Notes

Synthesis and Antitumor Activity of 10-Propargyl-10-deazaaminopterin

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Successive alkylation of dimethyl homoterephthalate with propargyl bromide and 2,4-diamino-6-(bromomethyl)pteridine followed by ester saponification at room temperature afforded 2,4-diamino-4-deoxy-10-carboxy-10-propargyl-10-deazapteroic acid. The 10-COOH was readily decarboxylated by heating in DMSO at a temperature of only 120 °C to yield the diamino-10-propargyl-10-deazapteroic acid intermediate. Coupling with diethyl L-glutamate and ester hydrolysis gave the title compound. The 10-propargyl analogue was about 5 times more potent than MTX as an inhibitor of growth in L1210 cells, but was only one-third as potent as an inhibitor of DHFR from L1210. The analogue was transported inward very effectively in L1210 cells showing a 10-fold advantage over MTX. At a dose of 36 mg/kg the 10-propargyl compound caused shrinkage of the E0771 solid murine mammary tumor to only 1% of untreated controls.

Since the announcement by Jones et al.[1] that the 10-*N*-propargyl analog of 5,8-dideazafolic acid (CB 3717) possessed potent inhibitory activity against thymidylate synthase, it has been of interest to study the effect of a 10-propargyl group in various folate analogs. Jackman and co-workers[2,3] have found that it was possible to retain or improve activity when the 2-amino group was replaced by hydrogen or methyl. Piper and co-workers[4] prepared 10-*N*-propargylaminopterin and found it to be more inhibitory than MTX against the DHFR enzyme and growth of L1210 cells in culture, while transport affinity in L1210 cells was similar to that of MTX. Activity against L1210 leukemia in mice was similar to that of MTX, but an unusually high dose of 288 mg/kg was required. Piper et al.[5] have reported enhanced transport and growth inhibitory activity against L1210 cells for 10-*N*-propargyl-5-deazaaminopterin analogs. However, the effect of substitution of a propargyl group at the 10-position in a 10-deaza folate compound has not been reported because of the difficulty inherent in the synthesis of a *C*-propargyl analog. We report herein a facile synthetic route for such analogs and a primary example, 10-propargyl-10-deazaaminopterin.

Chemistry

Previous synthetic methodology[6,7] for preparation of 10-alkyl-10-deazaaminopterin and related compounds has utilized steps that are incompatible with the presence of a propargyl group, e.g., hydrogenation bromination, strong acid media. One method that was potentially applicable to synthesis of a 10-*C*-propargyl analog was employed for 10-alkyl-8,10-dideazaaminopterin compounds.[8] This procedure involved cleavage of a 10-carbomethoxy group followed by decarboxylation at 180 °C as promoted by sodium cyanide in DMSO. We have further investigated this approach and found that initial saponification of the 10-$COOCH_3$ pteroate intermediate, with careful precipitation of the resulting acid at pH 6–7, allowed facile decarboxylation in DMSO solution at temperatures below 140 °C. Nearly quantitative yields of highly pure pteroic acid intermediates were obtained without the decomposition encountered in the NaCN–DMSO method. This improved technique was considered mild enough for synthesis of analogs with unsaturation at C-10.

Alkylation of the anion of dimethyl homoterephthalate (1) (Scheme I) with propargyl bromide as promoted by KH in DMF afforded the α-propargyl diester (2) in 83% yield. Subsequent treatment of an excess of the potassium salt of 2 in DMF by 2,4-diamino-6-(bromomethyl)pteridine[9] at −35 °C gave the dimethyl ester of 2,4-diamino-4-deoxy-10-propargyl-10-deazapteroic acid (3) in 90% yield. Saponification of 3 in 2-methoxyethanol containing aqueous NaOH over a 4-h period at room temperature readily yielded the diacid (4). When a solution of 4 in DMSO was heated at only 120 °C, decarboxylation was rapidly effected as shown by HPLC. The NMR spectrum in d_6-DMSO was consistent for the diamino pteroic acid intermediate (5) and did not suggest the presence of any 10-allenyl isomer. Coupling of 5 with diethyl L-glutamate by the mixed-anhydride method (isobutyl chloroformate) afforded the diethyl ester of 10-propargyl-10-deazaaminopterin (6). The diester was readily hydrolyzed to the target 10-propargyl-10-deazaaminopterin (7).

Biological Evaluation

10-Propargyl-10-deazaminopterin was initially evaluated for growth inhibition of L1210 murine leukemia cells in culture and for its ability to inhibit DHFR derived from L1210 cells. As shown in Table I it was found to be about one-third as potent as MTX for enzyme inhibition, a result that is consistent with extension of chain length beyond two carbon units. However, the propargyl compound was nearly 5-fold more potent than MTX as an inhibitor of growth in L1210 cells. This result prompted us to measure the transport properties for facilitated entry into the L1210 cells vs MTX. We were quite surprised to find about a

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Scheme I

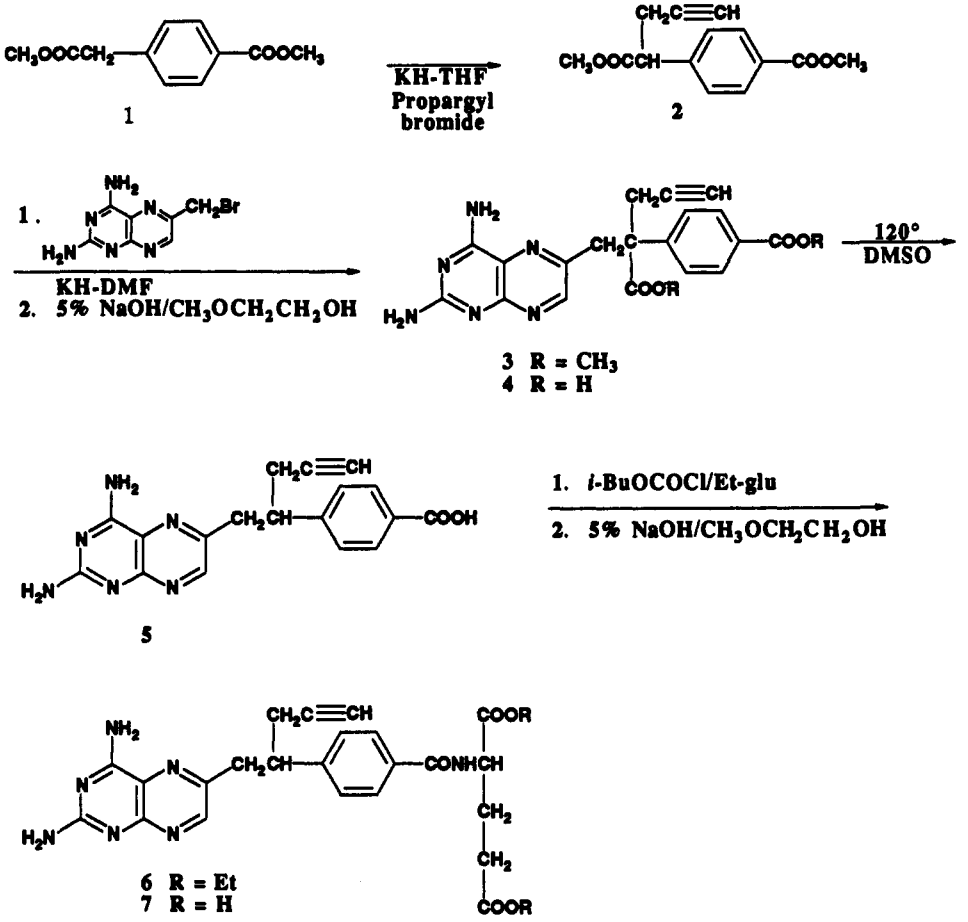

Table I. Biochemical and Growth Inhibition Data in L1210 Cells

compd	DHFR inhibn: K_i, pM[a]	growth inhibn: IC_{50}, nM[b]	transport influx: K_i, μM[b]
7	18.2 ± 4.0	2.0	0.45 ± 0.1
MTX	5.75 ± 1.0	9.50	4.2 ± 0.5
10-deazaaminopterin (10-DA)[c]	3.6	1.80	0.90
10-Me-10-DA[c]	4.1	1.34	0.95
10-Et-10-DA[c]	4.8	1.44	0.83
10-n-Pr-10-DA[c]	9.2	2.03	1.1
10-n-Am-10-DA[c]	>1000	251	1.0

[a] Enzyme derived from L1210; conducted at pH 7.3 and data analyzed by method of ref 12. [b] See ref 13 for methods. [c] Values obtained from previous runs and normalized to MTX control for comparison in this study (see refs 6 and 10).

10-fold transport advantage vs MTX for influx K_i as determined by competitive binding for the transport protein.

A comparison of the in vitro biological properties of the 10-propargyl analog 7 with other 10-alkyl-substituted 10-deazaaminopterins is also presented in Table I. For purposes of comparison, we have adjusted the values for 10-deazaaminopterin and its 10-methyl, 10-ethyl, 10-*n*-propyl, and 10-*n*-amyl analogs to reflect the values of the MTX controls obtained in those earlier studies.[6,10] Introduction of the propargyl group in 7 caused a substantial decrease in inhibition of the DHFR enzyme compared with the unsubstituted, 10-Me, and 10-Et compounds and was only half as effective as the 10-*n*-propyl analog. It is apparent that a chain length exceeding three carbons is detrimental to enzyme binding as evidenced by homologation to the 10-*n*-amyl analog which was inactive.

Table II. E0771 Solid Tumor Evaluation in BDF1 Female Mice[a]

dose (mg/kg)[b]	no. mice	average tumor vol (mm^3) day 10 (% T/C)	14	21
control	5	131 (100)	1232 (100)	2066 (100)
7(24)	3	62 (47)	19 (2)	204 (10)
7(36)	3	48 (31)	6 (1)	21 (1)[c]
MTX(3)	5	113 (86)	187 (15)	1260 (61)
MTX(6)	5	34 (26)	19 (2)	382 (18)
MTX(9)	5	4 (3)	19 (2)	310 (15)

[a] Subcutaneous tumor. [b] Dose schedule day 3, QDX 5 (ip). [c] One mouse was tumor free of two survivors.

Inhibition of L1210 cell growth was quite similar for 7 when compared to the other active analogs. This may be a reflection of the substantial improvement in inward transport which is about a factor of 2 better than the other analogs. Chain length of the 10-substituent does not appear to be a significant factor influencing the influx K_i of the 10-deaza analogs. However, the unsaturation present in the 10-propargyl analog is apparently responsible for the improved transport, possibly because of polarity effects relative to the binding to the transport protein.

The propargyl compound was further evaluated in the E0771 murine mammary tumor model in vivo. At a dose of 36 mg/kg compound 7 totally suppressed the growth of the tumor at the 14 and 21 day post treatment points. The compound was also quite effective at a 24 mg/kg dose at day 14, but some regrowth had commenced by day 21. At the 36-mg dose one tumor-free survivor was noted among the surviving animals. Methotrexate was not as effective in this assay at day 21 even at a dose of 9 mg/kg. Even

though this assay was conducted with an N of 3 animals per dose for 7, it is indicative that further study of this interesting analog is warranted. The E0771 solid tumor model is somewhat predictive for activity in human breast cancer as demonstrated by 10-ethyl-10-deazaminopterin (Edatrexate). This drug was highly effective in E0771 and has shown outstanding efficacy in the clinic with late-stage breast cancer.[11]

Experimental Section

Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN. Values were within 0.4% except as noted in parentheses following the element. The ^{1}H NMR spectra were taken on a Varian 400A or a JEOL FX90Q spectrometer. Mass spectra were run on a LKB 9000 GC–MS spectrometer or a Ribermag R10-10C MS system. Ultraviolet spectra were taken on a Perkin-Elmer 552 or Perkin-Elmer Coleman 575. Reverse-phase HPLC was run on a Waters Novapak C18 column eluted with 25% CH_3OH/75% 0.1 M NaH_2PO_4 buffer, pH 6.5.

α-Propargylhomoterephthalic Acid Dimethyl Ester (2). A mixture of 35% KH in oil (6.04 g, 35% w/w, 53 mmol) in 240 mL of sieve-dried THF was cooled to 0 °C. The cold mixture was treated with homoterephthalic acid dimethyl ester (10.0 g, 48 mmol). The mixture was stirred at 0 °C for 1 h. Propargyl bromide (53 mmol) was added, and the mixture stirred at 0 °C for 30 min and then at room temperature for 16 h. The resulting mixture was treated with 4.8 mL of 50% acetic acid and then poured into 480 mL of water. The mixture was extracted with ether (2 × 250 mL). The ether extracts were combined, dried over $MgSO_4$, and concentrated to a brown oil. Chromatography on 250 g of flash silica gel (10% ether in hexanes eluent) gave the product (9.9 g, 83%) as a white solid, mp 63–65 °C, after recrystallization from MeOH–H_2O: mass spectrum m/e 247 (M + H); IR (Nujol) C≡C—H, 3268 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 2H, C_6H_4), 7.40 (d, 2H, C_6H_4), 3.91 (s, 3H, $ArCOOCH_3$), 3.88 (dd, 1H, ArCH), 3.71 (s, 3H, $CHCOOCH_3$), 2.95 (dddd, 1H, CH_2), 2.64 (dddd, 1H, CH_2), 1.96 (dd, 1H, C≡CH). Anal. Calcd for $C_{14}H_{14}O_4$: C, H.

10-Propargyl-10-carbomethoxy-4-deoxy-4-amino-10-deazapteroic Acid Methyl Ester (3). A mixture of KH in oil (1.37 g, 35% w/w, 12 mmol) in dry DMF (10 mL) was cooled to −5 °C. The cold mixture was treated dropwise, with a solution of propargylhomoterephthalic acid dimethyl ester (2) (2.94 g, 12 mmol) in dry DMF (10 mL) and then stirred at 0 °C for 30 min. After cooling to −35 °C, a solution of 2,4-diamino-6-(bromomethyl)pteridine hydrobromide–0.2 2-propranol (1.32 g, 3.8 mmol) in dry DMF (20 mL) was added dropwise, while the temperature was maintained between −30 and −40 °C. The temperature was allowed to rise to 10 °C and the mixture was stirred for 2.5 h. The reaction was then adjusted to pH 7 by addition of solid CO_2. Concentration under high vacuum gave a residue which, however, was not soluble in common organic solvents and was therefore carried unpurified into the next step. The purity was acceptable by thin-layer chromatographic analysis. The crude weight recovery was 1.46 g (90%); mass spectrum m/e 420.

10-Propargyl-4-deoxy-4-amino-10-deazapteroic Acid (5). A solution of the dimethyl ester (3) (0.51 g, 1.2 mmol) in 2-methoxyethanol (5 mL) was treated with water (5 mL) and then 10% sodium hydroxide (5 mL). The solution was stirred at room temperature for 4 h and was adjusted to pH 6 with HOAc and concentrated under high vacuum to give a residue which was then dissolved in water (15 mL). Acidification to pH 6 resulted in a precipitate which was collected, washed with water, and dried in vacuo to leave 360 mg (76%); HPLC analysis indicated 85% purity; mass spectrum m/e 680 (M + H as the $(TMS)_4$ derivative) of the dicarboxylic acid (4).

Three decarboxylations of 4 were conducted on 86, 86, and 55 mg of material. In each case, the reaction aliquot was dissolved in 3 mL of dimethyl sulfoxide and immersed for a period of 5 min in an oil bath preheated to 120 °C. The runs were combined, and the solvent was removed in high vacuum. The residue was precipitated twice from dilute NH_4OH solution by addition of HOAc. HPLC analysis indicated 82% purity. The product was a tan solid (59 mg, 29%); mass spectrum 564 (M + H as the $(TMS)_4$ derivative).

10-Propargyl-10-deazaaminopterin Diethyl Ester (6). A solution of the acid (5) (100 mg, 0.29 mmol) in dry DMF (5 mL) was treated with triethylamine (0.28 mL, 2.0 mmol). After stirring at room temperature for 20 min, the solution was treated with isobutyl chloroformate (0.075 mL, 0.57 mmol). The mixture was stirred at room temperature for 1 h and then treated with L-glutamic acid diethyl ester hydrochloride (0.14 g, 0.57 mmol) and stirred for 2 h. The additions of isobutyl chloroformate and glutamate ester were repeated twice with one-quarter quantities of these reagents, and the final mixture was stirred for 15 h. The reaction was concentrated under high vacuum, and the residue was dissolved in $CHCl_3$ (10 mL) and washed with dilute NH_4OH and then water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on 10 g of flash silica gel (2% MeOH in $CHCl_3$).

Following chromatography, an aliquot was saponified; HPLC analysis indicated 93% purity. The product was obtained as a yellow foam 85 mg (55%): mass spectrum m/e 534 (M + H); ^{1}H NMR ($CDCl_3$) δ 8.5 (s, 1H, 7-H), 7.75 (d, 2H, C_6H_4), 7.28 (d, 2H, C_6H_4), 7.0 (br s, 1H, NH), 5.35 (br s, 1H, NH), 4.77 (m, 1H, NHC*H*), 4.10 and 4.25 (q, 4H, OCH_2), 3.46 (m, 2H, C-9CH_2), 3.23 (m, 1H, C-10H), 2.62 (m, 2H, C≡CCH_2), 2.46 (m, 2H, CH_2COOEt), 2.15 and 2.32 (m, 2H, glu-3CH_2), 2.04 (br s, 1H, C≡CH), 1.22 and 1.29 (t, 6H, CH_2CH_3).

10-Propargyl-10-deazaaminopterin (7). The diethyl ester (6) (83 mg, 0.16 mmol) was dissolved in 2-methoxyethanol (2 mL), and the solution was treated with water (1 mL) and then 10% NaOH (1 mL). The solution was stirred for 2 h at room temperature. The reaction mixture was diluted with 10 mL of H_2O, neutralized to pH 5 with HOAc to give a precipitate which was collected, and dried to leave 45 mg (61%) of a pale yellow solid; HPLC analysis indicated 95% purity; mass spectrum m/e 765 (as the $(TMS)_3$) derivative); UV (0.1 N NaOH) λ max 256 nm (ε 29 800), 372 (7000). Anal. Calcd for $C_{23}H_{23}N_7O_5 \cdot 2.5H_2O$: C, H, N.

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