Synthesis and Antifolate Evaluation of the 10-Propargyl Derivatives of 5-Deazafolic Acid, 5-Deazaaminopterin, and 5-Methyl-5-deazaaminopterin

J. R. Piper,*,† N. D. Malik,† M. S. Rhee,‡ J. Galivan,‡ and F. M. Sirotnak§

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201, and Memorial Sloan-Kettering Cancer Center, New York, New York 10021. *Received June 10, 1991*

5-Deaza-10-propargylfolic acid (**4**), an analogue of the thymidylate synthase (TS) inhibitor 10-propargyl-5,8-dideazafolic acid (PDDF, **1**), was prepared via alkylation of diethyl *N*-[4-(propargylamino)benzoyl]-L-glutamate (**7**) by 2-amino-6-(bromomethyl)-4(3*H*)-pyrido[2,3-*d*]pyrimidinone (**15**). Bromomethyl intermediate **15** was prepared from the corresponding hydroxymethyl precursor **14** by treatment with 48% HBr. Hydroxymethyl compound **14** was obtained by deamination of reported 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-methanol (**12a**) in refluxing 1 N NaOH. Both **12a** and its 5-methyl-substituted analogue **12b** were converted to versatile 6-bromomethyl intermediates **13a** and **13b** from which important antifolates may be readily derived. Alkylation of **7** by **13a,b** led to 10-propargyl-5-deazaaminopterin (**5**) and 5-methyl-10-propargyl-5-deazaaminopterin (**6**). As an inhibitor of TS from H35F/F cells, **4** gave an IC_{50} value showing it to be approximately 6-fold less inhibitory than PDDF (90 nM for **4** vs 14 nM for PDDF). In in vitro studies, IC_{50} (μM) values obtained for **4** vs L1210 and S180 of 1.50 and 2.35, respectively, were similar to those obtained for PDDF (2.61 and 1.97). Against HL60 cells, **4** was about 7-fold more cytotoxic than PDDF (IC_{50} values 0.72 and 5.29 μM). Inclusion of thymidine did not establish TS as the site of cytotoxic action for either **4** or PDDF in the cell lines used. In in vivo tests against L1210 in mice, **4** failed to show therapeutic effect. The 2,4-diamino compounds **5** and **6** were as potent inhibitors of DHFR from L1210 cells as MTX and 7- and 35-fold, respectively, more inhibitory than MTX toward L1210 cell growth. In mediated influx into L1210 cells, **5** and **6** were transported 2.7- and 8.5-fold, respectively, more readily than MTX. Against the EO771 mammary adenocarcinoma in mice, **6** produced greater antitumor effect than MTX. A dose of 36 mg/kg per day for 5 days caused no toxic deaths while the average tumor volume among 10 mice was reduced to 8–9% of that of the control, and 20% of the test animals were rendered tumor free.

Interest in 10-propargyl derivatives of folate analogues stem from findings that 10-propargyl-5,8-dideazafolic acid (**1**, PDDF or CB 3717) has exceptional antitumor activity attributable to inhibition of thymidylate synthase (TS).[1,2] Early results from clinical trials were promising, but toxicity problems prompted searches for analogues having improved therapeutic effect.[3-5] These efforts have led to remarkably improved agents modified in the 2-position and in the side chain.[3-6]

We and other investigators have explored modifications in the heterocyclic ring portion of PDDF.[7-9] Our work[7] and that of Ghazala and co-workers[8] resulted in independent syntheses of the full pteridine analogue 10-propargylfolic acid (**2**). Brixner and co-workers[9] reported synthesis of 10-propargyl-8-deazafolic acid (**3**). In this paper we report synthesis of 10-propargyl-5-deazafolic acid (**4**) along with 2,4-diamino analogues 10-propargyl-5-deazaaminopterin (**5**) and 10-propargyl-5-methyl-5-deazaaminopterin (**6**).

Our synthesis of the full pteridine analogue **2** was readily achieved by alkylation of diethyl *N*-[4-(propargylamino)-benzoyl]-L-glutamate (**7**) with 2-amino-6-(bromomethyl)-4(3*H*)-pteridinone (**8**). The bromomethyl compound **8** is a valuable synthon for side-chain analogues of folic acid, particularly those (such as **2**) bearing functional groups incompatible with conditions required for hydrolytic deamination of the corresponding 2,4-diaminopteridines.[7] Ghazala and co-workers recognized the value of **8** in this connection and initially tried to prepare **2** using **8**, but they did not identify suitable reaction conditions for alkylation of **7** by **8** and thereby missed the facility of the synthesis. They succeeded in preparing **2** using the Boon–Leigh route.[8] The 8-deaza analogue **3** was prepared by Brixner and co-workers[9] via its diethyl ester which was obtained in two ways: treatment of **7** with crude 2-amino-6-(bromomethyl)-4(3*H*)-pyrido[3,4-*d*]pyrimidinone (**9**) gave the ester of **3** in low (6.5%) overall yield, but direct treatment of diethyl 8-deazafolate with propargyl

† Southern Research Institute.
‡ New York State Department of Health.
§ Memorial Sloan-Kettering Cancer Center.

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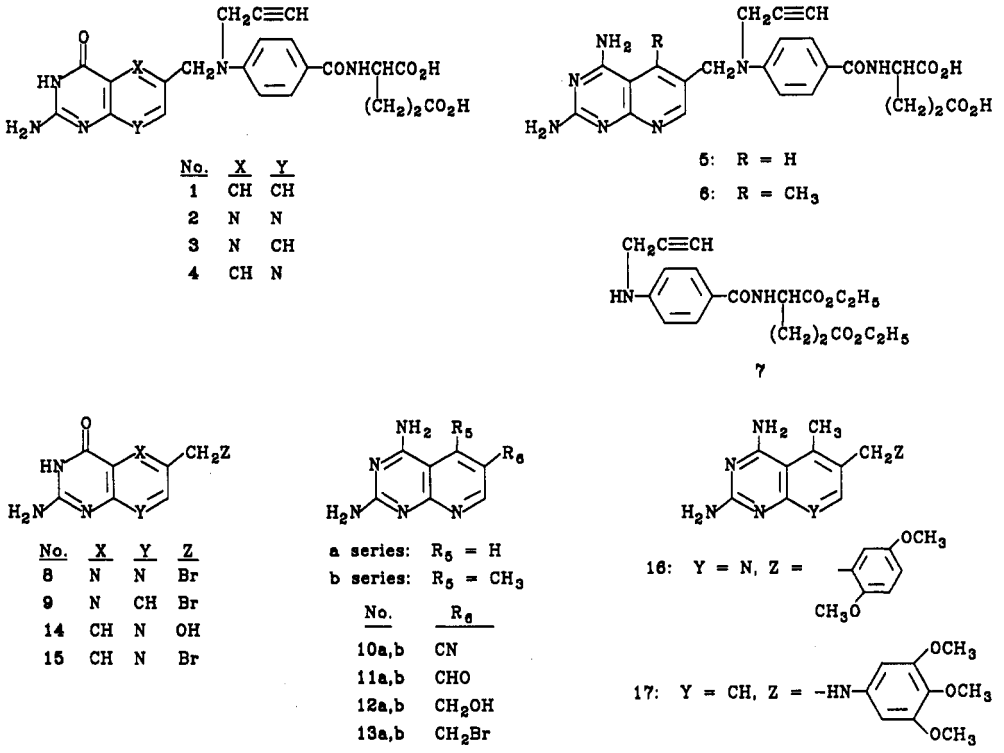
Scheme I



bromide afforded the 10-propargyl ester in 74% yield.

In the current work, 5-deaza analogue **4** and 2,4-diamino compounds **5** and **6** were prepared via previously reported 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-methanol (**12a**) and 2,4-diamino-5-methylpyrido[2,3-*d*]pyrimdine-6-methanol (**12b**). We earlier prepared 5-methyl compound **12b**[10] by borohydride reduction of the corresponding aldehyde **11b** using a straightforward adaptation of a procedure for conversion of the 5-unsubstituted aldehyde **11a** to **12a** reported by DeGraw and co-workers.[11] In this paper we describe improved procedures for preparing **11b** and **12b**; these improved procedures proved equally effective for preparing **11a** and **12a**.

Syntheses of **12a** and **12b** by different approaches were reported by Su and co-workers.[12] A key step in one of two routes used by the Su group involved a ring closure having positional isomer ambiguity with respect to the formation of **12b**. Later work by these investigators revealed that the compound which they initially thought to be **12b** was in fact the 7-methyl isomer.[13] Thus structures of compounds derived from this intermediate were also initially misassigned. These events accounted for the relatively poor antitumor activity of the substance thought to be 5-methyl-5-deazaaminopterin compared with the authentic compound. Su and co-workers used an unequivocal alternative route to synthesize authentic 5-methyl-5-deazaaminopterin, and we also synthesized it by yet another unambiguous route.[10]

The 6-hydroxymethyl compounds **12a** and **12b** were converted to bromomethyl compounds **13a** and **13b** by treatment with dry HBr in dioxane as described by Su and co-workers.[12-13] Slight modifications in the reported isolation techniques allowed the bromomethyl compounds to be obtained in high yields and purity. These products, which were isolated in contact with only dioxane and diethyl ester, are probably dihydrobromides instead of the unprotonated forms as they were regarded by Su and co-workers. Preparation of **13b** by this method was decidedly better than that which we reported earlier by treatment of **12b** with triphenylphosphine dibromide.[10] The higher purity of **13b** prepared by the HBr–dioxane procedure was evident in several standard comparisons and especially so in effectiveness in the planned use.

The 5-deaza intermediates **10a,b** through **13a,b** can be synthesized readily by the general route described in this report. This approach to these intermediates and subsequent target compounds compares favorably with other reported routes[11-14] in terms of brevity, facility, and efficacy. Although we reported some earlier experiments using the current methods,[10,15] the procedures given in this report are improvements over the early experiments. These intermediates, functionalized in the 6-position, represent synthons to important 5-deazapteridines. For example, from the **10a–13a** group, access is offered to glycinamide

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Table I. TS Inhibition Data for PDDF (1), 10-Propargylfolic Acid (2), and 8-Deaza (3) and 5-Deaza (4) Analogues[a]

compd	IC_{50}, nM[b]	TS source
2	3900 (13)[c]	*L. casei*
3	2400 (37)[d]	K562
4	90 (14)[e]	H35F/F

[a] Value for 1 given in parentheses. [b] Reference 5 lists 20 nM for 1 versus TS from L1210 cells that overproduce this enzyme. [c] Reference 8. [d] Reference 9. [e] Method described in ref 27; concentration of the substrate, 5,10-methylenetetrahydrofolic acid, was 0.16 mM which is 30-fold in excess of the K_m. Similar ratios were used in the assays for 2 and 3 where the substrate concentration (0.2–0.4 mM) exceeded the K_m values by 20- to 40-fold.

ribonucleotide formyltransferase inhibitors 5,10-dideazatetrahydrofolic acid[16,7] and 5-deazatetrahydrofolic acid.[18] The 5-methyl compounds **10b–13b** allow unambiguous routes to potent antitumor agents of the classical antifolate group such as 5-methyl-5-deazamethotrexate.[10,19] The **10b–13b** compounds also allow facile access to analogues of lipophilic antifolates of interest such as piritrexim (BW 30IU) (structure **16**)[20–24] and trimetrexate (**17**).[24–26] Piritrexim bears the (2,4-diamino-5-methyl-5-deaza-6-pteridinyl)methyl group; analogues bearing this group may be prepared by displacement reactions of **13b** or reductive condensation reactions of **10b** or **11b**. Similarly, analogues of trimetrexate, a lipophilic antifolate bearing the (2,4-diamino-5-methyl-5,8-dideaza-6-pteridinyl)methyl group, may also be prepared from **10b–13b**.

Table II. Growth Inhibition Data for PDDF (1) and 4 against Three Cell Lines

cell line	IC_{50}, μM (n = 2–3) ± SE[a]	
	PDDF	4
L1210	2.61 ± 0.79	1.50 ± 0.05
HL60	5.29 ± 0.49	0.721 ± 0.11
S180	1.97 ± 0.25	2.35 ● 0.07

[a] Methods reviewed in ref 19.

Table III. Comparison of the Biochemical Properties of MTX, 5-Deaza-10-propargylaminopterin (**5**), and 5-Methyl-5-deaza-10-propargylaminopterin (**6**) in L1210 Cells[a]

compd	DHFR inhibn, K_i (n = 3) ± SE, pM	cell growth inhibn, IC_{50} (n = 4) ± SE, nM	mediated transport (n = 3–4)	
			influx	efflux
			K_m ± SE, μM	k ● SE, min^{-1}
MTX	5.28 ± 0.8	4.51 ± 0.5	4.86 ± 0.5	0.21 ● 0.03
5	5.85 ± 1.0	0.64 ± 0.09	1.80 ± 0.3	0.23 ± 0.05
6	6.87 ± 0.9	0.13 ± 0.02	0.57 ± 0.04	0.24 ± 0.05

[a] Methods reviewed in ref 19.

With the aim of adapting the reported synthesis of 2[7] to the 5-deaza analogue **4**, hydrolytic deamination of **13a** was conducted in 48% HBr at 90–95 °C to give 2-amino-6-(bromomethyl)-4(3*H*)-pyrido[2,3-*d*]pyrimidinone (**15**). The coproduced NH_4Br proved difficult to remove. A better procedure consisted of deamination of the 6-hydroxymethyl precursor **12a** in refluxing 1 N NaOH to give 2-amino-4(3*H*)-oxopyrido[2,3-*d*]pyrimidine-6-methanol (**14**). Treatment of **14** with 48% HBr then replaced the hydroxyl group to give **15**.

Alkylation of diethyl *N*-[4-(propargylamino)benzoyl]-L-glutamate (**7**) by intermediates **16**, **13a**, and **13b** afforded the expected diethyl esters which were purified using silica gel chromatography (preparative TLC or column). Mild ester hydrolysis then led to pure target compounds **4**, **5**, and **6**.

Biological Studies. The inhibitory effect on the activity of TS by 10-propargyl-5-deazafolic acid (**4**) was measured in comparison with that of PDDF using TS derived from H35F/F cells. The enzyme activity was determined by spectrophotometric assay based on the change in molar absorbance at 340 nm due to conversion of 5,10-methylenetetrahydrofolate to dihydrofolate.[27,28] In this system the IC_{50} value found for **4** was 90 nM and that for PDDF was 14 nM. Thus **4** is approximately 6-fold less inhibitory than PDDF against TS from H35F/F cells. Previously reported IC_{50} values for PDDF against TS derived from other sources are 20 nM versus the enzyme from an L1210 variant that overproduces TS,[5] 37 nM against the enzyme from K562 cells,[9] and 13 nM against that derived from *L. casei*.[8] The IC_{50} for the analogue 8-deaza-10-propargylfolic acid (**3**) against TS from K562 cells was reported to be 2400 nM,[9] and that of the pteridine analogue, 10-propargylfolic acid (**2**), versus TS from *L. casei* was reported to be 3900 nM. The current and pre-

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viously reported results are summarized in Table I.

In commenting on the relatively poor inhibitory effect on TS by both the 8-deaza compound **3** and the pteridine **2**, Brixner and co-workers noted that it is conceivable that hydration about nitrogen of position 5 in compounds **2** and **3** could hinder the approach of a folate or 8-deazafolate to the tetrahydrofolate binding site of TS.[9] That concept, although tenuous, is sustained by the results at hand for 5-deaza analogue **4**. It appears that the replacement of the position-8 carbon by nitrogen does not greatly affect the capacity of **4** to inhibit TS in comparison with PDDF.

Compound **4** and PDDF were tested for their inhibitory effects on the growth of three cell lines (L1210, HL60, and S180), and the results are listed in Table II. Against the L1210 and S180 systems, **4** and PDDF produced similar effects. Against HL60, **4** was found to be about 7-fold more inhibitory than PDDF. Inclusion of thymidine in the growth-inhibition tests produced only slight increases in IC_{50} against L1210 and S180 for both PDDF and **4**, and added thymidine had virtually no effect on the ability of either compound to inhibit HL60 growth. The slight effect of thymidine addition raises questions pertinent to TS being the locus of the observed cytotoxicity of both **4** and PDDF in these cell lines. The fact that thymidine does reverse the cytotoxicity of PDDF against a particular L1210 variant[31] probably reflects the unique biochemical properties of that cell line as reported by other investigators.[29-31]

The results from the cytotoxicity tests prompted us to test **4** in vivo in mice bearing the L1210 tumor. The tests done at dose levels of 200 and 300 mg/kg administered subcutaneously daily for 5 days produced no toxic effect and no increase in life span compared with control. It is possible that this lack of activity by **4** is related to observed differences in response to PDDF by L1210 murine tumor systems from different sources. Treatment by PDDF of L1210 systems designated L1210/ICR and L1210/NCI led to long-term survivors in mice bearing the L1210/ICR line but only slight antitumor response in those bearing the L1210/NCI tumor.[29,30] The in vivo tests on **4** were done using a cell line more related to the L1210/NCI system than to the L1210/ICR.

The 2,4-diamino compounds **5** and **6** are, as expected, inhibitors of dihydrofolate reductase (DHFR), and they display potent antitumor activity. Both **5** and **6** exert virtually the same inhibitory effect against DHFR from L1210 cells as methotrexate (MTX), and both are more effective than MTX in inhibiting L1210 cell growth in vitro (see Table III). The in vitro results shown in Table III show the same trends with respect to improved influx and greater cytotoxicity due to introduction of a 5-alkyl substituent as observed in 5-deaza and 5-alkyl-5-deaza analogues of MTX.

In in vivo tests in mice against the EO771 mammary adenocarcinoma, **6** produced a greater antitumor effect than MTX (Table IV). A dose of 36 mg/kg per day for 5 days caused no toxic deaths while the average tumor volume among 10 mice was reduced to 8–9% of that of the control, and 20% of the test animals were rendered tumor free. Significant antitumor effectiveness was also observed at lower dose levels. Although **6** is somewhat less effective than 5-methyl-5-deazamethotrexate, it has the advantage of exerting antifolate effect over a large tolerated dose range. This property conceivably could afford some therapeutic advantage, but further studies will be required.

Table IV. Antitumor Effects of MTX and 5-Methyl-5-deaza-10-propargylaminopterin (**6**) against EO771 Mammary Adenocarcinoma[a]

compd	dose,[b] mg/kg	day of eval	AWC,[c] g	toxic deaths[d]/ total	ave tumor vol[e] mm³	ave tumor vol[e] T/C	tumor free/ total
control	–	7	+0.4	0/10	310	1.00	0/10
MTX	3	7	−0.4	0/10	230	0.74	0/10
6	36	7	−1.9	0/10	27	0.09	2/10
control	–	14	+1.7	0/10	2711	1.00	0/10
MTX	3	14	+0.8	0/10	2145	0.79	0/10
6	36	14	−0.6	0/10	212	0.08	2/10

[a] Methods used are described in ref 19. [b] Given once per day for 5 days starting 1 day after tumor implantation. [c] Average weight change (in grams) on day 7 or day 14. [d] Assessed on day of evaluation (day 7 or 14). [e] Assessed on day of evaluation; volume calculated in mm³ (as $^4/_3\pi r^3$); T/C = treated/control.

Experimental Section

Examinations by TLC were performed on Analtech precoated (250-μm) silica gel G(F) plates. HPLC assays were made with Waters Associates ALC-242 liquid chromatograph equipped with a UV detector (254 nm) and an M-6000 pump using a 30 × 0.29 cm C_{18} μBondapak column. Purity assays were done by reversed-phase in the isocratic mode with a mobile phase consisting of CH_3CN (10 or 15% by volume) in 0.1 M NaOAc (pH 3.6). Unless other conditions are specified, evaporations were performed with a rotary evaporator and a H_2O aspirator. Products were dried in vacuo (<1 mm) at 22–25 °C over P_2O_5 and NaOH pellets. Final products were dried and then allowed to equilibrate with ambient conditions of the laboratory. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Spectral determinations and elemental analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr. The ^1H NMR spectra data reported were determined with a Nicolet NMC 300 NB spectrometer using Me_4Si as internal reference. Chemical shifts (δ) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the electron-impact (EI) or fast-atom-bombardment (FAB) mode. UV spectra were determined with a Perkin-Elmer Model Lambda 9 spectrometer. Samples were first dissolved in 0.1 N NaOH, and the solutions were then diluted 10-fold with the medium given in the listings. Maxima are expressed in nanometers with the molar absorbance given in parentheses. Molecular weights used in all calculations conform with the composition listed with the indicated elemental analyses.

2,4-Diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (11a) and 2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (11b). The nitrile **10a** or **10b**[10] (0.200 mol) was dissolved in warm HCO_2H (1.0 L of 95–97%) in a three-necked flask equipped for mechanical stirring. Raney Ni (150 g of 50% slurry with H_2O) was then added with the aid of HCO_2H (200 mL). The stirred mixture was refluxed 2 h, cooled, and filtered. Raney Ni on the funnel was washed with portions of warm HCO_2H until the washings were colorless. The filtrate was concentrated under reduced pressure (bath to 50 °C) to a viscous orange semisolid. This residue was dissolved in boiling H_2O (2.8 L) with stirring, and the hot solution was clarified (Norit, Celite). The filtrate was then treated with mechanical stirring with concentrated NH_4OH solution to pH 8.5–9.0 to precipitate the aldehyde.

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The mixture was kept overnight in a refrigerator before the precipitate was collected and washed several times with 0.1 N NH_4OH followed by EtOH and finally with Et_2O. The product was dried in vacuo initially at 20–25 °C and then at 78 °C to give **11a,b** suitable for conversion to **12a,b**. The yield of **11a** was 62% and that of **11b** was 75%. Spectral data: **11a**, mass, *m/e* 190 MH^+; ^1H NMR (Me_2SO-d_6) δ 8.89 and 9.06 (2 d, C^5-H and C^7-H), 9.92 (s, CHO); **11b**, mass and ^1H NMR in agreement with previously reported data.[10]

2,4-Diaminopyrido[2,3-*d*]pyrimidine-6-methanol (12a). A suspension of the pulverized aldehyde **11a** (15.0 g, 79.3 mmol) in anhydrous MeOH (3.5 L) in a 5-L Morton (pleated) three-neck flask was mechanically stirred overnight to promote subdivision. The stirred mixture was then treated at 20–25 °C with $NaBH_4$ (2.0 g total) added in four approximately equal portions at intervals of 15 min. Stirring at 20–25 °C was continued for 5 h after the last addition of $NaBH_4$. Undissolved solid (6.6 g) filtered from the mixture consisted of unchanged **11a** along with unidentified material. The filtrate was diluted with H_2O (600 mL) and was then concentrated under reduced pressure (bath to 35 °C) until MeOH and most of the H_2O had been removed leaving a suspension of yellow solid **12a**. The mixture was kept 16 h in a refrigerator before the solid was collected to give **12a**·H_2O in 34% yield (5.70 g). Anal. ($C_8H_9N_5O$·1.1H_2O) C, H, N. Spectra data: mass, *m/e* 192, MH^+; UV λ_{max} 329 nm (ε 8590) at pH 1; 336 nm (ε 6340) at pH 7; 346 (ε 7100) at pH 13; ^1H NMR (Me_2SO-d_6) δ 4.50 (s, CH_2), 6.30 (s, NH_2), 7.50 (br s, NH_2), 8.32 and 8.60 (2 d, C^5-H and C^7-H).

2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-methanol (12b). Conversion of **11b** to **12b** by treatment with $NaBH_4$ was carried out as described above for the preparation of **12a**. From a run starting with 15.0 g (73.8 mmol) of **11b**, insoluble material removed before isolation of the product weighed 4.4 g and the yield of **12b**·0.5H_2O was 49% (7.75 g). Concentration of the filtrate gave a second crop of 1.33 g. The first crop gave the following characterization data. Anal. ($C_9H_{11}N_5O$·0.5H_2O) C, H; N: calcd, 32.69; found, 33.75. Spectral data: mass, *m/e* 206, MH^+; UV λ_{max} 317 nm (ε 7470) at pH 1; 329 nm (ε 5830) at pH 7; 342 nm (ε 7110), 270 (8720) at pH 13; ^1H NMR (Me_2SO-d_6) δ 2.65 (s, CH_3), 4.50 (s, CH_2), 6.18 (s, NH_2), 6.95 (br s, NH_2), 8.43 (s, C^7-H).

2-Amino-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidine-6-methanol (14). The procedure that follows is adapted from reported similar conversions.[9,12] A solution of **12a**·H_2O (3.95 g, 18.9 mmol) in 1 N NaOH (100 mL) was refluxed 10 h, cooled, filtered, and treated with 2 N HCl to pH 6 to precipitate **14**. After refrigeration (5 h) the precipitate was collected. The yield of hydrated **14** (see Anal.) was 80% (3.85 g). Spectral data: MS *m/e* 193, MH^+; UV, λ_{max} 243 nm (ε 21 500), 270 (10 300), 335 (7570) at pH 1; 268 nm (ε 12 600), 319 (6450) at pH 7; 243 nm (ε 21 700), 270 (10 400), 335 (7630) at pH 13; ^1H NMR (Me_2SO-d_6) δ 4.53 (d, CH_2), 5.29 (t, CH_2OH), 6.67 (br s, NH_2), 8.14 and 8.57 (2 d, C^5-H and C^7-H), 11.16 (br s, N^3-H). Anal. ($C_8H_8N_4O_2$·2.25H_2O) C; H: calcd, 5.49; found, 4.43; N: calcd, 24.08; found, 24.54.

6-(Bromomethyl)-2,4-diaminopyrido[2,3-*d*]pyrimidine (13a) and 6-(Bromomethyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine (13b). Samples of **12a** and **12b** used in these conversions were dried beforehand in vacuo at 110 °C over P_2O_5. The preparation of **13b** is illustrative. Dry HBr was introduced into a stirred mixture of **12b** (1.30 g, 6.34 mmol) in dry dioxane (100 mL) kept below 40 °C until saturation at 25 °C was attained. The mixture was stirred at about 25 °C in a stoppered flask while the solid gradually dissolved during about 2 h. After 20 h, the solution was added dropwise to stirred Et_2O (500 mL) to give a yellow solid. The product, collected with the aid of Et_2O and dried in vacuo at 40–50 °C, weighed 2.58 g. Spectral data: MS *m/e* 268 and 270, MH^+ for $C_9H_{10}BrN_5$; ^1H NMR (Me_2SO-d_6) 2.78 (s, CH_3), 4.94 (s, CH_2), 8.19 (s, NH_2), 8.77 (C^7-H), 9.45 (s, NH_2) with weak signals due to slight retention of dioxane and Et_2O. The weight of solid obtained along with the supportive spectral data suggest the product is **12b dihydrobromide** obtained in 95% yield. This product proved to be suitable for the conversion to **6** described below.

In the preparation of **13a**, the reaction mixture required filtration from a small amount of undissolved solid. Addition of the filtrate to Et_2O led to yellow solid, which was collected and dried to give **13a dihydrobromide** in 77% yield (3.00 g from 1.80 g, 9.41 mmol, of **12a**). Spectral data: MS *m/e* 254 and 256, MH^+ for $C_8H_8BrN_5$. This material proved to be satisfactory for conversion to **5**.

Samples of **13a,b dihydrobromides** thus isolated are stable in storage in desiccated containers in a refrigerator.

2-Amino-6-(bromomethyl)-4(3*H*)-pyrido[2,3-*d*]pyrimidinone (15). A solution of **14**·2.25H_2O (3.00 g, 12.9 mmol) in 48% HBr (180 mL) was kept 16 h at 20–25 °C and then for 2 h at 90–95 °C. The solution was then evaporated (bath to 50 °C) with the aid of several portions of EtOH added to the residue and also evaporated. The residue was finally stirred with EtOH to give the hydrobromide of **15** in 41% yield (1.85 g). Spectral data: MS *m/e* 255 and 257, MH^+. Anal. ($C_8H_7BrN_4O$·1.15HBr) C, H, N.

***N*-[4-[[[(2-Amino-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl]prop-2-ynyl]amino]benzoyl]-L-glutamic Acid or 10-Propargyl-5-deazafolic Acid (4).** A mixture of **15** (1.75 g, 5.03 mmol) and **7**[32] (1.80 g, 5.00 mmol) in Me_2NAc (50 mL) was stirred for 7 days at 20–25 °C in a stoppered flask protected from light. The mixture was then heated (bath at 90 °C) for 0.5 h. Nearly all insoluble material dissolved. The cooled mixture was filtered, and the filtrate was added to stirred H_2O (250 mL). Crude product which precipitated was collected, dried, and dissolved in DMF. This solution was treated with silica gel (Merck, 60A, 230–400 mesh; 5-fold weight of crude product), and the mixture was evaporated in vacuo (<1 mm, bath to 35 °C). The resulting dispersion was applied to a column of silica gel, and elution by $CHCl_3$–MeOH (initially 9:1 increasing to 4:1) followed. Fractions whose thin-layer chromatograms showed only a single UV-absorbing spot of R_f about 0.5 ($CHCl_3$–MeOH, 4:1) were combined and evaporated to give the **diethyl ester of 4** in 16% yield (420 mg). Spectral data: MS *m/e* 535, MH^+ for $C_{27}H_{30}N_6O_6$; ^1H NMR (Me_2SO-d_6) δ 1.15 (m, CH_3CH_2), 1.96, 2.06 (2 m, $CHCH_2CH_2$, nonequivalent), 2.41 (t, CH_2CO), 3.21 (s, C≡CH), 4.04 (m OCH_2CH_3), 4.30–4.40 (s due to $CH_2C{\equiv}CH$ overlapping m due to $CONHCH$), 4.72 (C^9H_2), 6.72 (br s, NH_2), 6.88 and 7.74 (2 d, C_6H_4), 8.12 and 8.58 (2 d, C^5-H and C^7-H), 8.36 (d, $CONHCH$), 11.28 (br s, N^3-H). For hydrolysis of the ester groups, this sample (408 mg, 0.76 mmol) was dissolved in MeOH (10 mL) containing 1 N NaOH (1.78 mL). The solution was kept at 20–25 °C for about 20 h. MeOH was then removed by evaporation, and the residue was dissolved in H_2O (10 mL). After 24 h at 20–25 °C, the aqueous solution was treated with 1 N HCl to pH 3.9 to precipitate **6**. The thick mixture was thinned with H_2O (~20 mL) and chilled for 4–5 h before the solid was collected to give **6**·1.6H_2O (310 mg) in 80% yield (based on diethyl ester). Spectral data: MS *m/e* 479, MH^+; UV λ_{max} 281 nm (ε 26 400), 351 (8000) at pH 1; 278 nm (ε 23 700), 298 (25 300) at pH 7; 243 nm (ε 24 000), 298 (24 000) at pH 13; ^1H NMR (Me_2SO-d_6) δ 1.92, 2.06 (2 m, $CHCH_2CH_2$, nonequivalent), 2.33 (t, CH_2CO_2H), 3.20 (s, C≡CH), 4.26–4.44 (s due to $NCH_2C{\equiv}CH$ overlapping m due to $NHCHCO_2H$), 4.72 (s, C^9H_2), 6.8 (br s, NH_2), 6.88 and 7.75 (2 d, C_6H_4), 8.12 and 8.59 (2 d, C^5-H and C^7-H), 8.26 (d, $CONHCH$). Anal. ($C_{23}H_{22}N_6O_6$·1.6H_2O) C, H; N: calcd, 16.57; found, 17.01.

***N*-[4-[[[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]prop-2-ynyl]amino]benzoyl]-L-glutamic Acid or 10-Propargyl-5-methyl-5-deazaaminopterin (6).** A solution of **13b**·2HBr (520 mg, 1.21 mmol) and **7**[32] (500 mg, 1.39 mmol) in Me_2NAc (10 mL) was kept at 20–25 °C while the conversion was monitored by TLC ($CHCl_3$–MeOH, 3:1). The product gave a UV-absorbing spot of R_f about 0.5. After 5 days, the solution was added dropwise to a stirred solution of $NaHCO_3$ (500 mg) in H_2O (100 mL) to precipitate crude **6**. The precipitate (530 mg) was stirred with Et_2O (50 mL) to remove unchanged **7**. The remaining solid (450 mg) was then applied in $CHCl_3$–MeOH (1:1) to two 20 × 20-cm Analtech 2-mm silica gel G(F) plates and chromatographed using $CHCl_3$–MeOH (3:1). The product band from each plate was extracted with $CHCl_3$–MeOH (1:1) to give pure **6** diethyl ester in 50% yield (380 mg). Spectral data: MS *m/e* 548, MH^+ for $C_{28}H_{33}N_7O_5$. For saponification, a solution of this ester (360 mg, 0.658 mmol) in MeOH (50 mL) was treated

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with 1 N NaOH (1.67 mL). The solution was kept at 20–25 °C for 24 h and then evaporated. The solid remaining was dissolved in H_2O (10 mL), and the aqueous solution was kept 24 h at 20–25 °C before it was filtered. The pale yellow solution (pH 11.5), now diluted to 30 mL, was treated with 1 N HCl to produce pH 3.9 and precipitate **6** as beige-colored solid, yield 79% (270 mg). Assay by HPLC showed the product to be homogeneous. Spectral data: MS *m/e* 492, MH^+; UV λ_{max} 226 nm (ϵ 40 500), 299 (27 900) at pH 1; 226 nm (ϵ 37 200), 296 (27 000) at pH 7; 226 nm (ϵ 35 900), 295 (27 200), 346 (8030) at pH 13; ^1H NMR (Me_2SO-d_6) δ 1.95, 2.05 (2 m, $CHCH_2CH_2$ nonequivalent), 2.30 (t, CH_2CO), 2.66 (s, CH_3), 3.20 (s, C≡CH), 4.20 (s, CH_2≡CH), 4.35 (m, CONHC*H*), 4.66 (s, C^9H_2N), 6.90 and 7.76 (2 d, C_6H_4), 8.16 (d, NH), 8.31 (s, C^7-H). Anal. ($C_{24}H_{25}N_7O_5 \cdot 1.67H_2O$) C, H, N.

***N*-[4-[[[(2,4-Diaminopyrido[2,3-*d*]pyrimidin-6-yl)methyl]prop-2-ynyl]amino]benzoyl]-L-glutamic Acid or 10-Propargyl-5-deazaaminopterin (5).** Alkylation of 7[32] by **13a**·2HBr in Me_2NAc was carried out as described above for the preparation of **6**. The intermediate diethyl ester of **5** was first isolated by preparative TLC ($CHCl_3$–MeOH, 3:1) on two plates to give nearly pure product (270 mg). This material was chromatographed as before (except on one plate) to give pure **5** diethyl ester in 36% yield (230 mg from 500 mg, 1.20 mmol, of **13a**·2HBr). Spectral data: MS *m/e* 534, MH^+ for $C_{27}H_{31}N_7O_5$. This ester (230 mg) was hydrolyzed (as described above for the preparation of **6**) to give **5**, homogeneous according to HPLC, in 70% yield (160 mg). Spectral data: MS *m/e* 478, MH^+; UV, λ_{max} 220 nm (ϵ 41 200), 301 (26 100) at pH 1; 222 nm (ϵ 37 000), 249 (20 500), 297 (24 900) at pH 7; 223 nm (ϵ 35 700), 250 (22 100), 297 (24 700), 347 (7310) at pH 13; ^1H NMR (Me_2SO-d_6) δ 1.93, 2.06 (two m, $CHCH_2CH_2$ nonequivalent), 2.31 (t, CH_2CO), 3.21 (s, C≡CH), 4.30–4.41 (s, CH_2C≡CH overlapping m due to CONHC*H*), 4.46 (s, C^9H_2N), 6.61 (br s, NH_2), 6.90 and 7.75 (2 d, C_6H_4), 7.70 (br s, NH_2), 8.20 (d, NH), 8.33 and 8.62 (2 d, C^5-H and C^7-H). Anal. ($C_{23}H_{23}N_7O_5 \cdot 1.5H_2O$) C, H, N.

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Structure–Activity Relationships of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine Analogues: Effect of Substitutions at the C-6 Phenyl Ring and at the C-5 Position on Anti-HIV-1 Activity

Hiromichi Tanaka,[†] Hideaki Takashima,[‡] Masaru Ubasawa,[‡] Kouichi Sekiya,[‡] Issei Nitta,[‡] Masanori Baba,[§] Shiro Shigeta,[§] Richard T. Walker,[∥] Erik De Clercq,[⊥] and Tadashi Miyasaka*,[†]

School of Pharmaceutical Sciences, Showa University, Hatanodai 1-5-8, Shinagawa-ku, Tokyo 142, Japan, Mitsubishi Kasei Corporation Research Center, Yokohama 227, Japan, Fukushima Medical College, Fukushima 960-12, Japan, Department of Chemistry, University of Birmingham, Birmingham B15 2TT, United Kingdom, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium. *Received June 3, 1991*

The effect of substitution on the pyrimidine moiety of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) and 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-2-thiothymine (HEPT-S) on anti-HIV-1 activity was investigated by synthesizing a series of 5-methyl-6-(arylthio) and 5-substituted-6-(phenylthio) derivatives. Preparation of the 5-methyl-6-(arylthio) derivatives was carried out based on either LDA lithiation of 1-[[2-(*tert*-butyldimethylsiloxy)ethoxy]methyl]thymine (**3**) and 1-[[2-(*tert*-butyldimethylsiloxy)ethoxy]methyl]-2-thiothymine (**4**) followed by reaction with diaryl disulfides or an addition–elimination reaction of 1-[[2-(*tert*-butyldimethylsiloxy)ethoxy]-methyl]-6-(phenylsulfinyl)thymine (**31**) with aromatic thiols. Preparation of the 5-substituted-6-(phenylthio) derivatives was carried out based on either C-5 lithiation of the 1-[[2-(*tert*-butyldimethylsiloxy)ethoxy]methyl]-6-(phenylthio)uracil (**41**) with LTMP or the LDA lithiation of 5-alkyl-1-[[2-(*tert*-butyldimethylsiloxy)ethoxy]methyl]-2-thiouracil derivatives **45–47**. Substitution at the meta position of the *C*-6-(phenylthio) ring by the methyl group improved the original anti-HIV-1 activity of HEPT, and introduction of two *m*-methyl groups to the phenylthio ring further potentiated the activity [EC_{50}: 6-[(3,5-dimethylphenyl)thio]-1-[(2-hydroxyethoxy)methyl]thymine (**28**), 0.26 μM; 6-[(3,5-dimethylphenyl)thio]-1-[(2-hydroxyethoxy)methyl]-2-thiothymine (**30**), 0.22 μM]. When the 5-methyl group was replaced by an ethyl or an isopropyl group, the anti-HIV-1 activity of HEPT was also improved remarkably [EC_{50}: 5-ethyl-1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-2-thiouracil (**48**), 0.11 μM; 5-isopropyl-1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)-2-thiouracil (**50**), 0.059 μM; 5-ethyl-1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-2-thiouracil (**54**), 0.12 μM; 5-isopropyl-1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-2-thiouracil (**56**), 0.063 μM]. 6-[(3,5-Dimethylphenyl)thio]-5-ethyl-1-[(2-hydroxyethoxy)methyl]thymine derivatives **51** and **57** and 6-[(3,5-dimethylphenyl)thio]-5-isopropyl-1-[(2-hydroxyethoxy)methyl]thymine derivatives **52** and **58** inhibited the replication of HIV-1 in the nanomolar concentration range.

Acquired immunodeficiency syndrome (AIDS)[1,2] is a systemic and fatal disorder that still evades curative therapy, though a number of therapeutic modalities have been proposed for the treatment of this disease.[3,4] The nucleoside derivative 3′-azido-3′-deoxythymidine (AZT)[5] is known to prolong survival in AIDS patients,[6] yet its treatment is sometimes associated with considerable side effects such as bone marrow suppression.[7] Furthermore, prolonged AZT treatment often leads to the emergence of AZT-resistant HIV-1 strains.[8] Therefore, there is a rel-

[†] Showa University.
[‡] Mitsubishi Kasei Corp.
[§] Fukushima Medical College.
[∥] University of Birmingham.
[⊥] Rega Institute.

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