# New Pathways from Pteridines. Design and Synthesis of a New Class of Potent and Selective Antitumor Agents



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Present day cancer chemotherapy can be said to have had its beginnings with the discovery that aminopterin and methotrexate (MTX), synthesized as potential antimetabolites of folic acid (Figure 1), induced remissions of

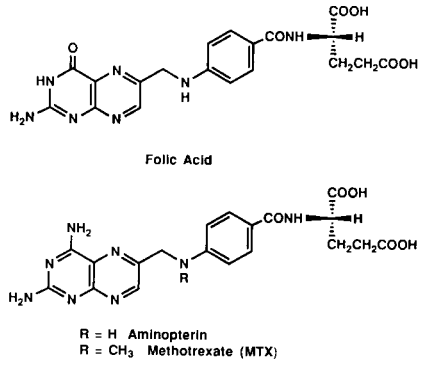
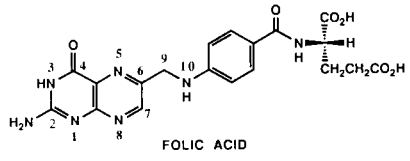



Figure 1

acute lymphoblastic leukemia [1-4]. The biological basis for the cytotoxicity of these two compounds was found to be their potent inhibitory activity towards dihydrofolate reductase (DHFR), an enzyme which regenerates tetrahydrofolate from dihydrofolate, and therefore maintains a cellular supply of this critical coenzyme. Tetrahydrofolate and its various modifications (see Figure 2) play critical roles

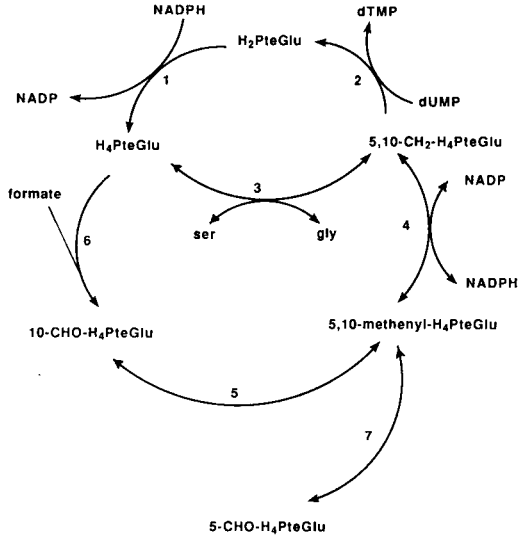



| | |
|---|---|
| Folic Acid | PteGlu |
| 7,8-Dihydrofolic Acid | $H_2$PteGlu |
| 5,6,7,8-Tetrahydrofolic Acid | $H_4$PteGlu |
| 5,10-Methylenetetrahydrofolic Acid | 5,10-$CH_2H_4$PteGlu |
| 5,10-Methenyltetrahydrofolic Acid | 5,10-$CHH_4$PteGlu |
| 5-Methyltetrahydrofolic Acid | 5-$CH_3H_4$PteGlu |
| 10-Formyltetrahydrofolic Acid | 10-$CHOH_4$PteGlu |
| 5-Formyltetrahydrofolic Acid | 5-$CHOH_4$PteGlu (Leucovorin) |
| 5-Formiminotetrahydrofolic Acid | 5-$CHNHH_4$PteGlu |

Figure 2

in a wide spectrum of cellular one-carbon transfer reactions - in the biosynthesis of both purine and pyrimidine nucleotides (and hence DNA), in the regeneration of methionine from homocys-teine, the interconversion of serine and glycine, and the catabo-lism of certain amino acids [5]. Figure 3 gives a much abbreviated summary of the diverse roles played by the tetrahydrofolate-requiring enzymes either in one-carbon transfer reactions, or in transformations among the folate cofactors themselves.

Although inhibition of any of these folate-requiring me-




Enzymes involved in reduced folate metabolism: 1, dihydrofolate reductase; 2, thymidylate synthase; 3, serine hydroxymethyltransferase; 4, 5,10-methylenetetrahydrofolate dehydrogenase; 5, cyclohydrolase; 6, 10-formyltetrahydrofolate synthetase; 7, $N^5$-formyltetrahydrofolate cyclodehydrase.

Figure 3

tabolic transformations might be predicted to have a severe effect upon cell growth, achievement of selectivity between normal cells and tumor cells is a more elusive goal. Methotrexate itself, although it has been in clinical use for some 40 years, is a non-se-lective cytotoxic agent; its extreme toxicity coupled with its in-effectiveness against many types of human cancers has severely limited its clinical effectiveness [5,6]. Furthermore, develop-ment of resistance to methotrexate by tumor cells remains a stubborn problem [7-16].

Extensive efforts over many years have been made to modify the aminopterin/MTX/folic acid structure in an effort to develop less toxic cofactor antagonists which might possess more selective transport and inhibitory properties and which might therefore be effective against a broader range of human cancers. A variety of deaza derivatives of folic acid/MTX/aminopterin have proven to be particularly intriguing, in part because their increased lipophilicity leads to more favorable transport properties [17,18]. One such deaza derivative, $N^{10}$-propargyl-5,8-dideaza-folic acid (CB3717), was shown to inhibit thymidylate synthase rather than DHFR, and has undergone extensive preclinical and clinical evaluation [19-27].

This lecture will describe some of our own efforts in the design and synthesis of certain folate antimetabolites.

This has been a research arena full of frustrations, distractions, and false leads, but extremely rich in challenges for synthetic heterocyclic chemistry. When we embarked upon this program a number of years ago, in collaboration with Prof. G. Peter Beardsley of the Yale University School of Medicine (one of my former Ph.D. students, and an active collaborator in the design program which led eventually to DDATHF), our initial objective was 5-deazaaminopterin (**1**, Figure 4). This compound was chosen as a potential chemotherapeutic agent not only because of considerations related to anticipated increased binding to DHFR, transport into tumor cells, and intracellular polyglutamation possibilities [28,29], but also because removal of the critical N-5 nitrogen should prevent utilization of this compound or its metabolites as surrogate cofactors. As will be seen from what follows, this initial foray into the design and synthesis of what we hoped would be an effective and selective antiproliferative agent proved to be a rather shaky one.

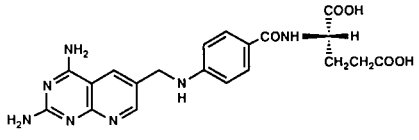



Figure 4

The synthesis of **1** seemed at first glance to be extremely straightforward. We readily prepared 2,4-diamino-6-methyl-5-de-azapteridine (**5**) by the reaction scheme shown in Figure 5. Thus, an aldol condensation between malononitrile and propion-aldehyde gave 1,1-dicyano-1-butene

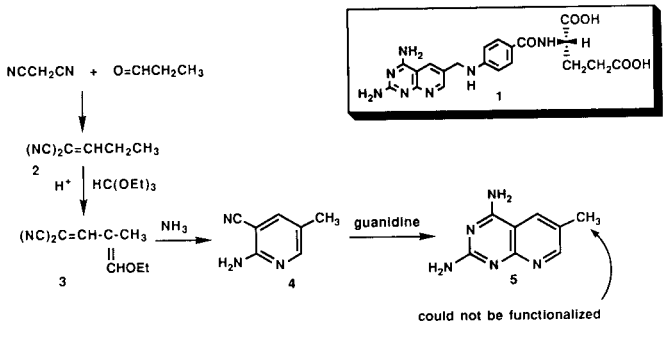



Figure 5

(**2**) which with triethyl orthoformate in the presence of an acid catalyst yielded the ethoxy-methylene derivative **3**. Treatment with ammonia resulted in Michael addition, loss of ethanol, and intramolecular cyclization of the resulting aminomethylene derivative across one of the two nitrile groups to give 2-amino-3-cyano-5-methylpyridine (**4**) which cyclized smoothly to **5** with guanidine. We assumed, on the basis of previous syntheses of folic acid [30,31], aminopterin [31-33] and MTX [31-35], that **5** would be a useful intermediate for functionalization at the methyl group for subsequent attachment of the requisite sidechain. However, the methyl group in **5** proved to be completely resistant to functionalization. Mild reaction conditions resulted in recovery of starting material, whereas harsher conditions consumed the substrate but gave no identifiable products. The unreactivity of this methyl group is reminiscent of difficulties encountered in attempts to functionalize 3-picoline [36], and is compounded in this case as a consequence of the extreme insolubility of **5** in organic solvents.

An alternative strategy for approaching our initial target compound **1** involved construction of the 5-deazapteridine nucleus complete with a C-6 substituent already suitably functionalized for further transformations. The sequence of reactions depicted in Figure 6 is mentioned here not

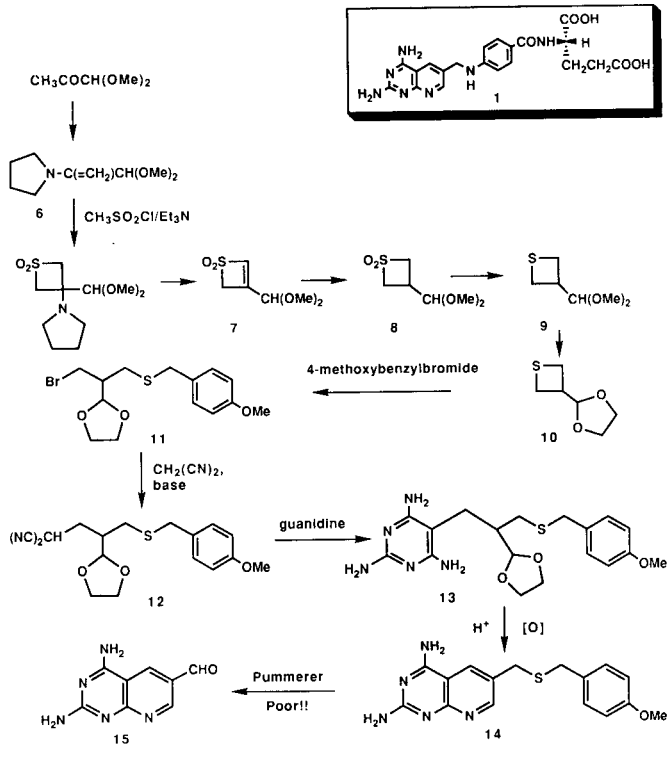



Figure 6

because of the efficiency of the overall synthesis (quite the reverse!) but rather because the strategy depicts some intriguing thietane chemistry which may be applicable in other synthetic situations. Very briefly, the pyrrolidine enamine of pyruvaldehyde dimethyl acetal (**6**) was converted in excellent yield to 3-(N-pyrrolidino)-3-formylthietane 1,1-dioxide dimethylacetal by cycloaddition with sulfene, formed in situ from mesyl chloride and triethylamine in methylene chloride. Quaternization with methyl fluorosulfonate was followed by elimination of N-methylpyrrolidine with triethylamine, and the resulting olefin (**7**) was reduced catalytically. The sulfone grouping in **8** was then reduced to the corresponding sulfide **9** with LAH. Transacetalization with ethylene glycol gave the thietane intermediate **10**, which upon sulfur alkylation with 4-methoxybenzyl bromide underwent smooth ring opening to the primary bromide **11**. Condensation with the sodium salt of malononitrile under Finkelstein conditions gave the mono-alkylated malononitrile **12** which was cyclized with

guanidine to the triaminopyrimidine **13**. Acidic hydrolysis under oxidative conditions resulted in cyclization and aromatization to give our desired C-6 functionalized 2,4-diamino-5-deazapteridine derivative **14**. Our reason for pursuing this rather laborious synthesis was our anticipation that the sulfide **14** should, upon oxidation to its sulfoxide, undergo a directed Pummerer rearrangement to give the long-sought aldehyde **15**. However, this strategy floundered at this critical last step, since the extreme insolubility of **14** made its characterization and functionalization extraordinarily difficult. This approach to the aldehyde **15**, which still appeared to be a most attractive intermediate for further conversion to **1**, was rather gratefully abandoned [17].

Our next approach was equally (and, in the event, unnecessarily) complex, but it was successful. Condensation of α-cyano-thioacetamide with 2-methyl-3-ethoxyacrolein gave 3-cyano-5-methyl-2(1H)-pyridinethione (**16**) (Figure 7). This was arylated on sulfur with 4-nitrofluorobenzene, and the resulting sulfide **17** was brominated under free radical conditions to give a mixture of mono-, di-, and tri-bromo derivatives. The desired mono-bromomethyl derivative was retrieved from this complex reaction mixture by addition of pyridine, which resulted in precipitation of the pyridinium salt **18**. This compound was subjected directly to the Kröhnke reaction (condensation with 4-nitrosodimethylaniline and acidic hydrolysis of the resulting nitrone) to give the aldehyde **19**. Aminolysis of its dimethylacetal **20** was effected with liquid ammonia in the presence of cupric bromide, and the resulting *o*-aminonitrile **21** was smoothly cyclized with guanidine in anhydrous methanol to **22**, the acetal of 2,4-diamino-6-formyl-5-deazapterin (**15**).

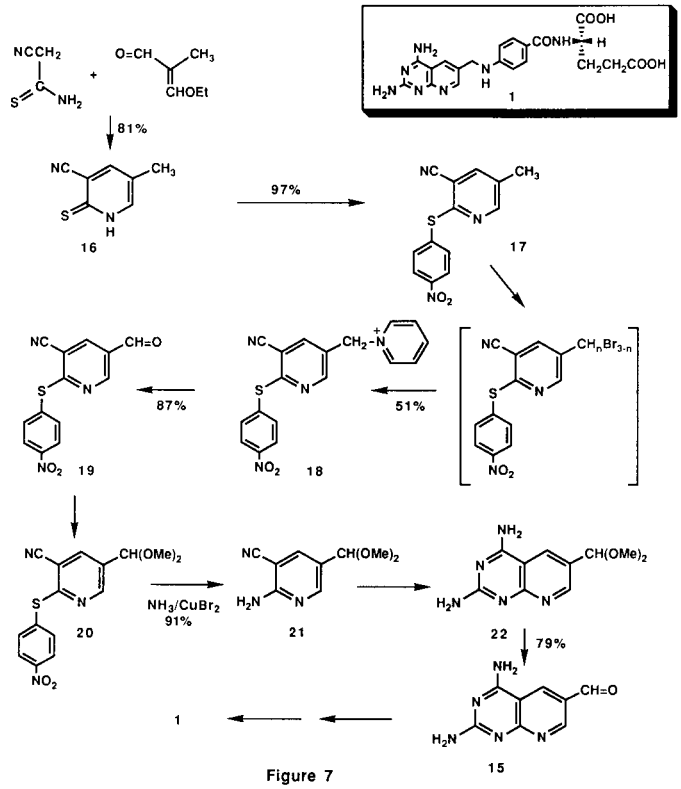



Figure 7

Our first target was now within our grasp. Acetal hydrolysis gave the aldehyde **15** which upon reductive amination with dimethyl 4-aminobenzoyl-L-glutamate and saponification of the resulting dimethyl ester finally yielded 5-deazaaminopterin (**1**). In retrospect, it is ironic that the critical aldehyde intermediate **15** can actually be prepared in a single step by reaction of 2,4,6-triaminopyrimidine with *impure* triformylmethane [37] (i.e., the crude reaction mixture from DMF/$POCl_3$/bromoacetic acid; the reaction with pure triformylmethane is unsuccessful [17]). Unfortunately, biological evaluation of this first target compound **1** showed it to be essentially equivalent to MTX, both as an inhibitor of DHFR, and as an antileukemic agent. No advantages over MTX were evident, and it was certainly less readily accessible!

Could the cytotoxicity, transport selectivity and tumor specificity of this type of folate antimetabolite be raised by a further increase in lipophilic character? In order to answer this question, we next examined [38] the synthesis of 5,10-dideazaaminopterin (**23**) (see Figure 8), since a useful intermediate for its preparation was already in hand as a result of the sequence of reactions just described. Thus, the mixture of mono-, di-, and tri-bromo derivatives obtained by bromination of 2-(4'-nitrophenylthio)-3-cyano-5-methylpyridine (**17**, Figure 7) was treated with triphenylphosphine. This resulted in direct crystallization from the reaction mixture of the triphenylphosphonium salt **24**, which was condensed with *t*-butyl 4-formylbenzoate under normal Wittig conditions (Figure 9). The resulting olefin **25** was readily converted into the *o*-aminonitrile **26** by reaction with liquid ammonia and cupric bromide at room temperature for 14 days. In our initial attempts to convert this intermediate on to **23**, we first reduced the olefinic double bond in **25** and then attempted guanidine cyclization. Under relatively mild conditions, only aroylation of the guanidine occurred; complex, inseparable mixtures were obtained under more forcing conditions. Similar results were obtained with N,N-dimethylguanidine, which is an effective replacement for guanidine in this type of ring annulation reaction [17]. We surmise that the failure of these guanidine cyclizations

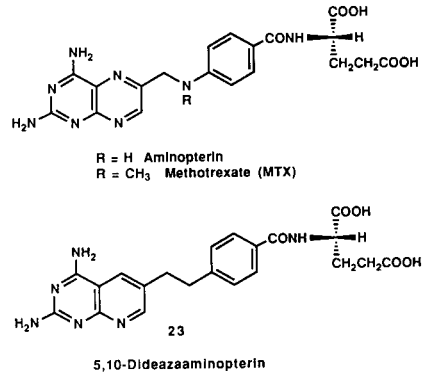



Figure 8

is probably due not only to the electrophilicity of the benzoate carbonyl grouping, but also to a decrease in the electrophilicity of the nitrile substituent in the pyridine ring (compared with analogous pyrazines) as a consequence of removal of one of the electronegative ring nitrogen atoms. Both of these factors could, in principle, be neutralized by employing the styryl aminonitrile **26** as a substrate for the guanidine pyrimidine ring annulation reaction, since retention of unsaturation between the pyridine and benzene moieties would serve both to decrease the electrophilicity of the benzoate carbonyl grouping, (which now would be a vinylogous urethane), and to increase the electrophilicity of the 3-cyano grouping. In the event, treatment of **26** with guanidine free base in refluxing *t*-butyl alcohol indeed resulted in smooth cyclization to **27**. Hydrolysis to the free acid **28** was smoothly accomplished with dry hydrochloric acid in nitromethane. Although the next transformation depicted in Figure 9 - coupling of the free acid with diethyl L-glutamate - looks

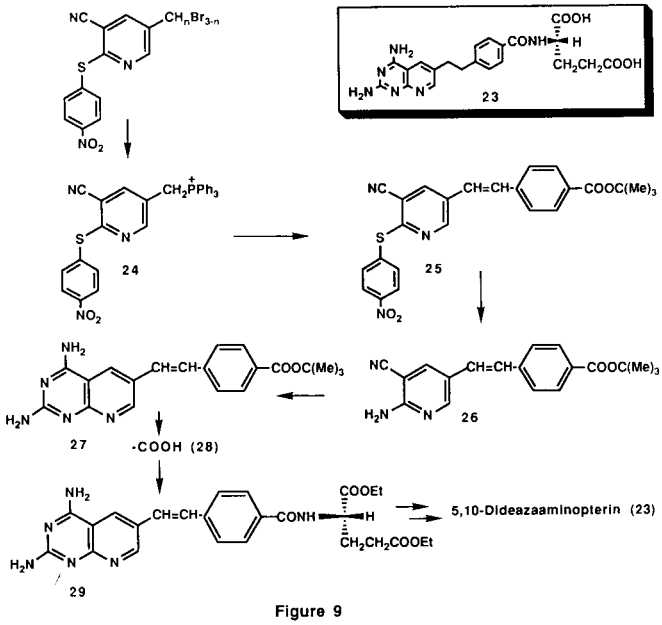



Figure 9

straightforward, it took us more than one year to accomplish. The carboxylic acid **28** is extraordinarily insoluble, and we constantly ran out of starting material. Coupling was finally achieved using diphenyl chlorophosphonate as the coupling agent in N-methylpyrrolidone as solvent. Conversion on to 5,10-dideazaaminopterin (**23**) was then straightforward. Once again, however, biological evaluation of this potential folate antimetabolite was disappointing. Compound **23** proved to be a potent inhibitor of DHFR and of L1210 murine leukemia cells in tissue culture, and it was an effective antileukemic agent in $BDF_1$ mice, but its activity was only comparable to that of methotrexate, and, like the 5-deaza analog **1**, it did not possess any advantages over MTX. We were therefore left with two non-selective MTX surrogates of comparable activity but which were (at least by the synthetic procedures utilized thus far), much less convenient to pre-

The *de novo* Purine Biosynthetic Pathway

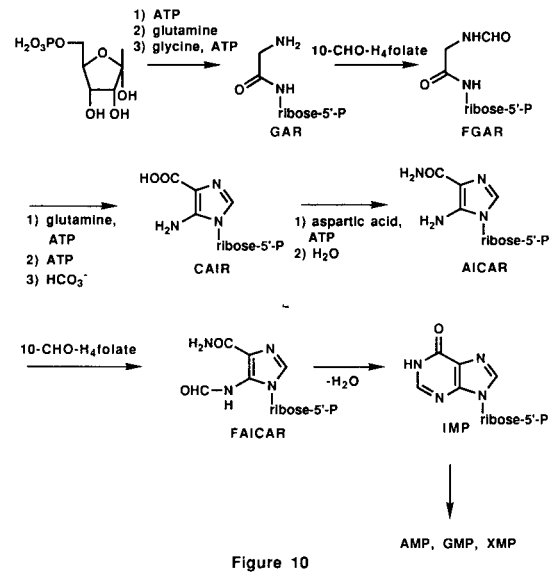



Figure 10

pare. Our program on the design and synthesis of folate antimetabolites as chemotherapeutic agents clearly needed revision.

It seemed worthwhile at this point to review once again the central role of the reduced folate cofactors in intermediary metabolism. Inhibition of DHFR may be, almost by definition, a frustrating arena in which to search for selective (non-toxic) inhibitors, since tetrahydrofolate coenzymes are crucial to so many metabolic transformations. The de novo purine synthetic pathway offers another possibility, however. As shown in Figure 10, a folate coenzyme (10-formyltetrahydrofolate) participates in two separate one-carbon transfer reactions involved in construction of the purine ring system. 5'-Phosphoribosylglycinamide (GAR) is formylated to 5'-phosphoribosyl-a-N-formylglycinamide (FGAR) (the enzyme involved is GAR TFase; this step introduces the eventual 8-carbon

The *de novo* Purine Biosynthesis Pathway

The GAR-to-FGAR Step

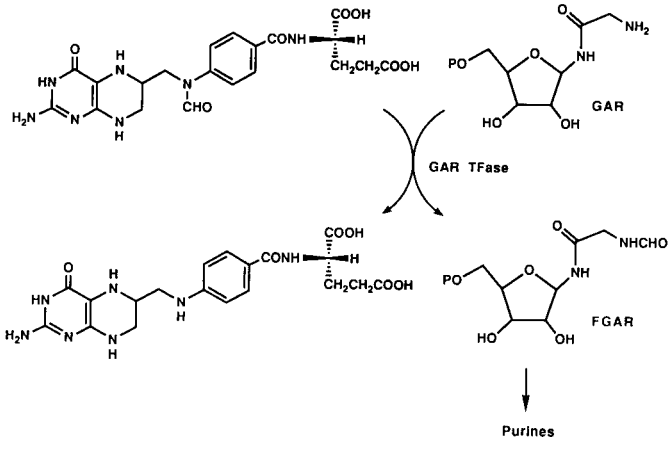
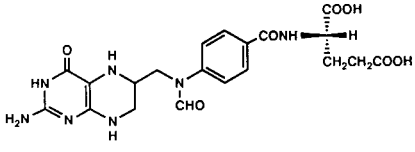


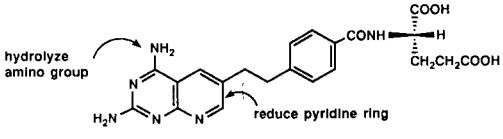

Figure 11

atom of the purine ring), and 5'-phosphoribosylamino-imidazolecarboxamide is formylated to 5'-phosphoribosyl-5-formamido-4-imidazolecarboxamide (FAICAR) (the enzyme involved is AICAR TFase; this step introduces the eventual 2-carbon atom of the purine ring). Dehydration of this latter intermediate gives inosine 5'-phosphate (IMP), from which the purine nucleotides, including ATP, are subsequently formed. The first of these two formyl transfer steps in de novo purine biosynthesis is shown in greater detail in Figure 11.

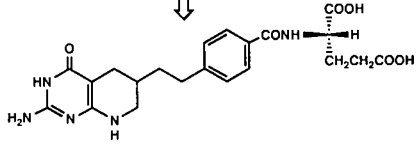
A comparison (see Figure 12) of the structure of the natural tetrahydrofolate cofactor involved in the above de novo purine synthetic pathway with our DHFR inhibitor **23** suggests that conversion of the latter to a close structural analog (and thus a potential inhibitor) of the former might be achieved by several simple structural modifications; i.e., by hydrolysis of the 4-amino grouping to a



Natural cofactor - the GAR-to-FGAR step




our DHFR inhibitor (5,10-dideazaaminopterin, 23)



5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid

DDATHF

Figure 12

cyclic lactam, and by reduction of the pyridine ring to its tetrahydro form. The resulting compound, 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF), possesses a number of predictable features which make it an attractive target for synthesis: it lacks nitrogens at positions 5 and 10, and thus could not be converted to substrates which might serve as surrogate cofactors in any of the metabolic tetrahydrofolate-dependent transformations discussed earlier; the absence of the 4-amino grouping means that DDATHF would be extremely unlikely to be an inhibitor of DHFR; as a tetrahydro derivative, DDATHF possesses a structural feature important for membrane transport and for polyglutamation by folylpolyglutamate synthetase (FPGS); and DDATHF, as a tetrahydropyridine rather than a tetrahydropyrazine derivative, should be exceptionally stable.

DDATHF was then synthesized [38] as shown in Figure 13 from the intermediate styryl derivative **28** in our

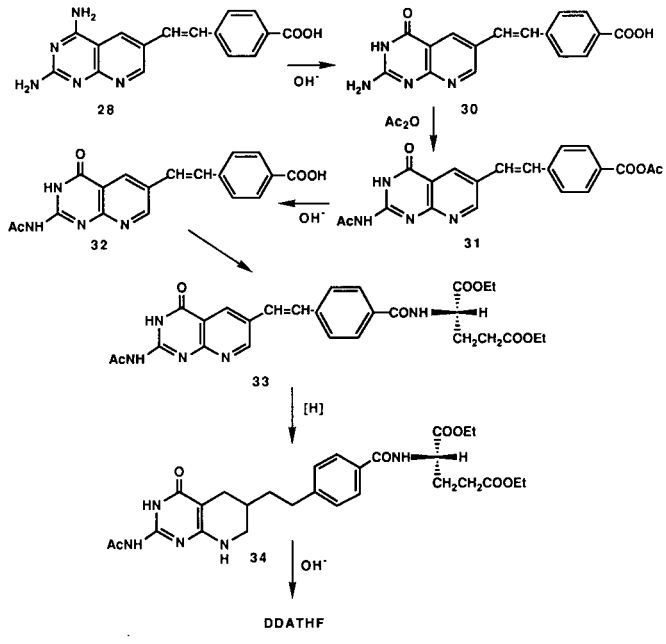



Figure 13

previously described synthesis of 5,10-dideazaaminopterin (**23**). Alkaline hydrolysis of the 4-amino grouping in **28** gave **30**, which was then acetylated to give the 2-acetamido mixed anhydride **31**. Selective hydrolysis afforded **32** which was coupled with diethyl L-glutamate with phenyl N-phenylphosphoramidochloridate as the coupling agent in N-methylpyrrolidone as solvent. Catalytic reduction of the resulting **33** then gave **34**, which was carefully saponified to DDATHF.

**Comparative Antitumor Activity of DDATHF vs. Methotrexate**

| Murine Solid Tumor Models | DDATHF | MTX |
|---|---|---|
| X-5563 Myeloma | +++ | ++ |
| CA 755 Adenocarcinoma | + | - |
| M 5 Ovarian Carcinoma | - | - |
| 6C3HED Lymphosarcoma | +++ | NT |
| Colon 26 Carcinoma | ++ | - |
| B-16 Melanoma | +++ | - |
| Lewis Lung Carcinoma | ++ | - |
| Madison Lung Carcinoma | ++ | - |
| C3H Mammary Carcinoma | +++ | - |
| Human Tumor Xenograft Models | | |
| LX-1 Lung Carcinoma | ++ | - |
| CX-1 Colon Carcinoma | + | - |
| MX-1 Mammary Carcinoma | + | - |
| HC1 Colon Carcinoma | +++ | - |
| GC3 Colon Carcinoma | +++ | - |
| VRC5 Colon Carcinoma | +++ | - |

Key: +++ 95-100% inhibition
++ 80-95% inhibition
+ 60-80% inhibition
- <60% inhibition
NT not tested

Figure 14

In vitro analysis showed that DDATHF inhibited the growth of a wide variety of tumor cell lines, and reversal studies demonstrated that its site of activity was GAR TFase, the enzyme required for the first of the formyl transfer steps in de novo purine biosynthesis. As expected, DDATHF was not a DHFR inhibitor. In vivo studies were even more exciting. DDATHF was shown to be extremely active against a broad range of solid tumors, and to be fully active against methotrexate-resistant tumors (see Figure 14). As an example, with B-16 melanoma DDATHF produced complete inhibition of tumor growth at 6.25 mg/kg/day for 10 days without evidence of host toxicity up to 100 mg/kg/day; methotrexate showed no significant activity against this tumor. These and further biochemical and pharmacological results have been summarized elsewhere [39-45]. DDATHF is now in Phase I clinical trial.

DDATHF as prepared is, of course, a mixture of two diastereomers (ddl and dll), and it was essential to achieve their separation in order to determine whether the above observed cytotoxic activity of "DDATHF" belonged to only one of the two diastereomers. Resolution of the 6-(*R*) and 6-(*S*) diastereomers was accomplished through fractional crystallization of d-10-(+)-camphorsulfonic acid salts, prepared from the N-2-acetyl diethyl ester of DDATHF (**34**); our overall strategy and results are summarized in Figure 15 [46]. It turned out that both diastereomers are essentially equiactive, although several of

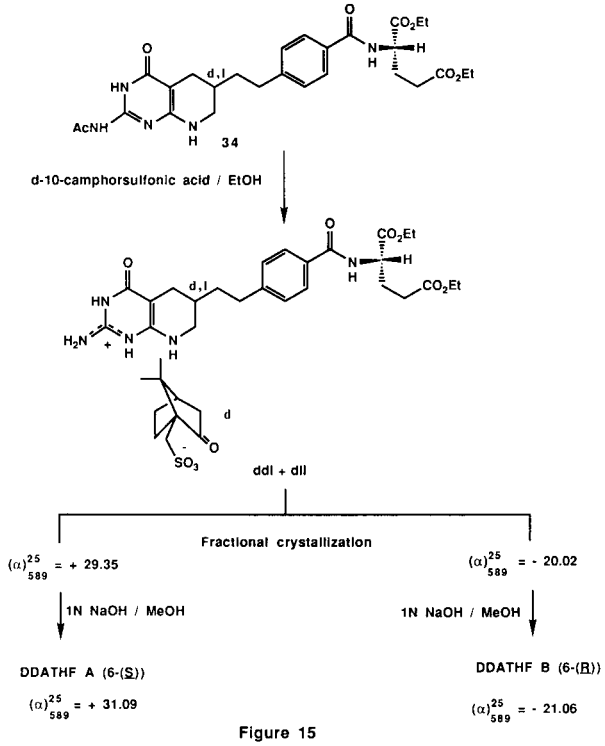



Figure 15

their biochemical characteristics differ in detail [47]. In all of the synthetic studies to be discussed below, therefore, we have dealt (whenever the situation arises) with diastereomeric mixtures, and initial biochemical evaluation of target compounds has been made on diastereomeric mixtures. Very recently, an asymmetric synthesis of both diastereomers of DDATHF has been achieved through exploitation of a lipase-catalyzed enantioselective esterification of 2-substituted 1,3-diols [48]. This work has made possible an assignment of the (*R*) configuration to diastereomer B (which is thus homochiral with naturally-occurring 6-(*S*)-tetrahydrofolic acid).

The extremely promising antitumor activity of DDATHF necessitated a much better synthesis (the one above was non-convergent and required 18 steps). We have now developed a number of additional total syntheses, some of which are described below; each of those illustrated has features which may be of general interest to those involved in heterocyclic synthesis.

A convergent and much more efficient approach to DDATHF is illustrated in Figure 16 [49]. Its key feature

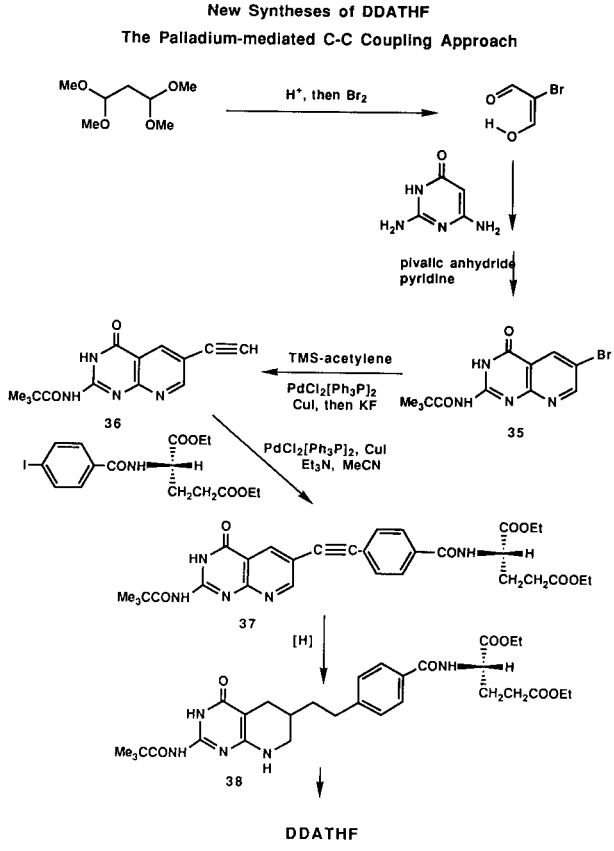



Figure 16

is a double exploitation of a palladium-catalyzed carbon-carbon bond coupling reaction to form the $C_6$-$C_9$ and $C_{10}$-aryl bonds. In its final form (there were many alternative pathways explored), 2-pivaloyl-6-bromo-5-deazapterin (**35**) emerged as a key intermediate; it was most efficiently prepared by condensation of 2,4-diamino-6(1H)-pyrimidone with bromomalonaldehyde, followed by pivaloylation [50]. This latter step is crucial; the pivaloyl protecting group confers remarkable solubility (words little known to workers in this field of heterocyclic chemis-

try) on what is otherwise an almost totally insoluble and thus chemically intransigent compound, and therefore makes possible a range of almost "normal" chemical transformations. Palladium-catalyzed coupling with trimethylsilylacetylene followed by desilylation gave **36** which was then subjected to a second palladium-catalyzed carbon-carbon coupling reaction, this time with diethyl 4-iodobenzoylglutamate, to yield **37**. Reduction of the triple bond and the pyridine ring to **38**, followed by careful alkaline deprotection of the 2-amino grouping and the glutamate esters, then led to DDATHF.

A further synthesis of DDATHF evolved from an extension of the condensation illustrated in Figure 16 for the synthesis of 6-bromo-5-deazapterin from bromomalonaldehyde and 2,4-diamino-6(1H)-pyrimidone. In principle, utilization of an appropriately substituted malonaldehyde could lead in a single step to a fully C-6 functionalized 5-deazapterin. The 4-arylbutyraldehyde derivative **39** was readily prepared by coupling of methyl 4-bromobenzoate with 3-butyn-1-ol using bis-triphenylphosphine, palladium chloride and copper(I) iodide in diethylamine as solvent, followed by reduction of the acetylenic triple bond and PCC oxidation (Figure 17). All attempts to α-formylate this intermediate to give the required malonaldehyde, however, were unsuccessful (DMF-DMA, triethyl orthoformate/$ZnCl_2$, acetic formic anhydride, the Vilsmeier reagent). A solution to this problem was suggested by some recent work by Sha [51,52]. Conversion of **39** to its condensation product **40** with malononitrile sufficiently activates the α-methylene group so that formylation with triethyl orthoformate to give **41** is now successful. We were able to show that this intermediate [53] functions as a malonaldehyde equivalent; condensation with 2,4-diamino-6(1H)-pyrimidone in 60% acetic acid gave the desired 5-deazapterin **45** analytically pure in 68% yield. An initial Michael addition of 2,4-diamino-6(1H)-pyrimidone to **41** gives **43**; this is followed by a second but intramolecular Michael addition which results in ring annulation to give **44**. Final aromatization by loss of malononitrile then yields **45**. It must be emphasized that every step in this pyridine ring construction reaction is facilitated by the greater electrophilicity of $C{=}C(CN)_2$ as compared with C=O. Activation of carbonyl groups in this manner seems worthy of further examination. Compound **45** was then converted to DDATHF as shown in Figure 17 [54].

Synthesis of the functionalized malonaldehyde **49** corresponding to **41** was achieved by what we have termed the isoxazole route, as depicted in Figure 18 [55]. 4-Formylisoxazole (**46**) was prepared from 4-methylisoxazole by dibromination with NBS followed by hydrolysis. The monobromo derivative formed concomitantly in the NBS bromination was likewise converted to **46** by hydrolysis to 4-hydroxymethylisoxazole, followed by PCC oxidation. The 4-styrylisoxazoles **48** (a mixture of cis and trans isomers) were then prepared either by Wittig con-

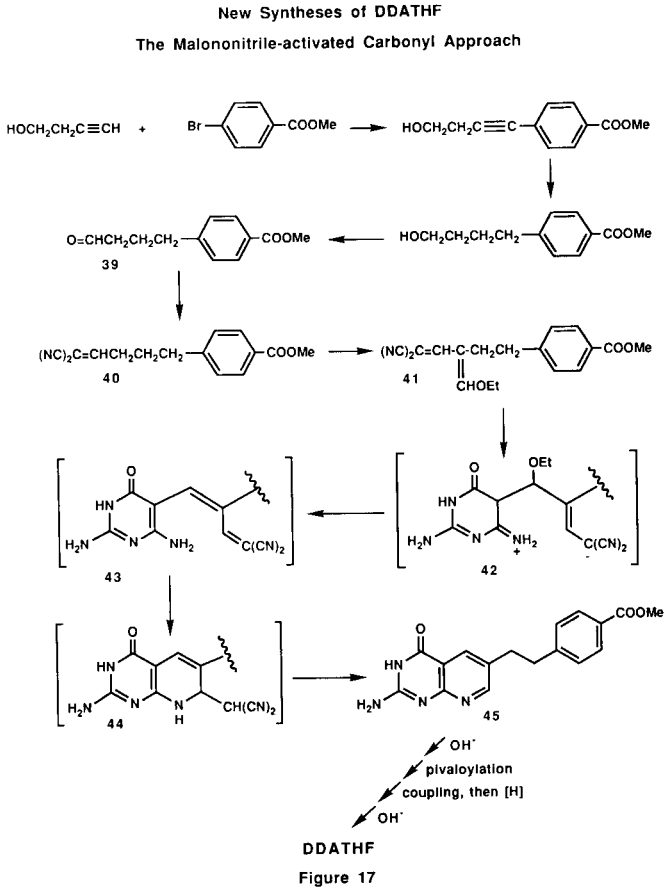



Figure 17

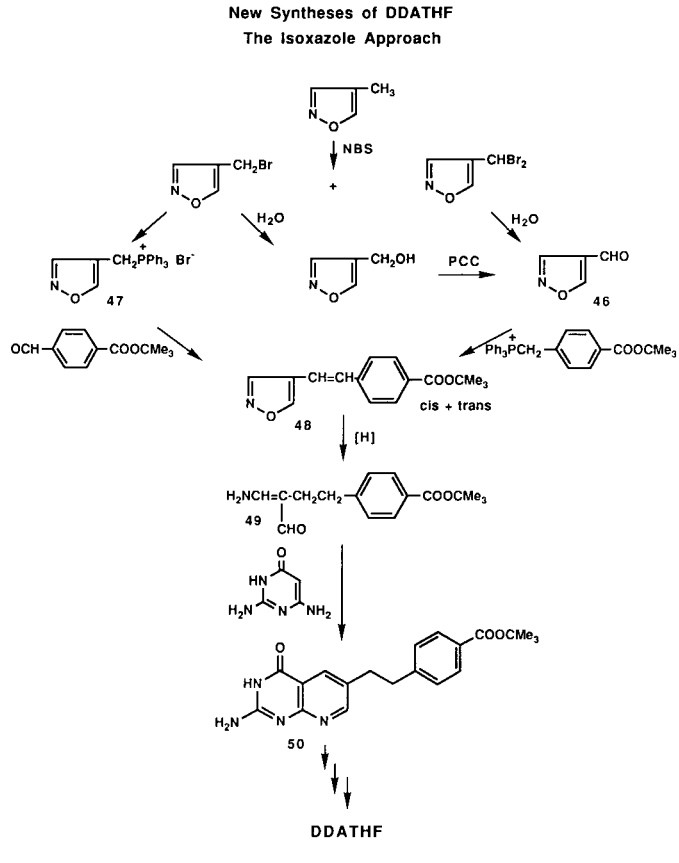



Figure 18

densation of 4-formylisoxazole with the Wittig reagent prepared from t-butyl 4-bromomethylbenzoate, or by condensation of the phosphonium salt **47** prepared from 4-bromomethylisoxazole with t-butyl 4-formylbenzoate. Catalytic reduction then gave the aminoacrolein **49** which was condensed with 2,4-diamino-6(1H)-pyrimidone in N-methylpyrrolidone to **50**. Further conversion of an equivalent intermediate to DDATHF was illustrated in Figure 17.

A totally different strategy for construction of the 5-deazapterin ring system is illustrated in Figure 19, and is based upon our extensive recent work on the utilization of 1,2,4-triazines as azadienes in Diels-Alder reactions with electron-rich dienophiles [56]. Methyl 4-(4'-oxobutyl)-benzoate (**39**, Figure 17) was converted to its pyrrolidine enamine **52** [57], which was then heated in anhydrous dioxane with 2-N-pivaloyl-7-methylthio-6-azapterin (**51**), prepared as outlined in Figure 19. A byproduct (displacement of the 7-methylthio substituent in **51** by pyrrolidine) was removed by filtration; chromatography of the filtrate then gave the Diels-Alder product **53** in modest yield. Raney nickel desulfurization yielded the 2-pivaloyl derivative of **45** (Figure 17) [58], identical with material prepared by the routes previously described.

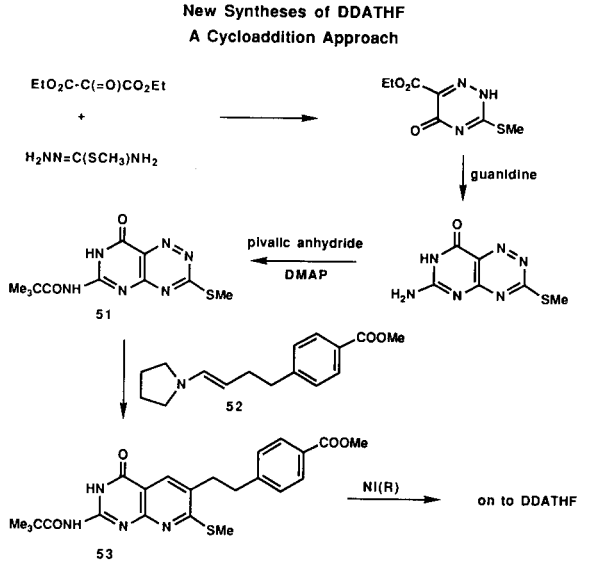



Figure 19

I would like to discuss in the remainder of this lecture the synthesis of a number of DDATHF analogs. These syntheses have been chosen both to illustrate the varieties of heterocyclic synthetic methodologies employed, and to outline the current state of our SAR studies on this new class of folate antimetabolite.

We have previously described several syntheses of 6-formyl-5-deazapterin (**56**) either (a) by condensation of 2,4-diamino-6(1H)-pyrimidone with triformylmethane, or (b) from **22** (see Figure 7) by alkaline hydrolysis of the 4-amino group followed by acidic hydrolysis of the acetal functionality [17]. A much improved synthesis of its 2-pivaloyl derivative **55** (essential because of its greatly improved solubility properties!) is illustrated in Figure 20. This synthesis takes advantage of our previous preparation of 2-pivaloyl-6-bromo-5-deazapterin (**35**), and the ease which this (soluble) compound undergoes palladium-catalyzed carbon-carbon coupling reactions with olefins. Thus, palladium-catalyzed coupling of **35** with styrene gave the styryl derivative **54**, which was ozonized in high yield to give **55** [50]. Reductive amination with diethyl 4-aminobenzoyl-L-glutamate then gave the protected 5-deazafolic acid derivative **57**. Initial attempts to reduce the pyridine ring in **57** were unsuccessful due either to facile hydrogenolysis of the benzylic carbon-nitrogen bond (with Pd/C or $PtO_2$) or to formation of a complex, inseparable mixture of products (with Rh/C). However, treatment of **57** with formic acetic anhydride gave the 10-formyl derivative **58** in quantitative yield, which smoothly underwent catalytic reduction of the pyridine ring ($PtO_2$ in glacial acetic acid) without concurrent benzylic cleavage. 5-Deaza-5,6,7,8-tetrahydrofolic acid (5-DATHF) (**59**) was then readily prepared (as a mixture of two diastereomers) upon mild alkaline hydrolysis [59]. This compound was at least as active as DDATHF both in vitro and in vivo (both diastereomers, which were separated by hplc, were almost equipotent). It thus appears that substitution of nitrogen for carbon at position 10 of DDATHF is not detrimental either to its overall biochemical properties or to its antitumor activity. It is also worth noting that N-10 formylation of **59** reduced its activity; conversion to an analog more closely resembling the natural cofactor for GAR TFase obviously did not have the anticipated conse-

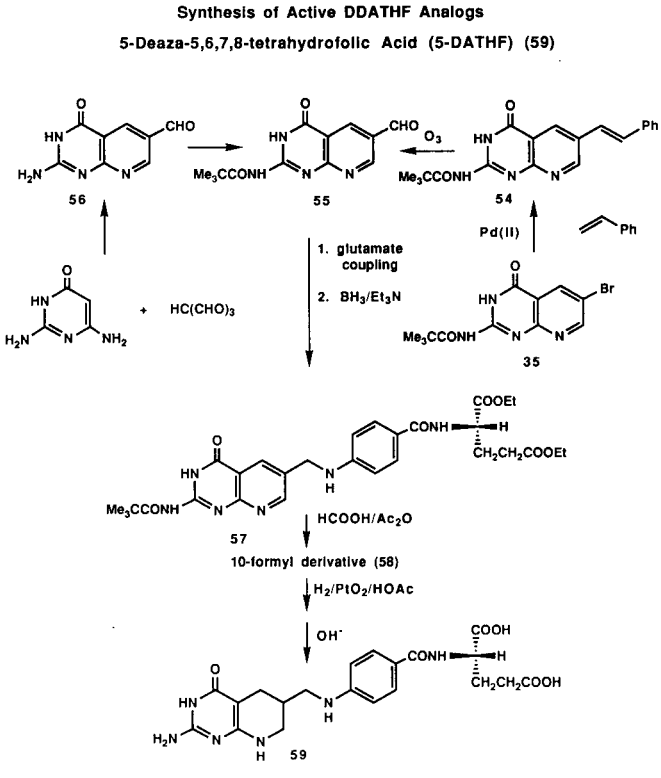



Figure 20

quence.

It remained of interest, however, to examine the effect of a substituent at C-10 of DDATHF itself, despite the synthetic complications associated with introduction of an additional chiral center. Figure 21 outlines our synthesis

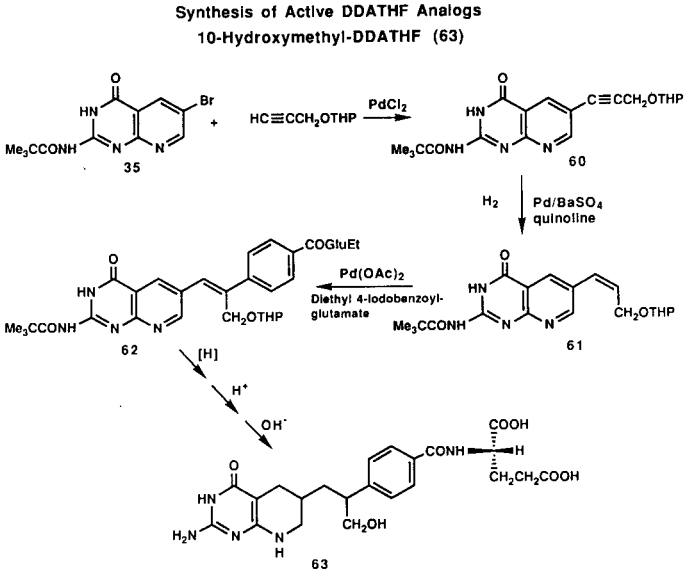



Figure 21

of 10-hydroxymethyl-DDATHF (**63**) [60]. Of particular interest here is the sequential exploitation of palladium-catalyzed carbon-carbon coupling reactions, both in the reaction of the (now ubiquitous) 2-pivaloyl-6-bromo-5-deazapterin **35** with the THP derivative of propargyl alcohol to give **60**, and in the regio- and stereochemically controlled arylation of the cis olefin **61**. This analog, even as a mixture of four diastereomers, proved to be exceptionally active as a cytotoxic agent. Although we have separated the four diastereomers on an hplc Cyclobond column, we have not yet determined whether the activity resides in only one of the diastereomers (this is the case with 10-methyl-DDATHF).

The palladium-catalyzed carbon-carbon coupling methodology has been extraordinarily useful throughout this investigation. As an additional example, consider the synthesis of homo-5-DATHF (**66**) as outlined in Figure 22. The target compound should have been readily available by reductive amination of diethyl 4-aminobenzoylglutamate with 2-pivaloyl-5-deazapterin carrying an acetaldehyde substituent at C-6, but the latter intermediate could not be prepared by any of the myriad of reactions attempted. We finally resorted to the rather simple scheme outlined in Figure 22. The N-vinyl intermediate **64**, which was readily prepared by palladium-catalyzed addition of vinyl acetate to diethyl 4-formylaminobenzoyl-L-glutamate, itself underwent palladium-catalyzed coupling with 2-pivaloyl-6-bromo-5-deazapterin (**35**) to give **65**, which was then elaborated to homo-5-DATHF (**63**) by standard chemistry [60]. And once again, the product proved to be exceptionally active as an antitumor agent. It was beginning to appear that every analog we could think of in this series would turn out to be active!

We sought guidance at this point for our synthetic work from a molecular mechanics study of DDATHF and possible analogs through use of the Macromodel package developed by Clark Still at Columbia University. Without going into details here, it appears that the 6-(*R*) and 6-(*S*) diastereomers of DDATHF have similar (but certainly not identical) lowest energy conformations, which can be described as extended L-forms in which the phenyl ring is almost orthogonol to the plane containing the pyrimidine ring [61]. This flexibility allows both the (*R*) (which possesses the "natural" C-6 configuration; this is the di-

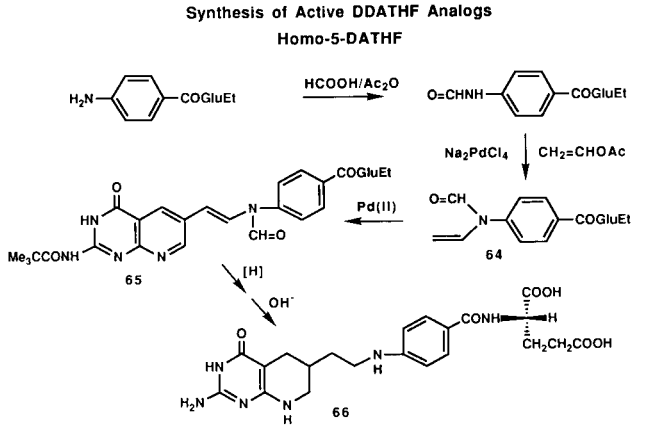



Figure 22

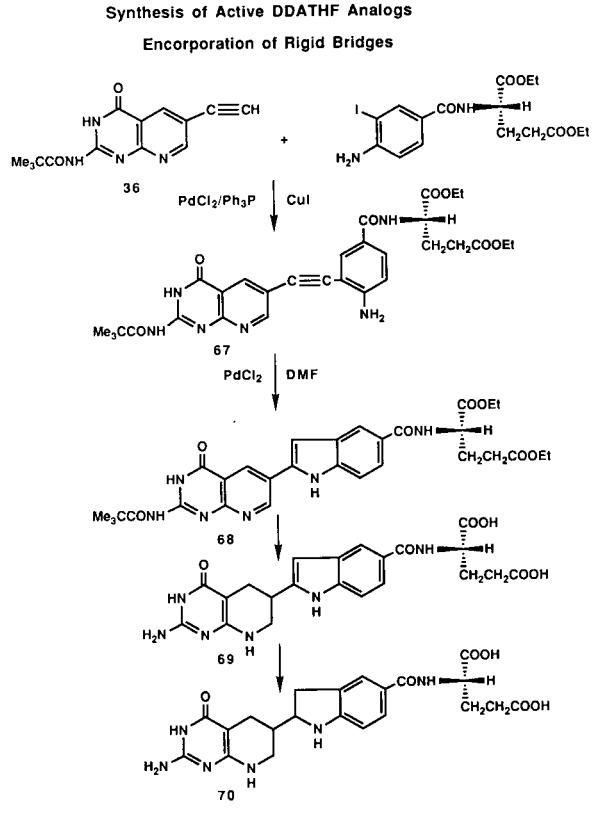



Figure 23

astereomer selected for clinical trial) and the (*S*) diastereomers to fit the proposed methotrexate binding site in FPGS [62].

In order to explore the possibility that the ability to adopt an L-shaped folded conformation might be related to antitumor activity, we prepared the pair of DDATHF analogs depicted in Scheme 23. Compound **69**, with an aromatic indole ring incorporated into the bridge between the 5-deazatetrahydropterin and benzoylglutamate moieties, appears to be somewhat more rigid than the reduced derivative **70**; the former is essentially devoid of activity, while the latter is almost as active as DDATHF itself. However, much further work will be required before any predictive significance can be attributed to such speculative conformational considerations.

The presence of a chiral C-6 position in DDATHF and in all of the analogs described thus far introduces inevitable chemical and (potentially, at least) biochemical complications. It was of obvious interest to see whether the "open-chain" derivative **71** (see Figure 24), in which this

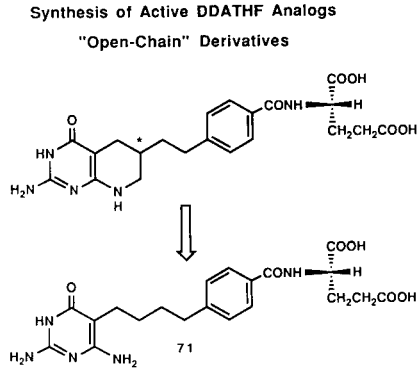



Figure 24

chiral center has been excised, would retain biological activity. Synthesis of this compound was extremely straightforward. Methyl 4-(4-hydroxybutyl)benzoate,

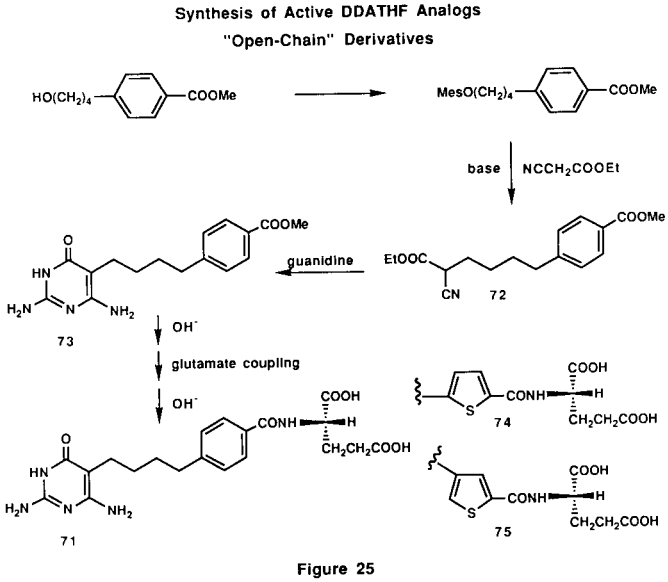



Figure 25

which had been prepared previously (see Figure 17), was converted to its mesylate, which was then used to alkylate ethyl cyanoacetate. Cyclization of the resulting monoalkylated ethyl cyanoacetate **72** with guanidine gave the C-5 substituted 2,4-diamino-6(1H)-pyrimidone **73**, which was then converted on to **71** by conventional steps [63]. This "open-chain" analog of DDATHF proved to be exceptionally interesting; despite its simplicity, it was almost as active an antitumor agent as DDATHF itself, is a superb substrate for FPGS (as is DDATHF), and is orally active. Other active "open-chain" analogs possessing a thiophene rather than a benzene ring in the bridge (**74** and **75**) have been prepared by similar methodology. In the meantime, another research group has prepared a similar "open-chain" analog of 5-DATHF; this compound is also a potent cytotoxic agent whose site of action is GAR TFase [64]. Because of the ease of synthesis of these acyclic analogs, and the absence of stereochemical complications thanks to deletion of the C-6 chiral center, considerable current effort is being devoted to further explorations of this intriguing lead.

The very high degree of antitumor activity and selectivity exhibited by DDATHF has served as a powerful stimulus for the development of new synthetic methods which would provide ready access to new types of analogs and derivatives. One arena which will not be discussed here today because of time restraints is concerned with the development of enantioselective synthetic routes to DDATHF and analogs. Another general challenge has been the design of flexible synthetic methods for the construction of the pyridopyrimidine nucleus. The projected route to 10-substituted DDATHF derivatives (**79**) described in Figure 26 is illustrative. The synthetic route to

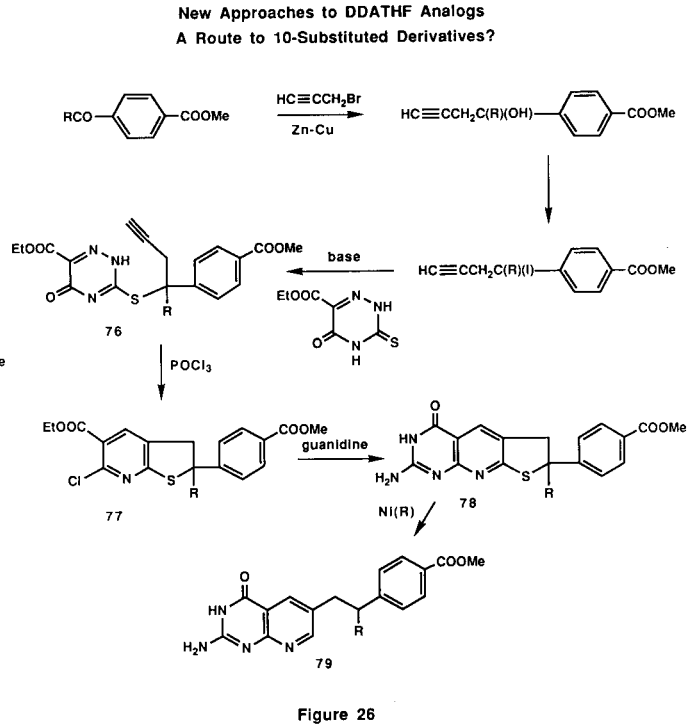



Figure 26

the 5-deazapterin ring system found in DDATHF which was outlined in Figure 19 utilized an intermolecular Diels-Alder reaction of a fused 1,2,4-triazine (a 6-azapterin) with an electron-rich dienophile (an enamine). Figure 26 illustrates an *intra*molecular Diels-Alder approach. The 1,2,4-triazine derivative (**76**) which carries a suitably designed dienophilic side chain tethered to the triazine ring through a sulfur linkage was prepared as shown. Chlorination of the triazinone **76** yields the corresponding 5-chloro derivative (not shown), which undergoes a spontaneous intramolecular Diels-Alder reaction to give the dihydrothieno[2,3-b]pyridine **77**. Guanidine pyrimidine ring annulation then gives **78**. In model systems (R = H), Raney nickel desulfurization smoothly leads to 6-substituted 5-deazapterins (**79**, R = H); future plans call for exploration of this strategy with R ≠ H, and the eventual construction of a series of 10-substituted DDATHF analogs modelled after the naturally-occurring GAR TFase folate cofactor.

In conclusion, I would like to express my heartfelt gratitude to my many talented students and collaborators-Prof. G. Peter Beardsley (at the Yale University School of Medicine): Zen-yu Chang, Donald J. Dumas, Stephen R. Fletcher, Thomas J. George, James M. Hamby, Peter J. Harrington, Philip M. Harrington, Evelyn P. Jackson, Dietmar Kuhnt, Keith F. McDaniel, Hshiou-Ting Liu, Shashank Otiv, David C. Palmer, Mona Patel, Maria Papadopoulou, Partha S. Ray, Annmarie Sabb, Thomas Schrader, Zdzislaw Szulc, Chi-ping Tseng, Markandu Vigneswaran, Loren Walensky, John C. Warner, George S. K. Wong, and Cheol-min Yoon (all at Princeton University): Prof. Richard G. Moran (at the University of Southern California); and Charles J. Barnett, Gerald B. Grindey, and Chuan J. Shih (at Eli Lilly & Company) - for their enthusiastic participation in and indispensible contributions to this project.

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