Synthesis of Conformationally-Constrained Glutamate Analogues of the Antitumor Agents DDATHF, LY254155, and LY231514

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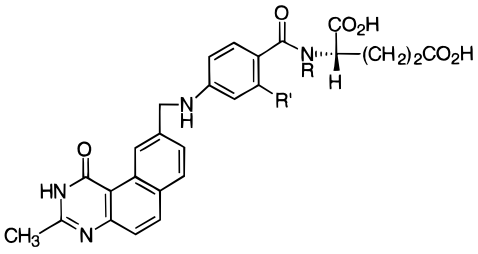
Received February 24, 1997[⊗]

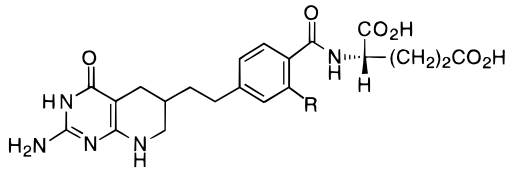
Analogues of the active antitumor agents DDATHF (**4**), LY254155 (**11**), and LY231514 (**14**) have been prepared in which the rotational flexibility of the benzoylglutamate amide linkage is constrained by incorporation of a methylene bridge between the glutamate amide nitrogen and the ortho position of the aromatic ring. Evaluation of the resulting isoindolinones as *in vitro* inhibitors of the growth of CCRF-CEM cells revealed that, although some analogues retained activity, in no case was cytotoxicity enhanced, and in some cases it was substantially reduced.

The concept of utilizing conformationally-constrained amino acids to explore active site binding parameters and other phenomena related to the geometry of substrate−enzyme interactions is now widely recognized and practiced.[1] In context with our long-standing interest in the synthesis of inhibitors of folate-dependent enzymes for use as antitumor agents, we were intrigued by a recent discovery by Burroughs-Wellcome scientists[2] that introduction of a 2′-fluoro substituent into the benzoquinazoline thymidylate synthase (TS) inhibitor **1** led to a significant increase in activity. Nuclear magnetic resonance studies showed that the glutamate moiety in the 2′-fluoro compound (**2**) was restrained into an in-plane conformation as a consequence of NH−F hydrogen bonding; this conformational restraint was simulated by the isoindolinone structure **3** (BW1843U89), which proved to be a superior inhibitor of cell growth as a consequence of increased substrate activity for folylpolyglutamate synthetase (FPGS) and for the reduced folate transport system.



1, R = R' = H
2, R = H, R' = F
3, R-R' = CH_2

Some time ago, during an extensive SAR study of derivatives of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, **4**) as antitumor agents, we had prepared its 2′-fluoro derivative **5**. The 2-fold increase in the IC_{50} of this compound as compared with DDATHF against



4, R = H (DDATHF)
5, R = F

CCRF-CEM cells[3] was viewed as due in part to improved activity of this 2′-fluoro derivative as a substrate for FPGS.[4] The present paper describes our synthetic efforts to prepare the isoindolinone derivatives **6**−**8** as conformationally-constrained glutamate analogues of DDATHF,[5] as well as the thienopyrrolidinone derivatives **9** and **10** as analogous conformationally-constrained glutamate analogues of the extremely active GARFT inhibitor and cytotoxic agent LY254155 (**11**).[6] Utilizing the isoindolinone moieties **16**, **26**, and **28**, we have also prepared compounds **12** and **13**, which represent conformationally-constrained glutamate derivatives of the multitargeted antifolate (MTA) and antitumor agent LY231514 (**14**).[7,8]

Compound **6** was prepared by two independent pathways. The first of these is outlined in Scheme 1. Using the method described by Marsham[9] for the conversion of methyl 4-nitro-2-methylbenzoate to diethyl 2-(2,3-dihydro-5-nitro-1-oxo-2(1*H*)-isoindolyl)-L-glutarate, the known methyl 4-iodo-2-methylbenzoate[10] (**15**) was converted to the isoindolinone **16** by free-radical bromination using NBS and dibenzoyl peroxide, followed by addition of

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1997.

(1) For leading references to the use of conformationally-constrained α-amino acids as bioactive agents and for conformational probes of active sites, see: (a) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699. (b) Liskamp, R. M. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *1*, 113. (c) Gainnis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244. (d) Mendel, D.; Ellman, J.; Schultz, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 4359. (e) Burgess, K.; Ho, K.-K.; Moye-Sherman, D. *Synlett* **1944**, 575. (f) Salaün, J.; Baird, M. S. *Curr. Med. Chem.* **1995**, *2*, 511.

(2) Duch, D. S.; Banks, S.; Dev, I. K.; Dickerson, S. H.; Ferone, R.; Heath, L. S.; Humphreys, J.; Knick, V.; Pendergast, W.; Singer, S.; Smith, G. K.; Waters, K.; Wilson, H. R. *Cancer Res.* **1993**, *53*, 810.

(3) Shih, C.; Grindey, G. B.; Gossett, L. S.; Moran, R. G.; Taylor, E. C.; Harrington, P. M. In *Chemistry and Biology of Pteridines 1989: Pteridines and Folic Acid Derivatives*; Curtius, H.-Ch., Ghisla, S., Blau, N., Eds.; Walter de Gruyter: New York, 1990; p 1035.

(4) Habeck, L. L.; Mendelsohn, L. G.; Shih, C.; Taylor, E. C.; Colman, P. D.; Gossett, L. S.; Leitner, T. A.; Schultz, R. M.; Andis, S. L.; Moran, R. C. *Mol. Pharmacol.* **1995**, *48*, 326.

(5) (a) For the original synthesis, see: Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1985**, *28*, 914. (b) Beardsley, G. P.; Moroson, B. A.; Taylor, E. C.; Moran, R. G. *J. Biol. Chem.* **1989**, *264*, 328. (c) Moran, R. G.; Baldwin, S. W.; Taylor, E. C.; Shih, C. *J. Biol. Chem.* **1989**, *264*, 21047. (d) Baldwin, S. W.; Tse, A.; Gossett, L. S.; Taylor, E. C., Rosowsky, A.; Shih, C.; Moran, R. G. *Biochemistry* **1991**, *30*, 1997. (e) For reviews of recent work, see: Taylor, E. C. *J. Heterocycl. Chem.* **1990**, *27*, 1. (f) Taylor, E. C. In *Chemistry and Biology of Pteridines and Folates*; Ayling, J. E., Nair, M. G., Baugh, C. M., Eds.; Plenum Press: New York, 1993; pp 387−408.

(6) (a) Shih, C.; Taylor, E. C. US Patent 4,882,334. (b) Habeck, L. L.; Leitner, T. A.; Shackelford, K. A.; Gossett, L. S.; Schultz, R. M.; Andis, S. L.; Shih, C.; Grindey, G. B.; Mendelsohn, L. G. *Cancer Res.* **1994**, *54*, 1021.

(7) (a) Taylor, E. C. US Patent 5,344,932, Sep 6, 1994. (b) Taylor, E. C.; Kuhnt, D.; Shih, C.; Rinzel, S. M.; Grindey, G. B.; Barredo, J.; Jannatipour, M.; Moran, R. G. *J. Med. Chem.* **1992**, *35*, 4450.

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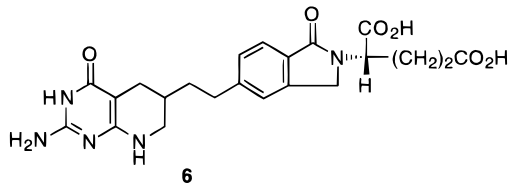
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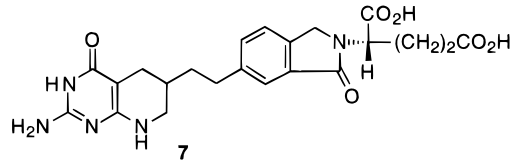
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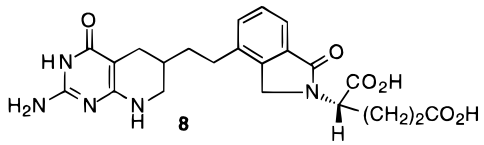
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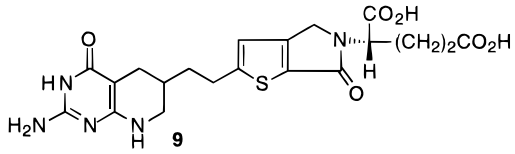
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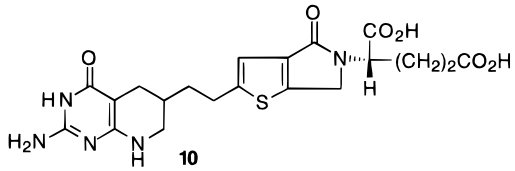
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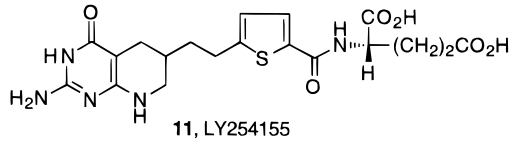
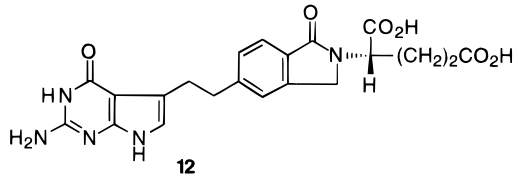

11, LY254155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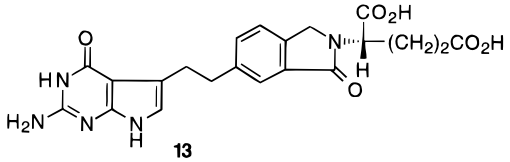
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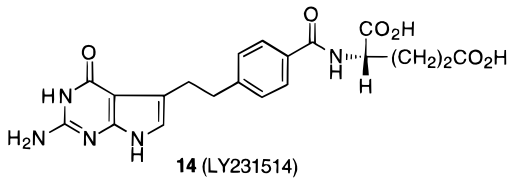
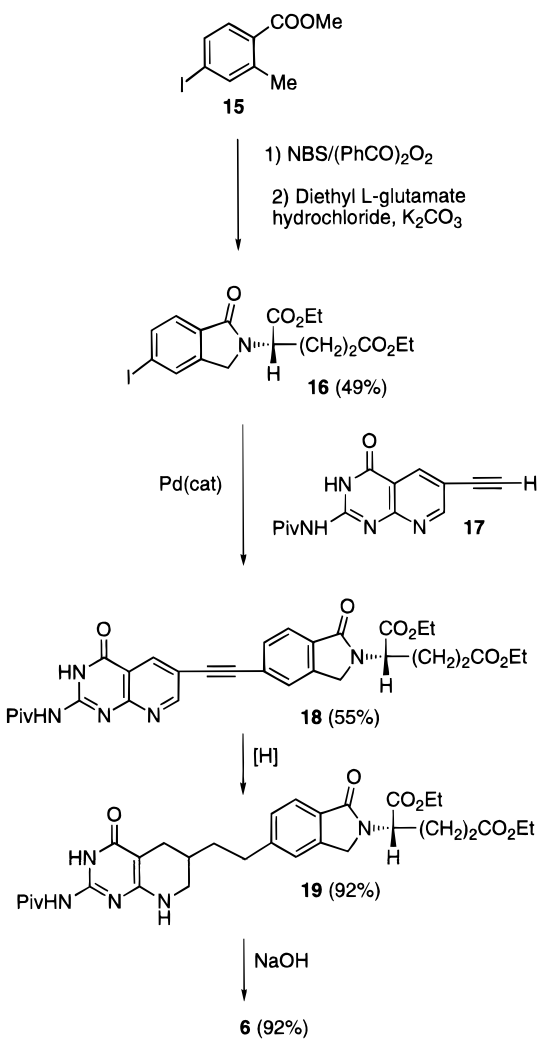

14 (LY231514)

diethyl L-glutamate in the presence of potassium carbonate. This compound underwent a palladium-catalyzed C−C coupling reaction with 2-pivaloyl-6-ethynyl-5-deazapterin (**17**)[11] to yield the disubstituted acetylene **18**. Catalytic hydrogenation of both the acetylenic bridge and the pyridine ring, using palladium-on-carbon catalyst in methanol as solvent, yielded **19**, which was saponified with dilute sodium hydroxide to give **6**.

The second route to **6** commenced with a Diels−Alder reaction of the commercially available Danishefsky diene

Scheme 1



20[12] with 4,4-diethoxybut-2-ynal (**21**)[13] to give the cyclohexadiene intermediate **22**,which underwent aromatization to 4-hydroxyphthalaldehyde **23** by treatment with 1 N HCl (Scheme 2). Stirring the phthalaldehyde **23** with diethyl L-glutamate hydrochloride in DMF at rt led to a mixture of the 5-hydroxyisoindolinone **24** (30% overall yield based on the Danishefsky diene **20**) and the isomeric 6-hydroxyisoindolinone derivative **25** (13% overall yield).[14] Treatment of **24** with triflic anhydride and collidine in

(8) For recent work characterizing **14** as a multitargeted antifolate (MTA), see: (a) Shih, C.; Gossett, L. S.; Gates, S. B.; MacKellar, W. C.; Mendelsohn, L. G.; Soose, D. J.; Patel, V. F.; Kohler, W.; Ratnam, M. *Proc. NCI−EORTC Symp. New Drugs in Cancer Ther., 9th* **1996**, *289*, 85. (b) Schultz, R. M.; Andis, S. L.; Chen, V. J.; Mendelsohn, L. G.; Patel, V. F.; Shih, C.; Houghton, J. *Ibid*. **1996**, *290*, 85. (c) Schultz, R. M.; Andis, S. L.; Bewley, J. R.; Chen, V. J.; Habeck, L. L.; Mendelsohn, L. G.; Patel, V. F.; Rutherford, P. G.; Self, T. D.; Shih, C.; Theobald, K. S.; Worzalla, J. F.; Houghton, J. *Proc. Am. Assoc. Cancer Res.* **1996**, *37*, 380. (d) Chen, V. J.; Bewley, J. R.; Gossett, L. S.; Shih, C.; Soose, D. J.; Patel, V. F.; Gates, S. B.; MacKellar, W. C.; Habeck, L. L.; Shackelford, K. A.; Mendelsohn, L. G.; Kohler, W.; Ratnam, M. *Ibid*. **1966**, *37*, 381.

(9) Marsham, P. R.; Jackman, A. L.; Hayter, A. J.; Daw, M. R.; Snowden, J. L.; O'Connor, B. M.; Bishop, J. A. M.; Calvert, A. H.; Hughes, L. R. *J. Med. Chem*. **1991**, *34*, 2209.

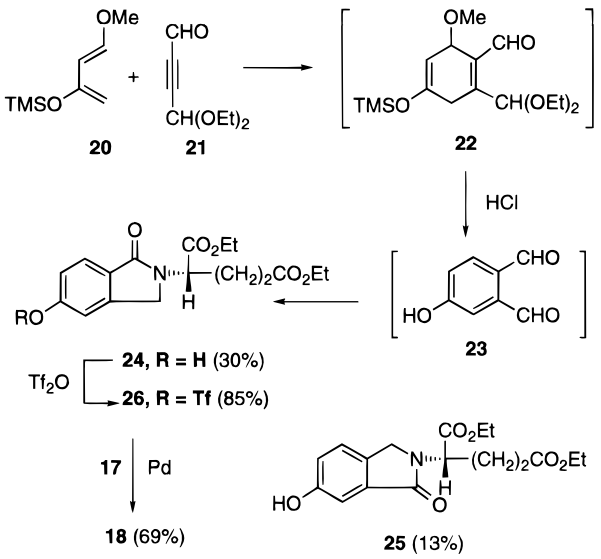
(10) Peltier, D. *Compt. Rend*. **1954**, *237*, 357.

(11) Taylor, E. C.; Wong, G. S. K. *J. Org. Chem*. **1989**, *54*, 3618.

(12) For a summary of Diels−Alder reactions utilizing the Danishefsky diene, see: Danishefsky, S. *Acc. Chem. Res*. **1981**, *14*, 400.

(13) For Diels−Alder reactions using aldehyde **21**, see: (a) Gustafsson, J.; Sterner, O. *J. Org. Chem*. **1994**, *59*, 3994. (b) Gorgues, A.; Simon, A.; Le Coq, A.; Hercouet, A.; Corre, F. *Tetrahedron* **1986**, *42*, 351. (c) A preliminary communication describing the synthesis of **6** starting with the above Diels−Alder reaction of **20** with **21** has been published: Taylor, E. C.; Zhou, P.; Jennings, L. D.; Mao, Z.; Hu, B.; Jun, J.-G. *Tetrahedron Lett*. **1997**, *38*, 521.

Scheme 2



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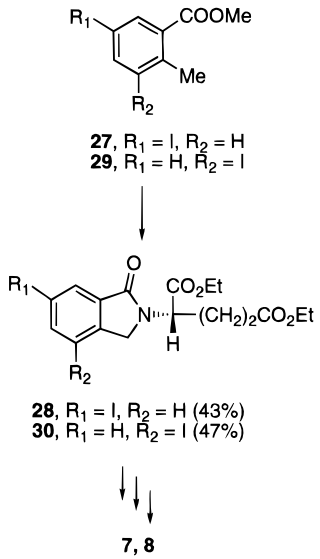

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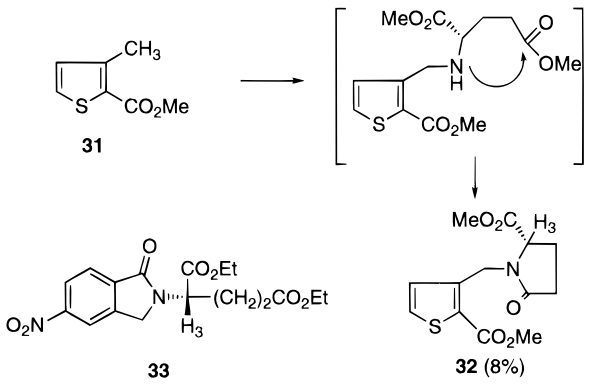
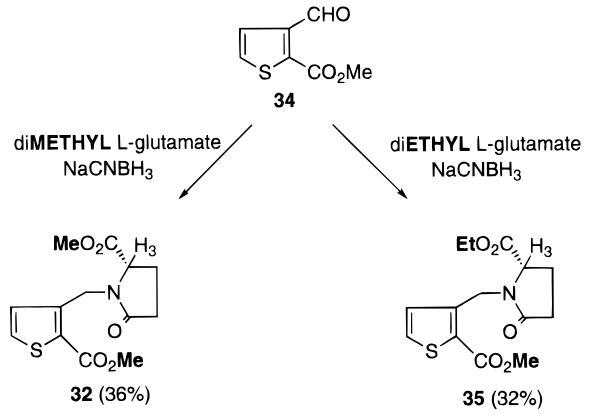


Scheme 5



methylene chloride solution gave the triflate **26**, which was then subjected to palladium-catalyzed coupling with 2-pivaloyl-6-ethynyl-5-deazapterin (**17**) to give the ethynyl-bridged intermediate **18**, identical in all respects with the sample of **18** prepared by the route outlined in Scheme 1.

The isomeric conformationally-constrained *m*-DDATHF derivative **7** was prepared in analogous fashion (Scheme 3) from the known methyl 5-iodo-2-methylbenzoate (**27**)[10] via the 6-iodoisoindolinone **28** as described above (see Scheme 1) for the synthesis of **6** from **15**. This series of conformationally-constrained analogues of DDATHF was completed with the synthesis of the isoindolinone **30** from methyl 3-iodo-2-methylbenzoate (**29**)[10] and its subsequent conversion to target compound **8** by a similar sequence of coupling, reduction, and hydrolysis steps.

Preparation of the thienopyrrolinone derivatives **9** and **10** posed a different challenge. An attempt to apply the above Marsham strategy to methyl 3-methylthiophene-2-carboxylate (**31**)—free-radical bromination with NBS and dibenzoylperoxide, followed by addition of dimethyl L-glutamate in the presence of potassium carbonate—instead gave the pyroglutamate **32** (see Scheme 4). It appears that the bond angle of 72° between C-2 and C-3 substituents in thiophene, which is substantially greater than the 60° ortho substituent bond angle in benzene,[15] results in diminished interaction between ortho substituents, thus favoring the observed intramolecular glutamate-to-pyroglutamate lactamization process over isoindolinone formation. The favored *s-trans* conformation of thiophene-2-carbonyl derivatives may also play a role in disfavoring isoindolinone formation.[16] The structure of the pyroglutamate **32** was confirmed not only by the pyroglutamate H_3 proton signal at 4.1 ppm, as contrasted with 4.95 ppm for the model isoindolinone **33** as reported by Marsham, but also by the sequence of reactions shown in Scheme 5. Thus, reductive amination of methyl 3-formylthiophene-2-carboxylate (**34**) with di*ethyl* glutamate yielded the mixed methyl ethyl ester **35**; by contrast, reductive amination of **34** with di*methyl* glutamate gave the same pyroglutamate dimethyl ester (**32**) as was obtained via the Marsham sequence discussed above.

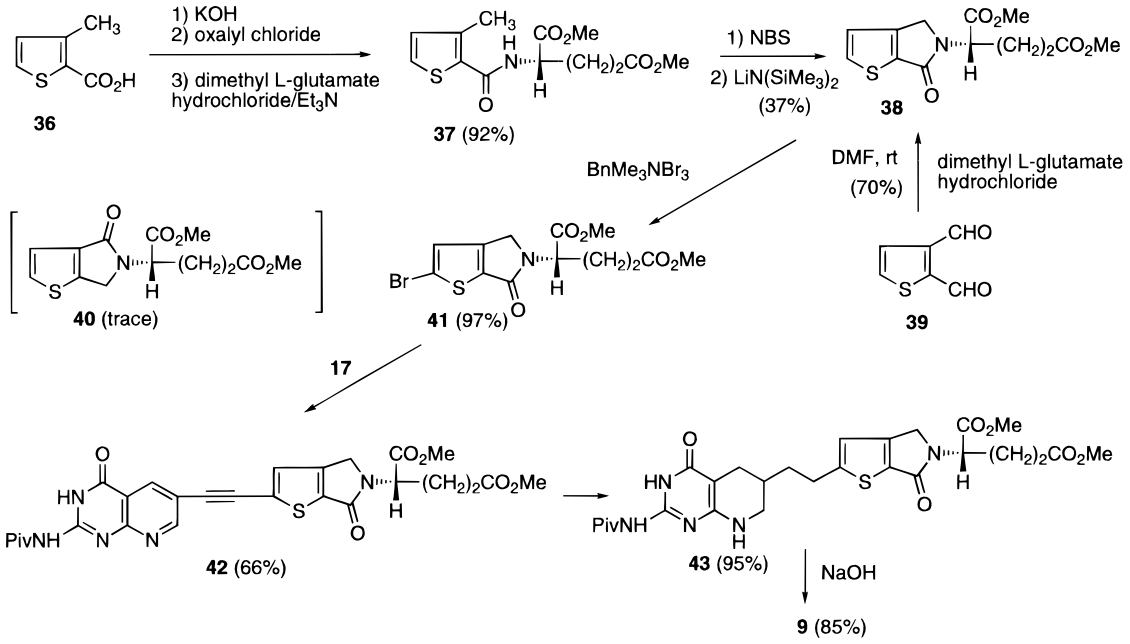
Since the sequential allylic bromination/alkylation/lactamization strategy had failed with the thiophene **31**, a reverse functionalization sequence was explored. 3-Methylthiophene-2-carboxylic acid (**36**) was converted to its potassium salt, which was stirred thoroughly for 1 h in benzene before addition of oxalyl chloride and one drop of DMF. To the resulting acid chloride was added dimethyl L-glutamate hydrochloride. The amide thus obtained (**37**) was then subjected to free-radical bromi-

(14) For the synthesis of N-substituted isoindolinones from *o*-phthalaldehyde and primary amines, see: (a) Takahashi, I.; Kawakami, T.; Hirano, E.; Yokota, H.; Kitajima, H. *Synlett* **1996**, 353 and references cited therein. (b) DoMinh, T.; Johnson, A. L.; Jones, J. E.; Senise, P. P., Jr. *J. Org. Chem*. **1977**, *42*, 4217.

(15) Bird, C. W.; Cheeseman, G. W. H. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds; Pergamon Press: Oxford, 1984; Vol. 4, p 1.

(16) Kaper, L.; De Boer, Th. J. *Recueil* **1970**, *89*, 825.

Scheme 6



nation with NBS and a catalytic amount of AIBN (Scheme 6). Without isolation, the resulting 3-(bromomethyl)thiophene was treated with lithium bis(trimethylsilyl)amide in the presence of tetrabutylammonium iodide. Workup then gave the desired 4*H*-thieno[2,3-*c*]-pyrrolinone derivative **38**, but only in 34% overall yield from **36**. Guilard's conditions (refluxing in xylene)[17] for the synthesis of thieno[2,3-*c*]pyrrolidinones from thiophene-2,3-dicarboxaldehyde (**39**) and primary amines were attempted using dimethyl L-glutamate as the primary amine, but again only decomposition was observed (*vide supra*). However, the milder conditions used earlier by us for the synthesis of **24** from **23** (see Scheme 2)—stirring in DMF solution at rt—were again successful and led to the desired thieno[2,3-*c*]pyrrolidinone **38** in 70% yield, together with a trace of its regioisomer **40**.

Treatment of **38** (from the two-step synthesis above) with benzyltrimethylammonium tribromide and zinc chloride[18] in acetic acid at room temperature gave exclusively the 5-bromo derivative **41** in 97% yield. Palladium-catalyzed coupling of **41** with 2-pivaloyl-6-ethynyl-5-deazapterin (**17**) to give **42** proved to be extremely sensitive to the palladium catalyst employed; the best results, which avoided what otherwise was predominant dimerization of **17**, employed palladium tetrakis(triphenyl)phosphine in acetonitrile in the presence of triethylamine and cuprous iodide. Application of the above sequence of bromination and subsequent coupling reactions to the thieno[2,3-*c*]pyrrolidinone **38** (along with a small amount of its isomer **40**) from the one-step Guilard reaction, followed by recrystallization of the crude coupling product from methanol, gave pure **42** in an overall yield of 64%. However, catalytic hydrogenation of **42** to **43** required 150−200 wt % of palladium-on-charcoal catalyst at 50 °C and 3 atm of hydrogen, presumably because of significant catalyst poisoning by the thiophene ring. Hydrolysis of **43** proceeded without incident to give the target constrained glutamate analog **9**.

An analogous strategy of intramolecular amide nitrogen alkylation was employed to prepare the isomeric constrained thiophene analog **10** of LY254155 (**11**). The requisite starting amide **45** was obtained by coupling of diethyl L-glutamate with 2-methylthiophene-3-carboxylic acid (**44**) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) with triethylamine as the base (Scheme 7). Allylic bromination with NBS followed by addition of lithium bis(trimethylsilyl)amide led to intramolecular N-alkylation with the formation of **46**. Regiospecific bromination at thiophene position 5 (to give **47**) was achieved with benzyltrimethylammonium tribromide in acetic acid in the presence of zinc chloride. Palladium-catalyzed coupling of **47** with 2-pivaloyl-6-ethynyl-5-deazapterin (**17**) was carried out in the usual manner; the resulting acetylene-bridged intermediate **48** was reduced catalytically to **49**, which was then saponified to the conformationally-constrained target compound **10**.

With the 5-iodoisoindolinone derivative **16** already in hand (see Scheme 1), a conformationally-constrained analog of the multitargeted antifolate and antitumor agent LY231514 (**14**) was also readily prepared. Thus, palladium-catalyzed coupling of **16** with (trimethylsilyl)-acetylene gave the (trimethylsilyl)ethynyl intermediate **50**, which was alternatively prepared by palladium-catalyzed coupling of (trimethylsilyl)acetylene with the triflate **26** derived from **24** (see Scheme 2). Desilylation of **50** with tetrabutylammonium fluoride in a mixture of ethanol and acetic acid led to the ethynylisoindolinone **51** (Scheme 8) in an overall yield of 80−82%. A second palladium-catalyzed coupling, this time between **51** and 2-pivaloyl-7-iodo-7-deazaguanine (**52**),[7] yielded **53**. Catalytic hydrogenation to **54** and subsequent saponification with 1 N NaOH then gave the constrained LY231514 analogue **12**.

Our final target compound was **13**, the isomer of **12** representing a conformationally-restricted analog of LY231514 (**14**) with the isoindolinone carbonyl group meta rather than para to the ethano bridge. Coupling

(17) Benachenhou, F.; Mesli, M. A.; El Borai, M.; Hanquet, B.; Guilard, R. *J. Heterocycl. Chem.* **1988**, *25*, 1531.

(18) Okamoto, T.; Kakinami, T.; Fujimoto, H.; Kajigaeshi, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 2566.

(19) The procedure for this assay has been described previously: Shih, C.; Gossett, L. S.; Worzalla, J. F.; Rinzel, S. M.; Grindey, G. B.; Harrington, P. M.; Taylor, E. C. *J. Med. Chem.* **1992**, *35*, 1109.

Scheme 7

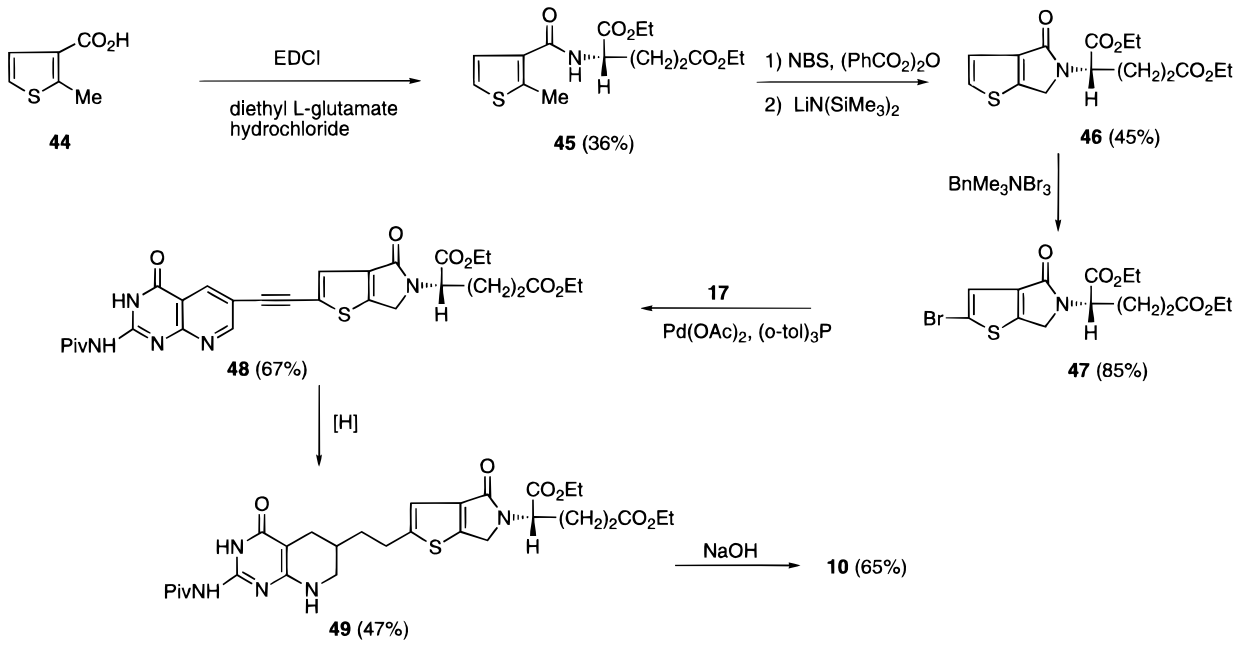


Table 1

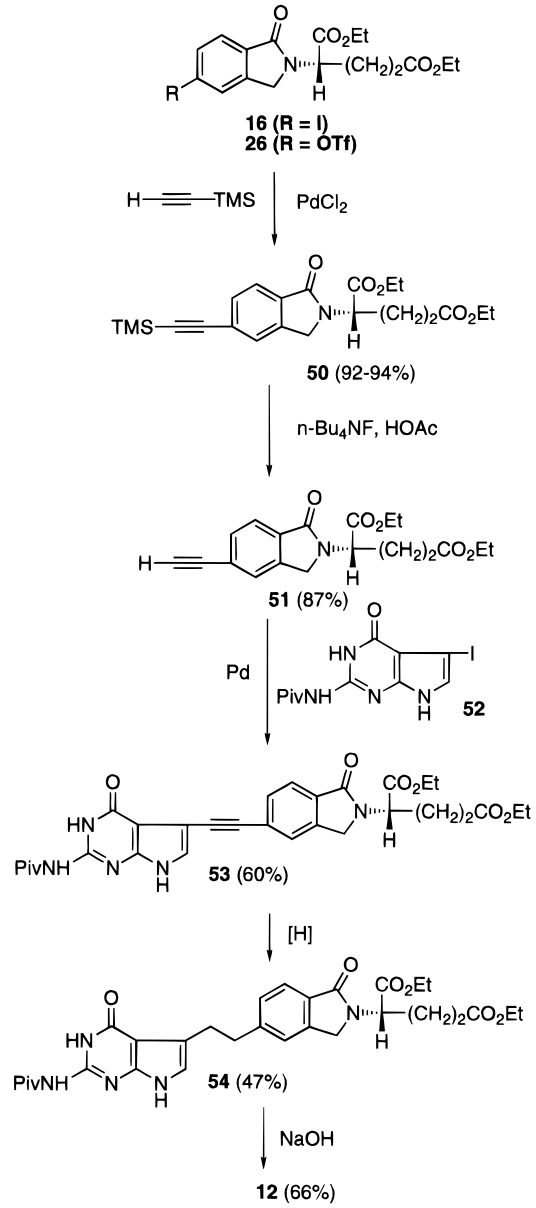
compd	cytotoxicity against CCRF-CEM cells[19] IC_{50} (μg/mL)
DDATHF (**4**)	0.007
6	0.014
m-DDATHF	0.015
7	0.05
8	>33.3
LY254155 (**11**)	0.001
9	0.011
10	0.0219
LY231514 (**14**)	0.007
12	1.6
13	5.1

of **28** with (trimethylsilyl)acetylene, deprotection to **55**, and a second palladium-catalyzed coupling, this time with **52** as the halogenated component, yielded the acetylenic intermediate **56** in reasonable (59%) yield (Scheme 9). Catalytic reduction of **56** to **60** followed by saponification then yielded the target conformationally-restricted analog **13** of LY231514 (Scheme 11).

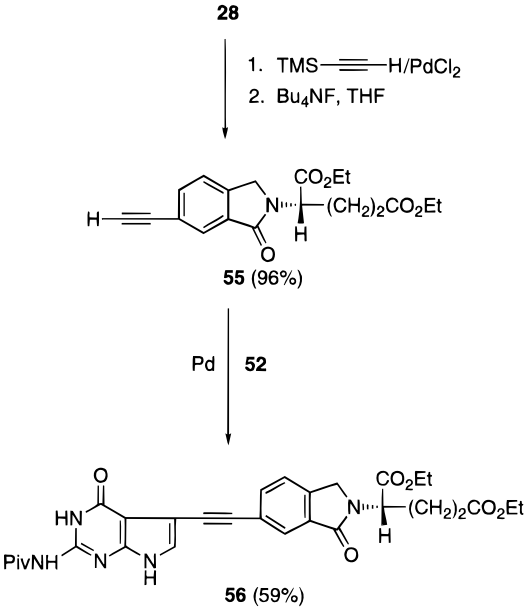
A somewhat more versatile route to **13** involved palladium-catalyzed coupling of tributylvinyl tin either with **28** or with the triflate prepared from **25** to give the styrene **57**, followed by a Heck reaction with 2-pivaloyl-7-iodo-7-deazaguanine (**52**) to give the ethylenic intermediate **58**. Alternatively, **58** could be prepared by reversing the order of these coupling reactions; thus, (tributylvinyl)tin and 2-pivaloyl-7-iodo-7-deazaguanine (**52**) yielded 2-pivaloyl-7-vinyl-7-deazaguanine (**59**), which coupled smoothly with **28** to give **58** (Scheme 10). Reduction of **58** to **60** followed by saponification then provided an alternate pathway to **13** (Scheme 11).

Preliminary biological evaluation of these analogs as inhibitors of CCRF-CEM cells in vitro revealed some striking consequences of constraining glutamate flexibility through formation of an isoindolinone ring. Table 1 compares the IC_{50} values of compounds **6**−**10**, **12**, and **13** with those of the corresponding nonconstrained parent antifolate. The conformationally-constrained analog **3** (BW1843U89) was more potent than the related acyclic compounds **1** and **2**.[2] In contrast, however, most of the conformationally-constrained compounds described in

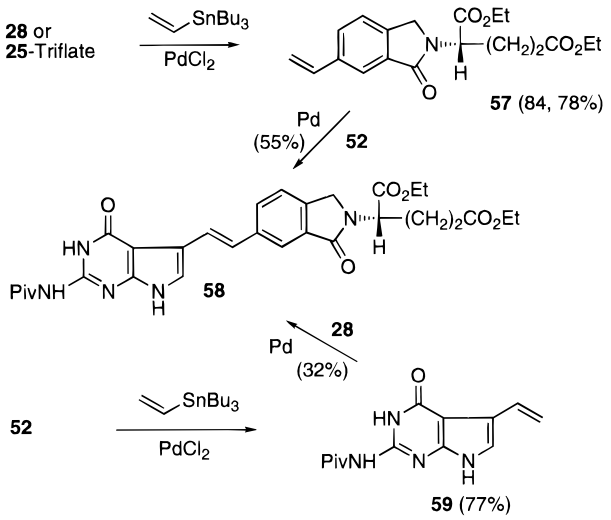
Scheme 8



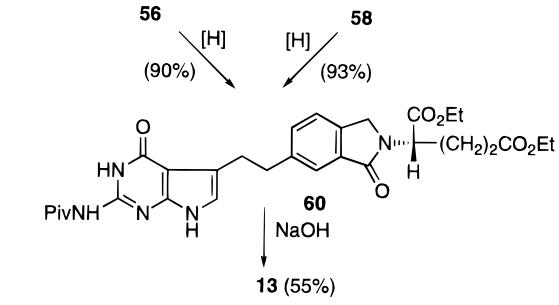
Scheme 9



Scheme 10



Scheme 11



this paper were less potent than their acyclic counterparts. Although **6** compares well in cell growth inhibitory activity with DDATHF itself, the meta isomer **7** is less active than meta-DDATHF. Constraining the isoindolinone ring through an ortho-bridge (compound **8**) totally eliminates cell growth inhibitory activity. Constraining the glutamate moiety in the very active 2′,5′-thiophene antifolate **11** to give **9** and **11** resulted in activity approximately comparable to the activity exhibited by DDATHF. In contrast to these results with constrained analogues of DDATHF and **11**, however, inhibitory activity was substantially reduced in the constrained pyrrolo[2,3-*d*]pyrimidine analogues **12** and **13** as compared with LY231514 (**14**). Further biological data, including enzyme inhibitory data against GAR FTase and substrate activity for FPGS, will be discussed in a later publication.

Experimental Section

Diethyl 2-(2,3-Dihydro-5-iodo-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (16). Methyl 4-iodo-2-methylbenzoate[10] (**15**, 43.8 g, 158 mmol), NBS (31.2 g, 175 mmol), and benzoyl peroxide (3.6 g, 15 mmol) were dissolved in 300 mL of benzene, and the mixture was heated under vigorous reflux for 4 h, cooled to rt, left overnight, and then filtered. The filtrate was evaporated under reduced pressure, and the crude product was dissolved in 600 mL of ether. The resulting solution was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The residual crude alkyl bromide was dissolved in 250 mL of dimethylacetamide, diethyl L-glutamate hydrochloride (75.75 g, 316 mmol), and anhydrous potassium carbonate (87.34 g, 632 mmol) were added, and the mixture was stirred overnight and then diluted with 1 L of water and extracted three times with EtOAc. The organic extracts were washed well with brine, 1 M $KHSO_4$, and water, dried over Na_2SO_4, and concentrated. The residual crude product was chromatographed using 20% EtOAc/hexane to give 34.7 g (49%) of **16** as a yellow oil that solidified upon standing. Recrystallization from toluene/hexane yielded colorless crystals: mp 88−89 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, 3 H), 1.26 (t, 3 H), 2.12−2.60 (m, 4 H), 3.95−4.12 (m, 2 H), 4.19 (q, 2 H), 4.38 (d, J = 18 Hz, 1 H), 4.58 (d, J = 18 Hz, 1 H), 5.02−5.18 (m, 1 H), 7.60 (d, J = 9 Hz, 1 H), 7.81 (d, J = 9 Hz, 1 H), 7.85 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.0, 24.9, 30.8, 46.2, 53.2, 60.5, 61.4, 98.5, 125.2, 131.2, 132.1, 137.2, 143.4, 168.2, 170.2, 172.0. Anal. Calcd for $C_{17}H_{20}INO_5$: C, 45.86; H, 4.53; N, 3.15. Found: C, 46.15; H, 4.49; N, 3.11.

Diethyl 2-(2,3-Dihydro-5-hydroxy-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (24) and Diethyl 2-(2,3-Dihydro-6-hydroxy-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (25). To a solution of 4,4-diethoxybut-2-ynal (**21**, 0.98 g, 6.32 mmol) in 3 mL of toluene was added the Danishefsky diene (**20**, 1.5 mL, 7.58 mmol, 1.2 equiv). The reaction mixture was heated to 80 °C and stirred for 20 h. After the mixture was cooled to rt, the solvent was removed under reduced pressure, 26 mL of THF/1 N HCl (1:1) was added, and the mixture was stirred at rt for 2 h. It was then diluted with 30 mL of ethyl acetate, the two layers were separated, and the aqueous layer was back-extracted with 2 × 30 mL of ethyl acetate. The combined extracts were washed with 30 mL of brine and dried over $MgSO_4$, and the solvent was removed by evaporation under reduced pressure. The crude 4-hydroxyphthalaldehyde was dissolved in 50 mL of DMF, diethyl L-glutamate hydrochloride (3.0 g, 12.64 mmol, 2.0 equiv) was added at 0 °C, and the reaction mixture was stirred at 0 °C for 1 h and then at rt overnight. The solvent was removed under reduced pressure, and the residual solid was dissolved in 50 mL of ethyl acetate, washed successively with 20 mL of 1 N HCl, 20 mL of water, and 20 mL of brine, and dried over $MgSO_4$. Removal of the solvent then gave a crude product that was purified by silica gel chromatography (eluting with 95:5 EtOAc/Et_3N) to give **24** (635 mg, 30%) and **25** (266 mg, 13%).

Compound **24**: oil; IR (neat) 3182, 2982, 1795, 1691, 1660, 1614, 1275, 1208 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (t, 3 H), 1.26 (t, 3 H), 2.10−2.50 (m, 4 H), 3.96−4.10 (m, 2 H), 4.19 (q, 2 H), 4.31 (d, J = 16.8 Hz, 1 H), 4.55 (d, J = 16.8 Hz, 1 H), 5.04−5.08 (m, 1 H), 6.93−6.96 (m, 2 H), 7.67 (d, J = 8.9 Hz, 1 H), 8.34 (br s, 1 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 14.0, 14.1, 25.0, 30.9, 47.0, 53.5, 60.9, 61.7, 109.6, 116.3, 122.4, 125.3, 144.2, 161.3, 170.2, 170.5, 172.5. Anal. Calcd for $C_{17}H_{21}NO_5$: C, 60.89; H, 6.31; N, 4.18. Found: C, 60.71; H, 6.31; N, 4.26.

Compound **25**: oil; IR (neat) 3244, 2983, 1736, 1695, 1667, 1626, 1471, 1267, 1213 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (t, 3 H), 1.26 (t, 3 H), 2.10−2.55 (m, 4 H), 3.95−4.10 (m, 2 H), 4.20 (q, 2 H), 4.32 (d, J = 16.2 Hz, 1 H), 4.54 (d, J = 16.4 Hz, 1 H), 5.06−5.11 (m, 1 H), 7.09 (dd, J_1 = 2.0 Hz, J_2 = 8.2 Hz, 1 H), 7.30 (d, J = 8.2 Hz, 1 H), 7.49 (d, J = 2.0 Hz, 1 H);

^{13}C NMR (68 MHz, $CDCl_3$) δ 13.9, 14.0, 24.9, 30.8, 46.7, 53.5, 60.8, 61.6, 109.9, 120.3, 123.6, 132.3, 132.6, 157.1, 170.0, 170.3, 172.4. Anal. Calcd for $C_{17}H_{21}NO_5$: C, 60.89; H, 6.31; N, 4.18. Found: C, 60.66; H, 6.41; N, 4.14.

Diethyl 2-[2,3-Dihydro-5-[(trifluoromethanesulfonyl)-oxy]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (26). To a solution of diethyl 2-(2,3-dihydro-5-hydroxy-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**24**) (170 mg, 0.507 mmol) in 4 mL of CH_2Cl_2 were added 0.08 mL (0.608 mmol, 1.2 equiv) of collidine and 0.1 mL (0.608 mmol, 1.2 equiv) of triflic anhydride at −78 °C. The reaction mixture was stirred for 30 min, quenched with 5 mL of water, and diluted with 30 mL of CH_2Cl_2. The two layers were separated, the aqueous layer was back-extracted with 15 mL of CH_2Cl_2, and the combined extracts were washed with 10 mL of brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude product was purified by silica gel chromatogaphy (eluting with 7:3 hexane/EtOAc) to give 201 mg (85%) of **26** as an oil: IR (neat) 2973, 1735, 1670, 1215 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (t, 3 H), 1.28 (t, 3 H), 2.05−2.50 (m, 4 H), 3.95−4.10 (m, 2 H), 4.21 (q, 2 H), 4.45 (d, J = 17.1 Hz, 1 H), 4.73 (d, J = 17.1 Hz, 1 H), 5.06−5.12 (m, 1 H), 7.37−7.42 (m, 2 H), 7.95 (d, J = 8.2 Hz, 1 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 13.9, 14.0, 25.0, 30.8, 46.6, 53.4, 60.6, 61.6, 116.5, 118.5, 121.5, 125.8, 131.6, 143.7, 151.6, 167.1, 170.2, 172.0. Anal. Calcd for $C_{18}H_{20}F_3NO_8S$: C, 46.25; H, 4.31; N, 3.10. Found: C, 46.52; H, 4.43; N, 3.05.

Diethyl 2-[2,3-Dihydro-5-[2-[2-(pivaloylamino)-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl]ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (18). Method A. To a flask containing 0.21 g (0.93 mmol) of $Pd(OAc)_2$, 0.57 g (1.86 mmol) of tri-*o*-tolylphosphine, 6.67 g (15 mmol) of **16**, 230 mL of MeCN, 8.5 g (84 mmol) of Et_3N were added 88 mg (0.46 mmol) of CuI and 3.78 g (14 mmol) of 2-pivaloyl-6-ethynyl-5-deazapterin (**17**). The solution was refluxed for 5.5 h, cooled to rt, filtered, and concentrated. The crude product was chromatographed using 2.5% MeOH/CH_2Cl_2 to give 4.50 g (55%) of **18** as a pale yellow solid: mp 207−208 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, 3 H), 1.26 (t, 3 H), 2.12−2.60 (m, 4 H), 3.95−4.12 (m, 2 H), 4.22 (q, 2 H), 4.38 (d, J = 17.5 Hz, 1 H), 4.64 (d, J = 17.5 Hz, 1 H), 5.02−5.18 (m, 1 H), 7.63−7.66 (m, 2 H), 7.86 (d, J = 7.5 Hz, 1 H), 8.40 (br s, 1 H), 8.63 (d, 1 H), 9.01 (s, 1 H), 12.10 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.1, 25.0, 26.8, 30.9, 40.4, 46.7, 53.4, 60.6, 61.6, 87.3, 92.6, 114.8, 116.9, 124.0, 125.7, 126.0, 131.5, 131.9, 138.5, 141.8, 149.5, 158.2, 161.0, 168.2, 170.5, 172.3, 180.7. Anal. Calcd for $C_{31}H_{33}N_5O_7$: C, 63.36; H, 5.66; N, 11.92. Found: C, 63.12; H, 5.66; N, 11.88.

Method B. To a solution of 200 mg (0.428 mmol) of **26** in 4 mL of DMF were added 25 mg (0.0214 mmol, 0.05 equiv) of $Pd(PPh_3)_4$, 0.3 mL (2.14 mmol, 5.0 equiv) of triethylamine, and 150 mg (0.556 mmol, 1.3 equiv) of 2-pivaloyl-6-ethynyl-5-deazapterin (**17**). The mixture was heated to 80 °C and stirred for 3 h, and the solvent was then removed under reduced pressure. Workup as described above gave 174 mg (69%) of **18**, mp 206−208 °C, identical with the sample of **18** prepared as described above by method A.

Diethyl 2-[2,3-Dihydro-5-[2-[2-(pivaloylamino)-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl]ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (19). A solution of **18** (4.4 g, 7.5 mmol) in 250 mL of MeOH was hydrogenated in the presence of 400 mg of 10% Pd−C under H_2 (50 psi) for 20 h. The solution was filtered, the filtrate was evaporated under reduced pressure, and the residual solid was purified by chromatography using 3% MeOH/CH_2Cl_2 to give an intermediate (3.5 g, 79%) in which (by NMR) the acetylenic bond had been reduced, but the pyridine ring was still aromatic. Further reduction of 3.4 g (5.75 mmol) of this intermediate in the presence of 350 mg of Pd−C (10%) in 80 mL of MeOH under H_2 (50 psi) for 2 days, followed by filtration and evaporation of the filtrate, gave **19** (3.2 g, 92%) as a colorless foam: mp 166−167 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 3 H), 1.23−1.29 (m, 3 H), 1.29 (s, 9 H), 1.60−1.90 (m, 3 H) 2.08−2.50 (m, 5 H), 2.70−3.08 (m, 4 H), 3.30−4.45 (m, 1 H), 3.95−4.07 (m, 2 H), 4.16−4.24 (q, 2 H), 4.33 (d, J = 21 Hz, 1 H), 4.57 (d, J = 21 Hz, 1H), 4.73 (br s, 1 H), 5.10 (m, 1 H), 7.27 (m, 2 H), 7.75 (d, J = 9 Hz, 1 H), 7.90 (br s, 1 H), 11.25 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.0, 24.9, 25.0, 26.7, 30.4, 30.8, 33.4, 40.1, 46.6, 46.7, 53.2, 60.5, 61.4, 122.6, 123.8, 128.2, 128.3, 129.5, 142.1, 146.6, 148.5, 158.4, 160.5, 169.0, 170.6, 172.2, 180.4. Anal. Calcd for $C_{31}H_{41}N_5O_7 \cdot 1.25H_2O$: C, 60.23; H, 7.09; N, 11.33. Found: C, 60.04; H, 6.70; N, 11.27.

2-[2,3-Dihydro-5-[2-(2-amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutaric Acid (6). A suspension of compound **19** (2.97 g, 5 mmol) in 60 mL of 0.5 N NaOH was stirred at rt for 3 days and filtered, and 2 N HCl was added dropwise to ∼pH 5−6. The resulting precipitate was collected by filtration, washed three times with cold water, and dried in a desiccator to give **6** (2.1 g, 92%) as a white solid: mp 195 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50−1.70 (m, 4 H), 1.78−1.90 (m, 1 H), 1.95−2.08 (m, 1 H), 2.12−2.30 (m, 3 H), 2.70−2.85 (m, 3 H), 3.18 (d, J = 6.5 Hz, 1 H), 4.44 (s, 2 H), 4.75−4.76 (m, 1 H), 5.94 (s, 2 H), 6.27 (s, 1 H), 7.34 (d, J = 7.5 Hz, 1 H), 7.44 (s, 1 H), 7.60 (d, J = 7.5 Hz, 1 H), 9.75 (br s, 1 H); FABMS calcd for $C_{22}H_{25}N_5O_6$ 455, found 456 (100) (M + 1). Anal. Calcd for $C_{22}H_{25}N_5O_6 \cdot 0.5HCl$: C, 55.78; H, 5.43; N, 14.78. Found: C, 55.53; H, 5.10; N, 14.41.

Diethyl 2-(2,3-Dihydro-6-iodo-1(2*H*)-oxoisoindolyl)-L-glutarate (28). This compound was prepared from methyl 5-iodo-2-methylbenzoate[10] (**27**) in 43% yield as a yellow oil following the procedure described above for the preparation of **16**: IR (film) cm^{-1} 3400 (br), 3280 (br), 2990 (m), 1974 (vst), 1694 (vst), 1453 (s), 1417 (s), 1311 (m), 1289 (s), 1203 (s); MS (EI) *m*/*z* (rel intensity) 445 (84), 400 (83), 372 (90), 326 (86), 298 (100); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.19 (t, 3 H), 1.26 (t, 3 H), 2.10−2.55 (m, 4 H), 3.97−4.15 (m, 2 H), 4.18 (q, 2 H), 4.37 (d, J = 18 Hz, 1 H), 4.56 (d, J = 18 Hz, 1 H), 5.10 (m, 1 H), 7.27 (d, J = 2.5 Hz, 1 H), 7.86 (d, J = 2.5 Hz, 1 H), 8.21 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.99, 14.03, 24.95, 30.83, 46.70, 53.32, 60.59, 61.53, 95.91, 124.65, 132.96, 133.80, 140.32, 140.90, 167.38, 170.29, 172.12; HRMS (EI) *m*/*z* calcd for $C_{17}H_{20}INO_5$ 445.0388, found 445.0378. Anal. Calcd for $C_{17}H_{20}INO_5$: C, 45.86; H, 4.53; N, 3.15. Found: C, 45.57; H, 4.44; N, 2.85.

2-[2,3-Dihydro-6-[2-(2-amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1(2*H*)-oxoisoindolyl]-L-glutaric Acid (7). Compound **28** was coupled with **17**, following the procedure given above for the preparation of **18**, to give **diethyl 2-[2,3-dihydro-6-[2-(pivaloylamino)-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate.** This compound was obtained in 45% yield as a pale brown foam: mp 208−210 °C; IR (KBr) cm^{-1} 3200 (br), 2900 (m), 1735 (vst), 1680 (vst), 1624 (vst), 1597 (vst), 1466 (s), 1447 (s), 1375 (s), 1244 (s), 1200 (s), 1148 (s), 732 (s); MS (EI) *m*/*z* (rel intensity) 587 (20), 530 (13), 440 (21), 382 (12), 170 (33), 77 (100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.20 (t, 3 H), 1.28 (t, 3 H), 1.35 (s, 9 H), 2.10−2.60 (m, 4 H), 3.90−4.15 (m, 2 H), 4.21 (q, 2 H), 4.47 (d, J = 18 Hz, 1 H), 4.67 (d, J = 18 Hz, 1 H), 5.05−5.15 (m, 1 H), 7.50 (d, J = 9 Hz, 1 H), 7.73 (d, J = 9 Hz, 1 H), 8.04 (s, 1 H), 8.42 (s, 1 H), 8.62 (s, 1 H), 8.99 (s, 1 H), 12.13 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 13.8, 24.7, 24.5, 30.6, 40.2, 46.8, 53.2, 60.3, 61.3, 85.7, 92.0, 114.5, 116.5, 122.1, 122.9, 126.7, 132.0, 134.6, 138.0, 141.8, 149.5, 157.8, 160.3, 167.8, 170.2, 172.1, 180.9; HRMS (EI) calcd for $C_{31}H_{33}N_5O_7$ 587.2380, found 587.2408. Anal. Calcd for $C_{31}H_{33}N_5O_7$: C, 63.36; H, 5.66; N, 11.92. Found: C, 63.59; H, 5.91; N, 11.70. Catalytic reduction of this ethynyl intermediate under the conditions described previously for the preparation of **19** from **18** gave **diethyl 2-[2,3-dihydro-6-[2-[2-(pivaloylamino)-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido-[2,3-*d*]pyrimidin-6-yl]ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate** in 75% yield as a colorless foam: mp 176−177 °C; IR (film) cm^{-1} 3400 (br), 3280 (br), 2930 (s), 2900 (s), 1797 (vst), 1685 (vst), 1647 (vst), 1572 (vst), 1478 (s); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.15−1.30 (overlapping triplets, 6 H), 1.29 (s, 9 H), 1.60−1.90 (m, 3 H), 2.10−2.55 (m, 5 H), 2.70−3.10 (m, 4 H), 3.40−3.55 (d, 1 H), 4.01−4.10 (m, 2 H), 4.16 (q, 2 H), 4.36 (d, J = 17.5 Hz, 1 H), 4.54 (d, J = 17.5 Hz, 1 H), 4.76 (br s, 1 H), 5.10 (m, 1 H), 7.38 (s, 2 H), 7.68 (s, 1 H), 8.00 (br s, 1 H), 11.25 (br s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.1, 25.0, 25.2, 26.9, 30.3, 30.9, 32.9, 34.9, 40.1, 46.0, 46.6, 53.2, 60.6, 61.5, 122.8, 123.2, 131.9, 132.2, 139.3, 142.3, 148.1, 158.0, 160.5, 169.2, 170.6, 172.3, 179.7; MS (EI) *m*/*z* (rel intensity) 595 (100), 550 (20), 495 (40), 250 (53). Anal. Calcd for $C_{31}H_{41}N_5O_7$: C,

62.51; H, 6.94; N, 11.76. Found: C, 62.70; H, 6.73; N, 11.79. Finally, hydrolysis of this penultimate intermediate following the procedure described above for the conversion of **19** to **6** yielded **7** in 83% yield as a colorless solid: mp 285 °C dec; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50−1.70 (m, 4 H), 1.78−1.90 (m, 1 H), 1.95−2.08 (m, 1 H), 2.12−2.30 (m, 3 H), 2.70−2.80 (m, 3 H), 3.18 (d, J = 6.5 Hz, 1 H), 4.43 (d, J = 10 Hz, 2 H), 4.76 (m, 1 H), 5.95 (s, 2 H), 6.27 (s, 1 H), 7.45−7.55 (m, 3 H), 9.75 (br s, 1 H). Anal. Calcd for $C_{22}H_{25}N_5O_6\cdot 2H_2O$: C, 53.76; H, 5.95; N, 14.25. Found: C, 53.74; H, 5.73; N, 14.03.

Diethyl 2-(2,3-Dihydro-4-iodo-1(2*H*)-oxoisoindolyl)-L-glutarate (30). A mixture of methyl 3-iodo-2-methylbenzoate[10] (**29**) (2.9 g, 10.5 mmol), *N*-bromosuccinimide (2.14 g, 12.07 mmol), and benzoyl peroxide (0.242 g, 0.1 equiv) in 50 mL of benzene was heated under vigorous reflux for 4 h. The reaction mixture was cooled and filtered, and the filtrate was evaporated under reduced pressure. The residual solid was dissolved in 100 mL of ether, the solution was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure, and the crude alkyl bromide was dissolved in 20 mL of dimethylacetamide. To this solution was added 5.03 g (2 equiv) of diethyl L-glutamate hydrochloride and 5.80 g (4 equiv) of anhydrous potassium carbonate, and the mixture was stirred at rt overnight, diluted with 200 mL of water, and extracted three times with ethyl acetate. The combined extracts were washed with brine, dried, concentrated, and chromatographed using 25% EtOAc/hexanes to give 2.19 g (47%) of **30** as a yellow oil that solidified upon standing. Recrystallization from toluene/hexane gave colorless crystals: mp 78−79 °C; IR (KBr) cm^{-1} 3000 (br), 1738 (vst), 1714 (vst), 1695 (vst), 1269 (vst), 1212 (vst), 1100 (s), 1021 (s), 744 (s); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.22 (t, J = 7.5 Hz, 3 H), 1.28 (t, J = 7.5 Hz, 3 H), 2.20−2.51 (m, 4 H), 4.04−4.11 (m, 2 H), 4.12−4.25 (m, 3 H), 4.50 (d, J = 18 Hz, 1 H), 5.12 (m, 1 H), 7.22 (t, J = 5 Hz, 1 H), 7.86 (d, J = 5 Hz, 1 H), 7.93 (d, J = 5 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1, 14.2, 25.0, 30.9, 50.9, 53.3, 60.7, 61.7, 95.3, 123.7, 130.0, 133.5, 140.7, 146.4, 168.7, 170.4, 172.2; MS (EI) *m/z* (rel intensity) 445 (30), 399 (40), 372 (66), 326 (52), 298 (100), 245 (50); HRMS (EI) *m/z* calcd for $C_{17}H_{20}INO_5$ 445.0388, found 445.0387. Anal. Calcd for $C_{17}H_{20}INO_5$: C, 45.86; H, 4.53; N, 3.15. Found: C, 45.51; H, 4.56; N, 3.21.

2-[2,3-Dihydro-4-[2-(2-amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutaric Acid (8). A flask containing 0.022 g (0.1 mmol, 4%) of $Pd(OAc)_2$, 0.060 g (0.2 mmol) of tri-*o*-toluylphosphine, and 1.055 g (2.37 mmol) of **30** was charged with 40 mL of MeCN and 1.39 g (13.75 mmol) of NEt_3. The solution was heated to reflux, and 9 mg (0.05 mmol) of CuI and 0.64 g (1 equiv) of **17** were added. The solution was heated under reflux for 2 h, after which time TLC indicated that the reaction was complete. The solution was cooled to rt, filtered, and concentrated. The crude product was chromatographed using 2.5% MeOH/CH_2Cl_2 to give 0.94 g (67%) of **diethyl 2-[2,3-dihydro-4-[2-[2-(pivaloylamino)-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl]ethynyl]-1-oxo-2-(1*H*)-isoindolyl]-L-glutarate** as a pale brown foam: mp 200−201 °C; IR (KBr) cm^{-1} 3200 (br), 2900 (m), 1797 (vst), 1699 (vst), 1624 (s), 1593 (s), 1462 (s), 1447 (s), 1145 (s), 749 (m); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.19 (t, 3 H), 1.30 (t, 3 H), 1.34 (s, 9 H), 2.34−2.41 (m, 4 H), 4.00−4.15 (m, 2 H), 4.18−4.23 (m, 2 H), 4.51 (d, J = 17.5 Hz, 1 H), 4.78 (d, J = 17.5 Hz, 1 H), 5.12 (m, 1 H), 7.51 (t, J = 5 Hz, 1 H,), 7.71 (d, J = 7.5 Hz, 1 H), 7.86 (d, J = 7.5 Hz, 1 H), 8.63 (s, 1 H), 8.99 (s, 1H), 12.5 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 14.0, 24.9, 26.7, 30.9, 40.4, 46.7, 53.3, 60.6, 61.6, 88.6, 90.0, 114.7, 116.4, 117.5, 124.2, 128.3, 132.1, 134.4, 138.4, 143.7, 149.7, 158.0, 160.3, 168.4, 170.4, 172.3, 180.9; MS (EI) *m/z* (rel intensity) 587 (44), 514 (34), 440 (68), 170 (80), 141 (100); HRMS (EI) *m/z* calcd for $C_{31}H_{33}N_5O_7$ 587.2380, found 587.2391. Anal. Calcd for $C_{31}H_{33}N_5O_7$: C, 63.36; H, 5.66; N, 11.92. Found: C, 63.59; H, 5.60; N, 11.76.

A solution of 0.294 g (0.5 mmol) of the above compound in 25 mL of MeOH was hydrogenated under 50 psi of H_2 with 0.011 g of 10% Pd−C for 18 h. When the reaction was complete, the solution was filtered to remove catalyst, and the solvent was evaporated under reduced pressure. The product was chromatographed using 3% MeOH/CH_2Cl_2 to give **diethyl 2-[2,3-dihydro-4-[2-[2-(pivaloylamino)-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl]ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate** (0.21 g, 70%) as a colorless foam: mp 150−152 °C; IR (film) cm^{-1} 3400 (br), 3280 (br), 2930 (s), 2900 (s), 1797 (vst), 1685 (vst), 1647 (vst), 1572 (vst), 1478 (s), 1204 (s); MS (EI) *m/z* (rel intensity) 595 (12), 298 (15), 263 (20), 170 (85), 141 (100); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.15−1.28 (overlapping t, 6 H), 1.29 (s, 9 H), 1.60−2.00 (m, 3 H) 2.15−2.60 (m, 5 H), 2.65−2.95 (m, 3 H), 3.00−3.15 (m, 1 H), 3.35−4.45 (m, 1 H), 4.01−4.07 (m, 2 H), 4.16−4.24 (q, J = 7.5 Hz, 2 H), 4.36 (d, J = 17.5, 1 H), 4.54 (d, J = 17.5 Hz, 1 H), 4.71 (m, 1 H), 5.10 (m, 1 H), 7.39 (m, 1 H), 7.69 (m, 1 H), 7.85 (s, 1 H), 11.25 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.2, 25.1, 25.3, 27.0, 29.4, 31.1, 33.4, 40.2, 46.2, 46.0, 53.4, 60.7, 61.6, 121.8, 128.5, 131.4, 131.8, 136.7, 140.1, 148.3, 158.1, 160.6, 169.5, 170.8, 172.4, 179.7; HRMS (EI) *m/z* calcd for $C_{31}H_{41}N_5O_7$ 595.3006, found 595.3014.

A solution of 0.149 g (0.25 mmol) of the above diethyl ester was dissolved in 2 mL of THF, 5 mL of 1 N NaOH was added, and the solution was stirred at rt for 2 days. The solution was concentrated to about 2 mL under reduced pressure, 2 mL of water was added, and 2 N HCl was added dropwise to precipitate the product. The solution was cooled at ca. 5 °C for 1 h and filtered, and the collected solid was washed (3×) with cold water and dried in a desiccator to give **8** (0.010 g, 13%) as a colorless solid: mp 195 °C dec; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50−1.80 (m, 4 H), 1.82−1.92 (m, 1 H), 2.08−2.18 (m, 1 H), 2.22−2.31 (m, 3 H), 2.54−2.83 (m, 3 H), 3.20−3.25 (m, 1 H), 4.48 (s, 2 H), 4.75−4.82 (m, 1 H), 5.97 (s, 2 H), 6.31 (s, 1 H), 7.43−7.46 (m, 2 H), 7.52 (d, J = 6 Hz, 1 H), 9.80 (br s, 1 H). Anal. Calcd for $C_{22}H_{25}N_5O_6\cdot 1.5HCl$: C, 51.80; H, 5.24; N, 13.72. Found: C, 51.76; H, 5.57; N, 13.56.

Methyl *N*-[(2-Carbomethoxythiophene-3-yl)methyl]-L-pyroglutamate (32). Method A. A mixture of methyl 3-methylthiophene-2-carboxylate (**31**)[20] (0.312 g, 2.00 mmol), *N*-bromosuccinimide (0.356 g, 2.0 mmol), and a catalytic amount of benzoyl peroxide in 10 mL of benzene was heated under reflux for 2 h. The reaction mixture was cooled, diluted with 15 mL of ether, and washed once with water. The organic phase was dried over $MgSO_4$ and evaporated to give crude methyl 3-(bromomethyl)thiophene-2-carboxylate as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 3 H), 4.93 (s, 2 H), 7.20 (d, J = 5 Hz, 1 H), 7.40 (d, J = 5 Hz, 1 H). A mixture of this crude 3-bromomethyl derivative, dimethyl L-glutamate hydrochloride (0.846 g, 4.0 mmol), and potassium carbonate (1.10 g, 8 mmol) was stirred for 40 h in 10 mL of dry, distilled DMF under argon at 40 °C. The solution was then diluted with 100 mL of water and extracted three times with EtOAc, the combined organic extracts were washed three times with brine and dried over Na_2SO_4 and the solvent was evaporated. The residual red liquid was flash chromatographed (66% EtOAc/hexane) to afford 0.046 g (8%) of **32** as a yellow oil, R_f = 0.325 (1.5% MeOH/CH_2Cl_2).

Method B. A mixture of dimethyl L-glutamate hydrochloride (0.85 g, 4.0 mmol) and methyl 3-formyl-2-thiophenecarboxylate[21] (**34**) (0.44 g, 2.6 mmol) was stirred in methanol (100 mL) and treated with triethylamine (1.01 g, 10 mmol). The mixture was stirred for 15 min. To the resulting homogeneous solution was added acetic acid (4 mL, 67.6 mmol) and activated 3 Å molecular sieves (5 g). After 15 h of stirring at rt, the mixture was heated to 50 °C, and $NaCNBH_3$ (1.28 g, 20 mmol) was added. After being heated at 50 °C overnight, the mixture was filtered, the filtrate was concentrated, and the residue was triturated with CH_2Cl_2. The organic layer was separated and washed with water, dried over $MgSO_4$, and concentrated. Purification of the residual material by silica gel chromatography using 10−70% ethyl acetate/hexanes as elute gave 0.12 g (36%) of **32** as a colorless oil: IR (neat) 1739, 1689 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.60−2.00 (m, 4 H), 3.69 (s, 3 H), 3.86 (s, 3 H), 4.13 (dd, J = 9.9, 2.7 Hz, 1 H), 4.76 (d, J = 15.0 Hz, 1 H), 5.04 (d, J = 15.0 Hz, 1 H), 7.09 (d, J = 5.1 Hz, 1 H),

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7.44 (d, J = 5.1 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.7, 28.9, 38.7, 51.6, 51.9, 59.0, 128.4, 130.3, 130.7, 143.7, 162.2, 172.0, 174.7; HRMS calcd for $C_{13}H_{15}NO_5S$ (M^+) 297.0671, found 297.0674. Anal. Calcd for $C_{13}H_{15}NO_5S$: C, 52.52; H, 5.09; N, 4.71. Found: C, 52.40; H, 5.24; N, 4.49.

Ethyl *N*-[(2-Carbomethoxythiophene-3-yl)methyl]-L-pyroglutamate (35). A mixture of diethyl L-glutamate hydrochloride (0.48 g, 2 mmol) and methyl 3-formyl-2-thiophenecarboxylate[21] (**34**) (0.14 g, 0.83 mmol) was stirred in dry THF (20 mL), triethylamine (1.01 g, 10 mmol) was added, and the reaction mixture was treated as described above to give 0.08 g (32%) of **35** as a colorless oil: IR (neat) 1739, 1700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (t, 3 H), 3.00−1.90 (m, 4 H), 3.83 (s, 3 H), 4.20−4.00 (m, 3 H), 4.75 (d, J = 15.1 Hz, 1 H), 5.02 (d, J = 15.1 Hz, 1 H), 7.07 (d, J = 5.2 Hz, 1 H), 7.41 (d, J = 5.2 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 23.0, 29.2, 39.0, 51.9, 59.4, 61.3, 128.6, 130.5, 1340.8, 144.0, 162.5, 171.5, 175.1; HRMS calcd for $C_{14}H_{17}NO_5S$ (M^+) 311.0827, found 311.0834. Anal. Calcd for $C_{14}H_{17}NO_5S$: C, 54.01; H, 5.50; N, 4.50. Found: C, 53.78; H, 5.59; N, 4.78.

Dimethyl *N*-(3-Methyl-2-thienylcarbonyl)-L-glutamate (37). The potassium salt of 3-methylthiophene-2-carboxylic acid[22] (**36**) was prepared by stirring 5.68 g (40 mmol) of **36** with 3.15 g (56.1 mmol) of KOH in 75 mL of refluxing MeOH for 10 min. The reaction mixture was evaporated to dryness, and the potassium salt was stirred in 175 mL of benzene for 1 h. The reaction mixture was cooled in an ice bath, one drop of DMF was added, and then oxalyl chloride (36.25 g, 285 mmol) was slowly added over a period of 45 min. The solution was stirred for 1 h at rt and evaporated to dryness, and excess oxalyl chloride was removed azeotropically by evaporation with the addition of $CHCl_3$. The resulting crude acid chloride was added to a stirred suspension of 8.45 g (40 mmol) of dimethyl L-glutamate hydrochloride and 12.15 g (120 mmol) of NEt_3 in 500 mL of CH_2Cl_2 at 0 °C. The mixture was then stirred at rt for 20 h, washed with aqueous $NaHCO_3$, 1 N HCl, and brine and dried over Na_2SO_4, and the solvent was evaporated. The crude product was chromatographed using 20% EtOAc/hexane to yield **37** (11.05 g, 92%) as a clear pale yellow oil: IR (film) cm^{-1} 3400 (br), 3280 (br), 1738, 1644, 1542, 1511, 1487, 1417, 1212; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07−2.20 (m, 1 H), 2.25−2.40 (m, 1 H), 2.40−2.60 (m, 2 H), 2.55 (s, 3 H), 3.67 (s, 3 H), 3.80 (s, 3 H), 4.81 (m, 1 H), 6.60 (br d, 1 H), 6.91 (d, J = 6 Hz, 1 H), 7.32 (d, J = 6 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.3, 26.7, 29.7, 51.3, 51.8, 52.1, 126.8, 130.1, 131.5, 141.1, 162.5, 171.9, 172.9; MS (EI) *m*/*z* (rel intensity) 299 (17), 267 (12), 240 (40), 174 (82), 125 (100); HRMS calcd for $C_{13}H_{17}NO_5S$ 299.0827, found 299.0809. Anal. Calcd for $C_{13}H_{17}NO_5S$: C, 52.16; H, 5.72; N, 4.68. Found: C, 51.95; H, 5.66; N, 4.61.

Dimethyl 2-(6-Oxo-4*H*-thieno[2,3-*c*]pyrrolin-5-yl)-L-glutarate (38). Method A. A mixture of dimethyl *N*-(3-methyl-2-thienylcarbonyl)-L-glutamate (**37**, 3.73 g, 12.47 mmol), NBS (2.66 g, 14.96 mmol), and a catalytic amount of AIBN was heated under reflux overnight in 100 mL of CCl_4. The solution was allowed to cool to rt, diluted with 150 mL of CH_2Cl_2, and washed with water. The solution was dried over Na_2SO_4 and evaporated to give 4.56 g of crude product. The ^{1}H NMR spectrum of this material indicated that it contained <10% of the starting material and no succinimide. The flask containing the crude product was swept with argon and charged with 100 mL of THF. Lithium bis(trimethylsilyl)-amide (12.4 mL of a 1.0 M solution in THF, 12.4 mmol) was added at −20 °C, and the solution was stirred at rt for 2 h and poured into 1 N HCl and then extracted three times with EtOAc. The combined organic phases were dried and concentrated to afford an oil. Flash chromatography (40% EtOAc−hexane) gave 1.37 g (37%) of **38** as a white solid, mp 58−60 °C, after recrystallization from hexane: R_f = 0.40 (1.5% MeOH−CH_2Cl_2); IR (film) cm^{-1} 1735, 1688, 1448, 13.98, 1201, 756; MS (EI) *m*/*z* (rel intensity) 297 (4), 265 (15), 238 (30), 206 (30), 178 (100), 125 (37), 69 (42); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10−2.25 (m, 1 H), 2.30−2.50 (m, 3 H), 3.59 (s, 3 H), 3.72 (s, 3 H), 4.32 (d, J = 17.1 Hz, 1 H), 4.54 (d, J = 17.1 Hz, 1 H), 5.00 (m, 1 H), 7.05 (d, J = 4.8 Hz, 1 H), 7.67 (d, J = 4.8 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.0, 30.4, 45.8, 51.5, 52.2, 53.5, 121.1, 134.2, 135.2, 151.9, 164.8, 170.9, 172.5; HRMS calcd for $C_{13}H_{15}NO_5S$ 297.0671, found 297.0659. Anal. Calcd for $C_{13}H_{15}NO_5S$: C, 52.52; H, 5.09; N, 4.71. Found: C, 52.44; H, 4.97; N, 4.59.

Method B. As an alternate procedure, 2,3-thiophenedicarboxaldehyde (**39**)[17] (2.0 g, 14.28 mmol) was mixed with dimethyl L-glutamate hydrochloride (6.4 g, 30 mmol) in 50 mL of DMF, and the mixture was stirred at rt for 24 h. Water was added to this reaction mixture, which was then extracted with EtOAc. The organic layer was washed with water several times, dried, and concentrated to give a dark brown oil. Column chromatography (40% EtOAc−hexane) gave 3.0 g (70%) of **38** as a yellow solid that appeared to contain a very small amount of the isomeric 4-oxo-6*H*-thieno[2,3-*c*]pyrroline derivative **40**.

Dimethyl 2-(6-Oxo-2-bromo-4*H*-thieno[2,3-*c*]pyrrolin-5-yl)-L-glutarate (41). A mixture of **38** (60 mg, 0.2 mmol), benzyltrimethylammonium tribromide (0.39 g, 1 mmol), and zinc chloride (0.1 g, 0.73 mmol) in 4 mL of acetic acid was stirred for 40 h at rt. The reaction mixture was quenched by the addition of 5% aqueous $NaHSO_3$ solution and extracted with CH_2Cl_2. The organic phase was washed with water several times, dried, and concentrated. The crude product was chromatographed (40% EtOAc−hexane) to give 73 mg (97%) of **41** as a yellow oil: IR (film) cm^{-1} 3400 (br), 1739, 1690, 1442, 1213, 1171; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02−2.19 (m, 1 H), 2.25−2.50 (m, 3 H), 3.55 (s, 3 H), 3.67 (s, 3 H), 4.23 (d, J = 17.1 Hz, 1 H), 4.46 (d, J = 17.1 Hz, 1 H), 4.90 (m, 1 H), 7.03 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.0, 30.4, 45.8, 51.5, 52.2, 53.5, 123.0, 123.9, 134.6, 150.8, 163.9, 170.8, 172.48; MS (EI) *m*/*z* (rel intensity) 377 (50), 375 (50), 345 (54), 343 (54), 318 (90), 316 (90), 258 (100), 256 (99); HRMS (EI) calcd for $C_{13}H_{14}{}^{79}BrNO_5S$ 374.9776, found 374.9766; calcd for $C_{13}H_{14}{}^{81}BrNO_5S$ 376.9755, found 376.9745. Anal. Calcd for $C_{13}H_{14}BrNO_5S$: C, 41.50; H, 3.75; N, 3.72. Found: C, 41.35; H, 3.77; N, 3.71.

Dimethyl 2-[5-[[2-(Pivaloylamino)-4(3*H*)-oxopyrido-[2,3-*d*]pyrimidin-6-yl]ethynyl]-6-oxo-4*H*-thieno[2,3-*c*]pyrrolin-5-yl]-L-glutarate (42). A mixture of **41** (2.48 g, 6.60 mmol), $Pd(PPh_3)_4$ (610 mg, 8 mol %), and Et_3N (4.6 mL, 5 equiv) in 100 mL of MeCN was stirred at rt for 15 min to give a clear solution. 2-Pivaloyl-6-ethynyl-5-deazapterin (**17**, 3.56 g, 13.2 mmol) and CuI (63 mg, 5 mol %) were added to this reaction mixture, which was then heated under reflux for 4 h, after which time TLC indicated that the reaction was complete. The solution was cooled to rt and concentrated. The crude product was chromatographed using 1.5% MeOH−CH_2Cl_2 to give 2.46 g (66%) of the product **42** as a pale brown foam that was recrystallized from MeOH: mp 195 °C dec; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.35 (s, 9 H), 2.10−2.30 (m, 1 H), 2.30−2.50 (m, 3 H), 3.62 (s, 3 H), 3.75 (s, 3 H), 4.33 (d, J = 17.5 Hz, 1 H), 4.56 (d, J = 17.5 Hz, 1 H), 5.00 (m, 1 H), 7.26 (s, 1 H), 8.35 (br s), 8.62 (s, 1 H), 8.97 (s, 1 H), 12.09 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.2, 26.8, 30.6, 40.5, 46.0, 51.8, 52.5, 53.8, 86.0, 91.9, 114.8, 116.2, 126.0, 131.8, 135.7, 138.4, 149.8, 150.7, 157.7, 157.8, 160.2, 164.3, 171.0, 172.7, 180.9 (1 carbon buried); MS (EI) *m*/*z* (rel intensity) 508 (33), 446 (55), 277 (100), 199 (47), 149 (50); HRMS calcd for $C_{27}H_{27}N_5O_7S$ 565.1631, found 565.1632. Anal. Calcd for $C_{27}H_{27}N_5O_7S\cdot0.5H_2O$: C, 56.43; H, 4.91; N, 12.20. Found: C, 56.70; H, 5.10; N, 12.01.

Dimethyl 2-[5-[[2-(Pivaloylamino)-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl]ethyl]-6-oxo-4*H*-thieno[2,3-*c*]pyrrolin-5-yl]-L-glutarate (43). To a solution of **42** (2.13 g, 3.77 mmol) in 170 mL of MeOH and 50 mL of CH_2Cl_2 was added 10% Pd−C (4.26 g) under N_2 atmosphere, and the mixture was hydrogenated under H_2 at 50 psi at 50 °C for 36 h. The solution was filtered, and the filtrate was concentrated and chromatographed (5% MeOH−CH_2Cl_2) to give 2.05 g (95%) of the product **43** as a pale yellow solid: mp 160 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 9 H), 1.70−2.00 (m, 4 H), 2.05−2.20 (m, 2 H), 2.30−2.50 (m, 2 H), 2.80 (m, 1 H), 2.90−3.10 (m, 3 H), 3.35 (m, 1 H), 3.61 (s, 3 H), 3.72 (s, 3 H), 4.22 (d, J = 17.5 Hz, 1 H), 4.45 (d, J = 17.5 Hz, 1 H), 4.75 (br s, 1 H), 5.00 (m, 1 H), 6.79 (s, 1 H), 7.89 (br s, 1 H), 11.26 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.1, 26.8, 28.3, 30.1, 30.5, 34.9, 40.2, 45.8, 45.9, 51.7, 52.4, 53.5, 88.8, 118.6,

(22) Blanchette, J. A.; Brown, E. V. *J. Am. Chem. Soc.* **1951**, *73*, 2779.

131.6, 148.7, 151.9, 156.6, 158.5, 160.5, 165.3, 171.3, 172.7, 180.8 (1 carbon buried); HRMS(FAB) calcd for $C_{27}H_{36}N_5O_7S$ 574.2335, found 574.2316. Anal. Calcd for $C_{27}H_{35}N_5O_7S$: C, 56.53; H, 6.15; N, 12.11. Found: C, 56.76; H, 6.15; N, 12.11.

2-[5-[(2-Amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-6-oxo-4*H*-thieno[2,3-*c*]pyrrolin-5-yl]-L-glutaric Acid (9). A solution of 0.920 g (1.6 mmol) of **43** was dissolved in 20 mL of 1 N NaOH and stirred at rt for 3 days. Addition of 0.5 N HCl dropwise to ~pH 3 resulted in precipitation of the product. The solution was cooled and filtered, and the collected solid was washed three times with cold water and dried in a desiccator to give 0.630 g (85%) of **9** as a colorless solid: mp 160 °C dec; IR (KBr) cm^{-1} 3363 (br, OH), 1693, 1644, 1553; ^1H NMR (500 MHz, DMSO-d_6) δ 1.50−1.70 (m, 4 H), 1.78−1.90 (m, 1 H), 1.95−2.08 (m, 1 H), 2.12−2.30 (m, 3 H), 2.75−2.82 (m, 1 H), 2.92−2.99 (m, 2 H), 3.18 (br d, 1 H), 4.34 (s, 2 H), 4.70 (m, 1 H), 5.93 (s, 2 H), 6.28 (s, 1 H), 7.02 (s, 1 H), 9.70 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.5, 25.3, 27.1, 27.8, 30.4, 34.8, 45.0, 46.2, 53.5, 81.8, 119.7, 130.9, 152.9, 153.2, 156.0, 158.8, 161.4, 164.6, 172.4, 173.6; HRMS(FAB) calcd for $C_{20}H_{24}N_5O_6S$ 462.1447, found 462.1441. Anal. Calcd for $C_{20}H_{23}N_5O_6S\cdot 0.5NaCl$: C, 48.95; H, 4.72; N, 14.27. Found: C, 48.86; H, 4.79; N, 14.22.

Diethyl *N*-(2-Methyl-3-thienylcarbonyl)-L-glutamate (45). A mixture of 2-methylthiophene-3-carboxylic acid[23] (**44**) (2.40 g, 16.9 mmol), diethyl L-glutamate hydrochloride (4.07 g, 17 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (3.26 g, 17 mmol) was stirred in CH_2Cl_2 (150 mL) at 0 °C. Triethylamine (2.5 g, 25 mmol) was added dropwise over 30 min, and then the reaction mixture was allowed to warm to rt. After being stirred overnight, the solution was washed with 0.05 N HCl followed by saturated $NaHCO_3$, dried over $MgSO_4$, and evaporated under reduced pressure. The crude product was chromatographed using 20% EtOAc/hexane to yield **45** (1.97 g, 36%) as a pale yellowish oil: IR (neat) 3358, 1731, 1647 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, J = 7.0 Hz, 3 H), 1.26 (t, J = 7.0 Hz, 3 H), 2.50−2.00 (m, 4 H), 2.67 (s, 3 H), 4.06 (q, J = 7.0 Hz, 2 H), 4.19 (q, J = 7.0 Hz, 2 H), 4.74−4.66 (m, 1 H), 6.68 (br d, J = 7.0 Hz, 1 H), 6.98 (d, J = 5.5 Hz, 1 H), 7.14 (d, J = 5.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.3, 14.0, 26.0, 29.8, 59.7, 60.6, 120.8, 126.0, 130.4, 144.4, 163.6, 171.3, 172.1; HRMS calcd for $C_{15}H_{21}NO_5S$ (M^+) 327.1140, found 327.1142. Anal. Calcd for $C_{15}H_{21}NO_5S$: C, 55.03; H, 6.47; N, 4.28. Found: C, 55.14; H, 6.41; N, 4.24.

Diethyl 2-(4-Oxo-6*H*-thieno[2,3-*c*]pyrrolin-5-yl)-L-glutarate (46). A solution of diethyl *N*-(2-methyl-3-thienylcarbonyl)-L-glutamate (**45**) (1.20 g, 3.67 mmol), NBS (0.75 g, 4.22 mmol), and a catalytic amount of benzoyl peroxide in benzene (60 mL) was heated under reflux overnight. The solution was allowed to cool to rt, diluted with 100 mL of ether, and washed with water, and the organic layer was dried over Na_2SO_4 and evaporated to give the crude (bromomethyl)thiophene. The ^1H NMR spectrum of this material indicated that it contained ~15% of the starting material. The flask containing the crude product was swept with argon, charged with 100 mL of THF, and cooled to −30 °C. Lithium bis(trimethylsilyl)amide (3.6 mL, 1.0 N in THF, 3.6 mmol) in 20 mL of dry THF was added dropwise to the stirred solution at −30 °C. The solution was allowed to warm to 0 °C and stirred at 0 °C for an additional 30 min. The reaction mixture was then poured into water and extracted with CH_2Cl_2. The combined organic phases were dried and concentrated to afford an oil. Flash chromatography (20−40% EtOAc−hexane) afforded 0.12 g (10%) of the starting material **45** and 0.53 g (45%) of **46** as a pale yellowish oil: IR (neat) 1724, 1689 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.0 Hz, 3 H), 1.24 (t, J = 7.0 Hz, 3 H), 2.50−2.00 (m, 4 H), 4.10−3.90 (m, 2 H), 4.17 (q, J = 7.0 Hz, 1 H), 4.40 (d, J = 17.2 Hz, 1 H), 4.68 (d, J = 17.2 Hz, 1 H), 5.10−4.90 (m, 1 H), 7.22 (d, J = 5.0 Hz, 1 H), 7.33 (d, J = 5.0 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.4, 13.6, 24.6, 30.0, 53.1, 60.0, 60.9, 119.5, 129.3, 138.9, 150.2, 165.0, 170.2, 170.2, 171.7; HRMS calcd for $C_{15}H_{19}NO_5S$ (M^+) 325.0984, found 325.0987. Anal. Calcd for $C_{15}H_{19}NO_5S$: C, 55.37; H, 5.89; N, 4.30. Found: C, 55.17; H, 5.61; N, 4.05.

Diethyl 2-(2-Bromo-4-oxo-6*H*-thieno[2,3-*c*]pyrrolin-5-yl)-L-glutarate (47). A mixture of **46** (0.49 g, 1.5 mmol), benzyltrimethylammonium tribromide (2.34 g, 6 mmol), and zinc chloride (0.49 g) in 50 mL of acetic acid was stirred overnight at rt. The reaction was quenched by the addition of 5% aqueous $NaHSO_3$ solution and extracted with CH_2Cl_2. The organic phase was washed with water, dried over Na_2SO_4, and concentrated, and the crude product was chromatographed (20% EtOAc−hexane) to give 0.51 g (85%) of **47** as a colorless oil: IR (neat) 1731, 1696 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.17 (t, J = 7.3 Hz, 3 H), 1.23 (t, J = 7.3 Hz, 3 H), 2.50−2.00 (m, 4 H), 4.10−3.92 (m, 2 H), 4.15 (q, J = 7.0 Hz, 2 H), 4.35 (d, J = 17.4 Hz, 1 H), 4.63 (d, J = 17.4 Hz, 1 H), 5.10−4.98 (m, 1 H), 7.21 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 24.9, 30.7, 46.1, 53.5, 60.4, 61.4, 115.9, 122.6, 138.5, 150.4, 164.1, 170.3, 171.9; HRMS calcd for $C_{15}H_{18}NO_5SBr$ (M^+) 403.0089, 405.0070, found 402.0094, 405.0068. Anal. Calcd for $C_{15}H_{18}NO_5SBr$: C, 44.56; H, 4.49; N, 3.46. Found: C, 44.48; H, 4.38; N, 3.43.

Diethyl 2-[5-[[2-(Pivaloylamino)-4(3*H*)-oxopyrido[2,3-*d*]pyrimidin-6-yl]ethynyl]-4-oxo-6*H*-thieno[2,3-*c*]pyrrolin-5-yl]-L-glutarate (48). A mixture of **47** (0.33 g, 0.82 mmol), 2-pivaloyl-6-ethynyl-5-deazapterin (**17**) 0.41 g, 1.5 mmol), $Pd(OAc)_2$ (0.022 g, 0.1 mmol), P(*o*-tolyl)$_3$ (0.061 g, 0.02 mmol), CuI (0.038 g, 0.2 mmol), and Et_3N (0.51 g, 5 mmol) in 30 mL of MeCN was sealed under argon in a heavy-walled Pyrex tube. The sealed tube was placed in an oil bath at 120 °C for 2.5 h. After being cooled to rt, the reaction mixture was concentrated and purified by preparative thin-layer chromatography with 1−5% MeOH/CH_2Cl_2 as the solvent to yield 0.32 g (67%) of **48** as a pale yellowish solid: mp 174−176 °C; IR (KBr) 3344−3028, 1739, 1668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, J = 7.0 Hz, 3 H), 1.26 (t, J = 7.0 Hz, 3 H), 2.50−2.10 (m, 4 H), 4.10−3.95 (m, 2 H), 4.19 (q, J = 7.0 Hz, 2 H), 4.44 (d, J = 17.5 Hz, 1 H), 4.73 (d, J = 17.5 Hz, 1 H), 5.10−4.95 (m, 1 H), 7.42 (s, 1 H), 8.40 (br s, 1 H), 8.58 (d, J = 1.1 Hz, 1 H), 8.95 (br s, 1 H), 12.1 (br s, 1 H); HRMS calcd for $C_{29}H_{31}N_5O_7S$ (M^+) 593.1944, found 593.1946. Anal. Calcd for $C_{29}H_{31}N_5O_7S$: C, 58.67; H, 5.26; N, 11.80. Found: C, 58.83; H, 5.35; N, 12.06.

Diethyl 2-[5-[[2-(Pivaloylamino)-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl]ethyl]-4-oxo-6*H*-thieno[2,3-*c*]pyrrolin-5-yl]-L-glutarate (49). A solution of **48** (60 mg, 0.1 mmol) in 100 mL of EtOH/CH_2Cl_2(3:1) was carefully added to a flask containing 5% Pd/C (0.5 g) under an atmosphere of N_2. The solution was hydrogenated under 50 psi of H_2 at 50 °C for 24 h. The solution was filtered, and the residue was concentrated and chromatographed (2% MeOH−CH_2Cl_2) to give 28 mg (47%) of the product **49** as a pale yellowish solid: mp 125 °C dec; IR (KBr) 3443−2784, 1732, 1720−1590 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.14 (t, J = 7.2 Hz, 3 H), 1.20 (t, J = 7.2 Hz, 3 H), 1.22 (s, 9), 3.40−1.60 (m, 13 H), 4.10−3.90 (m, 2 H), 4.19 (q, J = 7.2 Hz, 2 H), 4.30 (d, J = 17.2 Hz, 1 H), 4.58 (d, J = 17.2 Hz, 1 H), 4.90 (m, 1 H), 6.89 (s, 1 H), 7.88 (s, 1 H), 11.25 (br s, 1 H); HRMS calcd for $C_{29}H_{39}N_5O_7S$ (M^+) 601.2570, found 601.2570. Anal. Calcd for $C_{29}H_{39}N_5O_7S$: C, 57.89; H, 6.53; N, 11.64. Found: C, 57.61; H, 6.77; N, 11.46.

2-[5-[[2-(Pivaloylamino)-4(3*H*)-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl]ethyl]-4-oxo-6*H*-thieno[2,3-*c*]pyrrolin-5-yl]-L-glutaric Acid (10). A suspension of the ester **49** (20 mg) in NaOH (0.5 N, 3 mL) was stirred at rt for 3 days. Dropwise addition of 0.5 N HCl to pH 3 resulted in precipitation of a solid. The mixture was cooled, and the solid was collected by filtration, washed three times with cold water, and dried in a desiccator to give 10 mg (65%) of **10** as a pale yellowish solid: mp 240 °C dec; IR (KBr) 3600−3000, 1800−1500 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 3.30−1.50 (m, 13 H), 4.47 (s, 2 H), 4.60−4.70 (m, 1 H), 5.93 (s, 2 H), 6.30 (s, 1H), 6.95 (s, 1 H), 9.70 (br s, 1 H); FABHRMS calcd for $C_{20}H_{24}N_5O_6S$ ($M + 1$)$^+$ 462.1447, found 462.1432. Anal. Calcd for $C_{29}H_{39}N_5O_7S\cdot 0.6NaCl$: C, 48.37; H, 4.67; N, 14.10. Found: C, 48.52; H, 4.49; N, 13.97.

Diethyl 2-[2,3-Dihydro-5-[(trimethylsilyl)ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (50). Method A. Diethyl 2-(2,3-dihydro-5-iodo-1-oxo-2(1*H*)-isoindolyl)-L-glutarate

(23) (a) Steinkopf, W.; Jacob, H. *Ann.* **1935**, *515*, 273. (b) Campaigne, E.; Collins, C. J. *J. Heterocycl. Chem.* **1965**, *2*, 136.

(**16**, 4.45 g, 10 mmol) was dissolved in anhydrous MeCN (160 mL). $PdCl_2$ (177 mg, 1 mmol), triphenylphosphine (524 mg, 2 mmol), CuI (95 mg, 0.5 mmol), triethylamine (4.18 mL, 30 mmol), and (trimethylsilyl)acetylene (1.96 g, 20 mmol) were added. The reaction mixture was then heated to reflux for 5 h under argon. The resulting dark solution was evaporated to dryness under reduced pressure. The residue was chromatographed on a silica gel column (hexane/EtOAc = 1.5/1). The TLC homogeneous fractions were combined and evaporated under reduced pressure to give 3.82 g (92%) of **50** as a red oil: IR (neat) 2980, 2147, 1736, 1700, 1612, 1250, 1209 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.27 (s, 9 H), 1.19 (t, J = 7.0 Hz, 3 H), 1.26 (t, J = 7.0 Hz, 3 H), 2.05−2.50 (m, 4 H), 3.90−4.10 (m, 2 H), 4.19 (q, J = 14.3 Hz, 2 H), 4.36 (d, J = 16.5 Hz, 1 H), 4.60 (d, J = 16.9 Hz, 1 H), 5.06−5.11 (m, 1 H), 7.54−7.56 (m, 2 H), 7.78 (d, J = 8.1 Hz, 1 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 0.03, 14.2, 14.3, 25.3, 31.2, 46.9, 53.6, 60.9, 61.8, 97.3, 104.3, 123.9, 126.4, 126.9, 131.6, 132.1, 141.7, 168.6, 170.6, 172.4. Anal. Calcd for $C_{22}H_{29}CNO_5Si$: C, 63.59; H, 7.03; N, 3.37. Found: C, 63.48; H, 6.81; N, 3.33.

Method B. To a solution of 70 mg (0.15 mmol) of diethyl [2,3-dihydro-5-[(trifluoromethanesulfonyl)oxy]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (**26**) in 4 mL of DMF were added $Pd(PPh_3)_2Cl_2$ (6.3 mg, 0.009 mmol, 0.06 equiv), triethylamine (0.1 mL, 0.75 mmol, 5.0 equiv), and (trimethylsilyl)acetylene (0.04 mL, 0.3 mmol, 2 equiv). The mixture was heated to 60−65 °C and stirred for 2.5 h. The solvent was removed under reduced pressure, and the residual solid was purified by silica gel chromatography (eluting with 30% EtOAc in hexane) to give 58 mg (94%) of **50** as a red oil, identical in every respect with **50** prepared by method A.

Diethyl 2-(2,3-Dihydro-5-ethynyl-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (51). To a solution of diethyl 2-[2,3-dihydro-5-[(trimethylsilyl)ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (**50**, 55 mg, 0.13 mmol) in EtOH (2 mL) were added AcOH (0.2 mL) and *n*-Bu_4NF (0.66 mL, 0.66 mmol, 5 equiv). The mixture was stirred for 4 h at rt, the solvent was removed under reduced pressure, and the residual solid was purified by silica gel chromatography (eluting with 50% EtOAc in hexane) to give 39 mg (87%) of **51** as a red oil: IR (neat) 3260, 2982, 2097, 1735, 1696, 1619, 1211 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.19 (t, J = 7.3 Hz, 3 H), 1.26 (t, J = 7.3 Hz, 3 H), 2.10−2.50 (m, 4 H), 3.22 (s, 1 H), 3.95−4.12 (m, 2 H), 4.19 (q, J = 14.5 Hz, 2 H), 4.38 (d, J = 16.8 Hz, 1 H), 4.63 (d, J = 16.8 Hz, 1 H), 5.06−5.12 (m, 1 H), 7.54−7.60 (m, 2 H), 7.81 (d, J = 8.2 Hz, 1 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 14.1, 14.2, 25.1, 31.0, 46.7, 53.4, 60.7, 61.6, 79.5, 82.9, 123.9, 125.6, 126.5, 131.8, 132.0, 141.6, 168.3, 170.4, 172.3. Anal. Calcd for $C_{19}H_{21}N_1O_5.0.5H_2O$: C, 64.76; H, 6.29; N, 3.97. Found: C, 64.82; H, 6.23; N, 4.02.

Diethyl 2-[2,3-Dihydro-5-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (54). To a solution of 2-pivaloyl-7-iodo-7-deazaguanine[7] (**52**, 360 mg,1 mmol) in 5 mL of DMF were added diethyl 2-(2,3-dihydro-5-ethynyl-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**51**, 360 mg, 1.05 mmol), CuI (38 mg), triethylamine (0.3 mL), and $Pd(PPh_3)_4$ (100 mg). The mixture was stirred at rt for 4 h. The solvent was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column (2% MeOH in CH_2Cl_2 as eluent). The fractions containing product were again chromatographed, and the combined fractions were evaporated under reduced pressure to give **diethyl 2-[2,3-dihydro-5-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (53)** as a gray solid (345 mg, 60%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (t, 3 H), 1.26 (t, 3 H), 1.34 (s, 9 H), 2.21−2.45 (m, 4 H), 4.03 (m, 2 H), 4.20 (q, 2 H), 4.34 (d, J = 16.8 Hz, 1 H), 4.56 (d, J = 16.8 Hz, 1 H), 5.07 (m, 1 H), 7.10 (s, 1 H), 7.61 (s, 2 H), 7.78 (d, J = 7.9 Hz, 1 H), 8.72 (br, 1 H), 11.79 (s, 1 H).

To a solution of **53** (340 mg, 0.59 mmol) in 100 mL of 1:1 CH_2Cl_2/MeOH was added Pd−C (3%, 270 mg), and the mixture was hydrogenated at 50 psi for 3.5 h. The palladium catalyst was removed by filtration, and another 270 mg of the catalyst was added. The reaction mixture was hydrogenated for another 4 h and was evaporated to dryness under reduced pressure. The residue was separated on a silica gel column using 2% CH_2Cl_2/MeOH as eluent. After the evaporation of the combined fractions, **54** was obtained (150 mg, 47%) as a colorless solid: ^1H NMR (300 MHz, DMSO-d_6) δ 1.11 (t, 3 H), 1.16 (t, 3 H), 1.24 (s, 9 H), 2.21−2.45 (m, 4 H), 2.97 (t, J = 6.5 Hz, 2 H), 3.13 (t, J = 6.5 Hz, 2 H), 3.97 (m, 2 H), 4.13 (q, 2 H), 4.44 (d, J = 6.8 Hz, 1 H), 4.86 (m, 1 H), 6.86 (s, 1 H), 7.32 (d, J = 6 Hz, 1 H), 7.46 (s, 1 H), 7.60 (d, J = 6 Hz, 1 H). Anal. Calcd for $C_{30}H_{37}N_5O_7$: C, 62.16; H, 6.43; N, 12.08. Found: C, 62.16; H, 6.20; N, 12.09.

2-[2,3-Dihydro-5-[2-[2-amino-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutaric Acid (12). Diethyl 2-[2,3-dihydro-5-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (**54**, 120 mg, 0.21 mmol) was suspended in 4 mL of 1 N NaOH, and the suspension was stirred at rt for 3 days. The resulting clear solution was acidified with 1 N HCl to pH 4. The precipitate that formed was collected by filtration, washed with H_2O (3 × 2 mL), and dried under reduced pressure to give 60 mg (66%) of **12**: ^1H NMR (300 MHz, DMSO-d_6) δ 2.21−2.45 (m, 4 H), 2.89 (t, J = 6.6 Hz, 2 H), 3.08 (t, J = 6.6 Hz, 2 H), 4.55 (s, 2 H), 4.82 (m, 1 H), 6.70 (s, 1 H), 7.41 (d, J = 6 Hz, 1 H), 7.53 (s, 1 H), 7.75 (d, J = 6 Hz, 1H). Anal. Calcd for $C_{21}H_{21}N_5O_6$·1.0HCl: C, 53.00; H, 4.66; N, 14.72. Found: C, 53.00; H, 4.19; N, 15.01.

Diethyl 2-(2,3-Dihydro-6-ethynyl-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (55). To a solution of diethyl 2-(2,3-dihydro-6-iodo-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**28**, 0.92 g, 2.06 mmol) in anhydrous MeCN (20 mL) were added $PdCl_2$ (37.2 mg, 0.21 mmol), triphenylphosphine (110.2 mg, 0.42 mmol), CuI (20.9 mg, 0.11 mmol), triethylamine (0.88 mL, 6.3 mmol), and (trimethylsilyl)acetylene (0.412 g, 4.2 mmol), and the reaction mixture was heated to reflux for 5 h under argon. The resulting dark solution was evaporated to dryness under reduced pressure, and the residue was chromatographed on a silica gel column (7/3 hexane/EtOAc). The TLC homogeneous fractions were combined and were evaporated under reduced pressure to give 0.76 g (89%) of **diethyl 2-[2,3-dihydro-6-[(trimethylsilyl)ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate** as a red oil: ^1H NMR (300 MHz, $CDCl_3$) δ 0.27 (s, 9 H), 1.19 (t, 3 H), 1.26 (t, 3 H), 2.21−2.45 (m, 4 H), 4.03 (m, 2 H), 4.21 (q, 2 H), 4.39 (d, J = 16.5 Hz, 1 H), 4.57 (d, J = 16.5 Hz, 1 H), 5.08 (m, 1 H), 7.38 (d, J = 8.1 Hz, 1 H), 7.62 (d, J = 8.1 Hz, 1 H), 7.94 (s, 1 H). Anal. Calcd for $C_{22}H_{29}NO_5Si$: C, 63.59; H, 7.03; N, 3.37. Found: C, 63.62; H, 7.06; N, 3.46.

To a solution of diethyl 2-[2,3-dihydro-6-[(trimethylsilyl)-ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (0.415 g, 1 mmol) in MeOH (10 mL) were added (under argon) potassium fluoride (290 mg, 5 mmol) and HOAc (1 mL). The reaction solution was allowed to stand at rt for 4 h. The solvents were evaporated under reduced pressure. Water (50 mL) was added to the resulting residue, which was then extracted with Et_2O (3 × 40 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, and the organic layer was evaporated to dryness under reduced pressure. Compound **55** (330 mg, 96%) was obtained as a red oil after the combined fractions were evaporated under reduced pressure: ^1H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 3 H), 1.27 (t, 3 H), 2.19−2.45 (m, 4 H), 3.11 (s, 1 H), 4.04 (m, 2 H), 4.18 (q, 2 H), 4.45 (m, 2 H), 7.51 (d, J = 8.0 Hz, 1 H), 7.65 (d, J = 8.0 Hz, 1 H), 8.09 (s, 1 H); FABHRMS calcd for $C_{19}H_{21}NO_5$ 343.1420, found 343.1436. Anal. Calcd for $C_{19}H_{21}NO_5$.HOAc: C, 62.52; H, 6.25; N, 3.47. Found: C, 62.27; H, 5.89; N, 3.26.

Diethyl 2-[2,3-Dihydro-5-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl]ethynyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (56). To a solution of 2-pivaloyl-7-iodo-7-deaazaguanine (**52**, 360 mg, 1 mmol) in 5 mL of DMF were added diethyl 2-(2,3-dihydro-6-ethynyl-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**55**, 330 mg, 0.98 mmol), CuI (38 mg), triethylamine (0.3 mL), and $Pd(PPh_3)_4$ (100 mg). The mixture was stirred at rt overnight. The solvent was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column (9% MeOH in CH_2Cl_2 as eluent). The fractions containing product were again chromatographed, and the combined fractions were evaporated under reduced pressure to give **56** as a yellow solid (335 mg, 59%): ^1H NMR (300 MHz, DMSO-d_6) δ 1.11 (t, 3 H), 1.19 (t, 3 H), 1.26 (s, 9 H), 2.25−2.45 (m, 4 H), 3.95 (m, 2 H), 4.14 (q, 2 H), 4.51 (d, J =

4 Hz, 2 H), 4.91 (m, 1 H), 7.69 (m, 3 H), 10.90 (s, 1 H); FABHRMS calcd for $C_{30}H_{33}N_5O_7$ 575.2380, found 575.2378.

Diethyl 2-(2,3-Dihydro-6-ethenyl-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (57). Method A. Diethyl 2-(2,3-dihydro-6-iodo-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**28**, 200 mg, 0.45 mmol), (tributylvinyl)tin (315 mg, 1 mmol), and bis(triphenyl)-phosphinepalladium chloride (10 mg) were refluxed for 2.5 h under argon in toluene (10 mL). The mixture was evaporated to dryness in vacuo and chromatographed with silica gel (20/1 CH_2Cl_2/MeOH) to afford 130 mg (84%) of **57** as a white solid: mp 64−66 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.20 (t, 3 H), 1.27 (t, 3 H), 2.12−2.55 (m, 4 H), 3.95−4.12 (m, 2 H), 4.20 (q, 2 H), 4.39 (d, J = 16.8 Hz, 1 H), 4.63 (d, J = 16.8 Hz, 1 H), 5.09−5.15 (m, 1 H), 5.34 (d, J = 10.9 Hz, 1 H), 5.86 (d, J = 17.5 Hz, 1 H), 6.80 (dd, J_1 = 10.9 Hz, J_2 = 17.5 Hz, 1 H), 7.42 (d, J = 7.9 Hz, 1 H), 7.60 (d, J = 7.9 Hz, 1 H), 7.92 (s, 1 H). Anal. Calcd for $C_{19}H_{23}NO_5 \cdot 0.5CH_2Cl_2$: C, 60.39; H, 6.24; N, 3.61. Found: C, 60.47; H, 6.65; N, 3.19.

Method B. To a solution of 205 mg (0.61 mmol) of diethyl 2-(2,3-dihydro-6-hydroxy-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**25**) in 4 mL of CH_2Cl_2 was added 0.1 mL (0.73 mmol, 1.2 equiv) of collidine and 0.12 mL (0.73 mmol, 1.2 equiv) of triflic anhydride at −78 °C. The reaction mixture was stirred for 30 min, quenched with 5 mL of water, and diluted with 30 mL of CH_2Cl_2. The two layers were separated, the aqueous layer was back-extracted with 15 mL of CH_2Cl_2, and the combined organic extracts were washed with 10 mL of brine and dried over $MgSO_4$. Removal of solvent under reduced pressure gave a crude product that was purified by silica gel chromatography (eluting with 7:3 hexanes/EtOAc) to give 252 mg (89%) of **diethyl 2-[2,3-dihydro-6-[(trifluoromethanesulfonyl)oxy]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate** as a red oil: IR (neat) 2984, 1737, 1701, 1424, 1215 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (t, J = 7.3 Hz, 3 H), 1.28 (t, J = 7.3 Hz, 3 H), 2.10−2.50 (m, 4 H), 3.95−4.10 (m, 2 H), 4.21 (q, 2 H), 4.45 (d, J = 17.1 Hz, 1 H), 4.72 (d, J = 17.1 Hz, 1 H), 5.08−5.13 (m, 1 H), 7.49 (dd, J_1 = 2.3 Hz, J_2 = 8.2 Hz, 1 H), 7.58 (d, J = 8.6 Hz, 1 H), 7.77 (d, J = 2.3 Hz, 1 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 13.7, 13.8, 24.7, 30.6, 46.6, 53.4, 60.4, 61.5, 116.6, 118.4, 124.7, 124.8, 133.84, 141.3, 149.2, 166.9, 170.0, 171.9. Anal. Calcd for $C_{18}H_{20}F_3NO_8S$: C, 46.25; H, 4.31; N, 3.10. Found: C, 46.54; H, 4.24; N, 3.13. To a solution of 117 mg (0.25 mmol) of the above triflate in 3 mL of dioxane were added $Pd(PPh_3)_4$ (15 mg, 0.013 mmol, 0.05 equiv) and LiCl (32 mg, 0.75 mmol, 3.0 equiv). The reaction mixture was stirred for 5 min at rt, followed by addition of 0.1 mL (0.35 mmol, 1.4 equiv) of tributylvinyl tin. The reaction mixture was refluxed for 3 h, the solvent was removed under reduced pressure, and the residual solid was purified by silica gel chromatography (eluting with 30% EtOAc in hexane) to give 67 mg (78%) of **57**, mp 64−66 °C, identical in all respects with **57** prepared by method A.

2-Pivaloyl-7-ethenyl-7-deazaguanine (59). 2-Pivaloyl-7-iodo-7-deazaguanine (**52,** 1.44 g, 4 mmol), (tributylvinyl)tin (2.52 g, 8 mmol) and bis(triphenyl)phosphine palladium chloride (30 mg) were heated to 80 °C in dry DMF (10 mL) for 3 h under argon. The mixture was evaporated to dryness under reduced pressure, and the residual solid was purified by chromatography with silica gel (10% MeOH in CH_2Cl_2) to afford **59** as a gray solid (800 mg, 77%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (s, 9 H), 5.06 (d, J = 10 Hz, 1 H), 6.18 (d, J = 17.5 Hz, 1 H), 6.76 (dd, J_1 = 10 Hz, J_2 = 17.5 Hz, 1 H), 7.15 (s, 1 H), 10.84 (br, 1 H). Anal. Calcd for $C_{13}H_{16}N_4O_2 \cdot 0.3H_2O$: C, 58.77; H, 6.30; N, 21.09. Found: C, 59.11; H, 6.07; N, 20.67.

Diethyl 2-[2,3-Dihydro-6-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethenyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (58). Method A. To a solution of 2-pivaloyl-7-iodo-7-deazaguanine (**52**, 108 mg, 0.3 mmol) in 3 mL of DMF were added diethyl 2-(2,3-dihydro-6-ethenyl-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**57**, 103.5 mg, 0.3 mmol), triethylamine (0.084 mL), tri(*o*-tolyl)phosphine (38 mg), and palladium acetate (7.2 mg). The mixture was stirred at 80 °C overnight under argon. The solvent was evaporated under reduced pressure, and the residue was purified by chromatography on a silica gel column (5% MeOH in CH_2Cl_2 as eluent). The fractions containing the product were again chromatographed to give **58** (95 mg, 55%) as a gray solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, 3 H), 1.26 (t, 3 H), 1.34 (s, 9 H), 2.21−2.45 (m, 4 H), 4.05 (m, 2 H), 4.18 (q, 2 H), 4.42−4.56 (q, J = 16.5 Hz, 2 H), 5.07 (m, 1 H), 7.08 (s, 1 H), 7.28 (d, J = 14 Hz, 1 H), 7.35 (d, J = 4 Hz, 1 H), 7.62 (d, J = 14 Hz, 1 H), 7.69 (d, J = 4 Hz, 1 H), 7.91 (s, 1 H); FABHRMS calcd for $C_{30}H_{35}N_5O_7$ 577.2536, found 577.2513.

Method B. To a solution of 2-pivaloyl-7-ethenyl-7-deazaguanine (**59**, 92 mg, 0.35 mmol) in 3 mL of DMF were added diethyl 2-(2,3-dihydro-6-iodo-1-oxo-2(1*H*)-isoindolyl)-L-glutarate (**28**, 178 mg, 0.4 mmol), triethylamine (0.1 mL), $Pd(PPh_3)_4$ (30 mg), and CuI (10 mg). The mixture was stirred at 100 °C overnight under argon. The solvent was evaporated under reduced pressure, and the residue was purified by chromatography on a silica gel column (10% MeOH in CH_2Cl_2 as eluent) to give **58** (65 mg, 32%) as a gray solid, identical (TLC and ^{1}H NMR) with the product prepared from **57** as described above.

Diethyl 2-[2,3-Dihydro-6-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (60). Diethyl 2-[2,3-dihydro-6-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethenyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutarate (**58**, 70 mg, 0.12 mmol) was dissolved in 50 mL of 1:1 CH_2Cl_2/MeOH, Pd−C (5%, 50 mg) was added, and the mixture was hydrogenated at 50 psi for 3 h. The palladium catalyst was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified on a silica gel column, using 5% CH_2Cl_2/MeOH as eluent. Evaporation then yielded **60** (65 mg, 93%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (t, 3 H), 1.24 (t, 3 H), 1.32 (s, 9 H), 2.21−2.45 (m, 4 H), 2.90 (t, J = 6.5 Hz, 2 H), 3.15 (t, J = 6.5 Hz, 2 H), 4.04 (m, 2 H), 4.20 (q, 2 H), 4.40 (d, J =16 Hz, 1 H), 4.61 (d, J = 16 Hz, 1H), 5.11 (m, 1 H), 7.11 (s, 1 H), 7.40 (d, J = 6 Hz, 1 H), 7.63 (d, J = 6 Hz, 1 H), 8.11 (s, 1 H); FABHRMS cacld for $C_{30}H_{37}N_5O_7$ 579.2693, found 579.2672. Hydrogenation of **56** under the same conditions gave **60** (90% yield) identical (TLC, ^{1}H NMR) with **60** prepared by reduction of **58** as described above.

2-[2,3-Dihydro-6-[2-(2-amino-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]-1-oxo-2(1*H*)-isoindolyl]-L-glutaric Acid (13). A suspension of diethyl 2-[2,3-dihydro-6-[2-[2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl]ethyl]-1-oxo-2-(1*H*)-isoindolyl]-L-glutarate (**60**, 60 mg, 0.1 mmol) in 2 mL of 1 N NaOH was stirred at rt for 3 days. The resulting clear solution was acidified with 1 N HCl to pH 4, and the precipitate was collected by filtration, washed with H_2O (2 × 5 mL), and dried under reduced pressure to give 25 mg (55%) of **13**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21−2.45 (m, 4 H), 2.77 (t, J = 6.5 Hz, 2 H), 3.12 (t, J = 6.5 Hz, 2 H), 4.45 (s, 2 H), 4.82 (m, 1 H), 6.70 (s, 1 H), 7.51 (m, 3 H). Anal. Calcd for $C_{21}H_{21}N_5O_6 \cdot HCl$: C, 53.00; H, 4.66; N, 14.72. Found: C, 52.93; H, 4.95; N, 15.08.

Acknowledgment. We are deeply indebted to Eli Lilly & Company, Indianapolis, IN, for support of this work and for the *in vitro* cell growth inhibition evaluations.

JO9703459