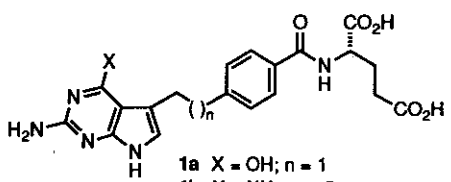
SYNTHESIS OF LY288601, A 5,6-DIHYDROPYRROLO[2,3-*d*]PYRIMIDINE BASED ANTIFOLATE COMPOUND RELATED TO LY231514§

Charles J. Barnett* and Thomas M. Wilson

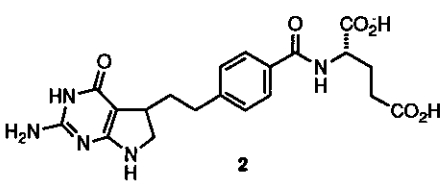
Chemical Process Research and Development Division, Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285-4813, U.S.A.

Abstract- An expeditious synthesis of LY288601 (**2**), the 5,6-dihydro analog of the pyrrolo[2,3-*d*]pyrimidine-based antifolate compound LY231514 (**1a**), is described. The synthesis proceeds in eight steps from *tert*-butyl 4-iodobenzoate and involves the elaboration of a 2-amino-4-hydroxypyrimidine ring onto an activated 3-carboalkoxy-2-pyrrolidinone via reaction with guanidine as a key step.

The deaza analogs of folic acid are an important new class of specific inhibitors of folate requiring enzymes for the treatment of cancer.[1] Progress in this area has been greatly facilitated by the pioneering synthetic work of Taylor and coworkers who first succeeded in synthesizing 5-deazafolic acid and 5,10-dideazatetrahydrofolic acid (DDATHF)[2] and have recently prepared and tested the pyrrolo[2,3-*d*]pyrimidine-based analog LY231514 (**1a**) in collaboration with a Lilly group.[3] LY231514 is currently being evaluated in phase I clinical trials.



1a X = OH; n = 1
1b X = NH_2; n = 2



2

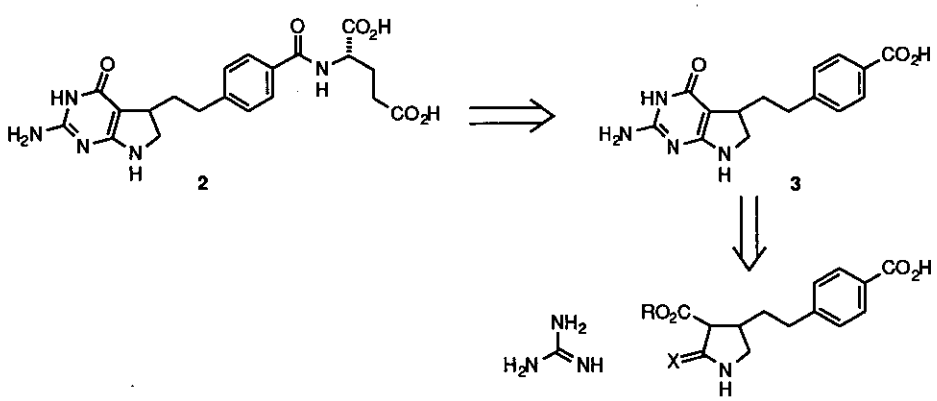
§ Dedicated to Professor Edward C. Taylor on the occasion of his seventieth birthday.

Akimoto and coworkers have independently developed a similar series of pyrrolopyrimidine-based antifolate compounds,[4] exemplified by TNP-351 (**1b**).[5] We have been interested in the preparation of LY288601 (**2**), the 5,6-dihydro-analog of LY231514, in connection with the exploration of structure-activity relationships in the LY231514 series. The recent report of the synthesis of **2** by the Akimoto group[6] prompts us to disclose our own approach to the synthesis of this compound.

Since methods have been developed for the synthesis of the pyrrolo[2,3-*d*]pyrimidines (**1**), partial reduction of these compounds (or their precursors) seemed to be an obvious approach to preparation of dihydro analogs. The 5-deazaguanine chromophore of LY231514 (**1a**) proved, however, to be remarkably stable toward reduction of the 5,6 bond of the pyrrole ring. For example, the model compound, 2-pivaloylamino-4-hydroxypyrrolo[2,3-*d*]pyrimidine, was not reduced under ordinary Parr conditions (50 psi hydrogen) with palladium or platinum catalysts,[7] and was recovered unchanged after several hours of exposure to 37.4 atmospheres of hydrogen in the presence of platinum oxide in acetic acid at 70 °C.[8] Triethylsilane in trifluoroacetic acid, a reagent generally useful for reducing indoles to indolines, was likewise ineffective in this case. Rather than attempt to activate the 5,6 double bond toward reduction by chemical modification of the 5-deazaguanine ring system,[9] we chose to take a different approach which would circumvent the problem.

Our strategy, depicted retrosynthetically in Scheme 1, was based on the extension of our successful synthesis of the 5-deaza-5,6,7,8-tetrahydropterin system required for DDATHF from a 3-carboethoxy-2-piperidone precursor.[10] Thus we recognized the pteroic acid analog **3** as a precursor to the target structure **2** which could be obtained by the reaction of guanidine with a suitably activated derivative of a 3-carboalkoxy-2-pyrrolidinone as

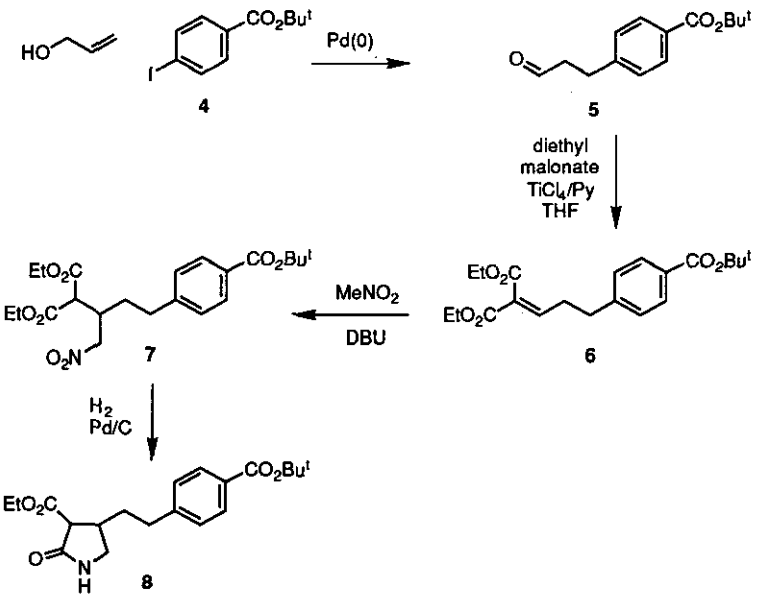
Scheme 1



shown. Anticipating that the aryl carboxyl group would have to be distinguished from the pyrrolidinone carboxyl during the guanidine cyclization, we planned to protect it as the *tert*-butyl ester in early intermediates and selectively unmask it just prior to the guanidine step. This approach would permit construction of the requisite 5,6-dihydro-5-deazaguanine ring system without resort to pyrrolopyrimidine intermediates, thus avoiding the difficult reduction problem.

The synthesis of the requisite carboethoxypyrrolidinone (**8**) is shown in Scheme 2. Thus palladium(0) mediated coupling of *tert*-butyl 4-iodobenzoate (**4**) with allyl alcohol provided aldehyde (**5**), substantially pure by nmr analysis, in 98% crude yield.[11] Knoevenagel condensation of **5** with diethyl malonate in the presence of

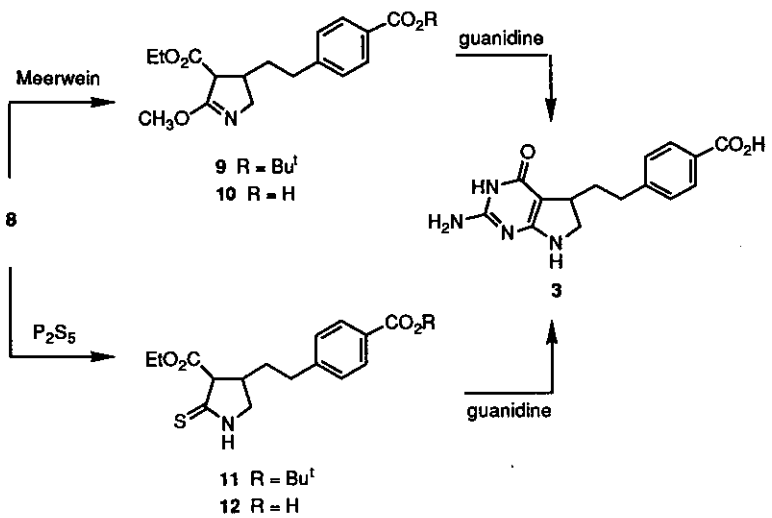
Scheme 2



titanium(IV) chloride[12] afforded alkylidinemalonate (**6**) which was smoothly converted without purification to **7** by treatment with nitromethane in the presence of DBU[13] in 72% overall yield for the three steps from **4**. Catalytic hydrogenation of **7** effected reduction of the nitro group and spontaneous cyclization to a single lactam ((±)-**8**) tentatively assigned *trans* stereochemistry based on known examples of the formation of γ-lactams from cyclization of aminoesters.[14] Crystalline **8** was easily isolated from the crude product by crystallization from ether - hexane, albeit in moderate yield (42%) .

Two approaches were taken to the preparation of the key intermediate (**3**) as shown in Scheme 3. In the first of

Scheme 3

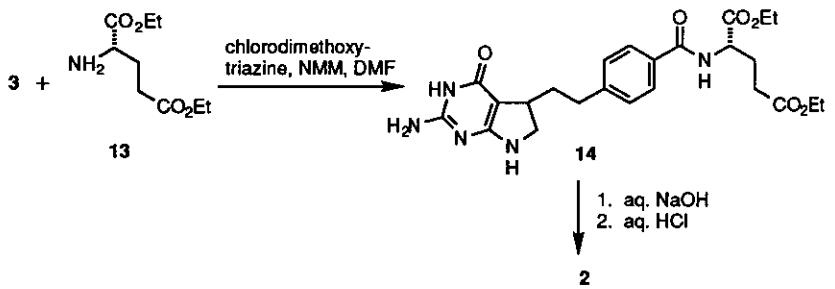


these, **8** was subjected to precedented[15] Meerwein alkylation with trimethyloxonium tetrafluoroborate in chloroform, affording the methyl imido ether (**9**) in 54% yield. Brief treatment of **9** with trifluoroacetic acid gave rise to the acid (**10**).[16] Heating **10** with guanidine[15] provided (±)-**3** in 58% overall yield (2 steps). No evidence was found for formation of products arising from reaction of guanidine with the aryl carboxyl groups in **10** or **3**.

Alternatively, we found that **8** could be smoothly and conveniently converted to the corresponding thiolactam (**11**) with phosphorous pentasulfide[17] in 77% yield (Scheme 3). Proceeding as previously, **11** was converted to the corresponding acid (**12**) by brief exposure to trifluoroacetic acid. It was fortunately not necessary to further activate the thiocarbonyl group in **12** prior to reaction with guanidine. Heating **12** in the presence of excess guanidine under conditions similar to the cyclization of the imido ether (**10**) provided (±)-**3** in 64% yield. To our knowledge this represents the first example of the formation of a fused pyrimidine ring by cyclization of an α-carboalkoxy thiolactam with guanidine. Sulfurization provides a more practical alternative to imido ether formation for activation of the lactam carbonyl group since the Meerwein reagent is both highly moisture-sensitive and expensive.

Coupling of **3** with diethyl L-glutamate (**13**) after activation with chlorodimethoxytriazine in the presence of *N*-methylmorpholine[18] afforded the known diester (**14**)[6b] in 52% yield (Scheme 4). Saponification of **14** with aqueous sodium hydroxide, followed by acidification, provided the target structure (**2**) as a 1:1 mixture of C-5 epimers[19] in 85% yield. Proton nmr data for **14** and **2** were in substantial agreement with data reported for the

Scheme 4



same epimeric mixture by the Takeda group.[6b]

Compound (**2**) has been reported to exhibit cytotoxic activity (IC_{50} 0.32 μg/ml) against KB cells.[6a] We have found that LY288601 (**2**) is also cytotoxic to CEM cells (IC_{50} 0.09 μg/ml) in tissue culture but was inactive in the L5178Y/TK-/HX- mouse tumor model at doses up to 200 mg/Kg.[20] Details of the biological testing of LY288601 will be reported elsewhere.

EXPERIMENTAL

General. ^1H and ^{13}C nmr spectra were determined on a General Electric QE-300 instrument at 300 and 75.5 MHz, respectively. Chemical shifts are reported in ppm (δ) down field from tetramethylsilane. Other spectral measurements were made using standard, commercially available instruments. Column chromatography was carried out with Silica Gel-60 (230-400 mesh, EM Sciences). Melting points are uncorrected.

3-(4-[2,2-Dimethylethyl]carboxyphenyl)-1-propanal (5).[11] To a mixture of 44.57 g (0.147 mol) of *tert*-butyl 4-iodobenzoate (**4**) and 12.8 g (0.221 mol) of allyl alcohol in 500 ml of dimethylformamide was added 1 g (0.004 mol) of palladium(II) acetate, 30.8 g (0.368 mol) of Na_2CO_3, and 47.25 g (0.147 mol) of tetrabutylammonium bromide. The resulting mixture was stirred at room temperature under nitrogen for 72 h at which time the reaction was deemed complete (tlc, silica gel, hexane-ether 3:2). The mixture was poured into 2 l of water and the resulting suspension extracted with hexane (4 X 450 ml). The combined extracts were washed with water, saturated NaCl solution, and dried (Na_2SO_4). Evaporation of the solvent afforded 33.7 g (98%) of **5**[21] as an oil sufficiently pure for further processing. A portion of the crude material was purified for analysis by silica gel chromatography (hexane - ethyl acetate 7:3). ^1H Nmr ($CDCl_3$) δ 9.82 (s, 1H), 7.92 (d, J = 9 Hz, 2H), 7.04

(d, J = 9 Hz, 2H), 2.99 (t, J = 7.8 Hz, 2H), 2.80 (t, J = 7.8 Hz, 2H), 1.50 (s, 9H); ^{13}C nmr ($CDCl_3$) δ 200.8, 165.6, 145.3, 130.3, 129.8, 128.2, 80.9, 44.9, 28.2, 28.1; ir ($CHCl_3$) 1707 cm^{-1}, ms (FD) m/z 234. Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.72; H, 7.82.

2-Ethylcarboxy-5-(4-(2,2-dimethylethyl)carboxyphenyl)-2-pentenoic acid ethyl ester (6). To 475 ml of THF cooled to -1.5 °C was added a mixture of 25.7 ml (45.14 g, 0.238 mol) of $TiCl_4$ in 57 ml of CCl_4 dropwise, with mechanical stirring, so that the temperature was kept <3.5 °C. To the resulting yellow suspension was added a mixture of 27.8 g (0.119 mol) of **5** and 19 g (0.119 mol) of diethyl malonate in 57 ml of dry THF while maintaining the temperature <3 °C by adjustment of the addition rate. To the resulting mixture was added 38.3 ml (37.7 g, 0.476 ml) of dry pyridine. The mixture was stirred for 1 h at 0-3 °C; then allowed to come to room temperature overnight. The reaction mixture was mixed with 750 ml of water and extracted with 300 ml of ether. The aqueous phase was extracted with ether (2 X 250 ml) and the extracts combined, washed with 250 ml portions of water, 0.5 N HCl, water, saturated $NaHCO_3$, saturated NaCl and dried (Na_2SO_4). Evaporation of the solvent gave 43.3 g of **6** as an oil, 85.7% pure (hplc). An analytical sample was prepared by flash chromatography (hexane - ethyl acetate 8:2). ^{1}H Nmr ($CDCl_3$) δ 7.90 (d, J = 8.1 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 6.97 (t, J = 8.4 Hz, 1H), 4.24 (m, 4H), 2.83 (t, J = 7.1 Hz, 2H), 2.64 (t, J = 7.1 Hz, 2H), 1.57 (s, 9H), 1.26 (m, 6H); ^{13}C nmr ($CDCl_3$) δ 165.7, 165.3, 163.9, 147.5, 145.2, 138.4, 129.8, 129.6, 128.2, 80.9, 64.9, 61.3, 61.2, 34.5, 31.0, 28.3, 14.1; ir ($CHCl_3$) 3018, 2892, 1710, 1611, 1296, 1167, 1115 cm^{-1}; ms (FD) m/z 377 (M^+). Anal. Calcd for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50. Found: C, 67.06; H, 7.58.

2-Ethylcarboxy-3-nitromethyl-5-(4-(2,2-dimethylethyl)carboxyphenyl)pentanoic acid ethyl ester (7). A solution of 42.2 g (0.113 mol) of **6**, obtained as indicated above, in 150 ml of nitromethane containing 1.5 g of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was stirred at room temperature overnight. The reaction mixture was concentrated to a small volume, then diluted with 300 ml of ether and with sufficient ethyl acetate to produce a clear solution. The resulting solution was washed with water, 0.5 M HCl, water, saturated aqueous $NaHCO_3$, water, saturated NaCl and dried (Na_2SO_4). Evaporation of the solvent afforded 47.4 g of **7** as an oil. Flash chromatography (silica, hexane-ethyl acetate 1:4) of the crude provided 37 g (72% based on **4**) of purified **7**. An analytical sample was prepared by additional flash chromatography. ^{1}H Nmr ($CDCl_3$) δ 7.90 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 8.1 Hz, 2H), 4.74 (dd, J = 13.6, 4.9 Hz, 1H), 4.55 (dd, J = 13.6, 4.9 Hz, 1H), 4.20 (m, 4H), 3.64 (d, J = 2.8 Hz, 1H), 2.92 (m, 1H), 2.74 (dd, J = 8.3, 7.8 Hz, 2H), 1.80 (m, 2H), 1.57 (s, 9H), 1.26 (m, 6H);

^{13}C nmr ($CDCl_3$) δ 167.8, 167.7, 165.6, 145.2, 130.5, 129.9, 128.2, 80.9, 62.0, 61.9, 52.7, 36.6, 33.0, 31.6, 28.2, 14.0; ir ($CHCl_3$) 2983, 1746, 1729, 1708, 1554, 1370, 1297, 1166, 1117 cm^{-1}, ms (FD) m/z 437 (M^+). Anal. Calcd for $C_{22}H_{31}NO_8$: C, 60.40; H, 7.14; N, 3.20 . Found: C, 60.45; H, 7.26; N, 2.99.

4-[2-(2-Oxo-3-carboethoxypyrrolidin-4-yl)ethyl]benzoic acid, 2,2-dimethylethyl ester (8). A mixture of 10.32 g of **7** and 1.5 g of platinum(IV) oxide in 200 ml of ethanol was hydrogenated in a Parr apparatus at an initial pressure of 50 psig H_2. Filtration of the catalyst and removal of the solvent in vacuo provided 9.3 g of crude product as an oil which was crystallized from a mixture of 50 ml of ether and 200 ml of hexane. After stirring overnight, the crystalline product was filtered, washed with hexane, and dried, affording 3.54 g (42%) of **8**, mp 98-104 °C. ^1H Nmr (CDCl3) δ 7.89 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 6.75 (s, 1H), 4.23 (q, J = 6.3 Hz, 2H), 3.54 (dd, J = 9.0, 8.3 Hz, 1H), 3.12 (d, J = 8.6 Hz, 1H), 3.00 (dd, J = 9.4, 7.4 Hz, 1H), 2.87 (m, 1H), 2.66 (t, J = 7.6 Hz, 2H), 1.86 (m, 2H), 1.57 (s, 9H), 1.28 (t, J = 6.3 Hz, 3H); ^{13}C nmr ($CDCl_3$) δ 173.2, 169.7, 165.6, 145.8, 130.1, 129.7, 128.0, 80.8, 62.6, 54.4, 46.5, 38.9, 35.1, 32.4, 28.1, 14.1; ir ($CHCl_3$) 3438, 3019, 2983, 2936, 1707, 1312, 1297, 1166, 1121 cm^{-1}; ms (FAB) m/z 362 (MH^+), 306 (100), 288 (22). Anal. Calcd for $C_{20}H_{27}NO_5$: C, 66.46; H, 7.53; N, 3.87. Found: C, 66.44; H, 7.77; N, 3.86.

4-(2-[2-Iminomethoxy-3-Carboethoxy-3H-4,5-dihydropyrrol-4-yl]ethyl)benzoic acid, 2,2-dimethylethyl ester (9). To a mixture of 3.61 g (10 mmol) of **8** and 5.03 g (60 mmol) of $NaHCO_3$ in 115 ml of chloroform (under nitrogen) was added 4.43 g (30 mmol) of trimethyloxonium tetrafluoroborate, the transfer being completed with 50 ml of chloroform. After stirring at room temperature for 4 h, 40 ml of water was added and the phases were separated. The aqueous phase was extracted with 40 ml of chloroform and the organic phases combined and dried ($MgSO_4$). Evaporation of the solvent in vacuo afforded 4.0 g of crude **9**. Purification by flash chromatography (silica gel, ether-hexane 4:1, ether, and ethyl acetate successively) provided purified 2.04 g (54%) of **9** as a clear, viscous oil, homogeneous by tlc (silica gel, 100% ether). ^1H Nmr ($CDCl_3$) δ 7.83 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 4.14 (q, J = 7.1 Hz, 2H), 3.82 (m, 1H), 3.75 (s, 3H), 3.26 (m, 2H), 2.71 (m, 1H), 2.60 (m, 2H), 1.77 (m, 2H), 1.52 (s, 9H), 1.22 (t, J = 7.1 Hz, 3H).

4-(2-[2-Iminomethoxy-3-Carboethoxy-3H-4,5-dihydropyrrol-4-yl]ethyl)benzoic acid (10). A mixture of 580 mg (1.54 mmol) of **9** and 10 ml of trifluoroacetic acid was stirred for 2 min to effect complete solution then immediately concentrated to dryness *in vacuo*. The residue was taken up in 2 ml of methanol and the resulting solution was slowly added to 15 ml of saturated aqueous sodium bicarbonate. The pH of the resulting mixture

was adjusted to 5.5 by addition of acetic acid and the product was extracted with ethyl acetate (3 X 10 ml). The organic extracts were combined, dried ($MgSO_4$), and concentrated to give 498 mg (100%) of **10** as an amorphous solid. ^{1}H Nmr ($CDCl_3$) δ 8.00 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 4.27 (q, J = 7.6 Hz, 2H) 3.93 (m, 1H), 3.84 (s, 3H), 3.35 (m, 2H), 2.90 (m, 1H), 2.79 (m, 2H), 1.86 (m, 2H), 1.28 (t, J = 7.6 Hz, 3H). This material was taken to the next step without further purification.

4-[2-(2-Amino-4,5,6,7-tetrahydro-4-oxo-3H-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoic acid (3) from 10. To a solution of sodium ethoxide in ethanol, prepared by cautious addition of 120 mg (5.0 mmol) of sodium hydride to 10 ml of anhydrous ethanol, was added 477 mg (5.0 mmol) of guanidine hydrochloride. The resulting mixture was heated to 50 °C for 20 min. The precipitated salt was filtered and to the resulting solution was added 404 mg (1.27 mmol) of **10**. The mixture was stirred until clear and then concentrated in vacuo to remove most of the alcohol. The mixture was heated to 90 °C at 10 torr for 90 min, then cooled to room temperature and diluted with 10 ml of water. The product was precipitated by addition of 6M HCl to pH 5.5 and filtered. The precipitate was washed with water, ethanol, and ether and vacuum dried at 50 °C, affording 218 mg (58%) of **3** as an amorphous solid, mp >290 °C. ^{1}H Nmr ($CDCl_3$) δ 13 (br s, 1H), 9.6 (br s, 1H), 7.79 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 8.2 Hz, 2H), 6.29 (s, 1H), 6.23 (s, 2H), 3.44 (t, J = 8.6 Hz, 1H), 3.03 (m, 2H), 2.62 (t, J = 7.7 Hz, 2H), 2.00 (m, 1H), 1.54 (m, 1H); ir (KBr) 3427, 3328, 3140, 2910, 1643, 1574, 1264 cm^{-1}; hrms (FAB) m/z calcd for $C_{15}H_{17}N_4O_3$ (MH^+): 301.1301. Found: 301.1298.

4-[2-(2-Thiocarbonyl-3-carboethoxypyrrolidin-4-yl)ethyl]benzoic acid 2,2-dimethylethyl ester (11). A mixture of 5.10 g (14.1 mmol) of **8** and 3.45 g (7.76 mmol) of phosphorus pentasulfide in 150 ml of THF was stirred at 60 °C for 30 min, cooled, filtered, and the solvent was removed by vacuum evaporation. The crude product thus obtained was purified by flash chromatography (silica gel, ethyl acetate-hexane 1:1), affording 4.11 g (77%) of **11** as an oil. ^{1}H Nmr ($CDCl_3$) δ 7.97 (br s, 1H), 7.90 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 4.26 (m, 2H), 3.82 (m, 1H), 3.53 (d, J = 7.2 Hz, 1H), 3.28 (dd, J = 10.8, 6.5 Hz, 1H), 2.90 (m, 1H), 2.68 (m, 2H), 1.85 (m, 2H), 1.57 (s, 9H), 1.31 (t, J = 7.2 Hz, 3H); ir ($CHCl_3$) 3420, 2983, 1732, 1707, 1517, 1471, 1393, 1371, 1313, 1297, 1166, 1121 cm^{-1}; hrms (FAB) m/z calcd for $C_{20}H_{28}NO_4S$ (MH^+): 378.1739. Found, 378.1729.

4-[2-(2-Thiocarbonyl-3-carboethoxypyrrolidin-4-yl)ethyl]benzoic acid (12). A mixture of 4.17 g (11.1 mmol) of **11** and 20 ml of trifluoroacetic acid was stirred at room temperature until a clear solution was

obtained. After an additional 5 min the mixture was concentrated in a rotary evaporator and the residue was dissolved in 10 ml of methanol. The resulting solution was carefully added to a suspension of 2 g of $NaHCO_3$ in saturated $NaHCO_3$. The pH was adjusted to 5.7 with acetic acid and the resulting mixture was extracted with ethyl acetate (5 X 20 ml). The combined extracts were dried ($MgSO_4$) and concentrated to give 3.87 g (109% crude yield) of **12** as a foam which was sufficiently pure for the preparation of **3**. Crystalline **12** was obtained from chloroform, mp 145-148 °C. ^1H Nmr (DMSO-d_6) δ 10.39 (s, 1H), 7.80 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 8.1 Hz, 2H), 4.06 (q, J = 7.1 Hz, 2H), 3.64 (dd, J = 10.9, 8.9 Hz, 1H), 3.46 (d, J = 8.5 Hz, 1H), 3.20 (dd, J = 10.9, 8.0 Hz, 1H), 2.64 (q, J = 8.0 Hz, 1H), 2.54 (t, J = 7.4 Hz, 2H), 1.70 (m, 2H), 1.12 (t, J = 7.1 Hz, 3H); ^{13}C nmr (DMSO-d_6) δ 198.6, 169.5, 167.0, 146.2, 129.1, 128.6, 128.1, 65.0, 60.4, 52.9, 40.8, 33.5, 32.6, 13.7; ir ($CHCl_3$) 3410, 2985, 1732, 1694, 1517 cm^{-1}; uv (EtOH) λ 236 nm (ε 14 900), 271 nm (ε 15 600); ms (FD) m/z 321 (M^+). Anal. Calcd for $C_{16}H_{19}NO_4S$: C, 59.79; H, 5.96; N, 4.36. Found: C, 59.77; H, 6.00; N, 4.37.

Preparation of 3 from 12. To a solution of sodium ethoxide in ethanol, prepared by reaction of 1.06 g (44.1 mmol) of sodium hydride and 50 ml of ethanol, was added 4.22 g (44.1 mmol) of guanidine HCl and the mixture was stirred at 50 °C for 20 min. The cooled suspension was filtered to remove NaCl and the filtrate was mixed with 2.83 g (8.81 mmol) of **12**. The resulting solution was concentrated *in vacuo* to remove most of the alcohol and the residue slowly heated to 90 °C while maintaining a vacuum of 10 torr. After heating 1 h the mixture was cooled and the reaction product was taken up in 75 ml of water. The pH was adjusted to 6 by addition of 6 M HCl and filtered. The precipitate was washed with 20 ml of water, 20 ml of methanol, and 40 ml of ether. After drying there was obtained 2.13 g (64%) of **3**, identical in all respects with an authentic sample prepared from **10** as described above.

N-[4-[2-(2-Amino-4,5,6,7-tetrahydro-4-oxo-3H-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid diethyl ester (14). To a solution of 1.91 g (6.36 mmol) of **3** and 0.63 g (6.3 mmol) of *N*-methylmorpholine (NMM) in 20 ml of DMF was added 1.12 g (6.36 mmol) of chlorodimethoxytriazine and the resulting mixture was stirred at room temperature. After 2 h an additional 0.3 g (2.97 mmol) of NMM and 0.22 g (1.25 mmol) of chlorodimethoxytriazine were added and the mixture was stirred for an additional 30 min. To the mixture was added 0.64 g (6.3 mmol) of NMM and 1.52 g (6.3 mmol) of diethyl-L-glutamate hydrochloride. After stirring overnight the resulting suspension was mixed with silica gel and dried under vacuum. The residue was added to a column of silica gel and eluted with CH_2Cl_2-EtOH 90:10 followed by CH_2Cl_2-EtOH 80:20.

There was obtained 1.62 g (52%) of purified **14**[6b] as a colorless powder, mp 111-114 °C. ^{1}H Nmr (DMSO-d_6) δ 9.55 (s, 1H), 8.60 (d, J = 7.4 Hz, 1H), 7.73 (d, J = 8.3 Hz, 2H) 7.27 (d, J = 8.3 Hz, 2H), 6.28 (s, 1H), 6.20 (s, 2H), 4.38 (m, 1H), 4.05 (q, J = 7.1 Hz, 2H), 3.99 (q, J = 7.1 Hz, 2H), 3.40 (m, 1H), 3.02 (m, 2H), 2.65 (t, J = 7.6 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.00 (m, 3H), 1.54 (m, 1H), 1.13 (t, J = 7.1 Hz, 3H), 1.11 (t, J = 7.1 Hz, 3H).

N-[4-[2-(2-Amino-4,5,6,7-tetrahydro-4-oxo-3H-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid (2). A solution of 1.50 g (3.09 mmol) of **14** in 25 ml of 1N NaOH was stirred at room temperature for 3 h. The solution was carefully acidified to pH 2.8 by addition of 6 M HCl and the resulting suspension filtered. The precipitate was washed with 20 ml of water, 10 ml of methanol, 10 ml of ether, and dried overnight at 50 °C, 10 torr. There was obtained 1.13 g (85%) of **2**[6b] as an amorphous solid. ^{1}H Nmr (DMSO-d_6) δ 12.20 (br s, 2H), 9.83 (br s, 1H), 8.48 (d, J = 7.7 Hz, 1H), 7.74 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 6.32 (br s, 2H), 6.28 (br s, 1H), 4.33 (m, 1H), 3.43 (m, 1H), 3.01 (m, 2H), 2.61 (t, J = 8.0 Hz, 2H), 2.30 (t, J = 7.4 Hz, 2H), 1.96 (m, 3H), 1.54 (m, 1H); ^{13}C nmr (DMSO-d_6) δ 173.7, 173.1, 169.4, 166.5, 159.0, 156.7, 146.2, 131.4, 127.9, 127.2, 89.1, 52.1, 49.3, 36.9, 35.5, 32.3, 30.5, 26.2; ir (KBr) 3366, 2931, 1639, 1503, 1451, 1091 cm^{-1}; ms (FAB) m/z 430 (MH^+); uv (EtOH) 220 nm (ε 27 660), 280 nm (ε 11 300).

REFERENCES AND NOTES

1. For a recent overview of the status of antifolate research related to antitumor therapy see: E. M. Berman and L. M. Werbel, *J. Med. Chem.*, **1991**, *34*, 479, and references cited therein.
2. E. C. Taylor, *J. Heterocycl. Chem.*, **1990**, *27*, 1, and references cited therein.
3. E. C. Taylor, D. Kuhnt, C. Shih, S. M. Rinzel, G. B. Grindey, J. Barredo, M. Jannatipour, and R. G. Moran, *J. Med. Chem.*, **1992**, *35*, 4450.
4. T. Miwa, T. Hitaka, H. Akimoto, and H. Nomura, *J. Med. Chem.*, **1991**, *34*, 555.
5. F. Itoh, O. Russelo, H. Akimoto, and G. P. Beardsley, *Proc. Am. Assn. for Cancer Research*, **1992**, *33*, Abstract 2434.
6. (a) H. Akimoto, T. Hitaka, T. Miwa, K. Yukishige, T. Kusanagi, and K. Ootsu, *Proc. Am. Assn. for Cancer Research*, **1991**, *32*, Abstract 1938. (b) H. Akimoto, H. Takenori, and T. Miwa, European

Patent Appl. 334 636 A2 (1989). Compound(**2**)is referred to as T-41440 in Takeda publications.

7. E. C. Taylor, personal communication.

8. C. J. Barnett and T. M. Wilson, unpublished observations.

9. A 2-amino-4(3*H*)-oxopyrrolo[2,3-*d*]pyrimidine bearing (removable) substituents at N-3 and N-7 was reported to undergo reduction of the 5,6-bond (in low yield) by hydrogenation over palladium on carbon. The preparation of the requisite substrate for this reduction required many steps (Reference 6b). An alternative synthesis of dihydro analogs of **1b** via borane reduction of a 6(5*H*)-oxopyrrolidinopyrimidine intermediates has been disclosed (reference 4).

10. C. J. Barnett and T. M. Wilson, *Tetrahedron Lett.*, **1989**, *30*, 6291.

11 Our procedure for **5** was based on the procedure of Taylor for the preparation of the corresponding methyl ester. E. C. Taylor, P. Gillespie, and M. Patel, *J. Org. Chem.*, **1992**, *57*, 3218. We thank Professor Taylor for providing the details of his method to us prior to publication.

12. W. Lehnert, *Tetrahedron Lett.*, **1970**, 4723. More conventional Knoevenagel conditions (piperidine acetate catalysis) were not suitable in this case due to extensive aldehyde self-condensation.

13. 1,8-diazabicyclo[5.4.0]undec-7-ene (Aldrich Chemical Company, Milwaukee, WI). For examples of the use of DBU as a catalyst in the Michael addition of nitoalkanes see: N. Ono, A. Kamimura, and A Kaji, *Synthesis*, **1984**, 226.

14. See, for example: A. Ontishenko, B. Buchholz, and H. Stamm, *Chem. Ber.*, **1986**, *119*, 2678. We have not pursued a more rigorous determination of the relative stereochemistry of **8** in view of the rehybridization of C-3 in the subsequent reaction with guanidine.

15. V. G. Granik, and R. G. Glushkov, *Khim.-Farm. Zh.*, **1967**, *1* 16 (*Chem. Abstr.*, **1968**, *68*, 12941z). See also reference 10.

16. The *tert*-butyl ester was cleaved to the acid prior to the guanidine cyclization step in order to protect the aryl carboxyl group more efficiently from attack by guanidine.

17. J. S. Petersen, G. Fels, and H. Rapoport, *J. Am. Chem. Soc.*, **1984**, *106*, 4539.

18. Z. J. Kaminski, *Tetrahedron Lett.*, **1985**, *26*, 2901.

19. We chose not to address the issue of C-5 stereochemistry of **2** prior to antitumor testing. Useful levels of

antitumor activity could easily be detected in the mixture even if only one of the isomers were active. Our synthesis provides opportunities for resolution or asymmetric induction which would not be available in approaches based on partial reduction of pyrrolo[2,3-*d*]pyrimidine precursors.

20. The *in vitro* and *in vivo* antitumor results were obtained by Dr. Gerald Grindey and his associates, Lilly Research Laboratories.

21. H. Akimoto, T. Hitaka, and T. Miwa, European Patent Appl. 402 903 A1 (1990). Compound (**5**) was described as a starting material, but no characterization data for it was presented therein, or, to our knowledge, elsewhere.

Received, 30th November, 1992