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SYNTHESIS OF A PYRIMIDO[4,5-*b*]AZEPINE ANALOG OF 5,10-DIDEAZA-5,6,7,8-TETRAHYDROFOLIC ACID (DDATHF)

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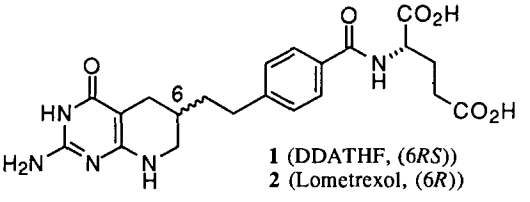
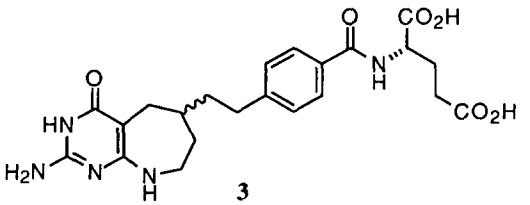
Abstract: The synthesis and biological evaluation of a pyrimido[4,5-*b*]azepine-based analog of DDATHF, a potential chemotherapeutic agent, are described.

The tetrahydrofolic acid analog DDATHF (**1**) exhibits potent, broad-spectrum antitumor activity and, as its 6*R* diastereomer (**2**, Lometrexol, LTX), is currently undergoing Phase II clinical evaluation for its potential use in cancer chemotherapy.[1,2] The cytotoxic effects of DDATHF,[3] and its poly-γ-glutamated intracellular metabolites,[4] result from inhibition of glycinamide ribonucleotide formyltransferase (GARFT), the enzyme which mediates the first formyl group transfer from the natural cofactor, 10-formyl-5,6,7,8-tetrahydrofolic acid, to the nascent ribonucleotide in purine de novo biosynthesis.[5]



1 (DDATHF, (6*RS*))
2 (Lometrexol, (6*R*))

In an effort to identify the structural features of DDATHF responsible for its growth-inhibitory activity, many analogs have been prepared and evaluated.[6] From this extensive structure-activity relationship (SAR) data have emerged an appreciation both of the elements of the pharmacophore, which must be present for recognition and binding,[7] and the regions of the molecule that are tolerant of modification, and into which changes may be introduced that lead to superior inhibitors.[8] As an extension of this SAR effort, a molecular modelling approach has been initiated in which proposed inhibitors are evaluated as substrates for the graphical representation of recombinant human monofunctional GARFT obtained by X-ray crystallography.[9] Since pyrimidine analogs of **1**, resulting from excision of the C-7 methylene group, exhibit in vitro (but not in vivo) cytotoxicity,[10] we have prepared **3**, in which expansion of the tetrahydropyridine ring of **1** by one methylene unit may impart additional conformational mobility while preserving the requisite bicyclic system.[11]



3

An assessment of the structural complementarity between **2** and **3a** (the 6R diastereomer of **3**) was made by superposition on the conformation adopted by **2** when bound by GARFT.[12] Following energy minimization, a comparison of the energy of the "bound" conformation of the new ligand with its lowest-energy structure, obtained by a conformational search routine, revealed that the desired (bound) conformer (Figure 1) is higher in energy by less than 3 kcal mol^{-1}, suggesting that no significant barriers exist which might prevent the ligand from assuming the orientation required for binding to GARFT.

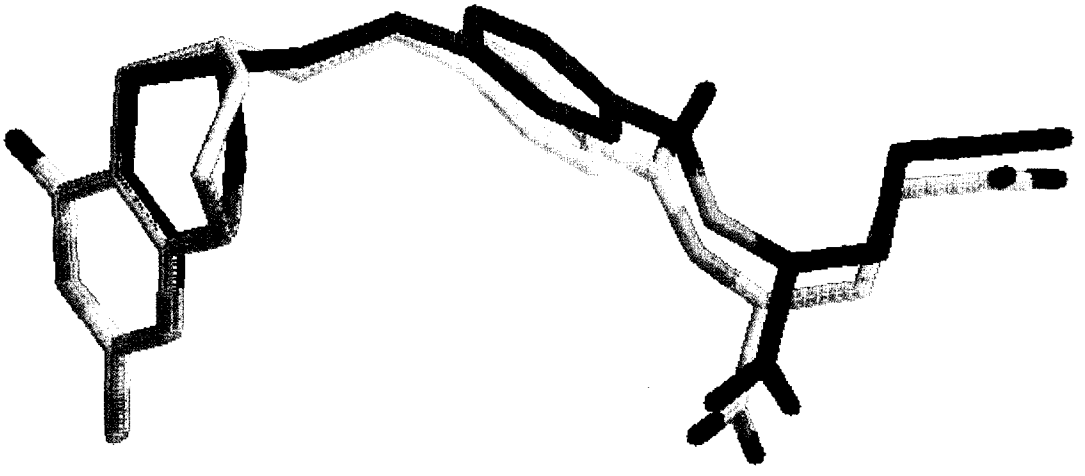
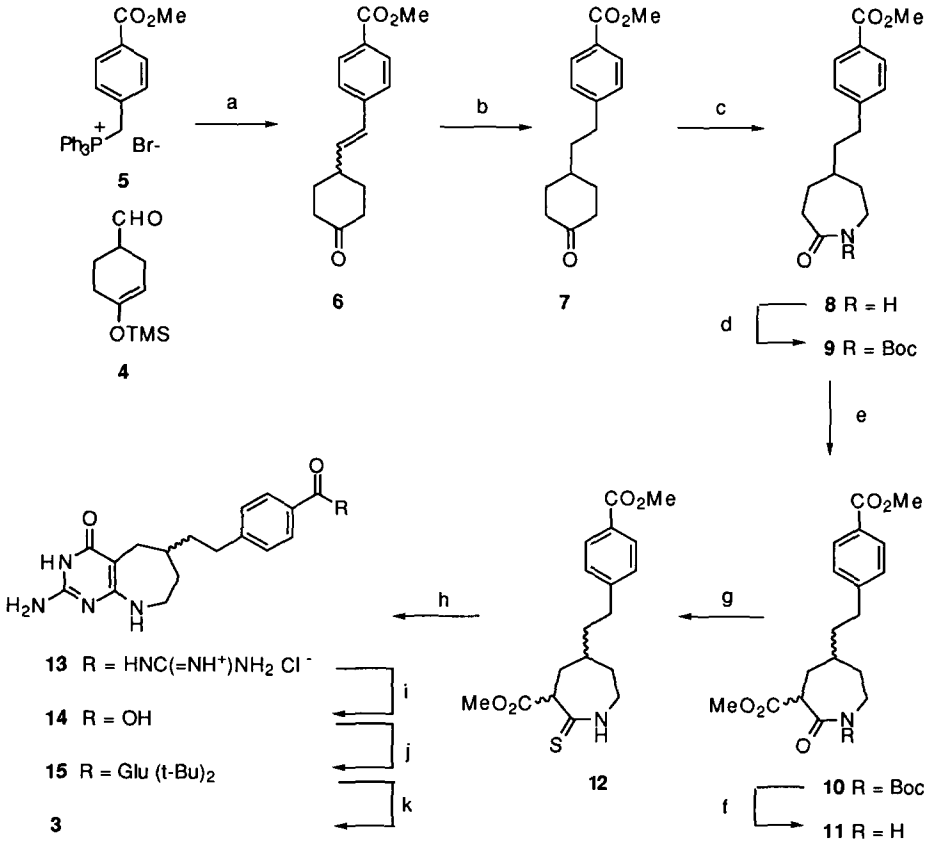


Figure 1. Superimposition of 2 and its pyrimidoazepine analog 3a. Hydrogen atoms have been omitted for clarity.

In this letter we report the synthesis of N-[4-[2-(2-amino-4(3H)-oxo-5,6,7,8-tetrahydropyrimido[4,5-*b*]azepin-6-yl)ethyl]benzoyl]-L-glutamic acid (**3**) as the first of a series of ring-expanded analogs of DDATHF. The synthesis of **3** (Scheme 1) commences with Wittig olefination of the known[13] aldehyde **4** with phosphonium salt **5** under conditions which effected in situ hydrolysis of the trimethylsilyl enol ether. Subsequent catalytic hydrogenation of the mixture of cis and trans olefins (**6**) afforded ketone **7** which, upon treatment with hydroxylamine O-sulfonic acid in formic acid,[14] underwent a Beckmann rearrangement to afford lactam **8**. Protection of **8** as its N-*t*-butoxycarbonyl derivative[15] (**9**) was followed by regioselective methoxycarbonylation using LHMDS and Mander's reagent[16] to give **10**. Deprotection of **10**, a mixture of diastereomers, followed by thionation of lactam **11** using phosphorus pentasulfide[2] gave thiolactam **12**. Treatment of **12** with guanidine free base under salt-free conditions followed by acidification gave the acyl guanidine **13**. Hydrolysis of **13** with 1 N NaOH provided the free acid **14** which, without purification, was subjected to 2-chloro-4,6-dimethoxy-1,3,5-triazine-promoted[17] coupling with the di-*t*-butyl ester of L-glutamic acid to afford **15**. Treatment of a solution of **15** in CH_2Cl_2 with TFA followed by basification with 1 N NaOH and acidification with 6 M HCl then gave **3** in 5% overall yield for the 11-step sequence beginning with **4**.

Scheme 1



Reagents: (a). DBU, THF, 8 h (75%); (b). H_2, Pd(C), EtOAc, 6 h, (100%); (c). hydroxylamine O-sulfonic acid, formic acid, 100 °C, 8 h, (71%); (d). $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2, 24 h, (100%); (e). LHMDS, methyl cyanoformate, THF, -78 °C, 8 h, (65%); (f). 20% TFA in CH_2Cl_2, 1 h, (95%); (g). P_4S_{10}, THF, 60 °C, 0.5 h, (80%); (h). guanidine hydrochloride, NaOMe, 90 °C, 10 torr, 1 h then H_2O, 6 M HCl (50%); (i). 1 N NaOH, 60 °C, 24 h (76%); (j). 2-chloro-4,6-dimethoxy-1,3,5-triazine, NMM, L-glutamic acid di-*t*-butyl ester hydrochloride, DMF, (70%); (k). 20% TFA in CH_2Cl_2, 8 h followed by 1 N NaOH, 6 M HCl, (78%).

Biological Evaluation

An in vitro cell growth inhibition assay of **3** against human T-cell derived lymphoblastic leukemia (CCRF-CEM) cells (Table 1) yielded an IC_{50} value of 47 nM. Reversal of the cytotoxicity of **3** could be effected by the addition of hypoxanthine (100 μM) and aminoimidazole carboxamide (AICA, 300 μM) but not by thymidine (5 μM), indicating that the locus of activity of **3** resides in the purine de novo biosynthetic pathway. Measurement of the affinity of **3** for murine trifunctional GARFT gave a K_i of 147 nm which is similar to the value obtained for **1** (also tested as a mixture of diastereomers)[18] against the enzyme obtained from L1210 cells. In the absence of data on the transport properties of **3**, its three-fold lower cytotoxicity may be attributed in large part to diminished affinity for folylpoly-γ-glutamyl synthetase (FPGS), the enzyme which converts folates and antifolates to the poly-γ-glutamated forms which are regarded as the active intra-

Table 1

Cellular Cytotoxicity, GARFT Inhibition, and FPGS Affinity of DDATHF, LTX, and Compound 3

Compound	IC_{50} (nM) [1]	K_i (nM) [2]	K_m (μM) [3]
DDATHF (**1**)	16	120	-
LTX (**2**)	15.2	59.7	16.4
3	47	147	39

1. Human CCRF-CEM lymphoblastic leukemia cells. Assay conditions are described in reference 18.
2. Trifunctional GARFT isolated from murine L1210 leukemia cells. Assay conditions are described in reference 18.
3. Hog liver FPGS. Assay conditions are described in reference 19.

cellular metabolites. The K_m value for conversion of **3** to its diglutamate by hog liver FPGS was determined to be 39 μM with a maximum velocity (V_{max}) of 797 nmol $h^{-1}mg^{-1}$. Comparison of the first order rate constants (k' values, defined as V_{max}/K_m) of **3** and **2** (V_{max} = 977 nmol $h^{-1}mg^{-1}$ and K_m = 16 μM)[19] indicates that **3** (k' = 20) is three times less efficiently polyglutamated than **2** (k' = 59).

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