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Synthesis of Pyrazolo[3,4-d]pyrimidine Analogues of the Potent Antitumor Agent N-{4-[2-(2-Amino-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (LY231514)

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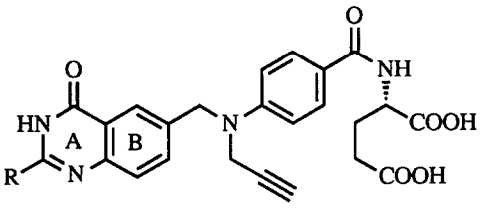
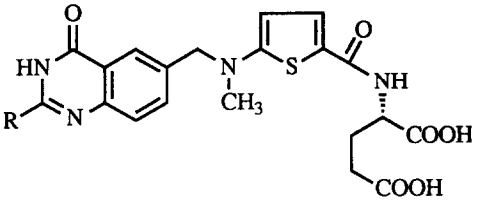
Abstract: *Several pyrazolo[3,4-d]pyrimidine analogues of the potent antitumor agent N-{4-[2-(2-amino-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (LY231514, 5) have been prepared. A principal synthetic step proved to be a palladium-catalyzed C-C coupling of the 5-halo-substituted pyrazolo[3,4-d]pyrimidines 12-15 with dimethyl 4-ethynylbenzoyl-L-glutamate (16). An additional pyrazolo[3,4-d]pyrimidine analogue of 5 possessing an isofolic acid bridge unit (-NHCH2-) was prepared by reductive alkylation of diethyl 4-formylbenzoyl-L-glutamate (31) with 2-methyl-5-amino-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (30). Only compound 26 proved to have in vitro cell growth inhibitory activity.*

Inhibition of thymidylate synthase (TS), which mediates the methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to give 2'-deoxythymidine-5'-monophosphate (dTMP) and is thus essential for *de novo* DNA biosynthesis, has long been recognized as a prime objective for the development of an effective antitumor chemotherapeutic agent.[1] Several TS inhibitors have emerged as clinical candidates. The earliest was the quinazoline antifolate CB3717 (**1**),[2] but this compound was later withdrawn from clinical trial because of the emergence of unexpected liver toxicity.[3] Second-generation quinazoline antifolates with greater water solubility (and thus lower toxicity) than CB3717 (e.g. the 2-desamino-,[4,5] 2-desamino-2-methyl-,[6-8] and 2-desamino-2-methyl thiophene[9-12] analogues **2**, **3** and **4**) have also reached clinical trial.

A significant departure from the quinazoline family of TS inhibitors involved replacement of the ring-B fused benzene ring by a fused pyrrole ring, coupled with removal of the nitrogen atom from the bridge. The lead compound in this new series of TS inhibitors is N-{4-[2-(2-amino-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (LY231514, **5**), which will shortly enter Phase I clinical trial.[13] The present paper describes the synthesis of several additional B-ring modifications of LY231514 in which the pyrrole ring is replaced by a pyrazole ring. Compounds **27** and **28** are analogues [with respect to the pyrimidine ring] of the second-generation quinazoline TS inhibitors **2-4**. Compound **33**, with an isofolic acid (-NHCH2-) bridge and a methyl group at C-2, is the

Dedicated with affection to Professor Charles W. Rees on the occasion of his retirement





1 R = NH_2
2 R = H
3 R = CH_3

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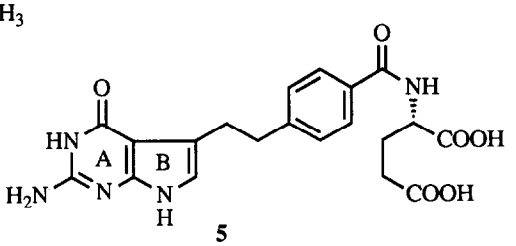

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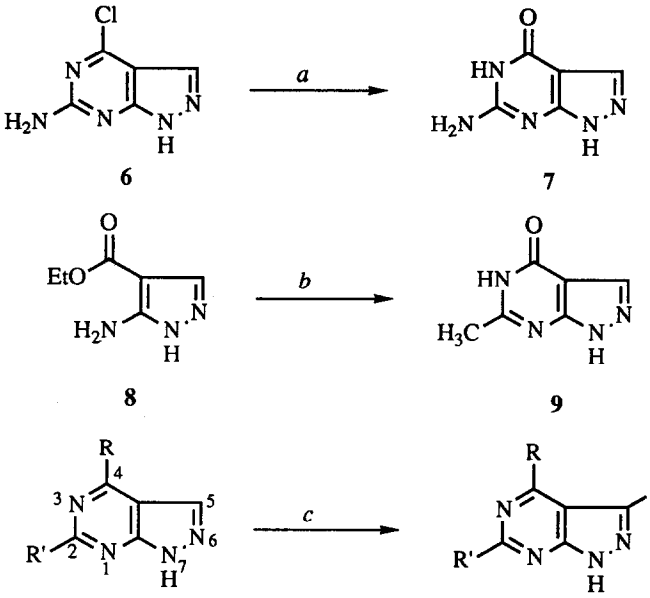
pyrazolo[3,4-d]pyrimidine analogue of the quinazoline TS inhibitor **29** which was recently reported by Marsham and coworkers[14] to be a slightly less potent TS inhibitor than its -CH_2NH- bridge isomer, although it exhibited similar cytotoxic activity. The 2,4-diamino derivative **26** may be considered as an analogue of the family of fused 2,4-diaminopyrimidine dihydrofolate reductase inhibitors which includes methotrexate and aminopterin.[15]

Over the past few years our group has extensively and effectively exploited palladium-catalyzed C-C coupling reactions of halo-substituted heterocycles with both acetylenic and olefinic moieties to prepare pterin, deazapterin, and pyrrolopyrimidine analogues of folic acid.[16] We have used an analogous approach for the preparation of our target pyrazolo[3,4-d]pyrimidine analogues.

2,4-Diaminopyrazolo[3,4-d]pyrimidine (**10**) was prepared according to the literature procedure,[17,18] while the 2-amino-4(3H)-oxo- and 2-methyl-4(3H)-oxo- analogues **7** and **9** were prepared as depicted in Scheme 1. Attempts to prepare **7** by alkaline hydrolysis of **10**, or by guanidine cyclization of 5-amino-4-carbethoxypyrazole (**8**), were unsuccessful, although **7** could be prepared in 89% yield by alkaline hydrolysis of **6**.[19] The 5-bromo derivatives **12**, **13**, and **15** were readily prepared by dropwise addition of bromine to an aqueous suspension of the appropriate precursors **7**, **9** and **11**. Bromination of **10** gave a mixture of mono- and di-bromo derivatives, but the 5-iodo derivative **14** was readily obtained in 81% yield by iodination of **11** with N-iodosuccinimide in DMF.

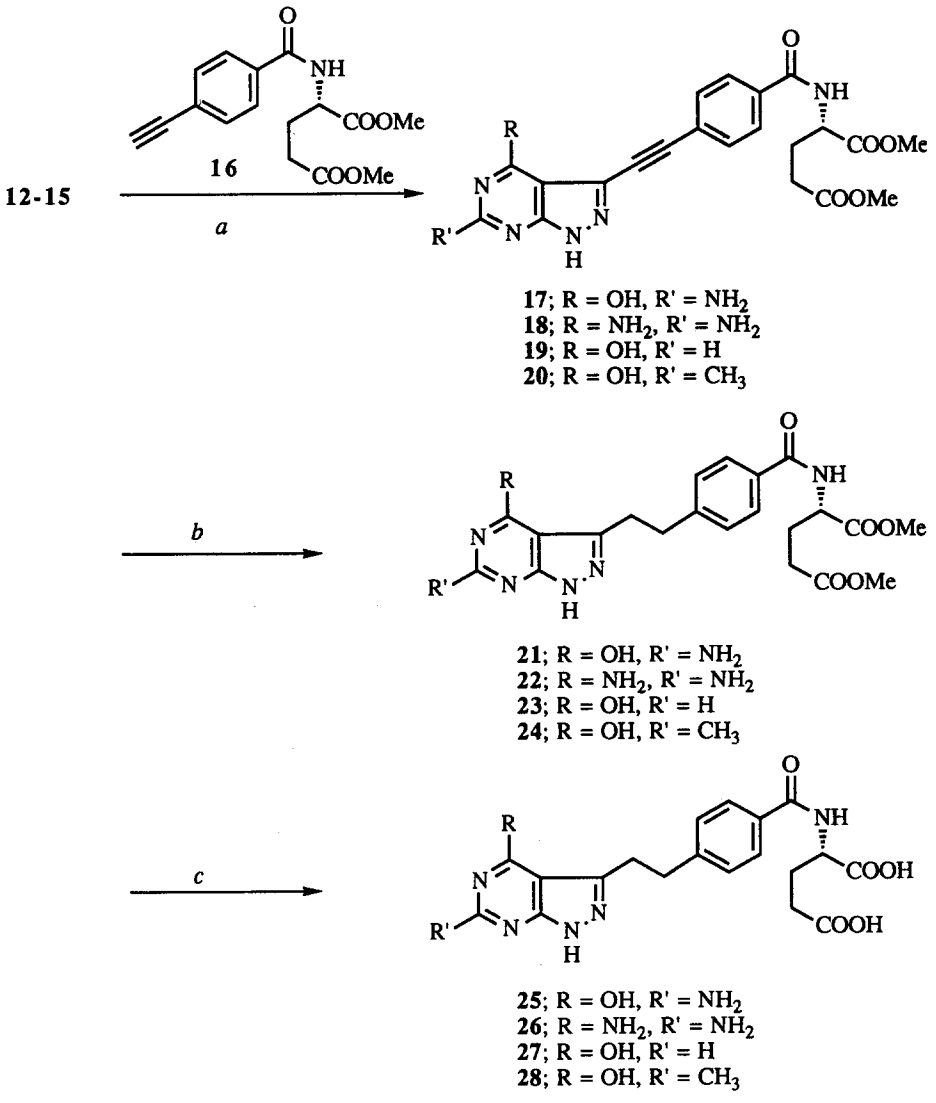
Although palladium-catalyzed coupling of **12-15** with dimethyl 4-ethynylbenzoyl-L-glutamate (**16**)[20] was successful in every case, the reaction conditions proved to be critical. Each of these couplings required tetrakis(triphenylphosphine)palladium(0) as the catalyst and DMF at 100-105 °C as solvent. Attempts to use $Pd(OAc)_2$ or $PdCl_2$ with triphenylphosphine led to very poor coupling yields, and gave the dimer of acetylene **16** as the major product. Hydrogenation of the acetylenic triple bond of the coupled products **17-20** using 10% Pd/C in trifluoroacetic acid as solvent led in fair to good yields to the reduced products **21-24**. Unfortunately, these hydrogenations required close to stoichiometric amounts of the palladium catalyst and prolonged reaction times. Final saponification

Scheme 1



(a) 2 *N* NaOH; (b) CH_3CN, HCl; (c) Br_2, H_2O or NIS, DMF

Scheme 2



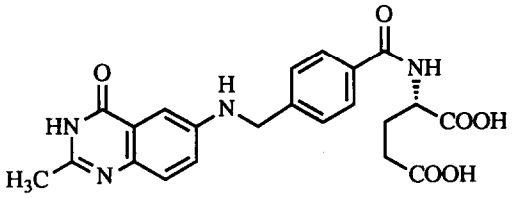
17; R = OH, R' = NH_2
18; R = NH_2, R' = NH_2
19; R = OH, R' = H
20; R = OH, R' = CH_3

21; R = OH, R' = NH_2
22; R = NH_2, R' = NH_2
23; R = OH, R' = H
24; R = OH, R' = CH_3

25; R = OH, R' = NH_2
26; R = NH_2, R' = NH_2
27; R = OH, R' = H
28; R = OH, R' = CH_3

(a) $Pd(PPh_3)_4$, CuI, NEt_3, DMF; (b) Pd/C, H_2; (c) 1*N* NaOH.

with aqueous sodium hydroxide, followed by acidification with acetic acid, then led to the desired target analogues **25-28**.



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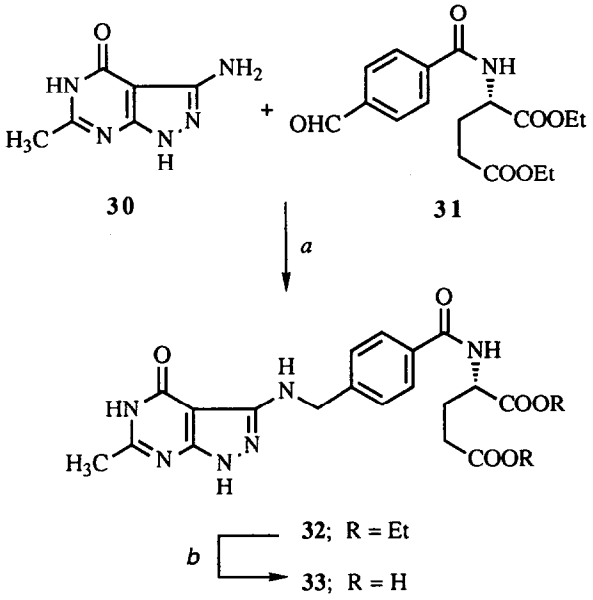
The isofolic acid analogue **33** was prepared by the general method developed by Hynes and Garett[21] for the synthesis of quinazoline isofolic acid analogues. Reductive amination (hydrogen and Raney nickel) of 2-methyl-5-amino-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**30**)[22] with diethyl 4-formylbenzoyl-L-glutamate (**31**)[21] led to intermediate **32**, which was readily saponified with dilute base to give **33** in 76% yield.

Scheme 3



(a) Raney-Ni, 70% AcOH, H_2; (b) 1 *N* NaOH.

In vitro cell growth inhibition studies with the above pyrazolo[3,4-d]pyrimidine folate analogues revealed that only **26** exhibited significant cytotoxic activity (IC_{50} = 0.018 μg/mL). Full details of the biological evaluation of these and further ring-B modified analogues of LY231514 will be reported independently.

Experimental Section

2-Amino-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (7). A solution of 2-amino-4-chloro-7H-pyrazolo[3,4-d]pyrimidine[19] (**6**, 3.18 g, 19 mmol) in 2 *N* NaOH (50 mL) was refluxed for 20 h. The solution was cooled to rt and adjusted to pH 5 with conc. HCl. The precipitated solid was collected by filtration, washed with water and dried, and the solid again boiled in 30% acetic acid, filtered, washed with water, and dried to give **7** as a light yellow solid (2.53 g, 89%). A small sample was recrystallized from aqueous DMF: mp > 320 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.43-6.56 (br s, 2 H), 7.75 (s, 1 H), 10.44 (s, 1 H), 12.76 (s, 1 H); EIMS, *m/z* (relative intensity) 151 (100), 111 (52), 97 (79); HRMS calcd for $C_5H_5N_5O$ *m/z* 151.0494, found 151.0501. Anal. calcd for $C_5H_5N_5O \cdot 0.3\ H_2O$: C, 38.37; H, 3.61; N, 44.74. Found: C, 38.46; H, 3.55; N, 44.96.

This compound has been prepared previously by a different method, but no physical or spectral properties were reported.[23]

2-Methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (9) was prepared by the general procedure described by Dave et al[24] for the cyclization of o-amino esters to fused 2-methyl-4(3H)-pyrimidinones. Thus, a stream of dry hydrogen chloride was passed through a suspension of 3-amino-4-carbethoxypyrazole (**8**, 1.55 g, 10 mmol) in CH_3CN (60 mL) for 6 h. The solvent was removed under vacuum, the solid residue was dissolved in water (10 mL), and the solution was adjusted to pH 9 with conc. NH_4OH. The mixture was cooled in an ice-bath, filtered, and the collected solid was washed with water and dried to give **9** as a white solid (0.94 g, 63%): mp > 280 °C (dec) (lit.[25] 336-338 °C dec., copper block); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.32 (s, 3 H), 7.97 (s, 1 H), 11.83-12.02 (br s, 1 H), 13.46-13.62 (br s, 1 H); EIMS, *m/z* (relative intensity) 150 (100), 135 (51), 110 (43); HRMS calcd for $C_6H_6N_4O$ *m/z* 150.0541, found 150.0548. Anal. calcd for $C_6H_6N_4O \cdot 0.3\ H_2O$: C, 46.33; H, 4.28; N, 36.02. Found: C, 46.58; H, 3.89; N, 36.04.

2-Amino-5-bromo-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (12). To a suspension of 2-amino-4-(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**7**, 1.51 g, 10 mmol) in water (50 mL), bromine (3.2 g, 10 mmol) was added dropwise and the mixture was stirred at rt for 1 h. The mixture was then heated on a boiling waterbath for an additional 1 h, cooled and the solid was collected by filtration, washed with water and dried to give **12** as a light tan-colored solid (1.82 g, 79%). A small sample was recrystallized from aqueous DMF: mp > 320 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.61 (br s, 2 H), 10.59 (s, 1 H), 13.00 (s, 1 H); EIMS, *m/z* (relative intensity) 231 (98), 229 (100); HRMS calcd for $C_5H_4BrN_5O$ *m/z* 230.9579, found 230.9569. Anal. calcd for $C_5H_4BrN_5O$: C, 26.11; H, 1.75; Br, 34.74; N, 30.45. Found: C, 26.34; H, 1.88; Br; 35.00; N, 30.22.

2,4-Diamino-5-bromo-7H-pyrazolo[3,4-d]pyrimidine (13). To a suspension of 2,4-diamino-7H-pyrazolo[3,4-d]pyrimidine (**10**, 1.5 g, 10 mmol) in water (50 mL), bromine (1.6 g, 10 mmol) was added, and the mixture was stirred at rt for 1 h. It was then heated on a boiling waterbath for an

additional 1 h, filtered hot, and the filtrate was evaporated to dryness. The light orange solid was taken up into boiling water (50 mL) and the pH of the solution was adjusted to 7 by 2 *N* sodium hydroxide. The solid which separated was collected by filtration, washed with cold water, and dried to give **13** as a light orange solid (1.42 g, 62%): mp 302-304 °C; ^{1}H NMR (DMSO-*d*$_6$, 300 MHz) δ 6.23 (s, 2 H), 6.29-7.10 (br s, 2 H), 12.76 (s, 1 H); EIMS, *m/z* (relative intensity) 230 (22), 228 (23); HRMS calcd for $C_5H_5BrN_6$ *m/z* 227.9758, found 227.9778. Anal. calcd for $C_5H_5BrN_6 \cdot 0.5\ H_2O$: C, 25.23; H, 2.54; N, 35.30; Br, 33.57. Found: C, 24.83; H, 2.46; N, 35.14; Br, 33.69.

5-Iodo-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (14). To a suspension of 4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**11**, 4.3 g, 32 mmol) in DMF (200 mL) was added *N*-iodosuccinimide (9.0 g, 40 mmol), and the mixture was stirred under nitrogen at 110 °C for 18 h. The solvent was evaporated at 50 °C on a rotary evaporator to a small volume (~20 mL)which was then poured into 5% acetic acid (100 mL). The precipitate was collected by filtration and dried to give **14** as a pale yellow solid (6.70 g, 81%): mp > 280 °C (dec.); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz) δ 7.98 (s, 1 H), 12.12 (s, 1 H), 14.01 (s, 1 H); EIMS, *m/z* (relative intensity) 262 (73), 179 (78), 165 (100); HRMS calcd for $C_5H_3IN_4O$ 261.9311, found 261.9333.

2-Methyl-5-bromo-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (15). 2-Methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**9**, 750 mg, 5 mmol) was brominated as described above to give **15** as a light yellow solid (923 mg, 81%): mp > 320 °C (dec); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz) δ 2.31 (s, 3 H), 12.11 (s, 1 H), 13.81 (s, 1 H); EIMS, *m/z* (relative intensity) 230 (79), 228 (80); HRMS calcd for $C_6H_5BrN_4O$ *m/z* 229.9647, found 229.9647. Anal. calcd for $C_6H_5BrN_4O$: C, 31.47; H, 2.20; Br, 34.89; N, 24.46. Found: C, 31.48; H, 1.95; Br, 35.02; N, 24.31.

Dimethyl *N*-{4-[2-(2-Amino-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (17). A mixture of 2-amino-5-bromo-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**12**, 230 mg, 1 mmol), dimethyl N-(4-ethynylbenzoyl)-L-glutamate (**16**, 606 mg, 2 mmol), cuprous iodide (50 mg, 0.26 mmol), tetrakis(triphenylphosphine)palladium(0) (100 mg, 0.087 mmol) and triethylamine (1.5 mL) in DMF (10 mL) was stirred under nitrogen at 105 °C for 18 h. The solvent was removed at 60 °C under reduced pressure, and the dark brown semi-solid residue was then chromatographed on silica gel eluting with 18% CH_3OH/CH_2Cl_2. Evaporation of the fractions containing the product gave **17** as a white solid (200 mg, 44%): mp 258-261 °C ^{1}H NMR (DMSO-*d*$_6$, 300 MHz) δ 1.97-2.11 (m, 2 H), 2.46 (t, 2 H, *J* = 7.0 Hz), 3.55 (s, 3 H), 3.62 (s, 3 H), 4.40-4.48 (m, 1 H), 6.56-6.60 (br s, 2 H, exchangeable with D_2O), 7.62 (d, 2 H, *J* = 8.3 Hz), 7.90 (d, 2 H, *J* = 8.3 Hz), 8.86 (d, *J* = 7.4 Hz, 1 H, exchangeable with D_2O), 10.63 (s, 1 H, exchangeable with D_2O) 13.15 (s, 1 H, exchangeable with D_2O). FAB HRMS Calcd for $C_{21}H_{21}N_6O_6$ (MH^+) *m/z* 453.1523, found 453.1515.

Dimethyl N-{4-[2-(2,4-Diaminopyrazolo[3,4-d]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (18). 2,4-Diamino-5-bromo-7H-pyrazolo[3,4-d]pyrimidine (**13**, 229 mg, 1 mmol), was coupled with dimethyl N-(4-ethynylbenzoyl)-L-glutamate (**16**, 606 mg, 2 mmol) as described above (but at 100 °C for 3 h; silica gel chromatography eluting with 10% CH_3OH/CH_2Cl_2) to give**18** as a light brown solid (200 mg, 47%): mp 185-187 °C; ^1H NMR (DMSO-d_6,300 MHz) δ 2.01-2.14 (m, 2 H), 2.44 (t, 2 H *J* = 7.4 Hz), 3.57 (s, 3 H), 3.64 (s, 3 H), 4.45-4.52 (m, 1 H), 6.17 (s, 2 H), 6.50-6.80 (br s, 2 H), 7.77 (d, 2 H, *J* = 8.3 Hz), 7.92 (d, 2 H, *J* = 8.3 Hz), 8.88 (d, 1 H, *J* = 7.5 Hz), 12.95 (s, 1 H); EIMS, *m/z* (relative intensity) 451 (23), 277 (84), 219 (36), 98 (86); HRMS calcd for $C_{21}H_{21}N_7O_5$ *m/z* 451.1604, found 451.1623. Anal. calcd for $C_{21}H_{21}N_7O_5$: C, 55.88; H, 4.69; N, 21.72. Found: C, 55.60; H, 4.52; N, 21.48.

Dimethyl N-{4-[2-(4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (19). 5-Iodo-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**14**, 2.62 g, 10 mmol) was coupled with dimethyl N-(4-ethynylbenzoyl)-L-glutamate (**16**, 3.03 g, 10 mmol) as described above (but at 85 °C for 3.5 h; silica gel chromatography eluting with 2% CH_3OH/CH_2Cl_2) to give **19** as a white solid (3.15 g, 73%): mp 105-107 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.95-2.19 (m, 2 H), 2.45 (t, 2 H *J* = 7.3 Hz), 3.56 (s, 3 H), 3.65 (s, 3 H), 4.46-4.51 (m, 1 H), 7.69 (d, 2 H, *J* = 8.1 Hz), 7.98 (d, 2 H, *J* = 8.1 Hz), 8.08 (s, 1 H), 8.95 (d, 1 H, *J* = 7.4 Hz), 12.28 (br s, 1 H), 14.15 (br s, 1 H); EIMS, *m/z* (relative intensity) 437 (1), 419 (3) 294 (17), 277 (100), 263 (41); HRMS calcd for $C_{21}H_{19}N_5O_6$ 437.1335, found 437.1352. Anal. calcd for $C_{21}H_{19}N_5O_6 \cdot 0.5\ H_2O$: C, 56.51; H, 4.52; N, 15.69. Found: C, 56.38; H, 4.43; N, 15.75.

Dimethyl N-{4-[2-(2-Methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (20). 2-Methyl-5-bromo-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**15**, 229 mg, 1 mmol) was coupled with dimethyl N-(4-ethynylbenzoyl)-L-glutamate (**16**, 606 mg, 2 mmol) as described above (but at 105 °C for 3 h; silica gel chromatography eluting with 4% CH_3OH/CH_2Cl_2) to give **20** as a white solid (280 mg, 62%). A small sample was recrystallized from aqueous CH_3OH: mp 265-267 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.95-2.11 (m, 2 H), 2.31 (s, 3 H), 2.44 (t, 2 H, *J* = 6.8 Hz), 3.55 (s, 3 H), 3.62 (s, 3 H), 4.41-4.48 (m, 1 H), 7.65 (d, 2 H, *J* = 8.3 Hz), 7.92 (d, 2 H, *J* = 8.3 Hz), 8.87 (d, 1 H, *J* = 7.4 Hz), 12.12 (s, 1 H), 13.90 (s, 1 H); EIMS, *m/z* (relative intensity) 451 (4), 433 (7), 307 (10) 291 (100), 277 (20), 264 (15); HRMS calcd for $C_{22}H_{21}N_5O_6$ *m/z* 451.1491, found 451.1482. Anal. calcd for $C_{22}H_{21}N_5O_6 \cdot 1\ H_2O$: C, 56.29; H, 4.94; N, 14.92. Found: C, 56.32; H, 4.69; N, 14.62.

Dimethyl *N*-{4-[2-(2-Amino-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (21). A mixture of **17** (250 mg, 0.55 mmol) and Pd-C (10%, 1.0 g) in trifluoroacetic acid (20 mL) was hydrogenated at 50 psi for 4 days. The catalyst was filtered off and the filtrate was evaporated to dryness under reduced pressure. The solid residue was chromatographed on silica gel (30 g, 2 x 18 cm, flash column), eluting with 10% CH_3OH/CH_2Cl_2. Fractions containing the product were combined and evaporated to give **21** as a white solid (140 mg, 56%): mp > 240 °C;

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.97-2.09 (m, 2 H), 2.39 (t, 2 H, J = 7.2 Hz), 2.93-3.14 (m, 4 H), 3.55 (s, 3 H), 3.60 (s, 3 H), 4.39-4.42 (m, 1 H), 6.54 (br s, 2 H, exchangeable with D_2O) 7.26 (d, 2 H, J = 7.9 Hz), 7.76 (d, 2 H, J = 7.9 Hz), 8.65 (d, 1 H, J = 7.4 Hz), 10.50 (s, 1 H, exchangeable with D_2O), 12.31 (s, 1 H, exchangeable with D_2O).

Dimethyl N-{4-[2-(2,4-Diamino-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (22). Compound **18** (200 mg, 0.44 mmol) was hydrogenated as described above (silica gel chromatography, eluting with 12% CH_3OH/CH_2Cl_2) to give **22** as a light yellow solid (114 mg, 56%): mp > 260 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.95-2.15 (m, 2 H), 2.44 (t, 2 H J =7.4 Hz), 3.01 (t, 2 H, J = 7.3 Hz) 3.35 (t, 2 H, J = 7.3 Hz), 3.57 (s, 3 H), 3.66 (s, 3 H), 4.45 (m, 1 H), 6.20-6.80 (br s, 4 H), 7.32 (d, 2 H, J = 7.9 Hz), 7.73 (d, 2 H, J = 7.9 Hz), 8.55 (d, 1 H, J = 7.4 Hz), 11.90-12.80 (s, 1 H); EIMS, *m/z* (relative intensity) 455 (18), 281 (26), 252 (19), 163 (32), 129 (28), 98 (80); HRMS calcd for $C_{21}H_{25}N_7O_5$ *m/z* 455.1917, found 455.1927.

Dimethyl N-{4-[2-(4(3H)-Oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (23). Compound **19** (1.50 g, 3.4 mmol) was hydrogenated as described above (silica gel chromatography, eluting with 4% CH_3OH/CH_2Cl_2) to give **23** as a white solid (1.18 g, 78%): mp 137-139 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.89-2.13 (m, 2 H), 2.44 (t, 2 H, J = 7.2 Hz), 3.12 (s, 4 H, Ar-CH_2-CH_2-Ar), 3.56 (s, 3 H), 3.62 (s, 3 H), 4.41-4.46 (m, 1 H), 7.29 (d, 2 H, J = 8.1 Hz), 7.76 (d, 2 H, J = 8.1 Hz), 7.95 (s, 1 H), 8.67 (d, 1 H, J = 7.4 Hz), 11.75-12.15 (br s, 1 H), 13.10-13.40 (br s, 1 H); EIMS, *m/z* (relative intensity) 441 (16), 298 (19), 267 (100); HRMS calcd for $C_{21}H_{23}N_5O_6$ *m/z* 441.1648, found 441.1645.

Dimethyl N-{4-[2-(2-Methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (24). Compound **20** (300 mg, 0.67 mmol) was hydrogenated as described above (silica gel chromatography, eluting with 8% CH_3OH/CH_2Cl_2) to give **24** as a white solid (125 mg, 41%): mp 205-207 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.92-2.11 (m, 2 H), 2.28 (s, 3 H), 2.41 (t, 2 H, J = 7.3 Hz), 3.07 (s, 4 H, Ar-CH_2-CH_2-Ar), 3.54 (s, 3 H), 3.60 (s, 3 H), 4.37-4.44 (m, 1 H), 7.26 (d, 2 H, J = 7.7 Hz), 7.73 (d, 2 H, J = 7.7 Hz), 8.63 (d, 1 H, J = 7.3 Hz), 11.87 (br s, 1 H), 13.07 (br s, 1 H); EIMS, m/e (relative intensity) 455 (18), 295 (23), 281 (100) 266 (36), 252 (96); HRMS calcd for $C_{22}H_{25}N_5O_6$ *m/z* 455.1804, found 455.1800.

***N*-{4-[2-(2-Amino-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (25).** A suspension of **21** (110 mg, 0.24 mmol) in 0.5 *N* NaOH (2 mL) was stirred at rt for 3 days. The solid material was filtered off and the filtrate was acidified with glacial acetic acid. The white precipitate was collected by filtration, washed with a little water, acetone, and dried to give **25** as a white solid (67 mg, 63%): mp > 240 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.87-2.06 (m, 2 H), 2.31 (t, 2 H, J = 7.3 Hz), 2.93-3.05 (m, 4 H), 4.30-4.38 (m, 1 H), 6.41 (br s, 2 H, exchangeable with D_2O), 7.25 (d, 2 H, J = 8.1 Hz), 7.74 (d, 2 H, J = 8.1 Hz), 8.47 (d, J = 7.5 Hz, 1 H, exchangeable

with D_2O), 10.37 (s, 1 H, exchangeable with D_2O), 12.32-12.40 (br s, 3 H, exchangeable with D_2O). FAB HRMS Calcd for $C_{19}H_{21}N_6O_6$ (MH^+) 429.1522, found 429.1501.

N-{4-[2-(2,4-Diamino-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (26). Compound **22** (114 mg, 0.25 mmol) was saponified in 1 *N* NaOH (1 mL) as described above to give **26** as a light yellow solid (67 mg, 63%): mp 227-229 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.89-1.96 (m, 2 H), 2.23-2.35 (m, 2 H) 2.98 and 3.12 (AA'BB', 4 H, J = 6.5 and 7.9 Hz),4.28 (m, 1 H), 5.87 (s, 2 H, exchangeable with D_2O), 6.60 (s, 2 H, exchangeable with D_2O), 7.32 (d, 2 H, J = 7.9 Hz), 7.73 (d, 2 H, J = 7.9 Hz), 8.17 (d, 1 H, J = 7.0 Hz, exchangeable with D_2O), 11.55-12.55 (br s, 2 H, exchangeable with D_2O); FAB HRMS calcd for $C_{19}H_{22}N_7O_5$ (MH^+) *m/z* 428.1682, found 428.1683.

N-{4-[2-(4(3H)-Oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (27). Compound **23** (200 mg, 0.45 mmol) was saponified in 1 *N* NaOH (1 mL) as described above to give **27** as a white solid (97 mg, 52%): mp 140-142 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.87-2.10 (m, 2 H), 2.33 (t, J = 7.4 Hz, 2 H), 3.11 (s, 4 H, Ar-CH_2-CH_2-Ar), 4.32-4.39 (m, 1 H), 7.27 (d, 2 H, J = 8.1 Hz), 7.77 (d, 2 H, J = 8.1 Hz), 7.95 (s, 1 H), 8.57 (d, 1 H, J = 7.7 Hz), 12.05 (br s, 2 H); FAB HRMS calcd for $C_{19}H_{20}N_5O_6$ (MH^+) *m/z* 414.1414, found 414.1444.

N-{4-[2-(2-Methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (28). Compound **24** (75 mg, 0.17 mmol) was saponified in 0.6 *N* NaOH (0.7 mL) as described above to give **28** (41 mg, 58%): mp > 260 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.86-1.98 (m, 2 H), 2.20-2.36 (m, 2 H), 2.27 (s, 3 H), 3.07 (s, 4 H, Ar-CH_2-CH_2-Ar), 4.25-4.33 (m, 1 H), 7.24 (d, 2 H, J = 8.1 Hz), 7.71 (d, 2 H, J = 8.1 Hz), 8.28 (d, 1 H, J = 7.2 Hz, exchangeable with D_2O), 11.84 (s, 1 H, exchangeable with D_2O), 12.70-13.55 (br s, 1 H, exchangeable with D_2O); FAB HRMS calcd for $C_{20}H_{22}N_5O_6$ (MH^+) *m/z* 428.1570, found 428.1597.

Diethyl N-{4-[*N*-1-(2-Methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)aminomethyl]benzoyl}-L-glutamate (32). A mixture of 5-amino-2-methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidine (**30**, 330 mg, 2 mmol) and diethyl N-(4-formylbenzoyl)-L-glutamate (**31**, 670 mg, 2 mmol) in 70% acetic acid (30 mL) was hydrogenated in the presence of Raney-nickel (450 mg) for 20 h. After the addition of charcoal, the catalyst was filtered off. The filtrate was evaporated to dryness and the crude product was chromatographed on silica gel, eluting with 8% CH_3OH/CH_2Cl_2. Fractions containing the product were combined and evaporated to yield **32** as a white solid (490 mg, 51%): mp 193-195 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.13 (t, 3 H, J = 7.2 Hz), 1.17 (t, 3 H, J = 7.2 Hz), 2.01-2.10 (m, 2 H), 2.26 (s, 3 H), 2.41 (t, 2 H, J = 7.5 Hz), 4.03 (q, 2 H, J = 7.2 Hz), 4.09 (q, 2 H, J = 7.2 Hz), 4.39-4.45 (m, 3 H), 6.11-6.20 (br s, 1 H), 7.42 (d, 2 H, J = 7.9 Hz), 7.77 (d, 2 H, J = 7.9 Hz), 8.63 (d, 1 H, J = 7.4 Hz), 11.64-11.71 (br s, 1 H), 12.05-12.12 (br s, 1 H); EIMS, *m/z* (relative intensity) 484 (2), 298 (12), 281 (56), 253 (100); HRMS calcd for $C_{23}H_{28}N_6O_6$ *m/z* 484.2070, found 484.2051.

N-{4-[*N*-1(2-Methyl-4(3H)-oxo-7H-pyrazolo[3,4-d]pyrimidin-5-yl)aminomethyl]benzoyl}-L-glutamic Acid (33). A solution of **32** (330 mg, 0.68 mmol) in 1 *N* NaOH (2 mL) was stirred at rt for 24 h. The solution was adjusted to pH 5 with glacial acetic acid and the solid product was collected by filtration, washed with water, acetone, and dried to give **33** as a white solid (223 mg, 76%): mp 220-222 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.88-2.08 (m, 2 H), 2.24 (s, 3 H), 2.32 (t, 2 H, J = 7.1 Hz), 4.32-4.40 (m, 1 H), 4.44 (d, 2 H, J = 5.4 Hz), 6.16 (t, 1 H, J = 5.4 Hz, exchangeable with D_2O), 7.41 (d, 2 H, J = 7.8 Hz), 7.78 (d, 2 H, J = 7.8 Hz), 8.47 (d, 1 H, J = 7.4 Hz, exchangeable with D_2O), 11.66 (s, 1 H, exchangeable with D_2O), 12.34-12.44 (br s, 1 H, exchangeable with D_2O); FAB HRMS calcd for $C_{19}H_{21}N_6O_6$ (MH^+) *m/z* 429.1522, found 429.1506.

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