A FISCHER-INDOLE APPROACH TO PYRROLO[2,3-d]PYRIMIDINES

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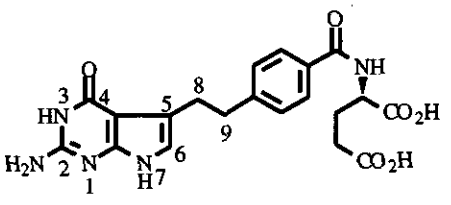
Abstract - Several new 5,6-disubstituted pyrrolo[2,3-d]pyrimidines have been prepared by thermolysis of ketone hydrazones of 2-amino-6-hydrazino-4(3H)-oxopyrimidine (**8**) (the Fischer-Indole Synthesis).

Thymidylate synthase (TS), which catalyzes the transformation of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP), is a critically important enzyme for *de novo* DNA biosynthesis.[1] We discovered recently that *N*-{4-[2-(2-amino-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (LY231514, **1**) is a potent cytotoxic agent for the treatment of solid tumors, primarily as a consequence of inhibition of TS.[2] As part of our ongoing efforts to delineate the structural features that determine activity for this class of antitumor agent, we describe herein three closely related structural analogs of **1** bearing substituents at the 6-position (**2**) or the 6-and 9-positions (**3** and **4**) which were prepared by a novel application of the classical Fischer-Indole Synthesis.[3]

A key step in the synthesis of the 6-methyl substituted analog (**2**) involved thermal indolization of the hydrazone (**9**) which was prepared as depicted in Scheme I. Palladium-catalyzed coupling ($PdCl_2/PPh_3$) of methyl 4-bromobenzoate with 4-pentyn-2-ol in diethylamine as solvent gave **5** in 60% yield. Hydrogenation using 5% palladium-on-carbon as catalyst gave the secondary alcohol (**6**) (96% yield),

which was then oxidized with PCC to the ketone (**7**) (75% yield).[4] Heating of **7** under reflux in 2-methoxyethanol with one equivalent of the hydrazine (**8**)[3b,5] afforded the requisite intermediate hydra-



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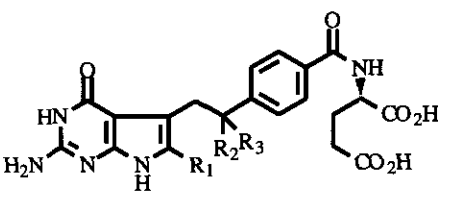


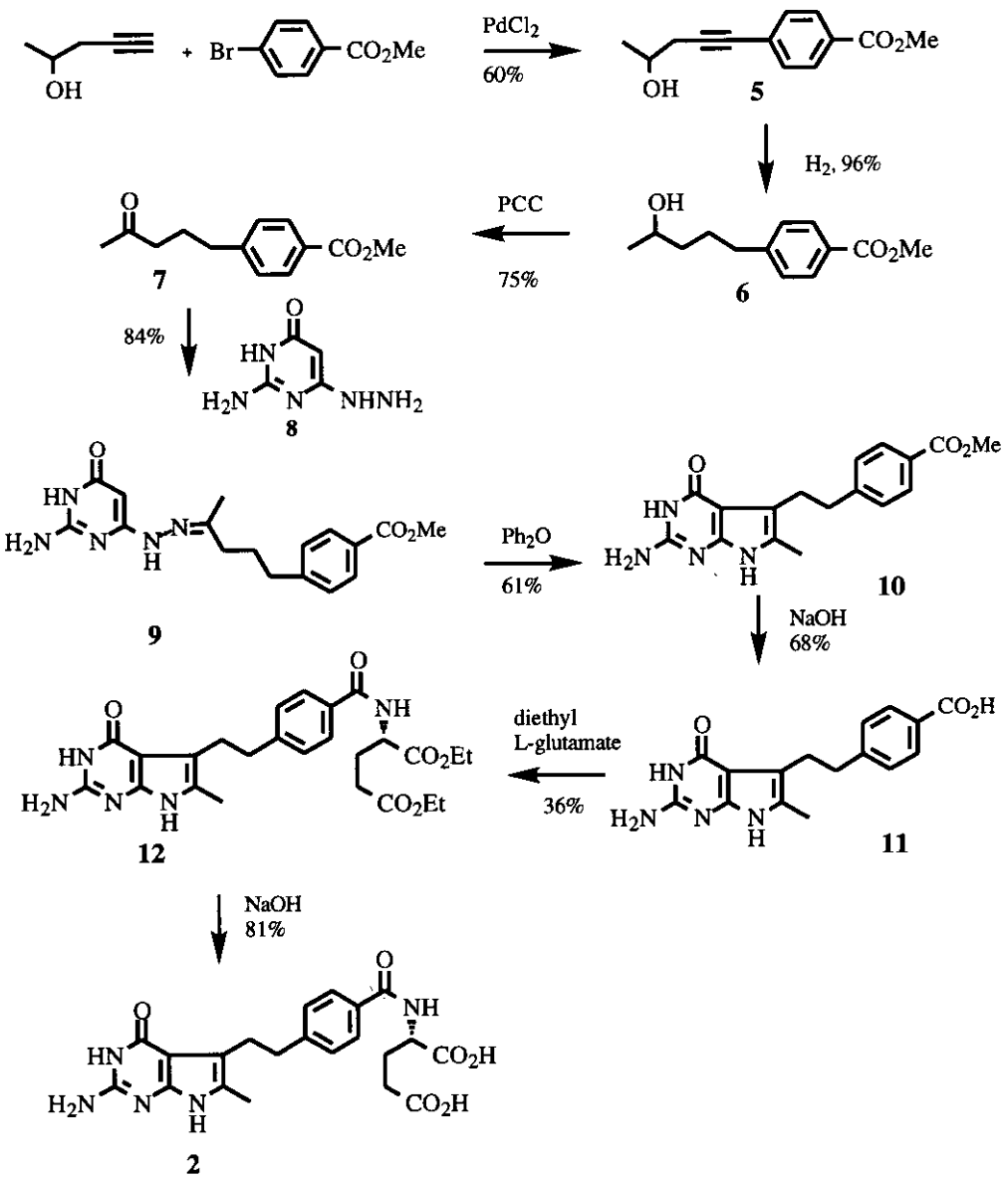
2 $R_1 = CH_3$, $R_2,R_3 = H$
3 $R_1,R_2 = CH_2CH_2$, $R_3 = H$
4 $R_1,R_2 = CH_2CH_2$, $R_3 = OH$

zonopyrimidine (**9**) in 84% yield. The key indole cyclization step was accomplished by thermolysis in refluxing diphenyl ether under argon. This thermal process was regioselective. Proton nmr analysis of the crude reaction mixture showed no evidence for a mixture of the possible C-5 and C-6 substituted pyrrolo[2,3-d]pyrimidine isomers. Saponification of the methyl benzoate (**10**) (68% yield) followed by coupling of the resulting benzoic acid (**11**) with diethyl L-glutamate using 6-chloro-2,4-dimethoxy-1,3,5-triazine as the coupling agent[6] gave the diethyl glutamate derivative (**12**) in 36% yield. Hydrolysis in NaOH followed by acidification with acetic acid then provided the desired 6-methyl analog (**2**) in 81% yield.

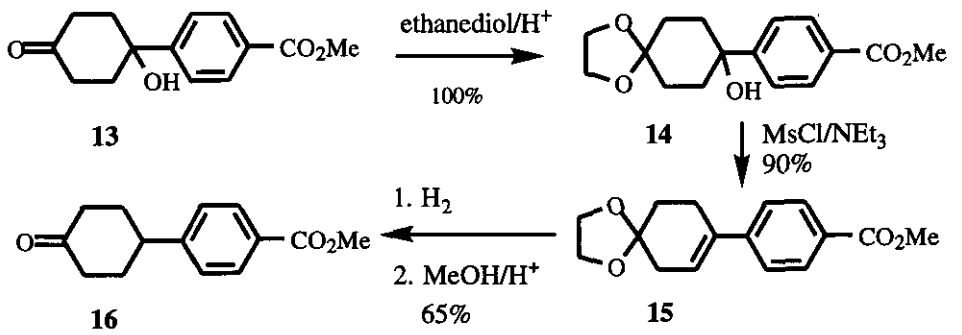
The 6,9-ethano analog (**3**), which was of interest as a conformationally constrained analog of **1**, was synthesized starting from the cyclohexanone (**16**) (Scheme II). This latter compound had been reported by DeGraw[7] to be available by palladium(100%)-catalyzed hydrogenolysis of the hydroxy ester (**14**), but in our hands this attempted reduction led only to the deprotected ketone (**13**). For that reason, a four-step synthesis of **16** was developed. Reprotection of the ketone functionality in **13** followed by mesylation ($MsCl/NEt_3$) led directly to the alkene (**15**), which upon hydrogenation on 5% palladium-on-carbon and final deprotection with acid gave the ketone (**16**) in 59% overall yield.

The hydrazone (**17a**), prepared in 73% yield from **8** and **16**, was heated in refluxing diphenyl ether to give the methyl benzoate (**18a**) (91%), which was then converted to the tricyclic analog (**3**) by the same

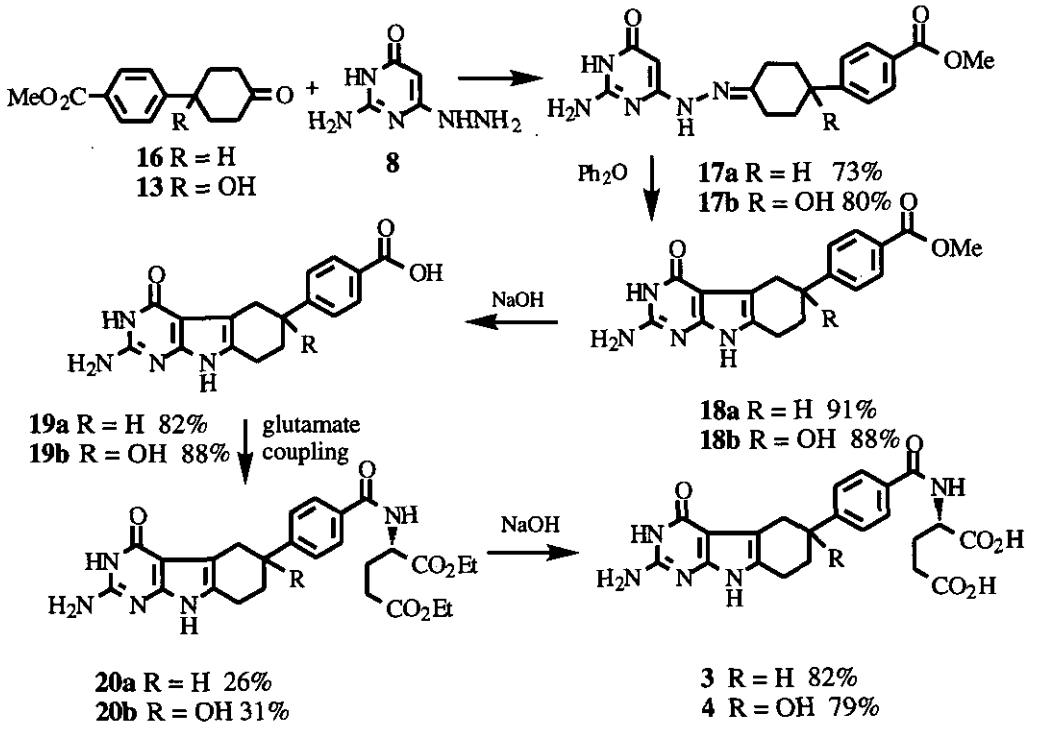
sequence of reactions described above for the conversion of **10** to **2**. The 6,9-ethano-9-hydroxy analog (**4**) was synthesized in the same manner starting with the ketone (**13**) (Scheme III).



Scheme I

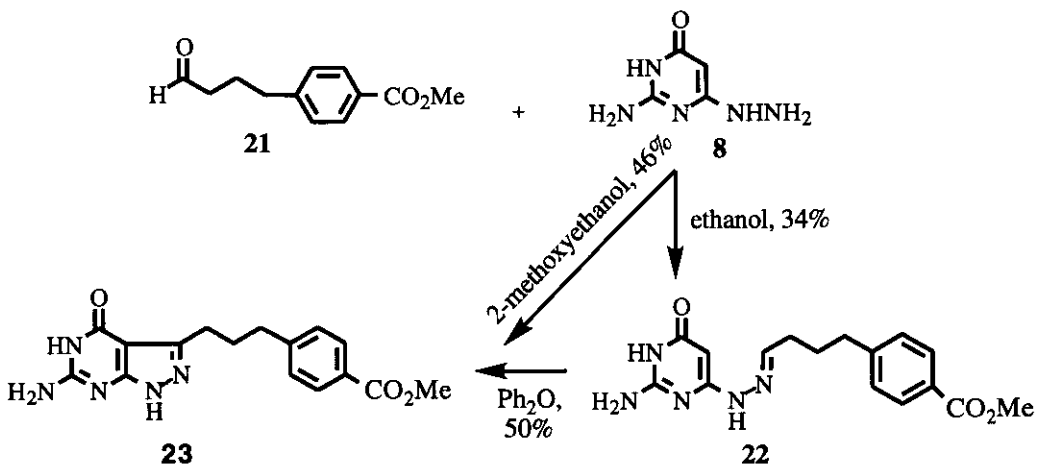


Scheme II



Scheme III

This successful application of the Fischer-Indole Synthesis to the pyrrolo[2,3-d]pyrimidine system suggested that it might be exploited for a simple alternative approach to **1** (LY231514). Accordingly, the hydrazone (**22**), readily prepared from the aldehyde (**21**)[4] and 2-amino-6-hydrazino-4(3H)-oxopyrimidine (**8**), was heated in diphenyl ether as described above for the converison of **9** to **10**. To



Scheme IV

our surprise, the product of this reaction proved to be the pyrazolo[2,3-d]pyrimidine (**23**) (Scheme IV). Attempts[3a] to effect indolization in the presence of acidic catalysts such as HBr·HOAc or PCl_3 were equally unsuccessful.

Preliminary *in vitro* biological evaluation of these three new analogs indicated that they were all extremely poor inhibitors of cell growth (Table 1). These results are consistent with our earlier observation that the C-6 regioisomer of **1** was also a poor cytotoxic agent,[8] and appear to suggest that substitution at C-6 effectively eliminates cytotoxic activity in this series of TS inhibitors.

Table 1 IC_{50} -CEM data

Cmps	**1**	**2**	**3**	**4**
IC_{50} μg/ml	0.007	4.9	>20	>100

EXPERIMENTAL

General. ^1H and ^{13}C nmr data were obtained on a General Electric QE-300 MHz instrument. Ir spectra were determined on a Nicolet FT-IR spectrometer. Melting points are uncorrected. High

resolution mass spectral data were determined by Dr. Dorothy Little on AEI MS-902 and Kratos MS50TC mass spectrometers. FAB mass determinations, elemental analyses and biological evaluations were performed by Eli Lilly and Co., Indianapolis, Indiana.

Methyl 4-(4-Hydroxypent-1-yn)benzoate (5): A mixture of 20.0 g of methyl 4-bromobenzoate (93.0 mmol), 7.9 g of pent-4-yn-2-ol (93.8 mmol), 0.082 g of $PdCl_2$ (0.46 mmol), 0.244 g of Ph_3P (0.93 mmol) and 0.178 g of CuI (0.93 mmol) in 250 ml of diethylamine was stirred under argon at room temperature for 18 h. Diethylamine was removed under reduced pressure and water was added to the residue. The mixture was extracted with benzene, the benzene extracts passed through a short silica pad to remove the catalyst, and the filtrate concentrated under reduced pressure. Recrystallization of the residue from benzene-hexanes gave 12.2 g (60%) of pure **5** as a white solid: mp 60 °C; ir (KBr) 3273, 3182, 2988, 2922, 2895, 1711, 1598 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.00 (d, J = 8.4 Hz, 2 H), 7.48 (d, J = 8.4 Hz, 2 H), 4.20-4.05 (m, 1 H), 3.93 (s, 3 H), 2.67 (dd, J = 16.8, 5.3 Hz, 1 H), 2.59 (dd, J = 16.8, 6.4 Hz, 1 H), 1.97 (d, J = 4.7 Hz, 1 H), 1.35 (d, J = 6.2 Hz, 3 H); ^{13}C nmr ($CDCl_3$) δ 166.4, 131.3, 129.2, 128.8, 128.1, 89.8, 81.9, 66.2, 52.0, 29.7, 22.3; HRms calcd for $C_{13}H_{14}O_3$ (M^+): 218.0943, found: 218.0955.

Methyl 4-(4-Hydroxypentyl)benzoate (6): To a Parr flask charged with 7.0 g (32.1 mmol) of **5** in 200 ml of ethanol was added 0.70 g (10% wt. equiv) of 5% Pd/C. Hydrogenation was carried out at 50 psi of hydrogen for 20 h. The reaction mixture was filtered through a silica gel pad and the filtrate was concentrated under reduced pressure. Purification of the residual material using silica gel chromatography with 4% ether/hexanes as the eluent gave 6.9 g (96%) of pure **6** as a colorless oil: ir (neat) 3628-3108, 1711, 1605 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.94 (d, J = 8.1 Hz, 2 H), 7.24 (d, J = 8.1 Hz, 2 H), 3.90-3.70 (m, 1 H), 3.89 (s, 3 H), 2.67 (t, J = 7.6 Hz, 2 H), 1.80-1.40 (m, 5 H), 1.18 (d, J = 7.2 Hz, 3 H); ^{13}C nmr ($CDCl_3$) δ 166.9, 147.8, 129.3, 128.1, 127.3, 67.2, 51.6, 38.4, 35.6, 26.9, 23.2; HRms calcd for $C_{13}H_{18}O_3$ (M^+): 222.1256, found: 222.1265.

Methyl 4-(4-Oxopentyl)benzoate (7): A 500 ml round-bottomed flask equipped with a magnetic stirrer was charged with 13.2 g (61.2 mmol) of PCC, 2.51 g (30.6 mmol) of NaOAc and 50 ml of CH_2Cl_2. To

this stirred solution was added dropwise 6.80 g (30.6 mmol) of **6** in 50 ml of CH_2Cl_2. After 12 h the black reaction mixture was diluted with ethyl ether, the resulting solution was filtered through a pad of Florisil, and the filtrate was concentrated under reduced pressure. Vacuum distillation of the residue (0.06 mm Hg, 130 °C) gave 5.11 g (75%) of **7** as a colorless oil: ir (neat) 1711, 1611 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.96 (d, *J* = 8.1 Hz, 2 H), 7.24 (d, *J* = 8.1 Hz, 2 H), 3.90 (s, 3 H), 2.67 (t, *J* = 7.4 Hz, 2 H), 2.44 (t, *J* = 7.3 Hz, 2 H), 2.12 (s, 3 H), 1.92 (tt, *J* = 7.4, 7.3 Hz, 2 H); ^{13}C nmr ($CDCl_3$) δ 207.1, 165.9, 146.3, 128.8, 127.5, 127.0, 50.9, 41.6, 34.0, 28.9, 23.8; HRms calcd for $C_{13}H_{16}O_3$ (M^+): 220.1099, found: 220.1108. Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.69; H, 7.38.

Methyl 4-{4-[(2-Amino-4(3H)-oxopyrimidin-6-yl)hydrazono]pentyl}benzoate (9): A mixture of the ketone (**7**) (1.10 g, 5.0 mmol) and 2-amino-6-hydrazino-4(3H)-oxopyrimidine[5] (0.71 g, 5.0 mmol) in 2-methoxyethanol (50 ml) was refluxed overnight, then cooled to room temperature and filtered. The filtrate was concentrated to ~10 ml, 100 ml of 1:1 ether/hexanes was added, and the resulting brownish solid was collected by filtration to afford 1.45 g (84%) of **9**: mp 242 °C; ir (KBr) 3375, 3334, 3121, 2948, 2862, 2722, 1711, 1645, 1598, 1525 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.94 (br s, 1 H), 8.72 (br s, 1 H), 7.85 (d, *J* = 7.9 Hz, 2 H), 7.34 (d, *J* = 7.9 Hz, 2 H), 6.15 (br s, 2 H), 5.06 (s, 1 H), 3.81 (s, 3 H), 2.64 (t, J = 7.0 Hz, 2 H), 2.19 (t, J = 7.0 Hz, 2 H), 1.85 (tt, J = 7.0, 7.0 Hz, 2 H), 1.81 (s, 3 H); ^{13}C nmr (DMSO-d_6) δ 166.2, 163.2, 163.0, 155.0, 151.2, 148.0, 129.2, 128.7, 127.2, 77.0, 52.0, 37.6, 34.7, 27.2, 15.7; HRms calcd for $C_{17}H_{21}N_5O_3$ (M^+): 343.1644, found: 343.1652. Anal. Calcd for $C_{17}H_{21}N_5O_3$: C, 59.46; H, 6.16; N, 20.40. Found: C, 59.64; H, 6.03; N, 20.38.

Methyl 4-[2-(2-Amino-6-methyl-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoate (10): A mixture of **9** (0.60 g, 1.75 mmol) in diphenyl ether (30 ml) was stirred under argon and heated under reflux for 5 h. After cooling to room temperature, hexanes (70 ml) were added and the precipitated solid was collected by filtration to give 0.35 g (61%) of **10** as a yellowish solid: mp 260 °C (decomp.); ir (KBr) 3501, 3355, 3168, 3128, 3055, 2909, 2849, 1711, 1652, 1618 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.48 (br s, 1 H), 10.10 (br s, 1 H), 7.80 (d, *J* = 7.4 Hz, 2 H), 7.23 (d, *J* = 7.4 Hz, 2 H), 5.96 (br s, 2 H), 3.80 (s, 3 H), 2.87 (t, *J* = 6.3 Hz, 2 H); 2.48 (t, *J* = 6.3 Hz, 2 H), 1.80 (s, 3 H); ^{13}C nmr (DMSO-d_6) δ 166.4,

158.9, 151.9, 150.4, 148.5, 129.0, 128.9, 127.0, 122.0, 112.0, 98.8, 51.9, 37.0, 26.4, 10.0; HRms calcd for $C_{17}H_{18}N_4O_3$ (M^+): 326.1378, found: 326.1390.

4-[2-(2-Amino-6-methyl-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoic Acid (11): A suspension of **10** (1.30 g, 4.0 mmol) in 30 ml of THF-H_2O (2:1) was added to 7 ml of 1 M aqueous NaOH solution. After 3 h of stirring at room temperature, the reaction mixture was concentrated in vacuo, and the residual aqueous solution was acidified with acetic acid. The solid which precipitated was collected by filtration, washed with water, and dried in a vacuum dessicator to give 1.10 g (88%) of **11** as an off-white solid: mp 195 °C (decomp.); ir (KBr) 3610-2855, 2870, 1740-1500 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.55 (br s, 1 H), 10.13 (br s, 1 H), 7.82 (d, J = 7.4 Hz, 2 H), 7.21 (d, J = 7.4 Hz, 2 H), 5.95 (br s, 2 H), 3.00-2.85 (m, 2 H), 2.85-2.70 (m, 2 H), 1.80 (s, 3 H); HRms calcd for $C_{16}H_{16}N_4O_3$ (M^+): 312.1223, found: 312.1196.

Diethyl *N*-{4-[2-(2-Amino-6-methyl-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (12): To a solution of 0.42 g (1.3 mmol) of **11** in 15 ml of anhydrous DMF was added 0.26 g (1.5 mmol) of 6-chloro-2,4-dimethoxy-1,3,5-triazine and 0.16 g (1.6 mmol) of *N*-methylmorpholine. The mixture was stirred at 0 °C under an argon atmosphere for 2 h. To the resulting homogeneous solution was added 0.16 g (1.6 mmol) of *N*-methylmorpholine and 0.38 g (1.6 mmol) of diethyl L-glutamate hydrochloride. The mixture was stirred at 0 °C for 2 h, and then at room temperature for 14 h, and the solvent was removed under reduced pressure. Purification of the residual solid by silica gel column chromatography with 5% MeOH/CH_2Cl_2 as the eluent gave 0.72 g of a 1:4 mixture of 2,4-dimethoxy-6(5H)-oxo-1,3,5-triazine and **12** (36%): ^1H nmr (DMSO-d_6) δ 10.50 (br s, 1 H), 10.05 (br s, 1 H), 8.82 (d, J = 8.4 Hz, 1 H), 7.75 (d, J = 9.0 Hz, 2 H), 7.20 (d, J = 9.0 Hz, 2 H), 5.95 (br s, 2 H), 4.45-4.35 (m, 1 H), 4.10 (q, J = 7.0 Hz, 2 H); 4.03 (q, J = 7.0 Hz, 2 H), 2.80-1.90 (m, 8 H), 1.82 (s, 3 H), 1.20 (t, J = 7.0 Hz, 3 H); 1.15 (t, J = 7.0 Hz, 3 H); HRms calcd for $C_{25}H_{31}N_5O_6$ (M^+): 497.2274, found: 497.2279.

N-{4-[2-(2-Amino-6-methyl-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (2) : To the 1:4 mixture of 2,4-dimethoxy-6(5H)-oxo-1,3,5-triazine and **12** (0.12 g, 0.22 mmol) in 20 ml of THF-H_2O (2:1) was added 5 ml of 1 M aqueous NaOH solution. After 2 h of stirring at room temperature, the reaction mixture was concentrated under reduced pressure, and the residual aqueous solution was acidified with acetic acid. The precipitated solid was collected by filtration, washed with water, and dried in a vacuum dessicator to give 0.081 g (82%) of **2** as an off-white solid: mp 210 °C (decomp.); ir (KBr) 3314, 3194, 2995, 2922, 1692, 1631, 1585 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.47 (br s, 1 H), 10.09 (br s, 1 H), 8.50 (d, J = 7.6 Hz, 1 H), 7.75 (d, J = 7.2 Hz, 2 H), 7.21 (d, J = 7.2 Hz, 2 H), 5.95 (br s, 2 H), 4.50-4.30 (m, 1 H), 2.95-2.80 (m, 2 H), 2.40-2.25 (m, 2 H), 2.20-1.84 (m, 4 H), 1.84 (s, 3 H); FABms calcd for $C_{21}H_{24}N_5O_6$ (MH^+): 442.1727, found: 442.1693.

4-(4-Carbomethoxyphenyl)-4-hydroxycyclohexanone (13): Following a published procedure,[7] the product we isolated proved to be **13** rather than **14**. Thus, a solution of 4-bromobenzonitrile (10 g, 55 mmol) in 260 ml of dry THF and 70 ml of dry hexanes under argon was cooled to -100 °C in a liquid nitrogen-Et_2O bath. n-Butyllithium (34.3 ml, 54.9 mmol, 1.6 M solution in hexanes) was added dropwise so that the internal temperature did not exceed -95 °C. The orange solution was stirred for an additional 10 min and then treated with a solution of 1,4-cyclohexanedione monoethylene ketal (8.57 g, 55 mmol) in 55 ml of dry THF, again carefully maintaining the temperature below -95 °C. The reaction mixture was stirred for 10 min at -95 °C, allowed to warm to room temperature, and poured into ice water (400 ml). The organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic extracts were dried over $MgSO_4$ and evaporated to give 13 g of a white crystalline solid.

A mixture of the above crystalline solid in 190 ml of 2-methoxyethanol and 190 ml of 2.5 N NaOH was heated to ~100 °C overnight. The solution was cooled in an ice bath, adjusted to pH 7 with conc. HCl, and evaporated to dryness. Water was added, and the pH was adjusted to 2 with 2 *N* HCl. The tan solid (11.6 g) was collected by filtration and washed with water.

A mixture of this tan solid in 500 ml of methanol and 8 ml of concentrated HCl was stirred at reflux for 4 h and then evaporated to dryness. The residue was treated with saturated $NaHCO_3$ and extracted with

$CHCl_3$. The organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness to give **13** (11.0 g, 80% over three steps) as a colorless solid, mp 112-113 °C; ir (KBr) 3401, 1711, 1691, 1678 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.00 (d, J = 9.6 Hz, 2 H), 7.48 (d, J = 9.6 Hz, 2 H), 3.95 (s, 3 H), 3.05-2.90 (m, 2 H), 2.50-2.10 (m, 7 H); ^{13}C nmr ($CDCl_3$) δ 211.8, 166.8, 152.5, 129.4, 128.6, 124.4, 71.6, 51.9, 38.1, 37.0; HRms calcd for $C_{14}H_{16}O_4$ (M^+): 248.1048, found: 248.1055; Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.48; H, 6.57.

4-(4-Carbomethoxyphenyl)cyclohex-3-enone Ethylene Ketal (15) : The ketone functionality in **13** was reprotected as follows: To a solution of **13** (5.2 g, 21.0 mmol) in benzene (100 ml), ethylene glycol (1.63 g, 26.3 mmol), and *p*-toluenesulfonic acid (1 g, 5.3 mmol) were added and the mixture was refluxed with water separation (Dean-Stark trap) for 2 h. The solvent was removed in vacuo, diethyl ether was added, and the mixture was washed with aq. $NaHCO_3$ and then with water. The organic phase was dried with $MgSO_4$ and the solvent was removed under reduced pressure to give the ketal (**14**) in quantitative yield.

A solution of MsCl (10.3 g, 90.0 mmol) in 50 ml of CH_2Cl_2 was added dropwise to a stirred solution of the ketal (**14**) (13.4 g, 45.9 mmol) and triethylamine (23 g, 227 mmol) in 250 ml of CH_2Cl_2 at 0 °C. The reaction mixture was stirred at 0 °C for 8 h, poured into water and extracted several times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated. Purification of the residual material by silica gel chromatography (using 20-50% ether/petroleum ether) gave 11.3 g (90%) of **15** as a pale yellow solid: mp 109 °C; ir (KBr) 1704, 1645 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.00 (d, J = 8.3 Hz, 2 H), 7.50 (d, J = 8.3 Hz, 2 H), 6.01 (m, 1 H), 4.05 (s, 4 H), 3.95 (s, 3 H), 2.65 (m, 2 H), 2.20 (m, 2 H), 1.95 (t, J = 6.8 Hz, 2 H); HRms calcd for $C_{16}H_{18}O_4$ (M^+): 274.1205, found: 274.1199. Anal. Calcd for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61. Found: C, 70.29; H, 6.87.

4-(4-Carbomethoxyphenyl)cyclohexanone (16) was prepared by hydrogenation of **15** followed by ketal cleavage. To a Parr flask charged with 5.7 g (20.8 mmol) of **15** in 150 ml of ethanol was added 0.57 g (10% wt. equiv) of 5% Pd/C. Hydrogenation was carried out at 50 psi of hydrogen for 20 h. The reaction mixture was filtered through a silica gel pad, and conc. HCl (3 ml) was added to the reaction

mixture. The resulting solution was then heated under reflux for 4 h and evaporated to dryness. The residue was triturated with saturated aq. $NaHCO_3$, and the resulting solution was extracted with $CHCl_3$. The $CHCl_3$ extracts were dried over $MgSO_4$, concentrated, and the residue purified by silica gel chromatography using 1:1 ether/hexanes as the eluent to give 3.10 g (65%) of **16**. Spectral data of **16** were in agreement with those previously reported.[7]

Methyl 4-{4-[(2-Amino-4(3H)-oxopyrimidin-6-yl)hydrazono]cyclohexyl}benzoate (17a): A mixture of **16** (1.45 g, 6.3 mmol) and 2-amino-6-hydrazino-4(3H)-oxopyrimidine (0.88 g, 6.2 mmol) in 2-methoxyethanol (70 ml) was refluxed overnight. The reaction mixture was allowed to cool to room temperature and filtered to afford 1.60 g (73%) of **17a** as a white solid: mp 270 °C (decomp.); ir (KBr) 3361, 3155, 2929, 2862, 2715, 1718, 1644, 1591, 1511 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.92 (br s, 1 H), 9.11 (s, 1 H), 7.87 (d, J = 8.2 Hz, 2 H), 7.40 (d, J = 8.2 Hz, 2 H), 6.16 (br s, 2 H), 5.09 (s, 1 H), 3.82 (s, 3 H), 3.08 (br d, J = 17.4 Hz, 1 H), 2.91 (br t, J = 10.5 Hz, 1 H), 2.50-2.30 (m, 2 H), 2.00-1.80 (m, 3 H), 1.75-1.40 (m, 2 H); HRms calcd for $C_{18}H_{21}N_5O_3$(M^+): 355.1619, found: 355.1638. Anal. Calcd for $C_{18}H_{21}N_5O_3$: C, 60.83; H, 5.96; N, 19.71. Found: C, 61.06; H, 5.92; N, 19.42.

Methyl 4-{4-[(2-Amino-4(3H)-oxopyrimidin-6-yl)hydrazono]-1-hydroxycyclohexyl}benzoate (17b): A mixture of **13** (1.6 g, 4.7 mmol) and 2-amino-6-hydrazino-4(3H)-oxopyrimidine (0.71 g, 5.0 mmol) in 2-methoxyethanol (50 ml) was heated under reflux for 15 h. The reaction mixture was allowed to cool to room temperature and filtered. The filtrate was concentrated to ~10 ml, 100 ml of 1:1 ether/hexanes was added, and the resulting light brown solid was collected by filtration to afford 1.40 g (80%) of **17b**: *mp 250 °C (decomp.); ir* (KBr) 3468, 3361, 3334, 3195, 2929, 2716, 1718, 1645, 1598, 1535 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.94 (br s, 1 H), 9.08 (s, 1 H), 7.88 (d, J = 8.5 Hz, 2 H), 7.63 (d, J = 8.5 Hz, 2 H), 6.16 (br s, 2 H), 5.32 (s, 1 H), 5.08 (s, 1 H), 3.84 (s, 3 H), 3.00-2.80 (m, 1 H), 2.75-2.50 (m, 1 H), 2.20-2.05 (m, 2 H), 2.00-1.60 (m, 4 H); ^{13}C nmr (DMSO-d_6) δ *166.1, 163.1, 155.1, 154.9, 154.9,* 153.6, 128.9, 127.7, 125.2, 76.8, 71.4, 52.0, 37.1, 30.6, 22.2; HRms calcd for $C_{18}H_{21}N_5O_4$ (M^+): 371.1593, found: 371.1599. Anal. Calcd for $C_{18}H_{21}N_5O_4$: C, 58.21; H, 5.70; N, 18.86. Found: C, 57.93; H, 5.85; N, 18.65.

Methyl 4-[2-Amino-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl)benzoate (18a): A mixture of **17a** (1.40 g, 3.9 mmol) in diphenyl ether (50 ml) was stirred under argon and heated under reflux for 2.5 h. After cooling to room temperature, hexanes (70 ml) were added, and the resulting precipitate was collected by filtration to afford 1.20 g (91%) of **18a** as a yellowish solid: mp 340-342 °C; ir (KBr) 3487, 3361, 3155, 2929, 2862, 2715, 1704, 1671, 1618, 1558 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.64 (br s, 1 H), 10.10 (br s, 1 H), 7.90 (d, J = 8.0 Hz, 2 H), 7.45 (d, J = 8.0 Hz, 2 H), 5.95 (br s, 2 H), 3.83 (s, 3 H), 3.05-2.85 (m, 2 H), 2.70-2.49 (m, 3 H), 2.00-1.80 (m, 2 H); HRms calcd for $C_{18}H_{18}N_4O_3$ (M^+): 338.1380, found: 338.1374. Anal. Calcd for $C_{18}H_{18}N_4O_3$: C, 63.89; H, 5.36; N, 16.56. Found: C, 63.71; H, 5.46; N, 16.64.

Methyl 4-[2-Amino-6-hydroxy-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl)benzoate (18b): A mixture of **17b** (1.36 g, 3.7 mmol) in diphenyl ether (70 ml) was stirred under argon and heated under reflux for 2.5 h. After cooling to room temperature, hexanes (70 ml) were added and the resulting precipitate was collected by filtration to afford 1.13 g (88%) of **18b** as a yellowish solid: mp 338-340 °C; ir (KBr) 3494, 3374, 3325, 3135, 3068, 2915, 1718, 1638, 1552 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.48 (br s, 1 H), 9.95 (s, 1 H), 7.76 (d, J = 8.4 Hz, 2 H), 7.50 (d, J = 8.4 Hz, 2 H), 5.80 (br s, 2 H), 5.05 (s, 1 H), 3.70 (s, 3 H), 2.87 (d, J = 17.0 Hz, 1 H), 2.70 (d, J = 17.0 Hz, 1 H), 2.60-2.48 (m, 1 H), 2.20-2.08 (m, 1 H), 2.05-1.90 (m, 1 H), 1.75-1.68 (m, 1 H); ^{13}C nmr (DMSO-d_6) δ 166.2, 158.8, 155.3, 151.7, 151.0, 128.7, 127.6, 125.5, 123.7, 109.0, 98.3, 71.6, 52.0, 36.8, 35.0, 19.5; HRms calcd for $C_{18}H_{18}N_4O_4$ (M^+): 354.1329, found: 354.1348. Anal. Calcd for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81. Found: C, 60.82; H, 5.09; N, 16.11.

4-[2-Amino-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl)benzoic Acid (19a): A mixture of **18a** (1.20 g, 3.5 mmol), 60 ml of THF-H_2O (2:1) and 10 ml of 1 M aqueous NaOH was stirred at room temperature for 4 h, the organic solvent was removed in vacuo, and the remaining aqueous solution was acidified by addition of acetic acid. The resulting precipitate was collected by filtration, washed with water, and dried in a vacuum dessicator to give 0.94 g (82%) of **19a** as an off-

white solid: mp >400 °C; ir (KBr) 3448, 3341, 3188, 2922, 1684, 1638, 1599 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.64 (br s, 1 H), 10.15 (s, 1 H), 7.84 (d, J = 8.2 Hz, 2 H), 7.30 (d, J = 8.2 Hz, 2 H), 5.92 (br s, 2 H), 3.05-2.85 (m, 2 H), 2.70-2.50 (m, 3 H), 2.00-1.80 (m, 2 H); HRms calcd for $C_{17}H_{16}N_4O_3$(M^+): 324.1224, found: 324.1227.

4-[2-Amino-6-hydroxy-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl)benzoic Acid (19b): A mixture of **18b** (1.3 g, 3.7 mmol), 7 ml of 1 M aqueous NaOH solution and 30 ml of THF-H_2O (2:1) was stirred at room temperature for 3 h, the organic solvent was removed in vacuo, and the residual aqueous solution was acidified with acetic acid. The light yellow precipitate which separated was collected by filtration, washed with water, and dried in vacuo to give 1.1 g (88%) of **19b**: mp ≥400 °C; ir (KBr) 3441, 3328, 3195, 3162, 2909, 1774-1598, 1558 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.60 (br s, 1 H), 10.12 (s, 1 H), 7.86 (d, J = 8.3 Hz, 2 H), 7.58 (d, J = 8.3 Hz, 2 H), 5.96 (br s, 2 H), 5.14 (s, 1 H), 3.01 (d, J = 16.5 Hz, 1 H), 2.83 (d, J = 16.5 Hz, 1 H), 2.75-2.57 (m, 1 H), 2.35-2.20 (m, 1 H), 2.20-2.05 (m, 1 H), 1.90-1.70 (m, 1 H); HRms calcd for $C_{17}H_{16}N_4O_4$ (M^+): 340.1171, found: 340.1172.

Diethyl N -{4-[2-Amino-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl]benzoyl}-L-glutamate (20a): To a solution of 0.64 g (2.0 mmol) of **19a** in anhydrous DMF was added 0.44 g (2.5 mmol) of 6-chloro-2,4-dimethoxy-1,3,5-triazine and 0.30 g (3.0 mmol) of *N*-methylmorpholine. The mixture was stirred at 0 °C under an argon atmosphere for 2 h. To the resulting homogeneous solution was added 0.30 g (3.0 mmol) of *N*-methylmorpholine and 0.72 g (3.0 mmol) of diethyl L-glutamate hydrochloride. The mixture was stirred at 0 °C for 2 h and then at room temperature for 14 h, the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with 5% MeOH/CH_2Cl_2 as the eluent to give 0.72 g of a 4:1 mixture of 2,4-dimethoxy-6(5H)-oxo-1,3,5-triazine and **20a** (26%): ^1H nmr (DMSO-d_6) δ 10.62 (br s, 1 H), 10.05 (s, 1 H), 8.84 (d, J = 8.4 Hz, 1 H), 7.80 (d, J = 8.5 Hz, 2 H), 7.40 (d, J = 8.5 Hz, 2 H), 5.95 (br s, 2 H), 4.30-4.18 (m, 1 H), 4.10 (q, J = 7.0 Hz, 2 H), 4.05 (q, J = 7.0 Hz, 2 H), 3.00-2.40 (m, 7 H), 2.00-1.80 (m, 4 H), 1.16 (t, J =7.0 Hz, 3 H), 1.14 (t, J = 7.0 Hz, 3 H); HRms calcd for $C_{26}H_{31}N_5O_6$ (M^+): 509.2276, found: 509.2256.

Diethyl N-{4-[2-Amino-6-hydroxy-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl]benzoyl}-L-glutamate (20b): A 100 ml round-bottomed flask, equipped with a magnetic stirrer and gas inlet, was charged with 0.87 g (2.6 mmol) of **19b**, 0.44 g (2.5 mmol) of 6-chloro-2,4-dimethoxy-1,3,5-triazine, 0.51 g (5.0 mmol) of *N*-methylmorpholine, and 20 ml of anhydrous DMF. The mixture was stirred at 0 °C under an argon atmosphere for 1 h. To the resulting homogeneous solution was added 0.51 g (5.0 mmol) of *N*-methylmorpholine and 1.20 g (5.0 mmol) of diethyl L-glutamate hydrochloride. The mixture was stirred at 0 °C for 2 h, then at room temperature for 14 h, and the solvent was removed under reduced pressure. Purification of the residual material by silica gel column chromatography with 5-50% MeOH/CH_2Cl_2 as the eluent gave 0.40 g (31%) of **20b** as a light yellow solid: mp 258 °C (decomp.); ir (KBr) 3335, 3222, 2982, 2928, 1718, 1645, 1612 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.59 (br s, 1 H), 10.06 (s, 1 H), 8.64 (d, J = 7.2 Hz, 1 H), 7.81 (d, J = 8.1 Hz, 2 H), 7.56 (d, J = 8.1 Hz, 2 H), 5.92 (br s, 2 H), 5.10 (s, 1 H), 4.48-4.35 (m, 1 H), 4.08 (q, J = 6.9 Hz, 2 H), 4.05 (q, J = 6.9 Hz, 2 H), 3.03 (d, J = 16.8 Hz, 1 H), 2.83 (d, J = 16.8 Hz, 1 H), 2.75-2.58 (m, 1 H), 2.49-1.80 (m, 7 H), 1.18 (t, J =6.9 Hz, 3 H), 1.15 (t, J = 6.9 Hz, 3 H); HRms calcd for $C_{26}H_{31}N_5O_7$ (M^+): 525.2223, found: 525.2241.

N-{4-[2-Amino-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl]benzoyl}-L-glutamic Acid (3): To the above 4:1 mixture of 2,4-dimethoxy-6(5H)-oxo-1,3,5-triazine and **20a** (0.14 g, 0.13 mmol) in 15 ml of THF-H_2O (2:1) was added 10 ml of 1 M aqueous NaOH solution and the resulting mixture was stirred for 2 h. The organic solvent was then removed under reduced pressure, and the remaining aqueous solution was acidified by addition of acetic acid. The resulting precipitate was collected by filtration, washed with water, and dried in vacuo to give 0.05 g (82%) of **3** as an off-white solid: mp 230 °C (decomp.); ir (KBr) 3600-2975, 2928, 1720-1600 cm^{-1}; ^1H nmr (DMSO-d_6) δ 10.60 (br s, 1 H), 10.16 (br s, 1 H), 8.35 (d, J = 8.1 Hz, 1 H), 7.85 (d, J = 8.2 Hz, 2 H), 7.35 (d, J = 8.27 Hz, 2 H), 5.99 (br s, 2 H), 4.40-4.20 (m, 1 H), 3.00-2.20 (m, 7 H), 2.05-1.80 (m, 4 H); FABms calcd for $C_{22}H_{24}N_6O_5(MH^+)$: 454.1727, found: 454.1686.

N-{4-[2-Amino-6-hydroxy-3,5,6,7,8,9-hexahydro-4-oxo-4H-pyrimido[4,5-b]indol-6-yl]benzoyl}-L-glutamic Acid (4): A mixture of **20b** (0.15 g, 0.29 mmol) in 9 ml of THF-H_2O (2:1) and 2 ml of 1 M aqueous NaOH was stirred for 2 h at room temperature, the organic solvent was removed in vacuo, and the residual aqueous solution was acidified with acetic acid. The solid which separated was collected by filtration, washed with water, and dried in vacuo to give 0.10 g (79%) of **4** as an off-white solid: mp 244 °C (decomp.); ir (KBr) 3335, 3228, 2929, 1791-1565, 1545 cm^{-1}; ^1H nmr (DMSO-d_6) δ 12.38 (br s, 2 H), 10.59 (s, 1 H), 10.07 (s, 1 H), 8.53 (d, J = 7.3 Hz, 1 H), 7.81 (d, J = 8.1 Hz, 2 H), 7.56 (d, J = 8.1 Hz, 2 H), 5.92 (br s, 2 H), 5.10 (s, 1 H), 4.40 (m, 1 H), 3.00 (d, J = 16.5 Hz, 1 H), 2.85 (d, J = 16.5 Hz, 1 H), 2.75-2.58 (m, 1 H), 2.40-1.70 (m, 7 H); FABms calcd for $C_{22}H_{24}N_5O_7$ (MH^+): *470.1676*, *found*: 470.1694.

Methyl 4-{4-[(2-Amino-4(3H)-oxopyrimidin-6-yl)hydrazono]butyl}benzoate (22): A mixture of methyl 4-(4-oxobutyl)benzoate[4] (2.06 g, 10.0 mmol) and 2-amino-6-hydrazino-4(3H)-oxopyrimidine (2.82 g, 20.0 mmol) in ethanol (100 ml) was heated under reflux for 15 h, then cooled to room temperature and filtered. The filtrate was concentrated to ~15 ml, and 100 ml of 1:1 ether/hexanes was added to the residue. The resulting brownish solid was collected by filtration to afford 1.11 g (34%) of **22**: mp 235 °C (*decomp.*); *ir* (KBr) 3307, 3162, 2942, 1718-1578, 1539 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.94 (br s, 1 H), 7.86 (d, J = 8.2 Hz, 2 H), 7.33 (d, J = 8.2 Hz, 2 H), 7.33 (m, 1 H), 6.23 (br s, 2 H), 4.97 (s, 1 H), 3.81 (s, 3 H), 2.80-2.60 (m, 2 H), 2.15-2.05 (m, 2 H), 1.95-1.85 (m, 2 H); HRms calcd for $C_{16}H_{19}N_5O_3$ (M^+): 329.1493, found: 329.1488.

Methyl 4-[3-(2-Amino-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)propyl]benzoate (23): A mixture of **22** (0.10 g, 0.30 mmol) in diphenyl ether (12 ml) was stirred under argon and heated under reflux for 30 min. After cooling to room temperature, hexanes (50 ml) were added, and the resulting precipitate was collected by filtration to afford 0.05 g (50%) of **23** as a yellowish solid: mp 260 °C (decomp.); ir (KBr) 3331-3125, 3164, 2920, 2860, 1720-1614 cm^{-1}; ^1H nmr (DMSO-d_6) δ 12.40 (br s, 1 H), 10.45 (s, 1 H), 7.83 (d, J = 7.3 Hz, 2 H), 7.32 (d, J = 7.3 Hz, 2 H), 6.46 (br s, 2 H), 3.80 (s, 3 H), 2.70-2.50 (m, 4 H), 2.00-1.85 (m, 2 H); ^{13}C nmr (DMSO-d_6) δ 166.2, 158.7, 157.2, 154.6, 148.0, 129.2,

128.7, 127.2, 97.0, 51.9, 34.6, 29.3, 27.3; HRms calcd for $C_{16}H_{17}N_5O_3$ (M^+): 327.1336, found: 327.1320.

Refluxing a mixture of methyl 4-(4-oxobutyl)benzoate and 2-amino-6-hydrazino-4(3H)-oxopyrimidine in 2-methoxyethanol for 8 h resulted in the formation of **23** in 46% yield.

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