# SYNTHESIS AND EVALUATION OF 8,10-DIDEAZATETRAHYDROFOLIC ACID AND DERIVATIVES

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**Abstract** - Syntheses of 8,10-dideazatetrahydrofolic acid and its 5-*N*-methyl and 5-*N*-formyl derivatives are reported. Hydrolysis of 2,4-diamino-4-deoxy-8,10-dideazapteroic acid in hot alkali afforded 8,10-dideazapteroic acid. Coupling with diethyl L-glutamate followed by saponification gave 8,10-dideazafolic acid. Hydrogenation in acidic media gave the tetrahydro compound, while hydrogenation in the presence of formaldehyde yielded the 5-*N*-$CH_3$ analog. The 5-*N*-$CH_3$ compound was more potent than 5,10-DDTHF as an inhibitor of growth for L1210 cells in culture. In contrast to 5,10-DDTHF, the locus of action was apparently unrelated to inhibition of GAR formyltransferase. Unfortunately, 5-$CH_3$-8,10-DDTHF was not active *in vivo* against an L1210 challenge in mice.

Recently, Taylor and coworkers[1] announced the synthesis of 5,10-dideazatetrahydrofolic acid (5,10-DDTHF). This compound was found to be a potent inhibitor of glycinamide ribotide (GAR) formyltransferase[2] as well as growth of L1210 cells in culture. The compound was also an effective antitumor agent in murine tumor models[3] and is undergoing clinical evaluation. Other reports[4,5] have indicated that considerable variation in structure, including ring B-opened analogs, was permissible without major loss of activity. However, it was also recently

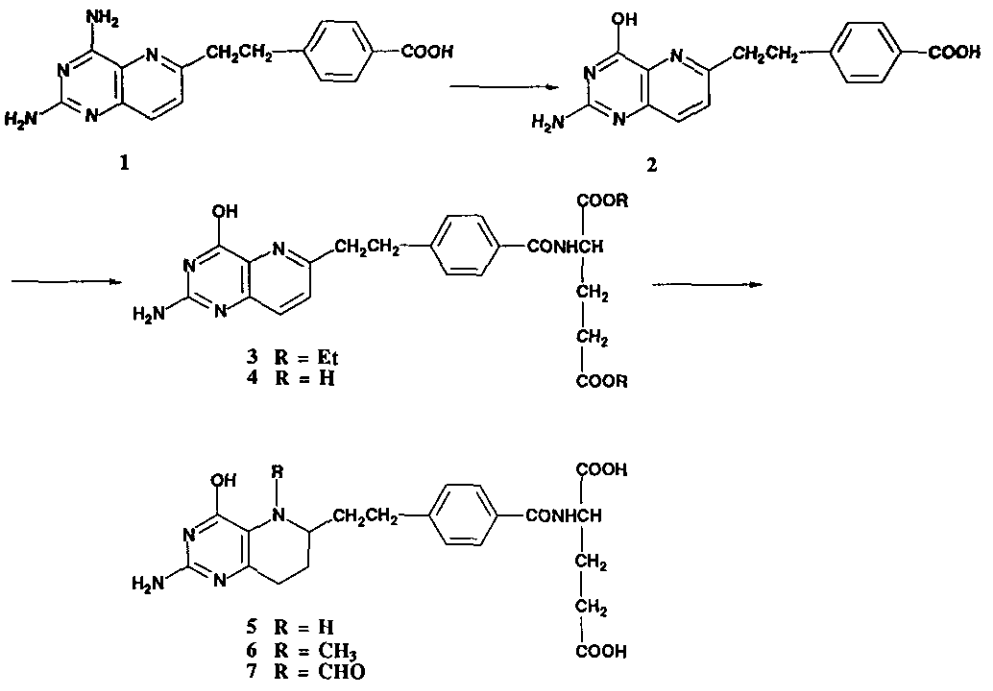
shown[6] that removal of *N*-5 is necessary and sufficient to confer GARTF inhibition. In light of these observations, we chose to investigate the 8,10-DDTHF analog as a further probe of the structure-activity relationships in this area. We report herein the synthesis and evaluation of 8,10-DDTHF and its 5-*N*-methyl and formyl derivatives.

## CHEMISTRY

The synthesis of 8,10-DDTHF and derivatives was readily accomplished by the method outlined in Scheme I. 2,4-Diamino-4-deoxy-8,10-dideazaaminopterin (**1**)[7] was treated with hot 10% NaOH to effect hydrolysis of the 4-$NH_2$ group affording 8,10-dideazapteroic acid (**2**). Compound (**2**) was obtained in 59% yield following crystallization of the disodium salt from cold 10% NaOH. Coupling of **2** with diethyl L-glutamate via the mixed anhydride procedure (isobutyl chloroformate) gave the diethyl ester of 8,10-dideazafolic acid (**3**) in only 13% yield following chromatography on silica gel. 8,10-Dideazafolic acid (**4**) was readily obtained in 77% yield following saponification of the diester with 1N NaOH in 2-methoxyethanol.

Hydrogenation of the folic acid (**4**) over $PtO_2$ in aqueous HOAc containing an equivalent of HCl proceeded rapidly at room temperature . The hydrochloride salt of 8,10-DDTHF (**5**) was obtained by evaporation of solvent following removal of the hydrogenation catalyst. When the hydrogenation was conducted in the presence of a small excess of formaldehyde, the HCl salt of the 5-*N*-methyl analog (**6**) was obtained following a similar isolation procedure.

The 5-*N*-formyl derivative (**7**) of 8,10-DDTHF was prepared by treating the NH compound (**5**) at 25° with formic-acetic anhydride. After treatment with $H_2O$ and evaporation of solvents, the product was obtained as a white solid in 66% yield.




**Scheme I**

## BIOLOGICAL RESULTS

In Table 1 the comparative activities of compounds (**5-7**) versus 5,10-DDTHF and methotrexate for inhibition of growth of *L1210 cells and inhibition of folate-related enzymes are shown.* The 5-*N*-$CH_3$ analog (**6**) was clearly the most active compound in terms of growth inhibition for L1210 cells in culture. In fact, **6** was approximately equipotent to 5,10-DDTHF, but both compounds were less potent than MTX by an order of magnitude. The 5-*N*-CHO compound (**7**) was a relatively weak inhibitor of cell growth for L1210.

Although **6** was slightly more potent than 5,10-DDTHF for growth inhibition, *the locus of activity does not* appear to be concerned with GAR formyltransferase. As shown in Table 1, 8,10-DDTHF and the 5-*N*-$CH_3$ analog were ineffective as inhibitors of this enzyme. In contrast, 5,10-DDTHF was quite effective, being some 4-5000 times more potent than compounds (**5**) or (**6**). Similarly, there was no evidence for activity against the *aminoimidazolecarboxamide ribotide formyl transferase* (AICR) enzyme. Although one could suggest that a polyglutamylated form of compound (**5**) or (**6**) was the active species following intracellular metabolism, the

exceptionally weak activity makes this appear doubtful. The above results indicate that the GAR enzyme is sensitive to the presence of the 8-*NH* group to achieve strong binding. Since we completed this work it became evident from the work of others[6] that an 8-*NH* is indeed necessary for GARTF activity. In fact, a recent x-ray crystal structure of *E. coli* GARTF complexed with a 5,10-DDTHF analog showed that the 8-*NH* is involved in a hydrogen bond to the enzyme.[8] Not surprisingly, compounds (**5**) and (**6**) were ineffective as inhibitors of dihydrofolate reductase or thymidylate synthase enzymes.

Even though compound (**6**) was quite effective against L1210 *in vitro* the analog was inactive against L1210 in mice at doses up to 200 mg/kg. We are unable to explain this lack of activity *in vivo* unless it was due to rapid clearance.

**Table 1**
**Cell Growth and Enzyme Inhibition[c,d]**

| Compound | L1210 Growth Inhibition[a] $IC_{50}$ nM | *L. casei* GAR Formyl Transferase[b] $IC_{50}$ nM | *L. casei* AICR Formyl Transferase[b] $IC_{50}$ nM |
|---|---|---|---|
| **5** | 96.8 | > 40,000 | > 40,000 |
| **6** | 38.2 | 53,000 | > 50,000 |
| **7** | 657.0 | — | — |
| **5,10-DDTHF** | 42.0 | 11.0 | — |
| **MTX** | 4.3 | — | — |

[a] See DeGraw *et al.* ref. 9 for methods.
[b] Assayed by the method of Smith *et al*, ref. 10
[c] L1210 DHFR inhibition was $IC_{50}$ >1 μM for **5** and **6**.
[d] Thymidylate synthase inhibition was $IC_{50}$ >10-4 M for **5** and **6**.

## EXPERIMENTAL SECTION

Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN. 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.

**8,10-Dideazapteroic Acid (2).** 2,4-Diamino-4-deoxy-8,10-dideazapteroic acid (**1**) (0.88 g, 2.8 mmol) was heated to 85-90°C with 15 ml of 10% NaOH solution under an argon atmosphere. Hplc analysis of the reaction indicated maximum conversion after 5 h. The reaction mixture was chilled at 5°C for 48 h. The resulting ppt. (disodium salt) was collected and washed with a small amount of ice-cold 10% NaOH. The ppt. was stirred with a little $H_2O$ and the pH adjusted to 5 by addition of HOAc. The suspended solid was stirred for 10 min, then collected and washed with a little $H_2O$ to yield 0.52 g (59%) of light grey solid after drying. *Anal.* Calcd for $C_{16}H_{14}N_4O_3 \cdot 0.5\ H_2O$: C, 60.2; H, 4.73; N, 17.5. Found: C, 60.2; H, 4.51; N, 17.5. Mass spectrum, calcd. 310. Found: M + 310.

**8,10-Dideazafolic Acid Diethyl Ester (3).** 8,10-Dideazapteroic acid (**2**) (0.52 g, 1.7 mmol) was stirred with 15 ml of dry DMF under an argon atmosphere. Triethylamine (0.69 g, 6.8 mmol) was added and the mixture was stirred for 15 min, then isobutyl chloroformate (0.87 g, 6.4 mmol) was added. After 1 h only partial solution was obtained. The mixture was sequentially treated with triethylamine (0.017 g, 1.7 mmol) and isobutyl chloroformate (0.23 g, 1.7 mmol). Complete solution occurred after 20 min. Triethylamine (0.81 g, 8.1 mmol) followed by diethyl-L-glutamic acid diethyl ester hydrochloride (1.94 g, 8.1 mmol) in 10 ml of DMF were added and the resulting solution was stirred for 20 h. The solvent was removed at 40° (0.1 mm) and the residue was dissolved in $CHCl_3$ then washed with $H_2O$ and dilute $NH_4OH$, dried over $Na_2SO_4$ and the solution evaporated *in vacuo*. The gummy residue was chromatographed on silica gel with $CHCl_3$ and $CH_3OH$-$CHCl_3$ elution. The product, 110 mg (13%) of yellow solid, was eluted with $CH_3OH$:$CHCl_3$, 2:98; *Anal.* Calcd for $C_{25}H_{29}N_5O_6 \cdot 0.5\ H_2O$: C, 59.5; H, 5.99; N, 13.9. Found: C, 59.3; H, 5.80; N, 13.6.

**8,10-Dideazafolic Acid (4).** 8,10-Dideazafolic acid diethyl ester (**3**) (110 mg, 0.22 mmol) was treated with 1 ml of 1N NaOH and 1 ml of 2-methoxyethanol. The solution was stirred for 15 h then the solvents were removed at 40° (0.1 mm). The residue was treated with 1 ml of $H_2O$ and acidified to pH 5.5 with HOAc. The resulting ppt. was collected, washed with $H_2O$ and dried to yield 74 mg (75%) of product as a yellow solid; *Anal.* Calcd for $C_{21}H_{21}N_5O_6 \cdot 2\ H_2O$: C, 53.1; H, 5.30; N, 14.7. Found: C, 53.4; H, 4.67; N, 14.5.

**8,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (5).** 8,10-Dideazafolic acid (**4**) (100 mg, 0.23 mmol) dissolved in 10 ml of HOAc, 9 ml of $H_2O$, and 2 ml (0.24 mmol) of 1% HCl solution was hydrogenated at atmospheric pressure over 25 mg of $PtO_2$. After 1 h, 15 ml of $H_2$ (theory 15 ml) had been taken up. The mixture

was filtered through Celite then taken to dryness at 0.1 mm pressure (40°). The residue was twice treated with 10 ml of $H_2O$ and taken to dryness each time to yield 100 mg (97%) of white solid; uv (pH 13) 238 nm, 290 (sh); *Anal.* Calcd for $C_{21}H_{25}N_5O_6 \cdot HCl \cdot 4\ H_2O$: C, 45.7; H, 6.16; N, 12.7. Found: C, 45.9; H, 5.68; N, 12.3. Mass spectrum, calcd. 443. Found: M + 443. Hplc analysis showed 96% purity detected at 254 and 300 nm.

**5-Methyl-8,10-dideaza-5,6,7,8-tetrahydrofolic Acid (6).** 8,10-Dideazafolic acid (**4**) (74 mg, 0.17 mmol) was dissolved in 8 ml of acetic acid and 2 ml of $H_2O$ containing 0.24 mmol of 12*N* HCl. $PtO_2$ (24 mg) was added and the mixture was hydrogenated at atmospheric pressure. Hydrogen uptake ceased after uptake of 15 ml of $H_2$ (theory 12.4 ml) and (0.018 ml, 0.20 mmol, of 35% formaldehyde) was added. Continued hydrogenation resulted in the rapid uptake of 4 ml of $H_2$ (theory 3.8 ml). The mixture was filtered through Celite and the solvent removed at 40°C (0.1 mm). The residue was twice treated with 2 ml of $H_2O$ and taken to dryness each time to leave 80 mg (99%) of white solid. Uv (pH 13) 238 nm, 290 (sh); *Anal.*Calcd for $C_{22}H_{27}N_5O_6 \cdot HCl \cdot H_2O$: C, 51.6; H, 5.90; N, 13.6. Found: C, 51.4; H, 5.82; N, 13.2. Mass spectrum, calcd 457. Found: M + 457.

**5-*N*-Formyl-8,10-dideaza-5,6,7,8-tetrahydrofolic Acid (7).** To a solution of 240 mg (0.55 mmol) of 8,10-dideazafolic acid (**4**) in 20 ml of 98% HCOOH was added 68 mg of $PtO_2$ and the mixture was stirred under one atm. of $H_2$ until the uv peak at 330 nm disappeared (48 h). The catalyst was removed by filtration. To the filtrate was added formic acetic anhydride (0.37 ml, 6.7 eq., prepared from $Ac_2O$–HCOOH at 50°C for 2 h) and the solution was kept at ambient temperature for 24 h. Another 0.37 ml of the mixed anhydride was added and the solution was allowed to stand for 48 h. The solution was then lyophilized under high vacuum to afford 305 mg of a tan solid. The material was stirred with 30 ml of $H_2O$ and centrifuged, followed by lyophilization of the supernatant to yield 229 mg of a white solid. The solid was treated with 40 ml of hot 2-PrOH and the solid was collected by filtration and dried to leave 167 mg (66%) of white solid; uv (pH 13) 240 nm, 285; *Anal.* Calcd for $C_{22}H_{25}N_5O_7 \cdot 2\ H_2O$: C, 52.1; H, 5.76; N, 13.8. Found: C, 51.8; H, 5.45, N, 14.1. Mass spectrum, calcd. for $(Me_3Si)_3$ 687. Found: 687.

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