Synthesis and Antifolate Activity of 8,10-Dideazaminopterin

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A synthesis of 8,10-dideazaminopterin, using 2,4-diamino-6-bromomethyl-8-deazapteridine (**2**) as a key intermediate, is described. Condensation of the triphenylphosphinylide derived from **2** with *p*-formylbenzoyl-L-glutamate afforded a 9,10-dehydro-8,10-dideazaminopterin ester intermediate **5**. Hydrogenation of the olefinic linkage and subsequent hydrolysis of the glutamate ester gave the title compound. 8,10-Dideazaminopterin was a potent growth inhibitor of folate dependent bacteria. It was 16 times more potent then methotrexate as an inhibitor of dihydrofolate reductase derived from L1210 leukemia cells, and showed strong activity against L1210 in mice.

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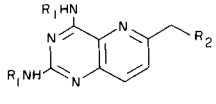
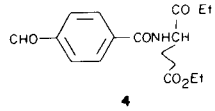
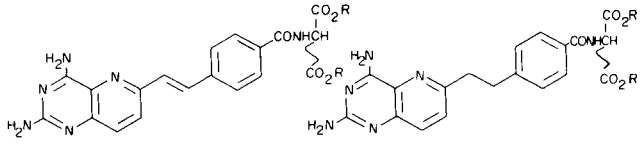
The synthesis and biological evaluation of analogues of methotrexate continues to be a major area of research in cancer chemotherapy. Recent attention has focused on deaza compounds which, amoung other effects, appear to have more desirable transport properties (2). A particular example is 10-deazaminopterin which, based on earlier results obtained for several murine tumor line (3), has now progressed to clinical trials. Replacement of nitrogen with carbon at position 8 of the pteridine nucleus in folate analogs produces compounds that are potent inhibitors of folate-dependent bacteria, or L-1210 murine leukemia (4,5). We have already reported (5) the preparation of 8,10-dideazafolic acid and we disclose here the first synthesis of 8,10-dideazaminopterin (**8**) and its activity against folate dependent bacteria and L-1210 leukemia in mice.



1 R_1 = H, R_2 = OH
2 R_1 = H, R_2 = Br
3 R_1 = Bz, R_2 = Br



4



5 R = Et
6 R = H

7 R = Et
8 R = H

The synthetic scheme utilized has a number of literature analogies and depends on the 6-bromomethyl compound (**2**) as a key intermediate. From a retro-synthetic standpoint, disconnection at the C9-C10 bridge provides the greatest degree of convergence and should allow also for ready extension to further analogs. As reported for the protected 2,4-diaminopteridine case (6), we were similarly unable to prepare **2** by bromination of the 6-Me compound, largely because of competitive dibromination. The 2,4-dibenzamido-6-bromomethyl compound **3** previously utilized for 8-deazafolate (4) was found to be a complex unresolvable mixture that was not suited for the present synthesis. However, a synthesis of **2** has recently been published (7) which, with some experimental modifications, was found to be adequate. The most significant departure from the published procedure is the use of dibromotriphenylphosphorane for conversion of alcohol **1** to the required bromide in place of phosphorus tribromide, as reported by Broom (6). Use of triphenylphosphine dibromide (6) (3.5 equivalents, DMF, room temperature, 1 hour) allowed us to isolate (**2**) as the hydrobromide salt (74%, from glacial acetic acid); ^{1}H-nmr (DMSO-d_6): δ 8.98 (br d NH_2), 8.00 (m, 7-H, 8-H, NH_2), 4.79 (s, CH_2); uv (*p*H 1): ($\epsilon \times 10^3$), 248 (20.2), 320 (8.78), 333 (sh, 7.03); (*p*H 13): 270 (11.05), 342 (6.3); ms: m/e (%) 255/253 (M^+, 8), 174 (100).

The phosphonium salt derived from **2** (triphenylphosphine, DMF, 60°, 1 hour) was allowed to react with diethyl *p*-formylbenzoyl-L-glutamate (**4**) (6) (room temperature, 20 hours) in the presence of sodium methoxide (2.5 equivalents) to give olefin **5** in 57% average yield; ^{1}H-nmr (DMSO-d_6): δ 8.6 (m, CONH), 8.0-7.2 (br m, 7-H, 8-H, olefin -CH = CH-, C_6H_4, NH_2), 6.2 (br s, NH_2) 4.4-3.6 (br m, ester CH_2O, -NHC*H*) 2.5-1.8 (br m, CH_2CH_2), 1.05 (m, ester CH_3); uv (*p*H 1): 225, 270, 318, 355; (*p*H 13): 220, 290, 330, 370; ms: m/e (%) 492 (M^+, 42), 419 (5), 290 (100). An impurity at m/e 478 (14%) was observed which we attribute to paritial trans-esterification between a glutamate ethyl ester and methoxide. Confirmation of this was obtained by

hydrolysis to the di-acid **6** (1 *M* sodium hydroxide, room temperature), which after conversion to a tetratrimethylsilyl derivative showed no trace of a peak at M-14 in the mass spectrum.

Reduction of the olefin linkage in **5** with platinum oxide in ethanol containing acetic acid (5-10% vol) gave **7**, purified by chromatography over silica-gel, (R_f 0.66 chloroform:methanol 6:1), in 65% yield; ^{1}H-nmr (DMSO-d_6): δ 8.6 (br d, amide), 7.75 and 7.3 (2 d, C_6H_4), 7.4 (m, 7-H, 8-H, NH_2), 6.1 (br s, NH_2), 4.35 (m, NHC*H*), 4.0 (m, ester CH_2), 3.1 (br s, 9-H, 10-H), 2.35 (m, CH_2CO_2Et), 2.0 (m, NH-CH-CH_2), 1.10 (m, ester CH_3); uv (*p*H 1): 225, 238 (sh), 245 (sh), 310 (sh), 322, 335; (*p*H 11): 238, 275 (sh), 340; ms: m/e 494 (M^+, 40), 421 (8), 292 (25), 174 (100). Apart from hydrogen uptake, the reaction can be conveniently monitored by either uv or tlc. Over reduction in this instance leads to formation of a more polar compound (R_f 0.23), presumably, the 5,6,7,8-tetrahydro derivative; uv (*p*H 1): 228, 300; (*p*H 13): 225, 242, 305; ms: m/e 498 (M^+). Interestingly, platinum in ethanol free of acetic acid failed to catalyze this reduction (8).

Hydrolysis of ester (**7**) (1 *M* sodium hydroxide, methoxyethanol, room temperature, 4.5 hours) gave the required di-acid **8** in 82% yield; ^{1}H-nmr (DMSO-d_6): δ 8.41 (br d, amide), 7.8 and 7.35 (2 d, C_6H_4), 7.5 (m, 7-H, 8-H, NH_2), 6.62 (br s, NH_2), 4.5 (m, NHC*H*), 3.15 (br s, 9-H, 10-H), 2.3 (m, CH_2 CO_2H), 2.05 (m, NHCHCH_2); uv (*p*H 1): 222 (36.0), 233 (sh, 33.7), 242 (sh, 31.1), 308 (sh, 6.8), 320 (8.6) 334 (sh, 6.9); (*p*H 11): 238 (34.3), 276 (sh, 10.3), 344 (5.8). Satisfactory elemental analyses (C, H, N) were obtained for all new compounds.

In Table I we present growth inhibition data against *Streptococcus faecium,* and *Lactobacillus casei,* dihydrofolate reductase (from L1210) inhibition and antitumor effect against L1210 in mice for compounds **6** and **8** in comparison with 10-deazaminopterin and methotrexate (MTX). The olefinic compound **6** was moderately active against the bacteria, **8** was four times as potent as MTX against *S. faecium* and equipotent against *L. casei.* Compound **8** was 16 times as potent an inhibitor of the reductase enzyme as MTX. Against L1210 leukemia in mice it produced an increase life span (ILS) of 189%, comparable to 10-deazaminopterin (184%). However, this effect was obtained at an LD_{10} dose of only 0.75 mg/kg, about one-twelfth of that required for 10-deazaminopterin. Further studies concerning biological activity of 8,10-dideazaminopterin (**8**) and analogs will be reported elsewhere.

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Table I

Biological Activity of 8,10-Dideazaminopterin and Δ^5-8,10-Dideazaminopterin

Compound	Growth Inhibition (ng/ml) (for 50% inhib) S. faecium (a)	L. casei (b)	Dihydrofolate Reductase Inhibition (c) K_i (m)	Antitumor (d) Effect ILS (%)
8	0.04	0.005	0.28×10^{-12}	+189
6	1.10	1.30	—	—
10-Deazaminopterin	0.20	0.01	3.57×10^{-12}	+184
Methotrexate	0.15	0.005	4.63×10^{-12}	+163

(a) ATCC 8043. (b) ATCC 7469. (c) Enzyme derived from L-1210. (d) Approximately LD_{10} dosage on a schedule every 2 days × 5; MTX, 12 mg/kg, 10-deazaminopterin, 9 mg/kg, 8,10-dideazaminopterin 0.75 mg/kg. Five mice per treated and control groups; average survival time of mice receiving 10^6 tumor cells was 7.1 ± 1 days.