-4-yl]benzoyl]-N^{δ}-hemiphthaloyl-L-ornithine (**6**) as analogues of N^{α}-(4-amino-4-deoxypteroyl)-N^{δ}-hemiphthaloyl-L-ornithine (**1**, PT523), a nonpolyglutamatable antifolate currently in advanced preclinical development. In a 72 h growth inhibition assay against cultures of CCRF-CEM human leukemic lymphoblasts, the IC_{50} of **2** and **6** was 0.69 ± 0.044 nM and 1.3 ± 0.35 nM, respectively, as compared with previously reported values 4.4 ± 0.10 nM for aminopterin (AMT) and 1.5 ± 0.39 nM for PT523. In a spectrophotometric assay of dihydrofolate reductase (DHFR) inhibition using dihydrofolate and NADPH as the cosubstrates, the previously unreported compounds **2** and the mixed 10*R* and 10*S* diastereomers of **6** had K_i values of 0.21 ± 0.05 pM and 0.60 ± 0.02 pM, respectively, as compared with previously reported values of 3.70 ± 0.35 pM for AMT and 0.33 ± 0.04 pM for PT523. Thus, while they were comparable to **1** and several of its previously studied analogues in their ability to bind to DHFR and inhibit the growth of CCRF-CEM cells, **2** and the mixed diastereomers of **6** were several times more active than AMT despite the fact that they cannot form γ-polyglutamylated metabolites of the type formed in cells from AMT and other classical antifolates with a glutamate side chain.

Introduction

N^{α}-(4-Amino-4-deoxypteroyl)-N^{δ}-hemiphthaloyl-L-ornithine (PT523, **1**, Figure 1) is an unusually potent antifolate originally synthesized in our laboratory[1] and currently in advanced preclinical development under the auspices of the Rapid Access to Intervention Development (RAID) program of the National Cancer Institute.

As noted in two recent reviews of laboratory studies published to date on the mode of action of **1**,[2a,b] the features that probably contribute most prominently to its high potency are tight binding to dihydrofolate reductase (DHFR) and efficient utilization of the reduced folate carrier (RFC), the membrane transport protein responsible for cellular uptake of both folates and classical antifolates. Initial interest in **1** as a potential drug was sparked by the observation that it could overcome a clinically relevant 10- to 30-fold level of resistance to the classical antifolate methotrexate (MTX) in cultured cells irrespective of whether this resistance was associated with enhanced expression of DHFR or diminished uptake via the RFC. In contrast to MTX and other classical antifolates, **1** is not a substrate for folyl polyglutamate synthetase (FPGS) and thus resembles nonclassical antifolates such as trimetrexate (TMX, Figure 2) and piritrexim (PTX, Figure 2), whose action is FPGS-independent. Unlike TMX and PTX, however, **1** is water-soluble at physiologic pH and is able to be conveniently administered by injection. Furthermore, **1** retains the ability to inhibit the growth of cells that have become resistant to TMX and a range of other lipophilic drugs by reason of increased P-glycoprotein expression.

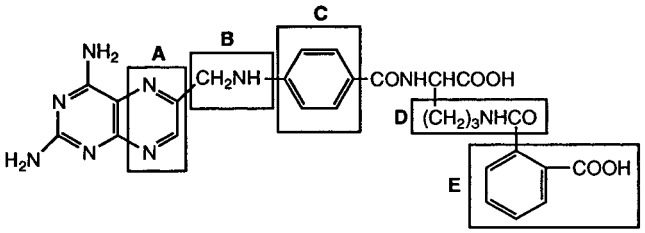

Figure 1. Structure of N^{α}-(4-amino-4-deoxypteroyl)-N^{δ}-hemiphthaloyl-L-ornithine (PT523, **1**).

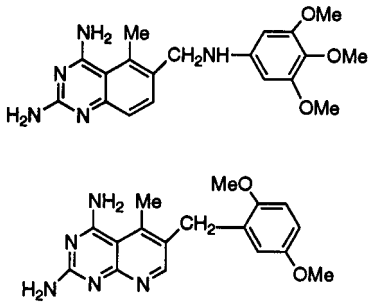

Figure 2. Structures of trimetrexate (TMX) and piritrexim (PTX).

Of further significance in terms of the clinical potential of **1** is that tumor cells selected for resistance to a number of the newer antifolates with a glutamate side chain, such as the thymidylate synthase inhibitor ICI D1694 (ZD1694, raltitrexed)[3] and the multitargeted antifolate LY231514,[4] were recently found to be only minimally cross-resistant to this drug.[5] Since the effects

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10.1021/jm010518t CCC: $22.00
Published on Web 03/16/2002

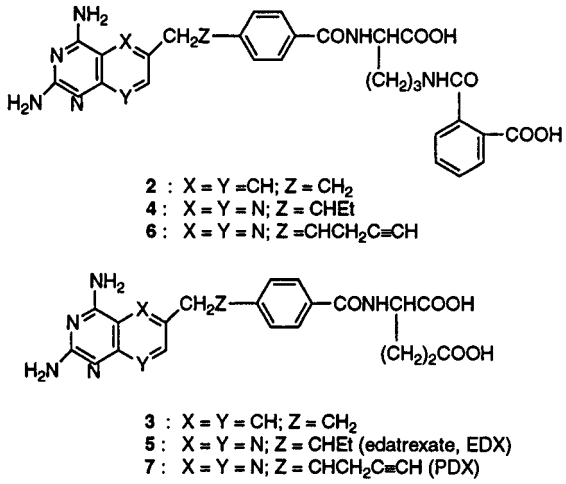


Figure 3. Structures of compounds **2**−**7**.

of raltitrexed and LY231514 are both highly dependent on cellular FPGS levels, and resistance to them is strongly correlated with FPGS expression, the fact that **1** is efficiently transported by the RFC[6a,b] and yet is not dependent on activation by FPGS for activity can be viewed as a very distinctive feature of its pharmacodynamics.

The superior in vitro activity of **1** relative to a number of other antifolates tested in the clinic over the last 10 years prompted us to undertake a series of structure optimization studies in which the molecule was formally dissected into five regions as shown in Figure 1. As a result of these studies, it was determined that (i) N^5 and/or N^8 could be replaced by carbon (region A);[7] (ii) N^{10} could also be replaced by carbon (region B);[8] (iii) the 1′,4′-substituted phenyl moiety could be 3′,5′-dichlorinated or replaced by a 1′,4′-substituted naphthyl moiety (region C);[8] (iv) the optimal number of CH_2 groups in the amino acid side chain was three (region D);[8−10] and (v) ortho substitution was preferred over meta or para substitution on the phthaloyl moiety (region E).[9]

The improved activity of the 5,8-dideaza- and 10-deaza analogues of **1** relative to the parent drug led us to speculate that replacement of all three nitrogen atoms (i.e., N^5, N^8, and N^{10}) by carbon might represent an optimal modification of regions A and B. Accordingly, we embarked on the synthesis of the previously undescribed compound N^{α}-[4-[2-(2,4-diaminoquinazolin-6-yl)ethyl]benzoyl]-N^{δ}-hemiphthaloyl-L-ornithine (**2**), which can be viewed as a nonpolyglutamatable analogue of 5,8,10-trideazaaminopterin (**3**).[11] In addition, because we had earlier synthesized the 10-ethyl-10-deaza derivative **4**[8] as a nonpolyglutamatable analogue of 10-ethyl-10-deazaaminopterin (edatrexate, EDX, **5**),[12a,b] we also prepared N^{α}-[4-[5-(2,4-diaminoteridin-6-yl)pent-1-yn-4-yl)]benzoyl]-N^{δ}-hemiphthaloyl-L-ornithine (**6**), a nonpolyglutamatable analogue of 10-propargyl-10-deazaaminopterin (PDX, **7**).[13a−d] Because of a better pharmacologic and therapeutic profile, **7** has recently been undergoing clinical trial as a potential successor for **5**. The structures of **2**−**7** are shown in Figure 3.

Results and Discussion

Retrosynthetic analysis suggested that a reasonable approach to the preparation of **2** would be via a Pd(0)-catalyzed coupling reaction between the previously undescribed compounds methyl L-2-(4-iodobenzamido)-5-phthalimidopentanoate (**8**) and 2,4-diamino-6-ethinylquinazoline (**9**). As an alternative route, Pd(0)-catalyzed coupling could also be carried out between methyl L-2-(4-ethinylbenzamido)-5-phthalimidopentanoate (**10**) and 2,4-diamino-6-iodoquinazoline (**11**) as had been done earlier in the published synthesis of **3**.[11] The structures of **8**−**11** are shown in Figure 4. After considering the merits of both routes, however, we chose the former because **9** had not, to our knowledge, been used before as a building block for the synthesis of DHFR inhibitors.

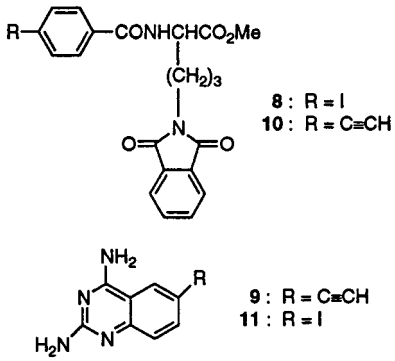
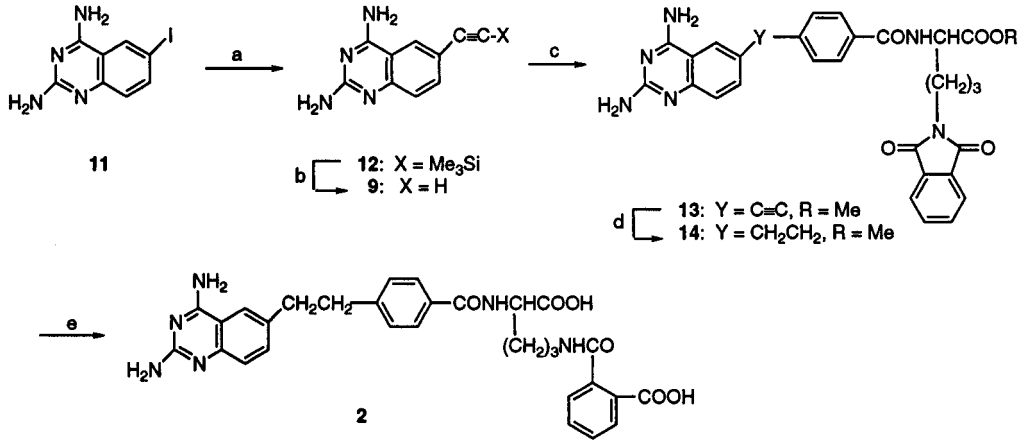


Figure 4. Structures of compounds **8**−**11**.

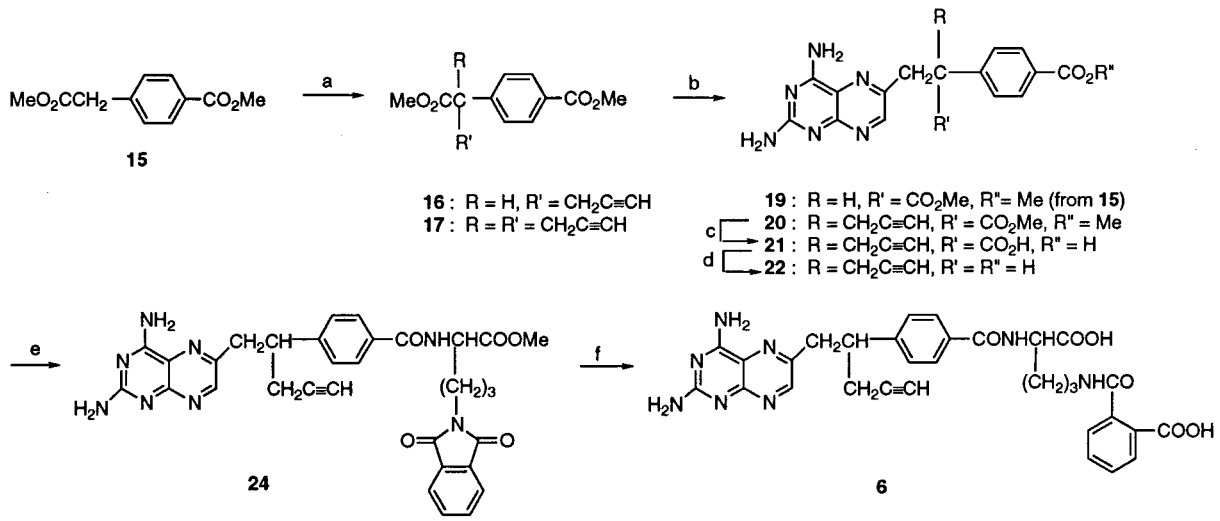
As shown in Scheme 1, access to **9** was conveniently achieved by reaction of **11** with trimethylsilylacetylene to obtain 2,4-diamino-6-(trimethylsilylethinyl)quinazoline (**12**), followed by desilylation with tetrabutylammonium fluoride (TBAF). The overall two-step yield of **9** from **11** was 62%. Compound **11** was obtained in 40% overall yield from 2-aminobenzonitrile by regioselective iodination with iodine monochloride, followed by annulation with chloroformamidine hydrochloride as described.[11,14] The synthesis of **8** was accomplished by straightforward mixed anhydride chemistry (*i*-BuOCOCl/Et_3N/DMF) from methyl L-2-amino-5-phthalimidopentanoate[9] and 4-iodobenzoic acid. A second Pd(0)-catalyzed reaction, this time between **8** and **9**, led to the ethinyl adduct **13**, which on catalytic hydrogenation (H_2/Pd-C) yielded **14**. Because of the formation of several unidentified side products during the second coupling reaction, purification of **13** by silica gel chromatography required careful monitoring of individual eluent fractions by TLC. The presence of these side products resulted in a yield of **13** that was somewhat low (33% versus 67% in the case of **12**). Whether this was due to the fact that the two coupling reactions used different iodide-alkyne pairs or different Pd catalysts and amine bases was not determined. To complete the synthesis, the triple bond was reduced catalytically, the methyl ester was cleaved, and the phthalimide ring opened in a single step with $Ba(OH)_2$ in wet MeOH, affording **2** as a tetrahydrate after preparative reversed-phase HPLC (C_{18} silica gel) followed by ion-exchange chromatography (DEAE-cellulose). The ^1H NMR spectrum of **2** contained the required signals for the CH_2CH_2 bridge and the C7 and C5 protons. Other peaks were appropriately assigned to the C8 proton, the A_2B_2 pattern on the phenyl ring, the ornithine aliphatic protons, and the protons of the hemiphthaloyl group. That the phthaloyl ring was in the open form was confirmed from the chemical shift of the aromatic protons, which in

Scheme 1[a]



[a] Reagents: (a) $Me_3SiC{\equiv}CH/Pd(OAc)_2/(2\text{-tolyl})_3P$, CuI, piperidine/DMF; (b) TBAF; (c) methyl L-2-(4-iodobenzamido)-5-phthalimidopentanoate (**8**)/$(Ph_3P)_4Pd$, CuI, Et_3N, DMF; (d) H_2/5% Pd−C; (e) $Ba(OH)_2$, MeOH−H_2O.

Scheme 2[a]

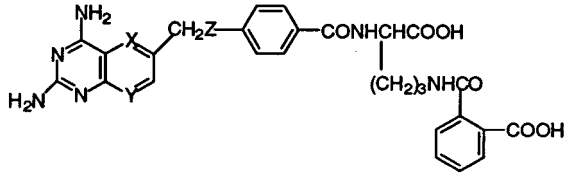


[a] Reagents: (a) $BrCH_2C{\equiv}CH$/NaH/THF; (b) 2,4-diamino-6-bromomethylpteridine hydrobromide (**18**·HBr)/NaH/DMF; (c) NaOH/$MeOCH_2CH_2OH$; (d) 120 °C/DMSO; (e) methyl L-2-amino-5-phthalimidopentanoate (**23**)/*i*-BuOCOCl/DMF; (f) $Ba(OH)_2$/aq MeOH.

characteristic fashion was at higher field than that of the closed form. A notable aspect of the spectrum of **2**, and of its ester **14**, was the consistently less deshielded character of the aromatic protons around the CH_2CH_2 bridge (i.e., C5−H, C7−H, and 3′,5′-H) in comparison with those around the C≡C bridge in **13**, which presumably reflects the linearity imposed on the latter by the triple bond.

The synthesis of **6** (Scheme 2) was patterned after the one used earlier[13a,b] to prepare **7** and recently adapted in our laboratory,[8] with small experimental modifications. Alkylation of the Na carbanion of dimethyl homoterephthalate (**15**) with propargyl bromide in THF solution as described was found to give mainly the desired monopropargyl derivative **16**. However, even though we replicated as closely as possible the process described in the literature,[13ab] we were unable to effect complete consumption of the starting material **15** or eliminate the formation of a small amount of the unwanted dipropargyl derivative **17** as judged by careful examination of mass spectral and ^1H NMR data. Because the separation of these compounds by silica gel chromatography on a multigram scale proved quite difficult, the crude mixture of **15**−**17** was used for the next step with the assumption that only **15** and **16** would be able to undergo further C-alkylation. A solution of the esters was added dropwise to a suspension of NaH in dry DMF kept at −5 to 0 °C, and the resulting mixture of carbanions was treated dropwise with a solution of the 2,4-diamino-6-bromomethylpteridine hydrobromide (**18**·HBr)[15] in DMF while carefully maintaining the temperature at −25 to −30 °C. The reaction was allowed to proceed for a total of 6 h while allowing the flask to warm first to −10 °C and then to room temperature. Column chromatography of the product on silica gel easily allowed **17** to be separated from the slower-moving alkylated products, **19** and **20**. However, separation of the latter two compounds was more difficult, requiring careful monitoring of small fractions of the eluent from the column to be monitored by TLC (cf. Experimental Section). The purified propargyl derivative **20** was hydrolyzed to the diacid **21** with 10% NaOH in 2-methoxyethanol, and the latter was converted to the key intermediate **22** by heating in DMSO at 120 °C for 30 min. Careful monitoring by HPLC showed that the hydrolysis step required 24 h, and that the decarboxylation required 30 min. For reasons we are unable to explain, both the hydrolysis and decar-

Table 1. Cell Growth Inhibition and DHFR Inhibition by PT523 Analogues Modified in the B-Ring and/or 9,10-Bridge



cmpd	X	Y	Z[a]	cell growth IC_{50} (nM, 72 h)[b,c]	DHFR binding K_i (pM)[b,d]
1 (PT523)	N	N	NH	1.5 ± 0.39	0.33 ± 0.04[e]
2	CH	CH	CH_2	0.69 ± 0.04	0.21 ± 0.05
4[f]	N	N	CHEt	1.2 ± 0.25	0.62 ± 0.10
6	N	N	CHPg	1.3 ± 0.35	0.60 ± 0.02
25	CH	CH	NH	0.64 ± 0.04	0.014 ± 0.005[g]
26	N	N	CH_2	0.53 ± 0.07	0.35 ± 0.06[f]

[a] Pg = propargyl. [b] Numbers are means ± SD for a minimum of three independent determinations. [c] Assays were carried out as in ref 8. [d] Assays were carried out as in ref 16. [e] A K_i of 0.35 ± 0.13 pM (n = 3) was reported previously for **1** in several earlier papers.[6b,7−10,16] [f] Data for compound **4** are taken from ref 6b. [g] K_i values we reported originally for **25** and **26** in ref 6b were 0.09 ± 0.03 pM and 0.18 ± 0.02 pM, respectively. The values listed here were obtained after repeating the assays with a more recently obtained batch of purified enzyme.

boxylation of **20** were found to be slower than reported by DeGraw and co-workers.[13a] However, the outcome was similar, with the overall two-step yield of **22** from **20** approaching 40%. To complete the synthesis, **22** was condensed with methyl L-2-amino-5-phthalimidopentanoate (**23**).[10] The resulting phthalimide **24** was purified by column chromatography on silica gel and was then treated with $Ba(OH)_2$ in aqueous MeOH to obtain **6** as a dihydrate. The presence of a propargyl group in **22**, **24**, and **6** was confirmed by the ^{1}H NMR spectra, which all featured a broad singlet at δ 2.7−2.8 corresponding to the acetylene proton. The broadness of this signal indicated that the product was likely to be a mixture of 10*R* and 10*S* diastereomers, as in the 10-methyl-10-deaza and 10-ethyl-10-deaza analogues we had made earlier.[8] The yield of **24** from **21** was only 13% despite the fact that two cycles of activation and amine addition were used. In the reported condensation of **21** with diethyl L-glutamate by DeGraw and co-workers,[13a] a total of three cycles were used, and the yield was 55%. While it is possible that a higher conversion of **21** to **24** would have been achieved if three or more rounds had been used, this was not investigated.

The ability of the 5,8,10-trideaza analogue **2** and the 10-propargyl-10-deaza analogue **6** to inhibit the growth of CCRF-CEM human leukemic lymphoblasts in culture during 72 h of continuous drug exposure was determined and compared with the results we had previously obtained with other analogues of **1** modified in the B-ring[7] or 9,10-bridge.[8] As shown in Table 1, the IC_{50} of **2** was 0.69 ± 0.04 nM as compared with 1.5 ± 0.39 nM for **1**, 0.64 ± 0.04 nM for the 5,8-dideaza analogue **25**, and 0.53 ± 0.07 nM for the 10-deaza analogue **26**. Thus, somewhat surprisingly, the effect of replacing N^{10} as well as N^5 and N^8 by carbon was not significantly different than that of either replacing N^5 and N^8 by carbon or replacing N^{10} by carbon. While we had hoped that the combined effect of these modifications might be greater than that of either one alone, it seems clear from these results that replacing all three nitrogen atoms by carbon does not, in fact, lead to any further increase in potency relative to **1**. In the case of the mixed diastereomers of 10-propargyl-10-deaza analogue **6**, the IC_{50} was found to be 1.3 ± 0.35 nM as compared with a previously obtained value of 1.2 ± 0.25 nM for **4** and 3.3 ± 0.36 nM for **5**.[8] It thus appears that, while the mixed diastereomers of **6** are somewhat more active than those of **5**, they are not significantly different from those of **4** under the conditions of our assay.

In addition to the cytotoxicity studies, we also examined the ability of **2** and the mixed diastereomers of **6** to inhibit reduction of dihydrofolate by NADPH in the presence of purified human recombinant DHFR. Using the same spectrophotometric assay procedure we used earlier to obtain K_i values for **1**[16] and other compounds in this series,[2b] we determined the K_i of **2** to be 0.21 ± 0.05 pM as compared with 0.014 ± 0.005 pM for **25** and 0.35 ± 0.06 pM for **26**. We conclude from these results that, while **2** is clearly a better DHFR inhibitor than **1**, replacement of N^5 and N^8 by carbon does little or nothing to enhance enzyme binding relative to **26**, whereas replacement of nitrogen by carbon at the 10-position actually leads, somewhat suprisingly, to a 2-fold decrease in competitive inhibition relative to **25**. In the case of **6**, a K_i of 0.60 ± 0.02 pM was obtained that was indistinguishable from that of the 10-ethyl analogue **4**. This result differed somewhat from what had been reported previously for the glutamate analogues **5** and **7**, for which there was as much as a 4-fold difference in K_i depending on the source of the enzyme and the pH of the assay.[13a,c] A possible explanation of the contrasting results between **4** and **6** versus and **5** and **7** is that the hemiphthaloylornithine derivatives come closer to being true stoichiometric inhibitors than the glutamate analogues, and thus may be a little less sensitive to minor changes in molecular structure.

In summary it would appear, on the basis of the results reported here for **2** and the diastereomeric mixture **6**, that further modification of **1** in the 9,10-bridge alone (region B in Figure 1) is not likely to yield significant changes in DHFR binding or cell growth inhibition. However, the possibility that other analogues of **1** with changes in more than one of the regions A, B, and C may be more active has not been ruled out and is being investigated in our laboratory.

Experimental Section

IR spectra were obtained on a Perkin-Elmer 281 double-beam spectrophotometer and UV spectra on a Perkin-Elmer 35 UV/visible instrument. Only IR peaks with ν values greater than 1200 cm^{-1} are listed; weak peaks and shoulders are omitted. ^{1}H NMR spectra were recorded at 200 MHz with a Varian VX200 instrument. Amino group protons did not always give rise to a well-defined signal and thus are in some instances omitted. Mass spectra were recorded in the Molecular Biology Core Facility of the Dana-Farber Cancer Institute using a Perkin-Elmer Voyager 4036 system (low-resolution) and in the Mass Spectrometry Laboratory, Department of Chemistry and Chemical Biology, Harvard University, using a MicroMass LCT instrument in the electrospray mode (high-resolution). TLC analyses were performed on Whatman MK6F silica gel plates, and spots were visualized under 254 nm illumination. The term "NH_4OH" in the experimental description refers to a solution of ca. pH 9 as determined with universal indicator paper. Column chromatography was done on Baker silica gel (regular grade, 60−200 mesh; flash grade, 40 μm particle size) or Whatman DEAE cellulose (DE-52).

Moisture-sensitive reactions were carried out in solvents that were of Sure-Seal grade (Aldrich, Milwaukee, WI), or had been stored over Linde 4A molecular sieves. HPLC separations were on C_{18} radial compression cartridges (Millipore, Milford, MA; analytical, 5 μm particle size, 5 × 100 mm; preparative, 15 μm particle size, 25 × 100 mm). Solids were generally dried at 50−80 °C over P_2O_5 in a drying apparatus attached to a vacuum pump. Melting points (not corrected) were determined on a Fisher-Johns hot-stage microscope or in open Pyrex capillary tubes in a Mel-Temp apparatus (Cambridge Laboratory Devices, Cambridge, MA). 2,4-Diamino-6-iodoquinazoline (**11**),[14] 2,4-diamino-6-bromomethylpteridine (**18**·HBr),[15] and methyl 2-L-amino-5-phthalimidopentanoate (**23**·HCl)[10] were prepared according to the literature. Other chemicals were purchased from Aldrich, Milwaukee, WI, or Fisher, Boston, MA. Microanalyses were performed by Robertson Laboratory, Madison, NJ, or Quantitative Technologies, Whitehouse, NJ, and were within ±0.4% of theory unless otherwise specified. The presence of fractional molar amounts of organic solvents such as $CHCl_3$ in analytical samples of lipophilic antifolate intermediates, even after overnight drying in vacuo at 60 °C, was confirmed by ^{1}H NMR whenever possible.

2,4-Diamino-6-(trimethylsilylethinyl)quinazoline (12). $Me_3SiC{\equiv}CH$ (4.91 g, 50 mmol) was added dropwise at room temperature to a mixture of **11** (2.86 g, 10 mmol), (*o*-tolyl)$_3$P (121 mg, 0.4 mmol), palladium(II) acetate (45 mg, 0.2 mmol), CuI (40 mg), and piperidine (8 mL) in dry DMF (12 mL), and the mixture was stirred under argon for 24 h. The DMF was evaporated under reduced pressure, and the residue was shaken with a mixture of NH_4OH (150 mL) and Et_2O (50 mL), then filtered and washed with H_2O until the washings were colorless. The residue was taken up in a minimum amount of 10:1:0.1 $CHCl_3$−MeOH−NH_4OH (system A), and the solution was applied onto a flash silica gel column packed and eluted with the same solvent. Fractions giving a single TLC spot with R_f 0.30 (silica gel, system A) were pooled and evaporated to obtain **12** as a yellow solid (1.73 g, 67%); mp >240 °C, with darkening above 200 °C; IR (KBr) ν 3340, 3260, 2950, 2160, 1650, 1640, 1620, 1550, 1500, 1480, 1450, 1370, 1320, 1280, 1250 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.22 (s, 9H, Me_3Si), 6.18 (br s, 2H, NH_2), 7.07−7.11 (d, 1H, C8−H), 7.34 (br s, 2H, NH_2), 7.42−7.47 (dd, 1H, C7−H), 8.15−8.16 (d, 1H, C5−H); MS *z*/*e* 257, calcd (M + 1) 257. Anal. ($C_{13}H_{16}N_4Si$·0.1H_2O) C, H, N.

2,4-Diamino-6-ethinylquinazoline (9). A 1 M solution of TBAF in THF (4.3 mL, 4.3 mmol) was added to a solution of **12** in THF (30 mL) under argon, and the mixture was stirred at room temperature for 24 h. The precipitated solid was filtered, washed with THF, and dried. The solid was then taken up in a minimum volume of system A, and the solution was applied onto a flash silica gel column which was packed and eluted with the same mixture. Fractions giving a single spot with R_f 0.24 (silica gel, system A) were pooled and evaporated to obtain a pale-yellow solid (0.71 g, 93%); mp >240 °C, darkening above 200 °C; IR (KBr) ν 3460, 3250, 3100, 2100, 1660, 1640, 1620, 1560, 1500, 1480, 1450, 1400, 1370, 1290, 1200 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 4.05 (s, 1H, C≡CH), 6.15 (br s, 2H, NH_2), 7.09−7.13 (d, 1H, C8−H), 7.34 (br s, 2H, NH_2), 7.45−7.50 (dd, 1H, C7−H), 8.16 (d, 1H, C5−H). MS *z*/*e* 185, calcd (M + 1) 185. Anal. ($C_{10}H_8N_4O$·0.1H_2O) C, H, N.

Methyl 2-L-(4-Iodobenzamido)-5-phthalimidopentanoate (8). To a solution of 4-iodobenzoic acid (3 g, 12.1 mmol) in dry DMF (50 mL) at room temperature were added Et_3N (5 g, 50 mmol) and *i*-BuOCOCl (1.65 g, 12.1 mmol), followed 1 h later by **23**·HCl (3.77 g, 12.1 mmol). Stirring at room temperature was continued for 16 h, the solvent was evaporated under reduced pressure, the residue was taken up in $CHCl_3$, and the solution was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was then taken up in 95:5 $CHCl_3$−MeOH (system B) and applied onto a column of silica gel (flash grade) which was packed and eluted with appropriate volumes of 98:2 $CHCl_3$−MeOH followed by system B. Fractions giving a single TLC spot with R_f 0.66 (silica gel, system B) were pooled and evaporated to obtain **8** as a white solid (3.78 g, 62%); mp 180−181 °C; IR (KBr) ν 3310, 2950, 1745, 1710, 1640, 1580, 1520, 1400, 1360, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.85−2.02 (m, 4H, β- and γ-CH_2), 3.60−3.73 (m, 2H, δ-CH_2), 3.77 (s, 3H, COOMe), 4.85 (m, H, α-CH), 6.80 (br s, 1H, NH), 7.52−7.56 (m, 2H, 2′- and 6′-H), 7.78−7.82 (m, 2H, 3′- and 5′-H), 7.69−7.84 (m, 4H, phthalimide ring); MS *z*/*e* 507, calcd (M + 1) 507. Anal. ($C_{21}H_{19}IN_2O_5$) C, H, N.

Methyl 2-L-[4-[2-(2,4-Diaminoquinazolin-6-yl)ethinyl]-benzamido]-5-phthalimidopentanoate (13). To a solution of **9** (300 mg, 1.63 mmol) in dry DMF (18 mL) were added **8** (916 mg, 1.81 mmol), $(Ph_3P)_4Pd(0)$ (188 mg, 0.16 mmol), CuI (12 mg), and Et_3N (0.3 mL). The mixture was stirred under argon at room temperature for 24 h, the solvent was evaporated under reduced pressure, and the residue was washed with H_2O, redissolved in a minimum volume of 10:1 $CHCl_3$−MeOH (system C), and chromatographed on a silica gel column which was packed and eluted with system C. Separation was monitored by TLC using 10:1:0.1 $CHCl_3$−MeOH−Et_3N (system D). Fractions giving a single spot with R_f 0.3 were pooled and evaporated to obtain **13** as a yellow solid (0.3 g, 33%); mp >150 °C dec; IR (KBr) ν 3350, 3200, 2940, 2200, 1700, 1640, 1600, 1560, 1500, 1440, 1400, 1220 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.59−1.79 (m, 4H, β- and γ-CH_2), 3.62 (m, 5H, COOMe and δ-CH_2), 4.45 (m, 1H, α-CH), 7.05 (br s, 2H, NH_2), 7.30−7.34 (d, 1H, C8−H), 7.60−7.64 (m, 2H, 3′- and 5′−H), 7.73−7.78 (m, 1H, C7−H), 7.82−7.86 (m, 4H, phthalimide ring), 7.87−7.92 (m, 2H, 2′- and 6′-H), 8.14 (br s, 2H, NH_2), 8.39 (d, 1H, C5−H), 8.60 (d, 1H, CONH). Anal. ($C_{31}H_{26}N_6O_5$·0.3$CHCl_3$) C, H, N.

Methyl 2-L-[4-[2-(2,4-Diaminoquinazolin-6-yl)ethyl]-benzamido]-5-phthalimidopentanoate (14). A solution of **13** (100 mg, 0.18 mmol) in a mixture of CH_2Cl_2 (20 mL) and MeOH (20 mL) was shaken under H_2 (50 lb/in^2) in the presence of 5% Pd-C (120 mg) in a Parr apparatus for 18 h. The reaction was filtered (Celite), the filter cake was washed with MeOH, and the combined filtrate and wash solution were evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel packed and eluted with system C. Fractions giving a single TLC spot with R_f 0.22 (silica gel, system D) were pooled and evaporated to obtain **14** as a pale-yellow solid (62 mg, 62%); mp 138−139 °C; IR (KBr) ν 3350, 3200, 2950, 1710, 1650, 1630, 1560, 1540, 1500, 1440, 1400 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.69−1.77 (m, 4H, β- and γ-CH_2), 2.98 (m, 4H, C9−C10 bridge), 3.60 (m, 5H, COOMe and δ-CH_2), 4.43 (m, 1H, α-CH), 7.28−7.32 (m, 3H, C8−H, 3′- and 5′-H), 7.40 (br s, 2H, NH_2), 7.56−7.62 (m, 1H, C7−H), 7.73−7.78 (m, 2H, 2′- and 6′-H), 7.82−7.86 (m, 4H, phthalimide ring), 8.06 (d, 1H, C5−H), 8.50 (br s, 2H, NH_2), 8.80 (d, 1H, CONH); MS *z*/*e* 567, calcd (M + 1) 567. Anal. ($C_{31}H_{30}N_6O_5$·1.3$CHCl_3$) C, H, N.

***N*$^{\alpha}$-[4-[2-(2,4-Diaminoquinazolin-6-yl)ethyl]benzoyl]-*N*$^{\delta}$-hemiphthaloyl-L-ornithine (5,8,10-Trideaza-PT523) (2).** Solid $Ba(OH)_2$ (56 mg, 0.17 mmol) was added to a stirred solution of **14** (50 mg, 0.088 mmol) in MeOH (30 mL) and H_2O (30 mL), and the mixture was stirred at room temperature for 48 h, followed by addition of solid NH_4HCO_3 (100 mg), and continued stirring for 30 min. The $BaCO_3$ precipitate was removed and the solvent evaporated under reduced pressure. The residue was taken up in H_2O and the solution freeze-dried to a white solid. Preparative HPLC (C_{18} silica gel, 12% MeCN in 0.1 M NH_4OAc, pH 7.5, 10 mL/min), followed by anion-exchange chromatography (DEAE-cellulose, extensive H_2O wash, then 0.2 M NH_4HCO_3), afforded analytically pure **2** as a white solid (20 mg, 40%); mp >200 °C dec, with softening ca. 180 °C; HPLC (as above except that the flow rate was 1 mL/min) 32 min; IR (KBr) ν 3340, 3200, 2950, 1650, 1600, 1530, 1490, 1380 cm^{-1}; UV (pH 7.4) λ_{max} 233 nm (ϵ 55950), 323 (4630); ^{1}H NMR (DMSO-d_6) δ 1.70−1.95 (m, 4H, β- and γ-CH_2), 2.89 (m, 4H, C9−C10 bridge), 3.25 (m, 2H, δ-CH_2), 4.37 (m, 1H, α-CH), 7.09−7.17 (m, 3H, C8−H, 3′- and 5′-H), 7.33−7.38 (m, 4H, phthaloyl ring), 7.55 (m, 1H, C7−H), 7.76−7.80 (m, 2H, 2′- and 6′-H), 7.86 (m, 1H, C5−H), 8.04 (br s, 2H, NH_2), 8.34 (br m, 3H, NHCO and NH_2); MS (high-resolution) *z*/*e* 571.2292, calcd (M + 1) 571.2305. Anal. ($C_{30}H_{30}N_6O_6$·4H_2O) C, N; H: calcd, 5.97; found, 5.48.

Methyl 2-L-[[5-(2,4-Diaminopteridin-6-yl)pent-1-yn-4-yl)]benzamido]-5-phthalimidopentanoate (24). Step 1. A solution of **15** (3.1 g, 15.1 mmol) in dry THF (4.5 mL) was added to a suspension of NaH (60% in mineral oil, 0.66 g, 16.6 mmol) in dry THF (11 mL) which had been precooled to 0 °C in an ice-bath. Propargyl bromide (1.85 g, 16.6 mmol) was then added, and stirring was continued at 0 °C for 30 min, then at room temperature for 16 h. The reaction mixture was quenched with 50% AcOH (1.5 mL) and poured into H_2O (200 mL). Extraction with Et_2O (3 × 100 mL), drying ($MgSO_4$), and evaporation gave an oil, which was applied onto a silica gel column for flash chromatography using appropriate volumes of 95:5, 94:6, 93:7, and 90:10 hexanes−EtOAc as eluents. Fractions were carefully monitored by TLC, and those giving a single spot with R_f 0.35 (9:1 hexanes−EtOAc) were pooled and evaporated to obtain a white solid (2.5 g). Although this solid consisted mainly of the monopropargyl derivative **16** according ^{1}H NMR and MS analysis, small amounts of unchanged **15** and the dipropargyl derivative **17** were still evident. Nonetheless, this material was taken directly to the next step.

Step 2. A stirred suspension of NaH (60% in mineral oil, 488 mg, 12.2 mmol) in dry DMF (10 mL) was cooled to −5 °C and treated dropwise with a solution of impure **16** from several pooled batches (3 g, assumed to contain 12.2 mmol) in dry DMF (10 mL). The reaction mixture was stirred at 0 °C for 30 min, then cooled to between −25 °C and −30 °C (dry ice/ acetone bath) while a solution of **18**·HBr (1.5 g, 4.0 mmol) in dry DMF (20 mL) was added dropwise. The temperature was raised to −10 °C over 2 h, and stirring at −10 °C was continued for another 2 h. The mixture was then allowed to come to room temperature and stirred for a final 2 h. The pH was adjusted to 7 with small pieces of dry ice, the solvent was evaporated under high vacuum, and the residue was washed with EtOAc and then with H_2O to obtain a yellowish-brown powder, which was chromatographed on silica gel with 96:4 $CHCl_3$−MeOH as the eluent. Evaporation of fractions containing a single spot (TLC: R_f 0.46, silica gel, 9:1 $CHCl_3$−MeOH, system E) afforded **20** as a yellow solid (0.79 g, 46%); mp >200 °C, darkening above 170 °C; IR (KBr) ν 3450, 3280, 3100, 2950, 1715, 1620, 1550, 1440, 1280 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.84 (m, 1H, C≡CH), 2.84−2.93 (m, 2H, CH_2C≡CH), 3.59 (s, 3H, aliphatic COOMe), 3.72 (m, 2H, 9-CH_2), 3.83 (s, 3H, aromatic COOMe), 7.5−7.9 (m, 4H, phenyl protons), 8.45 (s, 1H, C7−H); MS *z*/*e* 421, calcd (M + 1) 421.

A slightly slower-moving, but resolvable band on the silica gel column was also isolated and identified as the nonpropargylated compound **19**; TLC: R_f 0.44 (silica gel, system E); MS *z*/*e* 383; calcd (M + 1), 383.

Step 3. To a suspension of **20** (0.78 g, 1.9 mmol) in 2-methoxyethanol (10 mL) was added H_2O (10 mL) followed by 10% NaOH (7.8 mL). The mixture was stirred at room temperature for 24 h, while monitoring the progress of the reaction by HPLC (C_{18} silica gel, 1% to 25% MeCN in 0.1 M NH_4OAc, pH 7.5, 30 min gradient, 1 mL/min). Analysis after 4 h showed the diacid **21** as a peak at 16 min [MS: *z*/*e* 393, calcd (M + 1), 393] and a second product, probably consisting of a mixture of monoacids, as a slower peak at 26 min [MS: *z*/*e* 407, calcd (M + 1) 407]. After 24 h, when only the fast peak could be seen, AcOH was added dropwise until the pH decreased to ca. 8. The solvents were removed under high vacuum, the residue was taken up in H_2O (25 mL), the solution was cooled in an ice-bath, and the pH was adjusted to 5.5 with 6 N HCl. The precipitate was collected, washed with H_2O, and dried in vacuo to obtain **21** as a beige solid (470 mg, 64%); mp >200 °C dec, darkening above 180 °C; IR (KBr) ν 3350, 2950, 1640, 1550, 1490, 1420, 1350, 1250 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.82 (m, 1H, C≡CH), 2.73−2.97 (m, 2H, CH_2C≡CH), 3.67 (m, 2H, 9-CH_2), 6.66 (br s, 2H, NH_2), 7.5−7.9 (m, 4H, phenyl protons), 8.45 (s, 1H, C7−H); MS *z*/*e* 393, calcd (M + 1) 393. An additional 250 mg of **21** was recovered by lyophilization of the mother liquor, and the two batches were combined and used directly in the next step.

Step 4. Compound **21** (750 mg, 1.9 mmol) was dissolved in DMSO (15 mL), and the solution was immersed in an oil bath preheated to 120 °C. The progress of the decarboxylation was monitored by HPLC (C_{18} silica gel, 1% to 25% MeCN in 0.1 M NH_4OAc, pH 7.5, 30 min gradient, 1 mL/min). The peak corresponding to **21**, at 16 min, was gradually replaced by a slower peak at 26 min. After 30 min, the reaction mixture was cooled to room temperature, and the solvent was removed under high vacuum. The residue was taken up in dilute NH_4-OH, and the solution was cooled in an ice-bath and acidified to pH 4 with 6 N HCl. The precipitated solid was filtered, washed with H_2O, and dried in vacuo to obtain **22** as a yellow powder (400 mg, 60%); mp >200 °C dec; IR (KBr) ν 3450, 3280, 2950, 1630, 1545, 1440, 1370, 1260 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.78 (m, 1H, C≡CH), 2.59−2.78 (m, 2H, CH_2C≡CH), 3.1 (m, 2H, 9-CH_2), 3.66 (m, 1H, C10−H), 6.52 (br s, 2H, NH_2), 7.38 (m, 3′- and 5′-H), 7.81 (m, 2H, 2′- and 6′-H), 8.37 (s, 1H, C7−H); MS: *z*/*e* 349, calcd (M + 1) 349. This material was used directly in the next step.

Step 5. *i*-BuOCOCl (235 mg, 1.72 mmol) was added to a solution of **22** (300 mg, 0.86 mmol) and Et_3N (353 mg, 3.5 mmol) in dry DMF (20 mL), and the solution was stirred at room temperature for 1 h, followed by addition of **23**·HCl (536 mg, 1.72 mmol). Stirring was continued for 2 h, followed by addition of a second portion of *i*-BuOCOCl (117 mg, 0.86 mmol) and, after 30 min, a second portion of **23**·HCl (268 mg, 0.86 mmol). The reaction mixture was then stirred for 20 h, whereupon the solvent was removed under high vacuum, and the residue was washed with H_2O and chromatographed on a silica gel column (20 g, 2 × 20 cm) packed and eluted with $CHCl_3$−MeOH (98:2, then 93:7). Fractions giving a single TLC spot with R_f 0.48 (silica gel, system E) were evaporated, the residue was redissolved in a small volume of system E, and the solution was added to excess Et_2O. The precipitated solid was isolated by centrifugation, washed with Et_2O, and dried in a vacuum oven at 60 °C to obtain **24** as a yellow powder (67 mg, 13%); mp 145 °C dec; IR (KBr) ν 3460, 3360, 3280, 2950, 1760, 1710, 1700, 1600, 1550, 1520, 1430, 1400, 1350, 1200 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.67−1.74 (m, 4H, β- and γ-CH_2), 2.76 (m, 1H, C≡CH), 2.56−2.74 (m, 2H, CH_2C≡CH), 3.17 (m, 2H, 9-CH_2), 3.58 (m, 6H, COOMe, δ-CH_2, C10−H), 4.38 (m, 1H, α-CH), 6.5 (br s, 2H, NH_2), 7.35 (m, 2H, 3′- and 5′-H), 7.69 (m, 4H, 2′- and 6′-H, NH_2), 7.80−7.85 (m, 4H, phthalimide ring), 8.38 (d, 1H, C7−H), 8.6 (d, 1H, CONH); MS *z*/*e* 607, calcd (M + 1) 607. Anal. ($C_{32}H_{30}N_8O_5$·0.6H_2O) C, H, N.

***N*$^{\alpha}$-[4-[5-(2,4-Diaminopteridin-6-yl)pent-1-yn-4-yl]benzoyl]-*N*$^{\delta}$-hemiphthaloyl-L-ornithine (10-propargyl-10-deaza-PT523) (6).** Compound **24** (30 mg, 49 mmol) was dissolved in MeOH (20 mL) to which were then added H_2O (20 mL) and $Ba(OH)_2$ (46 mg, 1.46 mmol). The mixture was stirred at room temperature for 24 h, treated with NH_4HCO_3 (100 mg), and stirred for another 30 min. The $BaCO_3$ precipitate was removed, and the solvents were evaporated under reduced pressure. The residue was taken up in a small volume of H_2O, and the solution lyophilized. The resulting product was dried in vacuo at 60 °C to obtain **6** as a yellow solid (26 mg, 87%); mp >180 °C dec; HPLC 25.5 min (C_{18} silica gel, 1% to 25% MeCN in 0.1 M NH_4OAc, pH 7.5, 30 min gradient, 1 mL/min); IR (KBr) ν 3400, 2950, 1635, 1530, 1500, 1440, 1370, 1300, 1210 cm^{-1}; UV (pH 7.4) λ_{max} 254 (ϵ 37000), 371 (7050); ^{1}H NMR (DMSO-d_6) δ 1.58−1.92 (m, 4H, β- and γ-CH_2), 2.76 (m, 1H, C≡CH), 2.56−2.74 (m, 2H, CH_2C≡CH), 3.20 (m, 4H, 9-CH_2, δ-CH_2), 3.63 (m, 1H, 10-CH), 4.39 (m, 1H, α-CH), 6.58 (br s, 2H, NH_2), 7.40 (m, 2H, 3′- and 5′-H), 7.45−7.65 (m, 4H, phthaloyl ring), 7.60 (br s, 2H, NH_2), 7.76 (m, 2H, 2′- and 6′-H), 8.30 (m, 1H, phthaloyl CONH), 8.39 (d, 1H, C7−H), 8.47 (d, 1H, CONH); MS *z*/*e* 611, calcd (M + 1) 611. Anal. ($C_{31}H_{30}N_8O_6$·2H_2O) C, H, N.

Acknowledgment. This work was supported in part by Grants CA25394 and CA70349 from the National Cancer Institute (DHHS). Technical assistance in obtaining cytotoxicity data against CCRF-CEM cells was

provided by Dr. Ying-Nan Chen. Competitive DHFR inhibition constants were determined with the help of Mr. Gregory Yurasek.

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JM010518T