SYNTHESIS OF 4-[(8-AMINO-6-BENZYL-10(9*H*)-OXO-4,5-DIHYDRO-(6*H*)-ISOXAZOLO[4,3-*d*]PYRIMIDO[4,5-*b*]AZEPIN-3-YL]BENZOYL-L-GLUTAMIC ACID. A NOVEL PYRIMIDOAZEPINE-BASED FOLIC ACID DERIVATIVE

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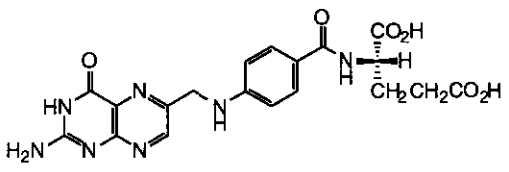
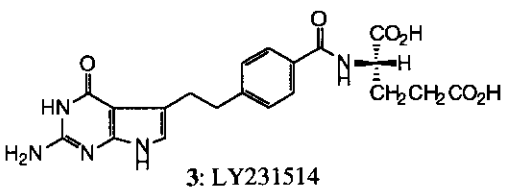
Abstract - Synthesis of the titled compound (**13**) is described using an intramolecular 1,3-dipolar cycloaddition reaction as the key step.

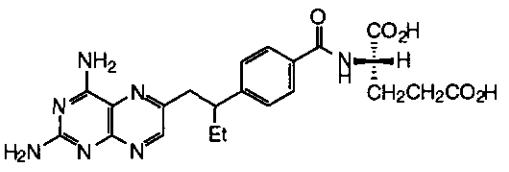
The design and synthesis of folic acid (**1**) based antimetabolites as potential inhibitors of certain enzymes which are involved in the biosynthesis of nucleotides has gained prominence over the past decade. [1-3] In particular, the enzymes dihydrofolate reductase (DHFR), thymidylate synthase (TS), and glycinamide ribonucleotide formyltransferase (GARFT) have become attractive biochemical target sites for antifolates as potential anticancer agents. There are currently several antifolates at various stages of clinical development. [4] For example, 10-ethyl-10-deazaaminopterin (Edatrexate [5], **2**) is an inhibitor of DHFR, while the pyrrolopyrimidine LY231514 [6] (**3**) inhibits TS and Lometrexol [7] (**4**) is known to exert its cytotoxic effect *via* inhibition of GARFT.



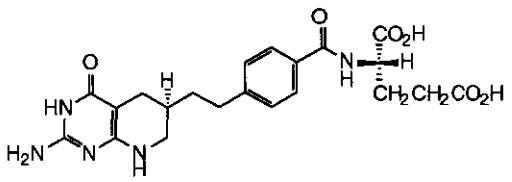
1: Folic Acid



3: LY231514



2: Edatrexate



4: Lometrexol

Despite the many hundreds of folic acid analogs which have been prepared over the past several decades there have been no reports of pyrimidoazepine based folic acid derivatives. We have initiated a program aimed at the synthesis of such compounds with the aim of discovering a more selective, less toxic, antitumor agent than the ones currently in clinical trials. In this paper we report the synthesis of the pyrimidoazepine based folic acid derivative (**13**) using an intramolecular 1,3-dipolar cycloaddition methodology.

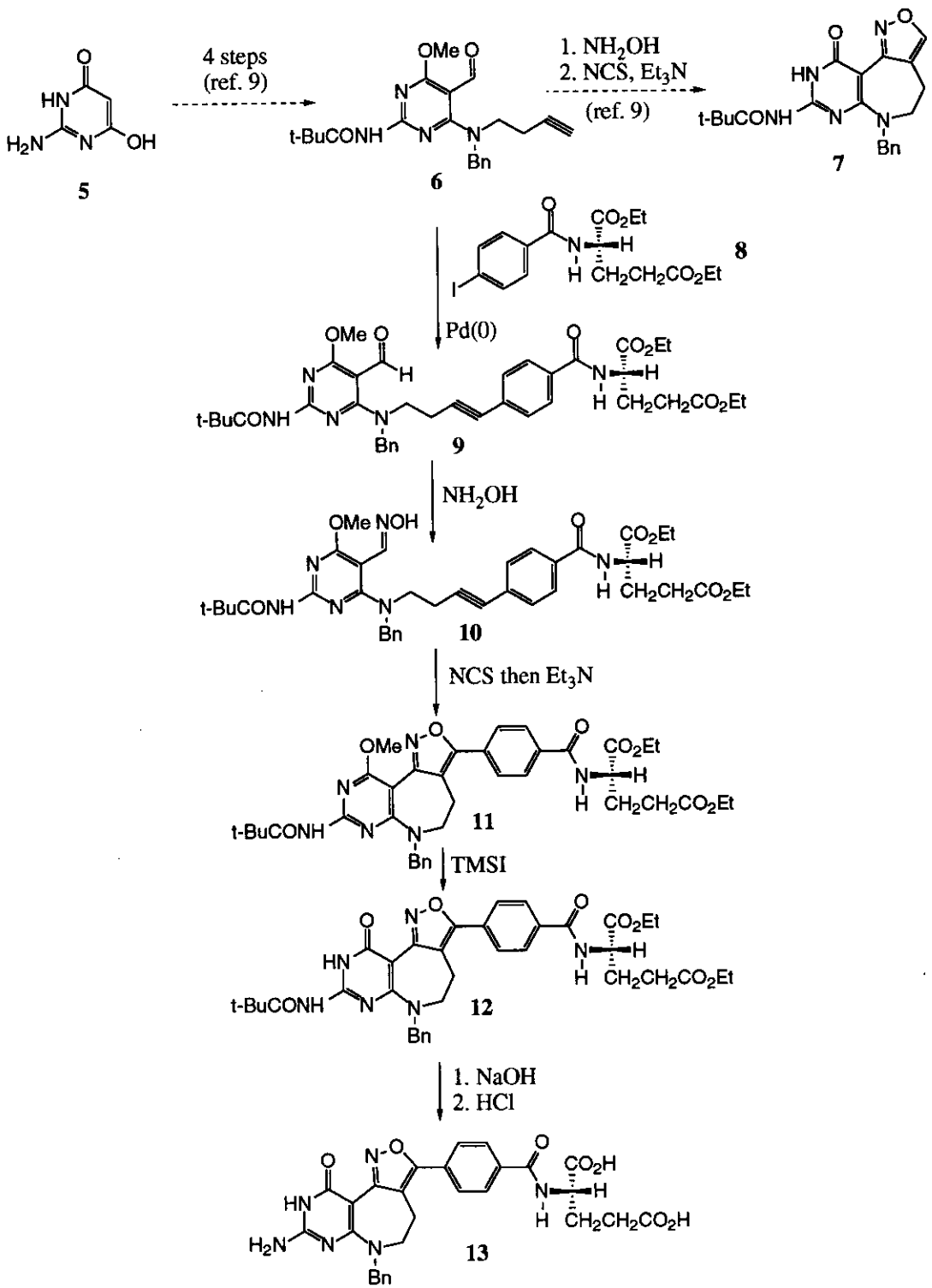
Recently, we have developed a synthetic strategy to the pyrimidoazepine system using the intramolecular nitrile oxide cycloaddition methodology. [8,9] Thus, we have reported the preparation of the pyrimidoazepine (**7**) from 2-amino-4,6-dihydroxypyrimidine (**5**) *via* the formylpyrimidine derivative (**6**) (Scheme).[9] Here we report the utilization of this intramolecular nitrile oxide cycloaddition methodology as a key step to prepare the pyrimidoazepine-based folic acid derivative (**13**).

Our synthetic studies began with the palladium catalyzed coupling reaction of **6** with diethyl 4-iodobenzoylglutamate [10] (**8**). This provided the alkyne (**9**) in 58% yield after purification by chromatography on silica gel (Scheme). Reaction of **9** with hydroxylamine in ethanol at room temperature afforded the expected oxime (**10**) in 93% yield. Treatment of this oxime with NCS in methylene dichloride at room temperature followed by treatment with triethylamine led to the desired intramolecular 1,3-dipolar cycloaddition reaction (between the *in situ* generated nitrile oxide and the alkyne) to give the cycloadduct (**11**). This was isolated in 63% yield. Reaction of **11** with iodotrimethylsilane in refluxing methylene dichloride followed by quenching with methanol, led to cleavage of the methyl ether and gave the pyrimidin-4-one derivative (**12**) in 92% yield. Finally, treatment of **12** with 1% NaOH for 14 days followed by treatment with HCl gave the desired pyrimidoazepine-based folic acid derivative (**13**) in 73% yield. Compound (**13**) is currently under evaluation as an inhibitor of cell growth in a number of tumor cell culture assays.

EXPERIMENTAL

Melting points were determined in open capillary tubes using a Thomas-Hoover apparatus and are uncorrected. The proton (300 MHz) and carbon (75 MHz) NMR spectra were recorded on a Varian VXR-300 spectrometer. Chemical shifts are expressed in parts per million (ppm) downfield from internal tetramethylsilane. Column chromatography was performed on Merck silica gel 60 (240-400) mesh; silica gel plates were routinely used for tlc determinations. Elemental analyses were performed by Desert Analytics, Tucson, AZ, and were within $\pm$0.4% of the theoretical values. Mass spectra were obtained by using electrospray ionization technique on a Micromass Inc., Platform II single quadrapole mass spectrometer.

Scheme


4 steps
(ref. 9)
1. NH2OH
2. NCS, Et3N
(ref. 9)
5
6
7
8
Pd(0)
9
NH2OH
10
NCS then Et3N
11
TMSI
12
1. NaOH
2. HCl
13

Diethyl 4-[4-*N*-benzyl-(5-formyl-6-methoxy-2-pivaloylaminopyrimidin-4-yl)amino]but-1-ynyl]-benzoyl-L-glutamate (9). A mixture of **6** [9] (2.46 g, 6.21 mmol), diethyl *N*-(4-iodobenzoyl)-L-glutamate (**8**) [10] (2.94 g, 6.8 mmol), palladium chloride (0.25 g, 1.4 mmol), triphenylphosphine (0.734 g, 2.8 mmol), copper(I) iodide (0.532 g, 2.8 mmol), and triethylamine (20 mL) in acetonitrile (30 mL) was heated under reflux under nitrogen for 5 h. The solution was cooled to room temperature and filtered through a pad of Celite. The solvent was removed by evaporation under reduced pressure, and the residue was partitioned between methylene chloride and water. The organic layer was dried over anhydrous magnesium sulfate, filtered and the solvent was removed by evaporation under reduced pressure. The residue was chromatographed on silica gel eluting with 50% ethyl acetate in hexane. The fractions containing the pure product were combined and removal of the solvent gave 2.25 g (58%) of a colorless solid: mp 63-65 °C; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.12 Hz), 1.22 (t, 3H, J = 7.11 Hz), 1.24 (s, 9H), 2.07 (m, 1H), 2.22 (m, 1H), 2.39 (m, 2H), 2.69 (t, 2H, J = 6.5 Hz), 3.70 (t, 2H, J= 6.75 Hz), 3.95 (s, 3H), 4.01 (dq, 2H, J = 1.62, 7.10 Hz), 4.15 (q, 2H, J = 7.1 Hz), 4.68 (m, 1H), 4.48 (s, 2H), 7.05 (d, 1H, J = 7.52 Hz), 7.20 (m, 5H), 7.30 (d, 2H, J = 8.42 Hz), 7.65 (d, 2H, J = 8.48), 7.78 (br s, 1H), 9.97 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.03, 14.05, 18.64, 27.01, 27.29, 30.39, 40.35, 49.09, 52.33, 54.57, 54.70, 60.73, 61.63, 81.51, 89.93, 97.91, 126.86, 127.06, 127.31, 127.65, 128.49, 131.56, 132.43, 136.64, 156.65, 163.58, 166.38, 171.78, 173.15, 174.22, 175.48, 184.65. Anal. Calcd for $C_{38}H_{45}N_5O_8$: C, 65.22; H, 6.48; N, 10.0. Found: C, 64.91; H, 6.28; N, 9.99.

Diethyl 4-[4-*N*-benzyl-(6-methoxy-5-oximinomethyl-2-pivaloylaminopyrimidin-4-yl)amino]but-1-ynyl] benzoyl-L-glutamate (10). A mixture of **9** (1.8 g, 2.57 mmol), hydroxylamine hydrochloride (0.18 g, 2.57 mmol), pyridine (0.23 mL, 2.8 mmol) and ethanol (50 mL) was stirred at room temperature under nitrogen for 5 h. The solvent and excess pyridine were removed by evaporation under reduced pressure and the residue was partitioned between methylene dichloride and water. The organic layer was separated, dried over anhydrous magnesium sulfate and the solvent was removed under reduced pressure to give 1.72 g (93 %) of a pale yellow solid. An analytical sample was obtained by chromatography on silica gel, eluting with 50% ethyl acetate in hexane to give a colorless solid; mp 73-74 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.15 Hz), 1.21 (t, 3H, J = 7.12 Hz), 1.23 (s, 9H), 2.07 (m, 1H), 2.21 (m, 1H), 2.38 (m, 2H), 2.70 (t, 2H, J = 6.74 Hz), 3.65 (t, 2H, J = 6.91 Hz), 3.86 (s, 3H), 4.02 (dq, 2H, J = 1.62, 7.20 Hz), 4.15 (q, 2H, J = 7.16), 4.69 (m, 1H), 4,75 (s, 2H), 7.19 (m, 6H), 7.28 (d, 2H, J = 8.46 Hz), 7.63 (d, 2H, J = 8.42 Hz), 7.77 (s, 1H), 7.98 (s, 1H), 9.55 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.03, 14.05, 18.52, 27.03, 27.36, 30.41, 40.20, 49.07, 52.32, 54.42, 60.75, 61.68, 81.38, 90.45, 91.24, 126.91, 127.12, 127.40, 127.19, 128.47, 131.51, 132.34, 137.34, 154.77, 163.97, 166.46, 171.93, 173.15, 169.31, 175.58, 144.19. Anal. Calcd for $C_{38}H_{46}N_6O_8$: C, 63.85; H, 6.49; N, 11.76. Found: C, 63.82; H, 6.30; N, 11.57.

Diethyl 4-[(6-benzyl-10-methoxy-8-pivaloylamino)-4,5-dihydro-6*H*-isoxazolo[3,4-*d*]pyrimido[4,5-*b*]azepin-3-yl] benzoyl-L-glutamate (11). A mixture of **10** (1.556 g, 2.17 mmol),

N-chlorosuccinimide (0.29 g, 2.17 mmol), and anhydrous methylene chloride (50 mL) was stirred at room temperature, under nitrogen, for 1.5 h. Triethylamine (0.3 mL, 2.2 mmol) was then added and the mixture was stirred for a further 2 h. Water (10 mL) was added and the organic layer separated, dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo. The residue was chromatographed on silica gel eluting with 50% ethyl acetate in hexane to give 0.96 g (63%) of a colorless solid; mp 93-95 °C; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.21 Hz), 1.22 (t, 3H, J = 7.21 Hz), 1.26 (s, 9H), 2.09 (m, 1H), 2.23 (m, 1H), 2.40 (m, 2H), 2.78 (m, 2H), 3.36 (m, 2H), 4.03 (dq, 2H, J = 1.46, 7.18 Hz), 4.09 (s, 3H), 4.16 (q, 2H, J = 7.04 Hz), 4.70 (m, 1H), 4.98 (s, 2H), 7.20 (d, 1H, J = 7.62 Hz), 7.25 (m, 5H), 7.65 (d, 2H, J = 8.42 Hz), 7.76 (br s, 1H), 7.83 (d, 2H, J = 8.42 Hz); 13C NMR (CDCl3) d 14.08, 14.11, 25.95, 26.91, 27.42, 30.46, 40.27, 49.94, 52.51, 54.66, 54.88, 60.85, 61.72, 87.37, 113.58, 126.56, 127.40, 127.64, 128.46, 128.50, 130.99, 134.12, 137.61, 153.78, 157.51, 162.36, 164.77, 166.16, 170.21, 171.76, 173.31, 175.68. Anal. Calcd for $C_{38}H_{44}N_6O_8.H_2O$: C, 62.45 H, 6..34; N, 11.50 Found: C, 63.57; H, 6.21; N, 11.23.

Diethyl 4-[(6-benzyl-10(9*H*)-oxo-8-pivaloylamino)-4,5-dihydro-6*H*-isoxazolo[3,4-*d*]pyrimido[4,5-*b*]azepin-3-yl]benzoyl-L-glutamate (12). To a solution of **11** (0.5 g, 0.7 mmol) in anhydrous methylene chloride (20 mL) was added dropwise iodotrimethylsilane (1.0 mL, 7.0 mmol) and the mixture was heated under reflux for 4 h. The reaction mixture was quenched with methanol (5 mL) and filtered through a pad of Celite. The filtrate was concentrated in vacuo and the residue was partitioned between methylene dichloride and water. The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was removed. The residue was chromatographed on silica gel eluting with 10% methanol in methylene chloride. The fractions containing the pure product were combined. The solvent was removed to give 0.43 g (92%) of a colorless solid: mp 155-157 °C; ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H, J = 7.3 Hz), 1.20 (t, 3H, J = 7.3 Hz), 1.24 (s, 9H), 2.44 (m, 2H), 2.46 (m, 2H, overlapping with DMSO peak), 3.39 (m, 2H), 4.09 (dq, 2H, J = 1.5, 7.52 Hz), 4.14 (q, 2H, J = 7.16 Hz), 4.46 (m, 1H), 5.04 (s, 2H), 7.38 (m, 5H), 7.82 (d, 2H, J = 7.74 Hz), 8.03 (d, 2H, J = 8.14 Hz), 8.92 (d, 1H, J = 4.30 Hz), 10.67 (br s, 1H), 11.46 (br s, 1H). Anal. Calcd for $C_{37}H_{42}N_6O_8.H_2O$: C, 62.0; H, 6.19; N, 11.72. Found: C, 61.84; H, 6.29; N, 11.54.

4-[(8-Amino-6-benzyl-10(9*H*)-oxo)-4,5-dihydro-(6*H*)-isoxazolo[3,4-*d*]pyrimido[4,5-*b*]azepin-3-yl] benzoyl-L-glutamic Acid (13). A suspension of **12** (0.15 g, 0.214 mmol) in 1% sodium hydroxide (4 mL) was stirred for 14 days. The mixture was filtered and the filtrate was acidified with two drops of concentrated hydrochloric acid. The resulting solid was collected by filtration at the pump, washed with water and dried in vacuo to give 0.088 g (73%) of a colorless solid: mp > 260 °C; ^{1}H NMR (DMSO-d_6) δ 2.05 (m, 1H), 2.36 (m, 1H), 2.92 (m, 2H), 4.14 (m, 1H), 4.87 (s, 2H), 6.61 (br s, 2H), 7.33 (m, 5H), 7.77 (d, 2H, J = 7.84 Hz), 8.02 (d, 2H, J = 8.14 Hz), 8.78 (d, 1H, J = 7.08 Hz), 10.21 (br s, 1H), 12.25 (br, 2H), 2H must be hidden in DMSO peak. MS (electrospray) m/z: 559 (MH^+). Anal. Calcd for $C_{28}H_{26}N_6O_7.H_2O$: C, 58.33; H, 4.89; N, 14.58. Found: C, 58.52; H, 4.78; N, 14.38.

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