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Synthesis of 10-Substituted "Open-Chain" Analogues of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF, Lometrexol)

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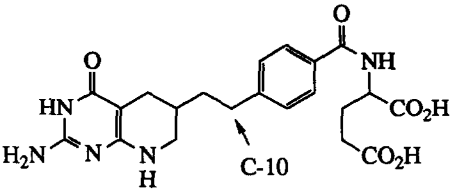
Abstract: *Several novel and very potent folate antimetabolites, structurally based upon our previously described "open-chain" version of DDATHF but carrying 1-carbon substituents in the 10-position, have been synthesized. A key synthetic sequence involving a palladium-catalyzed C-C coupling reaction, oxymercuration, and Wittig olefination constitutes a new route to α-branched 4-styrene carboxylic acids. Classical construction of the pyrimidine ring from the key intermediate **6** followed by glutamate coupling furnished **12**, which upon hydrolysis furnished the 10-methenyl derivative **13**. The 10-methenyl functionality in **12** was further modified to afford the 10-methyl-, 10-hydroxymethyl- and 10-dihydroxyboromethyl derivatives **22**, **3** and **25** respectively; double bond isomerization led to the 10-methyl-9,10-didehydro analog **20**. Preliminary in vitro cell culture screening showed that many of these "open-chain" analogs rivaled DDATHF itself as cytotoxic agents, and were about ten times more active than the parent "open-chain" DDATHF analog lacking a C-10 substituent. Surprisingly, however, compounds **13** and **22** were inactive in vivo.*

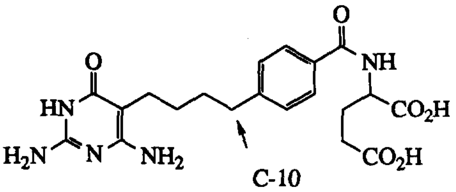
INTRODUCTION

The design and synthesis of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (**1**, DDATHF) in 1985 by Taylor and coworkers[1] represents a potential breakthrough in antifolate cancer chemotherapy. DDATHF exhibits broad and selective antitumor activity, and has been shown to inhibit glycinamide ribonucleotide formyltransferase (*GARTF)*, an enzyme that catalyzes the first of two formyl transfer reactions in *de novo* purine biosynthesis. Because DDATHF is not a DHFR inhibitor, it is fully effective against tumors resistant to Methotrexate. DDATHF is currently in Phase I clinical trials.

Over the past four years, many DDATHF analogs have been synthesized in an attempt to uncover the structural requirements for GARFT inhibition. These structure-activity studies have led, *inter alia*, to the synthesis of the so-called "open chain" analog **2** of DDATHF[2] in which the C-7 methylene carbon has been deleted, thereby eliminating C-6 chirality, yet retaining almost complete cytotoxicity. Introduction of carbon substituents at C-10 of DDATHF leads to significant increases in activity, perhaps because such analogs resemble more closely than DDATHF itself the natural cofactor for GARFT (10-formyl-5,6,7,8-tetrahydrofolic acid). The 10-hydroxymethyl derivative of DDATHF is, in fact, the most potent DDATHF analog yet known,[3] although as synthesized it consisted of a mixture of four diastereomers (only one of which is active).[4] The above considerations motivated us to pursue the synthesis of the 10-hydroxymethyl derivative **3** of the "open-chain" DDATHF analog **2**.



1, DDATHF

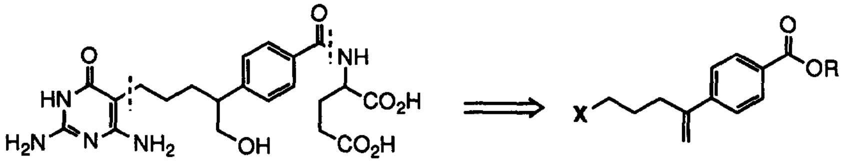


2, "Open-chain" DDATHF

We report in this paper a novel and highly efficient route to DDATHF "open-chain" analogs substituted in the 10-position by a C-1 substituent, as well as a new method for the synthesis of α-branched 4-styrene carboxylic acids.

RESULTS AND DISCUSSION

Retrosynthetic analysis suggested structure **A** as a key intermediate for the synthesis of the 10-hydroxymethyl analog **3** (Figure 1). Taylor and Harrington[2] have demonstrated that the requisite pyrimidine ring can be constructed in a two-step sequence from such a precursor (alkylation of ethyl cyanoacetate followed by ring closure with guanidine); glutamate coupling with the aromatic carboxylic acid would then complete the formal synthesis. We approached the synthesis of the potential precursor **A** as follows:



3 **A**

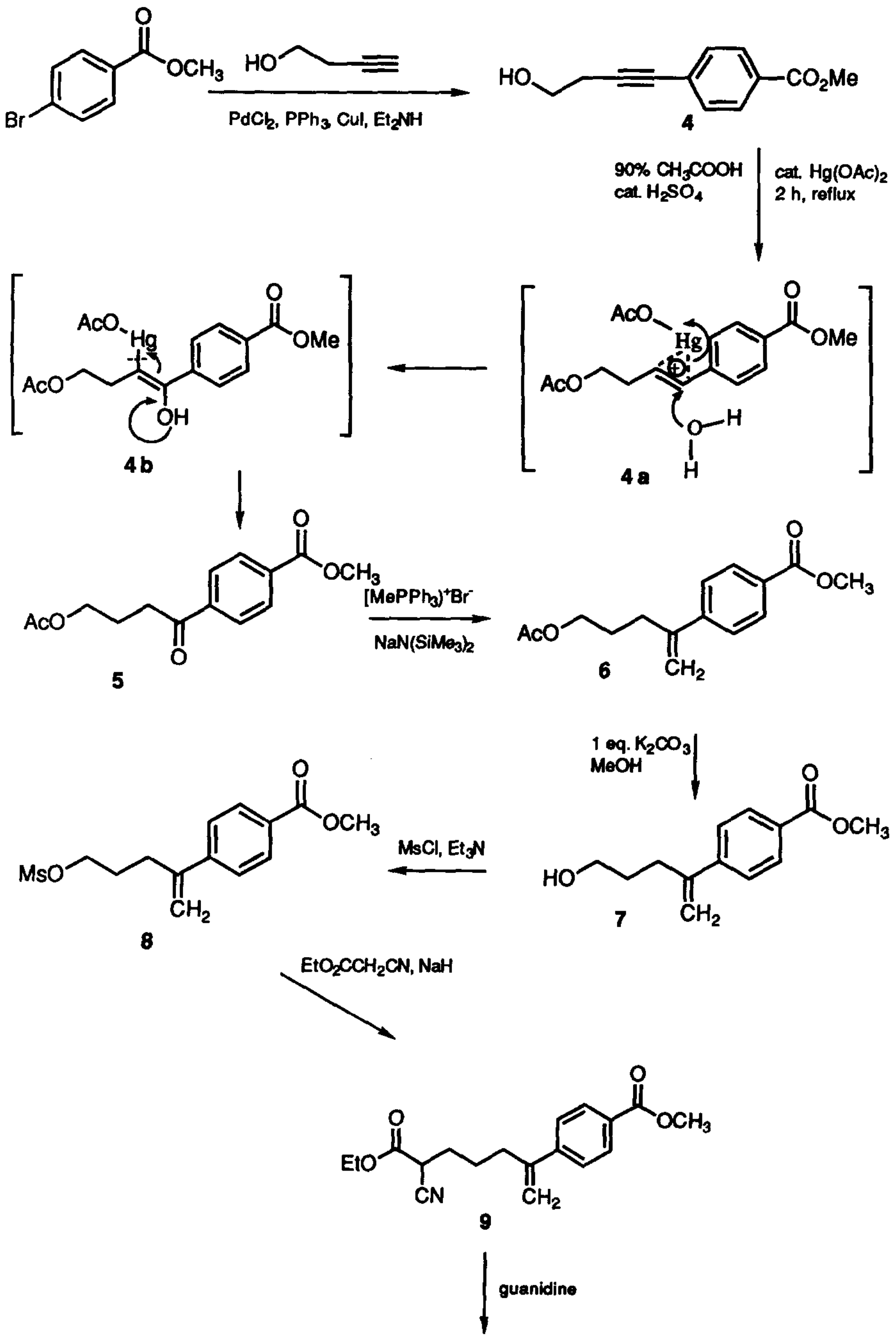
X = leaving group , R = alkyl

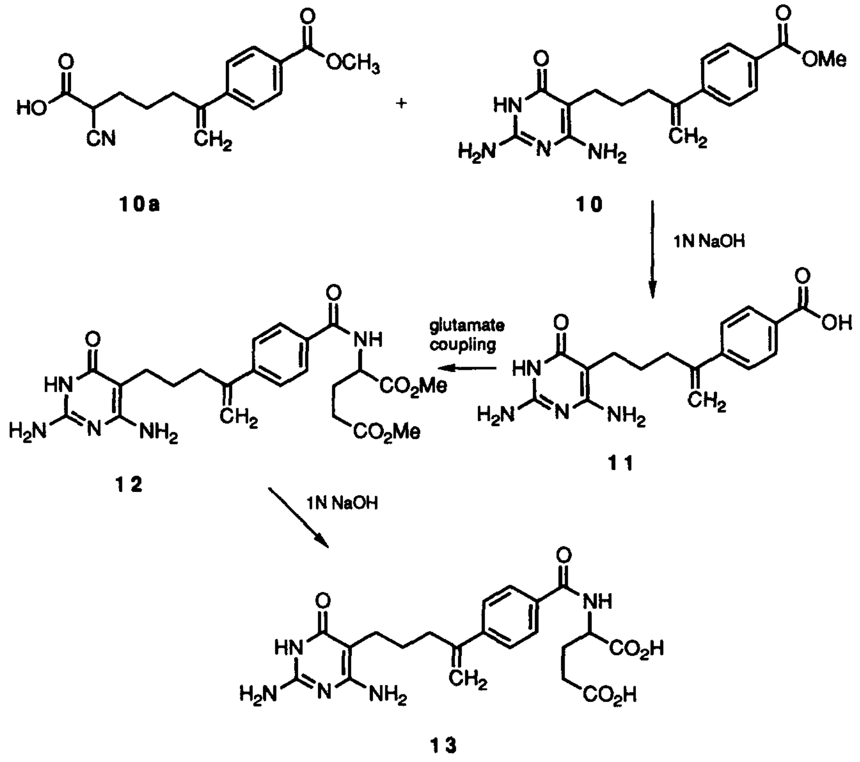
A palladium-catalyzed coupling of 3-butyn-1-ol with methyl 4-bromobenzoate gave the alcohol **4** (83%),[2] which underwent mercury-catalyzed hydration of the triple bond to furnish **5** in 61% yield (Scheme 1). Complete regioselectivity was observed, with none of the isomeric β-carbonyl product detected. This result is in accord with the proposed mechanism for this hydration[5] in which the positive charge in the intermediate mercuronium acetate (**4a**) is preferentially stabilized in the benzylic position so that nucleophilic attack by water leads to the enol **4b**. Mercury-carbon cleavage then gives the aralkyl ketone. An important feature of this reaction is simultaneous O-acetylation, giving the protected intermediate **5** for the subsequent Wittig olefination step (*vida infra*).

Clean conversion of **5** to the methenyl derivative **6** (76.7%) was achieved at room temperature by use of methyltriphenylphosphonium bromide and sodium hexamethyldisilazide in anhydrous THF.[6] The only detectable byproduct was a small amount of an ester (**6a**; see Experimental) formed by transesterification of **6** with some deacetylated **6** formed under the strongly basic conditions of the Wittig reaction.

In an equimolar version of Rapoport's deacetylation reaction[7] using potassium carbonate in anhydrous methanol, the O-acetyl group in **6** was selectively removed without (apparent) cleavage of the methyl ester,

Scheme I


PdCl2, PPh3, CuI, Et2NH
4
90% CH3COOH
cat. H2SO4
cat. Hg(OAc)2
2 h, reflux
4 a
4 b
5
[MePPh3]+Br-
NaN(SiMe3)2
6
1 eq. K2CO3
MeOH
7
MsCl, Et3N
8
EtO2CCH2CN, NaH
9
guanidine



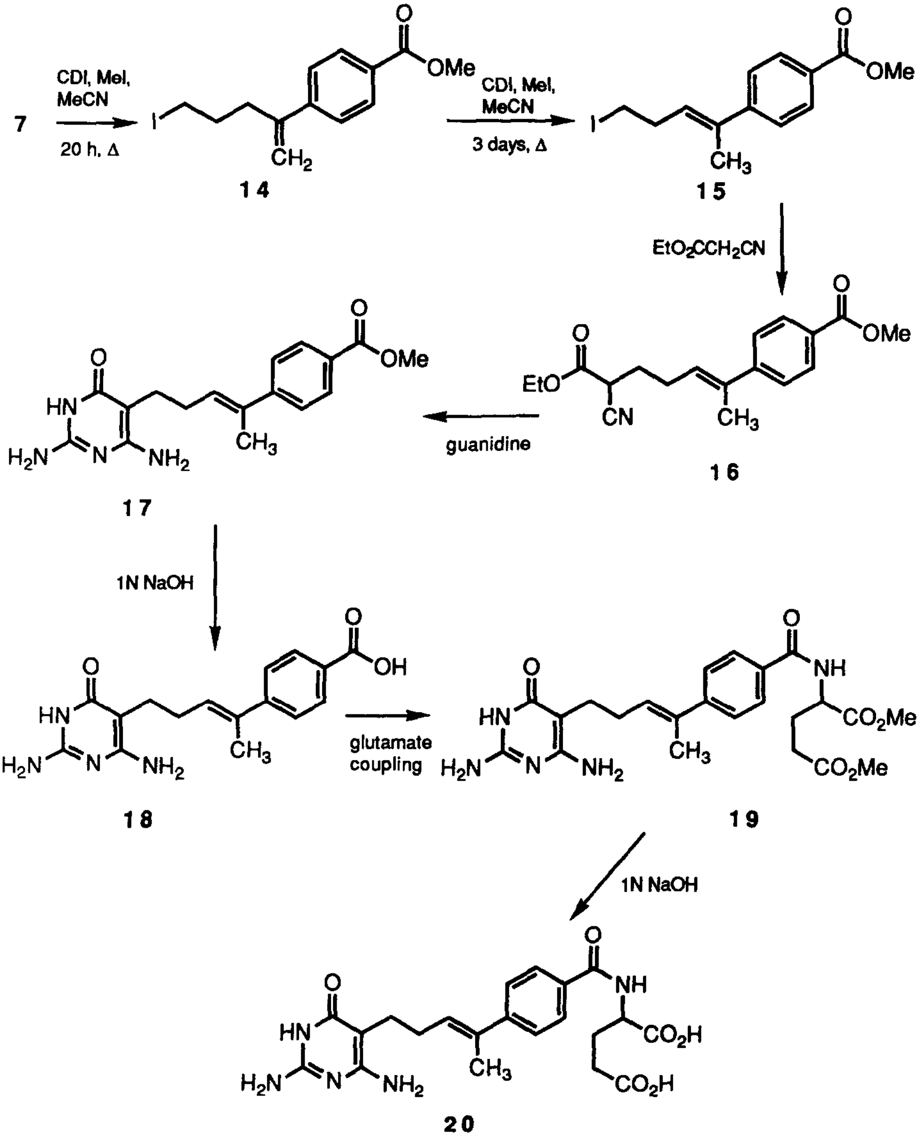
giving **7** in quantitative yield. This simple procedure is strongly recommended for the selective "saponification" of esters (other than methyl esters) in the presence of a methyl ester (which is, of course, also hydrolyzed, but is reformed under the reaction conditions). For example, the oxymercuration product **5** was smoothly converted to its deacylated derivative under the same conditions. It should be noted that the reverse process - methyl ester cleavage with retention of the O-acetyl group - can be effected by heating **6** in acetic acid.

The terminal hydroxyl group of **7** was converted to its mesylate **8** in quantitative yield. Alkylation of the sodium salt of ethyl cyanoacetate with **8** proceeded smoothly to give **9** (67%). Cyclization with the free base of guanidine in refluxing methanol then gave the desired pyrimidine intermediate **10** (67%), along with some deesterified starting material (**10a**). [Deesterification of esters with guanidine, leading to N-alkylguanidines and the carboxylic acid, is well known]. For unknown reasons, the guanidine ring annulation reaction is extremely concentration-dependent, with more concentrated reaction mixtures leading to dramatically improved yields (10% to 67% - see Experimental).

Alkaline cleavage of the benzoate ester **10**, followed by glutamate coupling of the resulting carboxylic acid **11** using 2,4-dimethoxy-6-chloro-1,3,5-triazine/N-methylmorpholine as the coupling agent,[8] gave **12** (70%), a potentially versatile precursor for a number of target 10-substituted "open-chain" DDATHF analogs. Final hydrolyis of **12** with 1 N aqueous sodium hydroxide followed by acidification with acetic acid yielded the glutamic acid derivative **13**. The 10-methenyl "open-chain" analog **13** was thus obtained in an overall yield of 8.0% over 11 steps.

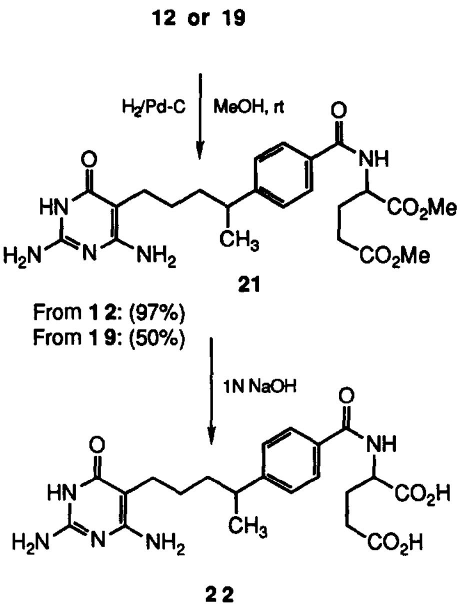
An alternate route to the intermediate **10** by direct alkylation of 2,4-diamino-6(1H)-pyrimidinone with the terminal primary iodide **14** was also considered. This latter compound was prepared in one step from **7** using the indirect iodination procedure of Kamijo.[9] Although the alkylation reaction gave a complex mixture of products, which resulted in abandonment of this alternate synthetic strategy, it was fortuitously observed that prolonged heating of the above iodination reaction mixture (carbonyl diimidazole and methyl iodide in acetonitrile) led to smooth double bond isomerization to give the 9,10-dehydro derivative **15** in 91% yield (97% E). The 10-methyl-9,10-dehydro "open-chain" DDATHF analog **20** was then prepared from **15** as summarized in Scheme 2. Hydrogenation of **12** proceeded smoothly at 50 psi of hydrogen in methanol solution in the

Scheme 2



presence of Pd/C as catalyst to give the 10-methyl derivative **21** in 97% yield (Scheme 3). Compound **21** could also be prepared by catalytic reduction of the 9,10-dehydro derivative **19**, but much longer hydrogenation times were required, and yields were lower. Saponification of the glutamate esters in **21** with 1N NaOH then gave the 10-methyl "open-chain" analog **22**.

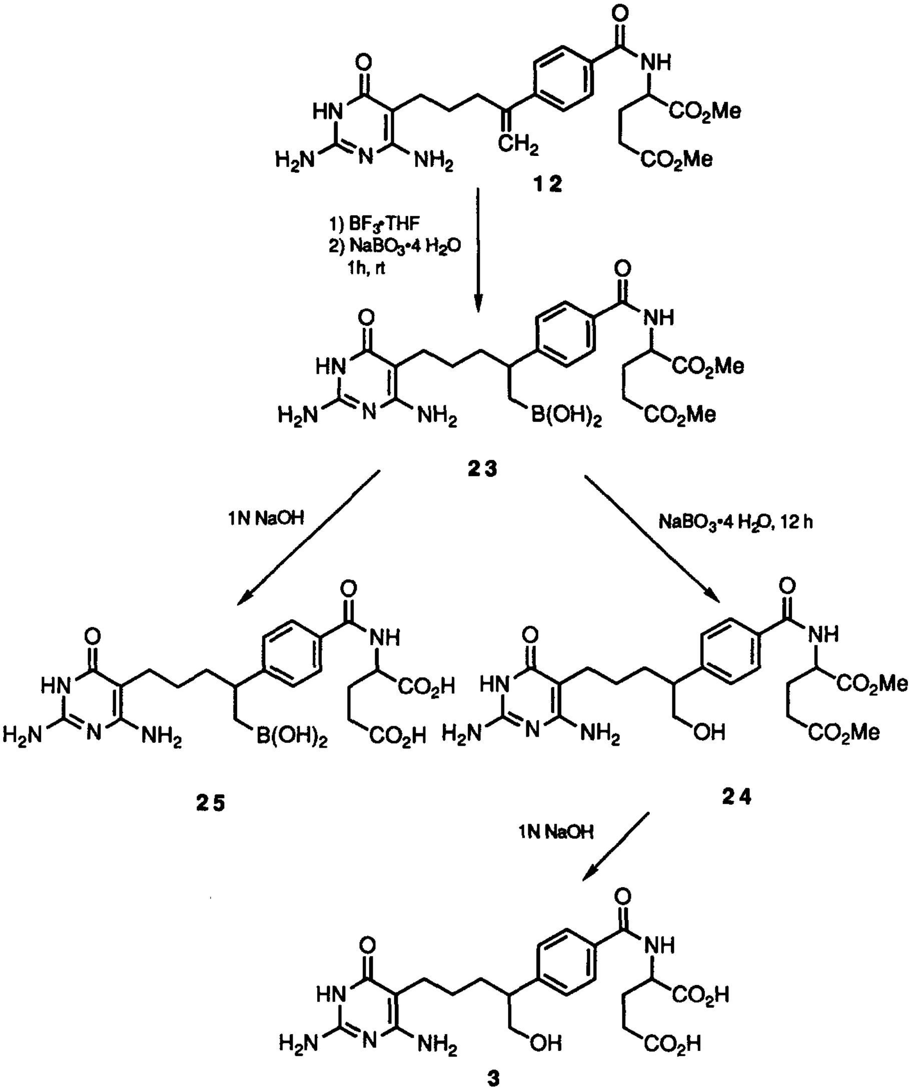
Scheme 3



Hydroboration of **12** with borane in THF yielded an intermediate borane adduct which was oxidatively cleaved with aqueous $NaBO_3$[10] (without hydrolysis of the glutamate esters) to give the 10-hydroxymethyl derivative **24** (51%) (Scheme 4). Despite the use of more than six equivalents of the borane reagent, only moderate yields (51%) of **24** could be obtained, presumably because of extensive chelation of borane with the pyrimidine ring in **12**. By contrast, hydroboration of the model compound **6** went to completion with only one equivalent of borane. Interruption of the hydroboration-perboration reaction one hour after addition of $NaBO_3$ gave a stable intermediate containing boron; continued stirring with $NaBO_3$ resulted in complete conversion to the 10-hydroxymethyl precursor **24**. On the basis of spectral and microanalytical data, we suggest that the borane-containing intermediate mentioned above is the boronic acid **23**. Mild saponification of **23** and **24** with 1N sodium hydroxide gave the glutamic acids **25** and **3** respectively.

The preparation of additional 10-substituted "open-chain" DDATHF analogs, such as the 10-azidomethyl-, 10-aminomethyl-, and 10-formyl- derivatives, from the methenyl intermediates **6**, **10** and **12** is currently in progress.

Scheme 4



BIOLOGICAL ACTIVITY

In vitro cell culture screening of the 10-methyl- (**22**), 10-hydroxymethyl (**3**) and 10- $CH_2B(OH)_2$ (**25**) derivatives (each as a 50:50 mixture of two diastereomers), and the 10-methenyl (**13**) and 9,10-dehydro-10-methyl- (**20**) analogs (which are single enantiomers) indicated that all but **20** were effective cytotoxic agents with IC_{50}-values of 0.006 mg/ml, 0.003 mg/ml, 0.036 μg/ml, 0.006 mg/ml, and 0.1 mg/ml respectively against human T-cell derived lymphoblastic leukemia (CCRF-CEM) cells (cf. DDATHF, with an IC_{50} value of 0.007 mg/ml). The dramatic decrease in activity resulting from introduction of a double bond into the bridge (e.g., **20**) confirms previous observations[11] that bridge rigidity decreases cytotoxic activity. Cell culture reversal studies performed on compounds **13** and **22** indicated that their cytotoxicity could be reversed by the addition of hypoxanthine (100 μM) and AICA (300 μM), but not by addition of thymidine (5 μM). Thus these open-chain DDATHF analogs, like DDATHF itself, inhibit purine *de novo* biosynthesis and are inactive against thymidylate synthase and dihydrofolate reductase. Very surprisingly, however, no *in vivo* activity was observed. Compounds **13** and **22** were tested against the 6C3HED lymphosarcoma murine tumor model up to 200 mg/kg and 100 mg/kg (ip, daily x 10) respectively, without any evidence of activity.

EXPERIMENTAL SECTION

Methyl 4-(4-Hydroxy-1-butynyl)benzoate (4). Compound **4** (138.9 g) was prepared from methyl 4-bromobenzoate (178.6 g) according to Taylor et al.[2] The yield was improved by stirring for 3 days at rt, extracting with diethyl ether and filtering over silica gel, eluting with ethyl acetate/hexanes (1:1), to obtain 82.8% of pure **4** as a light yellow solid.

Methyl 4-(4-Acetoxy-1-oxobutyl)benzoate (5). A 500 mL flask equipped with a gas inlet and reflux condenser was charged with 10.0 g (1.0 eq) of **4** in 200 mL of 90% aqueous acetic acid, 2.4 g (0.15 eq) of mercuric acetate and 1.0 mL of concentrated sulfuric acid. The reaction mixture was refluxed for 2 h under a nitrogen atmosphere (oil bath temperature ~140 °C). After cooling to rt, the precipitated mercury salts were filtered off, and the solution was extracted with CH_2Cl_2 (3x). The acidic organic extract was neutralized to pH 7 with saturated sodium bicarbonate solution, the organic layer was separated, and the aqueous layer subsequently extracted with CH_2Cl_2. The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to yield crude **5**. Purification was carried out by flash chromatography, eluting with ethyl acetate/hexanes (1:2), to afford 7.9 g (61%) of pure **5** as a colorless solid, mp 61-62 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.08 (s, 3H, $C\mathit{H}_3$-CO); 2.10 (quin, 2H, CH_2-$C\mathit{H}_2$-CH_2, J = 7.5 Hz); 3.12 (t, 2H, $C\mathit{H}_2$-CO, J = 7.5 Hz); 3.96 (s, 3H, $C\mathit{H}_3O$); 4.20 (t, 2H, $C\mathit{H}_2O$, J = 7.5 Hz); 8.02 (d, $2H_{arom}$, J = 9 Hz); 8.18 (d, $2H_{arom}$, J = 9 Hz). HRMS, found 233.0812 (M^+ - CH_3O), calcd for $C_{13}H_{13}O_5$ 233.0814. Anal. Calcd for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10; O, 30.27. Found: C, 63.69; H, 5.89; O, 29.99.

Methyl 4-(5-Acetoxypent-1-en-2-yl)benzoate (6). To a suspension of 90 g (1.1 eq) of methyl triphenylphosphonium bromide in 1.5 L of anhydrous THF was added 240 mL of 1.0 M solution of sodium hexamethyldisilazide (1.05 eq) in THF. The mixture was stirred at rt for 1 h to ensure complete formation of the bright yellow ylid. A solution of 60 g (1.0 eq) of **5** in 120 mL of anhydrous THF was added dropwise, and the reaction mixture was stirred for 2 h at rt. After filtration of the precipitated sodium bromide/triphenylphosphine oxide, the resulting THF solution was washed with water. The aqueous layer was extracted with diethyl ether (3x), and the combined organic layers were dried over $MgSO_4$ and evaporated to dryness. Flash chromatography, eluting with ethyl acetate/hexanes (1:10), yielded 45.7 g (76.7%) of **6** as a colorless oil, and 1.6 g of an ester **6a** (2%) as a yellow solid: mp 32 °C; (**6**): ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.80 (quin, 2H, CH_2-$C\mathit{H}_2$-CH_2, J = 7.5 Hz); 2.04 (s, 3H, $C\mathit{H}_3$-CO); 2.62 (t, 2H, $C\mathit{H}_2$-C=, J = 7.5 Hz); 3.94 (s, 3H, $C\mathit{H}_3O$); 4.10 (t, 2H, $C\mathit{H}_2O$, J = 7.5 Hz); 5.20 (s, $1H_{olef}$); 5.41 (s, $1H_{olef}$); 7.48 (d, $2H_{arom}$, J = 9 Hz); 8.01 (d, $2H_{arom}$, J = 9 Hz). HRMS, found 231.1020 (M^+ - CH_3O), calcd for $C_{14}H_{15}O_3$ 231.1021. Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.69; H, 6.92. Found: C, 68.90; H, 7.14. (**6a**): ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.80 (quin, 2H, $[CH_2\text{-}C\mathit{H}_2\text{-}CH_2]_a$, J = 7.5 Hz); 1.95 (quin, 2H, $[CH_2\text{-}C\mathit{H}_2\text{-}CH_2]_b$, J = 7.5 Hz); 2.04 (s, 3H, $[C\mathit{H}_3\text{-}CO]_b$); 2.62 (t, 2H, $[C\mathit{H}_2\text{-}C{=}]_a$, J = 7.5 Hz); 2.70 (t, 2H, $[C\mathit{H}_2\text{-}C{=}]_b$, J = 7.5 Hz); 3.94 (s, 3H, $[C\mathit{H}_3O]_a$); 4.10 (t, 2H, $[C\mathit{H}_2O]_a$, J = 7.5 Hz); 4.38 (t, 2H, $[C\mathit{H}_2O]_b$, J = 7.5 Hz); 5.20 (s, $[1H_{olef}]_a$); 5.24 (s, $[1H_{olef}]_b$); 5.41 (s, $[1H_{olef}]_a$); 5.43 (s, $[1H_{olef}]_b$); 7.45-7.53 (m, $[2H_{arom}]_a$ + $[2H_{arom}]_b$, J= 9 Hz); 7.96-8.07 (m, $[2H_{arom}]_a$ + $[2H_{arom}]_b$, J= 9 Hz). HRMS, found 450.2015 (M^+), calcd for $C_{27}H_{30}O_6$ 450.2042.

Methyl 4-(5-Hydroxypent-1-en-2-yl)benzoate (7). To a solution of 12.5 g (1.0 eq) of dry potassium carbonate in 2 L of anhydrous methanol was added a solution of 24.0 g (1.0 eq) of **6** in 500 mL of anhydrous methanol. The reaction mixture was stirred at rt for 2 h, and 182.4 mL of 1 N HCl (2.0 eq) was then added.

The resulting neutral solution was extracted with CH$_2$Cl$_2$ (2x), and the organic extracts were dried over MgSO$_4$ and concentrated under reduced pressure to afford 20.0 g of pure **7** (99.2%) as a colorless solid, mp 28-30 °C; ^{1}H-NMR (CDCl$_3$, 300 MHz): δ 1.56 (s (broad), 1H, O*H*); 1.75 (quin, 2H, CH$_2$-C*H*$_2$-CH$_2$, *J* = 7.5 Hz); 2.60 (t, 2H, C*H*$_2$-C=, *J* = 7.5 Hz); 3.68 (t (broad), 2H, C*H*$_2$OH, *J* = 7.5 Hz); 3.94 (s, 3H, C*H*$_3$O); 5.20 (s, 1H$_{olef}$); 5.42 (s, 1H$_{olef}$); 7.48 (d, 2H$_{arom}$, *J* = 9 Hz); 8.00 (d, 2H$_{arom}$, *J* = 9 Hz). HRMS, found 220.1094 (M$^+$), calcd 220.1099. Anal. Calcd for C$_{13}$H$_{16}$O$_3$: C, 70.89; H, 7.32. Found: C, 70.62; H, 7.29.

Methyl 4-(5-Methylsulfonyloxypent-1-en-2-yl)benzoate (8). A solution of mesyl chloride (19.6 g, 1.25 eq) in 500 mL of anhydrous diethyl ether was added dropwise to a solution of **7** (30.0 g, 1.0 eq) and triethylamine (17.4 g, 1.25 eq) in anhydrous diethyl ether at 0 °C (ice bath). After 15 min, the ice bath was removed and the mixture was stirred under nitrogen for 4 h at rt. The precipitated triethylamine hydrochloride was extracted with saturated ammonium chloride solution and the aqueous layer was subsequently washed with diethyl ether (3x). The combined organic layers were dried over MgSO$_4$ and concentrated under reduced pressure to afford 40.6 g (quantitative) of **8** as a colorless oil; IR (NaCl) 2950, 1720, 1610, 1440, 1350, 1280, 1180, 1120, 970, 840, 790, 730 cm^{-1}; ^{1}H-NMR (CDCl$_3$, 300 MHz): δ 1.92 (quin, 2H, CH$_2$-C*H*$_2$-CH$_2$, *J* = 7.5 Hz); 2.70 (t, 2H, C*H*$_2$-C=, *J* = 7.5 Hz); 3.02 (s, 3H, C*H*$_3$-SO$_3$); 3.96 (s, 3H, C*H*$_3$O); 4.24 (t, 2H, C*H*$_2$O, *J* = 7.5 Hz); 5.22 (s, 1H$_{olef}$); 5.44 (s, 1H$_{olef}$); 7.48 (d, 2H$_{arom}$, *J* = 9 Hz); 8.01 (d, 2H$_{arom}$, *J* = 9 Hz). HRMS, found 267.0683 (M$^+$ - CH$_3$O), calcd for C$_{13}$H$_{15}$O$_4$S 267.0691.

Methyl 4-(6-Carboethoxy-6-cyanohex-1-en-2-yl)benzoate (9). A 1 L three-necked round-bottomed flask equipped with a reflux condenser, addition funnel, and gas inlet was torched dry, cooled to rt, and charged with 21.1 g (5.0 eq) of 80% sodium hydride in 500 mL of anhydrous THF. The mixture was brought to 0 °C (ice bath) and an anhydrous THF solution of 82.5 mL (5.5 eq) of ethyl cyanoacetate was added dropwise under a nitrogen atmosphere. The mixture was stirred vigorously while warming up to rt until hydrogen evolution was no longer observed (30 min). After addition of 42.0 g (1.0 eq) of **8** in 200 mL of anhydrous THF, the reaction mixture was refluxed for 36 h. Saturated ammonium chloride solution (1 L) was then added and the mixture was extracted with diethyl ether (3x). The organic extracts were dried over MgSO$_4$ and concentrated under reduced pressure. Excess ethyl cyanoacetate was completely removed by Kugelrohr distillation at 60 °C (1.0 mm Hg) (ethyl cyanoacetate shows the same R$_f$ value as the product). Flash chromatography, eluting with ethyl acetate/hexanes (1:3), yielded 30.0 g (67.5%) of pure **9** as a colorless oil; ^{1}H-NMR (CDCl$_3$, 300 MHz): δ 1.28 (t, 3H, C*H*$_3$-CH$_2$O, *J* = 7.5 Hz); 1.64 (quin, 2H, CH$_2$-C*H*$_2$-CH$_2$, *J* = 7.5 Hz); 1.96 (dt, 2H, C*H*$_2$-CH, *J* = 7.5 Hz); 2.60 (t, 2H, C*H*$_2$-C=, *J* = 7.5 Hz); 3.44 (t, 1H, C*H*-CN, *J* = 7.5 Hz); 3.92 (s, 3H, C*H*$_3$O); 4.22 (q, 2H, CH$_3$-C*H*$_2$O, *J* = 7.5 Hz); 5.19 (s, 1H$_{olef}$); 5.41 (s, 1H$_{olef}$); 7.42 (d, 2H$_{arom}$, *J* = 9 Hz); 8.00 (d, 2H$_{arom}$, *J* = 9 Hz). HRMS, found 315.1458 (M$^+$), calcd for C$_{18}$H$_{21}$O$_4$N 315.1470.

Methyl 4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-1-en-2-yl]benzoate (10). A 250 mL round-bottomed flask equipped with a gas inlet, a reflux condenser, and an addition funnel was charged with 21.7 mL of 25% sodium methoxide solution (2.0 eq) in 50 mL of anhydrous methanol, followed by addition of 5.76 g (60.3 mmol, 1.25 eq) of dry guanidine hydrochloride. The reaction mixture was stirred at rt for 30 min. After addition of 15.2 g (48.2 mmol, 1.0 eq) of **9** in 50 mL of anhydrous methanol, the solution was refluxed for 12 h under a nitrogen atmosphere. The solution was cooled to rt and the excess sodium methoxide neutralized by addition of 2.7 mL (1.0 eq) of glacial acetic acid. The solution was then concentrated under reduced pressure and the resulting precipitate was filtered off, washed with cold chloroform/methanol (1:1), and dried to afford 10.65 g (67.3%) of pure **10** as a colorless solid, mp 127 °C; ^{1}H-NMR (DMSO-d^6, 300 MHz): δ = 1.40 (quin, 2H, CH$_2$-C*H*$_2$-CH$_2$, *J* = 7.5 Hz); 2.25 (t, 2H, C*H*$_2$-pyr, *J* = 7.5 Hz); 2.60 (t, 2H, C*H*$_2$-C=, *J* = 7.5 Hz); 3.84 (s, 3H, C*H*$_3$O); 5.10 (s, 1H$_{olef}$); 5.36 (s, 1H$_{olef}$); 5.62 (s (broad), 2H, N*H*$_2$); 5.93 (s (broad), 2H, N*H*$_2$); 7.53 (d, 2H$_{arom}$, *J* = 9 Hz); 7.84 (d, 2H$_{arom}$, *J* = 9 Hz); 9.86 (s (broad), 1H, N*H*). HRMS, found 328.1530 (M$^+$), calcd for C$_{17}$H$_{20}$N$_4$O$_3$ 328.1535.

The mother liquor was evaporated to dryness, and the byproduct (**10a**) was recrystallized from ethyl acetate, filtered, washed with ethyl acetate/diethyl ether, and dried under high vacuum to afford 3.5 g (25%) of pure **10a**, mp 98 °C; ^{1}H-NMR (DMSO-d^6, 300 MHz): δ 1.54 (quin, 2H, CH$_2$-C*H*$_2$-CH$_2$, *J* = 7.5 Hz); 1.80 (dt, 2H, C*H*$_2$-CH, *J* = 7.5 Hz); 2.45 (t, 2H, C*H*$_2$-C=, *J* = 7.5 Hz); 3.22 (t, 1H, C*H*-CN, *J* = 7.5 Hz); 3.81 (s, 3H, C*H*$_3$O); 5.10 (s, 1H$_{olef}$); 5.33 (s, 1H$_{olef}$); 7.42 (d, 2H$_{arom}$, *J* = 9 Hz); 7.87 (d, 2H$_{arom}$, *J* = 9 Hz). HRMS, found 243.1265 (M$^+$- CO$_2$), calcd for C$_{15}$H$_{17}$N$_1$O$_2$ 243.1259.

4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-1-en-2-yl]benzoic Acid (11). A 250 mL round-bottomed flask was charged with 14.2 g (43.2 mmol, 1.0 eq) of **10** in 130 mL of 1 N aqueous NaOH (3.0 eq). The mixture was warmed to 60 °C for 2 h and was stirred at rt overnight. After removing the solid impurities by filtration, the clear filtrate was acidified with 8.2 mL (1.1 eq) of glacial acetic acid. The resulting precipitate was filtered and dried under high vacuum to afford 12.16 g (89.4%) of pure **11** as a colorless solid, mp 243 °C; ^{1}H-NMR (DMSO-d^6, 300 MHz): δ 1.40 (quin, 2H, CH_2-$C\mathit{H}_2$-CH_2, *J* = 7.5 Hz); 2.22 (t, 2H, $C\mathit{H}_2$-pyr, *J* = 7.5 Hz); 2.56 (t, 2H, $C\mathit{H}_2$-C=, *J* = 7.5 Hz); 5.14 (s, $1H_{olef}$); 5.39 (s, $1H_{olef}$); 5.68 (s (broad), 2H, $N\mathit{H}_2$); 5.92 (s (broad), 2H, $N\mathit{H}_2$); 7.53 (d, $2H_{arom}$, *J* = 9 Hz); 7.88 (d, $2H_{arom}$, *J* = 9 Hz); 9.82 (s (broad), 1H, N*H*). HRMS, found 314.1366 (M^+), calcd for $C_{16}H_{18}N_4O_3$ 314.1379.

Dimethyl *N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-1-en-2-yl]benzoyl}-*L*-glutamate (12). A 100 mL round-bottomed flask equipped with a gas inlet was charged with 6.0 g (12.7 mmol, 1.0 eq) of **11** and 2.51 g (2.73 mL, 24.8 mmol, 1.3 eq) of *N*-methylmorpholine in 50 mL of anhydrous DMF. The mixture was stirred vigorously at rt for 10 min, and 4.01 g (22.9 mmol, 1.2 eq) of 2,4-dimethoxy-6-chloro-1,3,5-triazine was then added neat. After 40 min of stirring at rt, 2.89 g (3.15 mL, 28.6 mmol, 1.5 eq) of *N*-methylmorpholine was introduced, followed by 236 mg (1.12 mmol, 1.3 eq) of dry dimethyl *L*-glutamate hydrochloride (the hydrochloride immediately dissolved; after 5 min triazinone precipitated from solution). Stirring was continued overnight at rt under a nitrogen atmosphere. After removing the solvent under reduced pressure (50 °C water bath, ~7 mm Hg), the crude oil was dissolved in chloroform and extracted with saturated aqueous sodium bicarbonate to remove unreacted acid, triazine and triazinone. The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness. Purification was carried out by flash chromatography, eluting with methanol/chloroform (1:10), to afford 6.26 g (69.6 %) of pure **12** as a colorless solid, mp 79-80 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.41 (quin, 2H, CH_2-$C\mathit{H}_2$-CH_2, *J* = 7.5 Hz); 2.00-2.55 (m, 8H, 4 x -$C\mathit{H}_2$-); 3.56 (s, 3H, $C\mathit{H}_3O$); 3.68 (s, 3H, $C\mathit{H}_3O$); 4.68 (dt, 1H, C*H*, *J*= 6, 7.5 Hz); 4.84 (s (broad), 2H, $N\mathit{H}_2$); 4.97 (s, $1H_{olef}$); 5.18 (s, $1H_{olef}$); 5.86 (s (broad), 2H, $N\mathit{H}_2$); 7.29 (d, $2H_{arom}$, *J* = 9 Hz); 7.62 (m, (broad), 1H, $N\mathit{H}_{glu}$); 7.64 (d, $2H_{arom}$, *J* = 9 Hz); 11.20 (s (broad), 1H, $N\mathit{H}_{pyr}$). HRMS, found 471.2081 (M^+), calcd for $C_{23}H_{29}N_5O_6$ 471.2118.

***N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-1-en-2-yl]benzoyl}-*L*-glutamic Acid (13).** A 10 mL round-bottomed flask was charged with 100 mg (0.212 mmol) of **12** dissolved in 1.0 mL of 1 N aqueous sodium hydroxide. The solution was stirred overnight at rt (after 1 h a clear solution was observed), and 0.12 mL (2.0 eq) of glacial acetic acid was then added dropwise (pH = 4.0) as the solution was stirred vigorously. The precipitate was filtered off and subsequently washed three times with 1 mL of cold water. The product was dried under high vacuum at rt to afford 67 mg (71.2%) of pure **13** as a colorless solid, mp 153 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 1.43 (quin, 2H, CH_2-$C\mathit{H}_2$-CH_2, *J* = 7.5 Hz); 1.85-2.15 (m, 2H, $C\mathit{H}_2$-CH, *J* = 7.5 Hz); 2.21 (t, 2H, $C\mathit{H}_2$-pyr, *J* = 7.5 Hz); 2.36 (dt, 2H, $C\mathit{H}_2$-CO_2H, *J* = 7.5 Hz); 2.56 (t, 2H, $C\mathit{H}_2$-C=, *J* = 7.5 Hz); 4.36 (dt, 1H, C*H*, *J* = 6, 7.5 Hz); 5.15 (s, $1H_{olef}$); 5.39 (s, $1H_{olef}$); 5.69 (s (broad), 2H, $N\mathit{H}_2$); 5.96 (s (broad), 2H, $N\mathit{H}_2$); 7.47 (d, $2H_{arom}$, *J* = 9 Hz); 7.83 (d, $2H_{arom}$, *J* = 9 Hz); 8.57 (d, 1H, $N\mathit{H}_{glu}$, *J* = 6 Hz); 9.84 (s (broad), 1H, $N\mathit{H}_{pyr}$); 12.65 (s (broad), 2H, 2 x $CO_2\mathit{H}$). HRMS (FAB), found 444.18695 (M^+ + H), calcd for $C_{21}H_{26}N_5O_6$ 444.1883.

Methyl 4-(5-Iodopent-1-en-2-yl)benzoate (14). A 250 mL round-bottomed flask equipped with a gas inlet was torched dry, cooled to rt and charged with 1.0 g (4.54 mmol) of **7** in 100 mL of anhydrous acetonitrile, 1.1 g (1.5 eq) of N,N'-carbonyl diimidazole (CDI), and 1.4 mL (5.0 eq) of methyl iodide. After stirring for thirty min at rt, the solution was refluxed for 20 h under a nitrogen atmosphere. The reaction mixture was quenched with saturated ammonium chloride solution and extracted with diethyl ether (3x). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was passed through a silica pad, eluting with ethyl acetate/hexanes (1:3), to afford 1.36 g (90.7%) of pure **14** as a colorless oil; ^{1}H-NMR ($CDCl_3$, 300 MHz); δ 1.95 (quin, 2H, CH_2-$C\mathit{H}_2$-CH_2, *J*= 7.5); 2.69 (t, 2H, $C\mathit{H}_2$-C=, *J*= 7.5Hz); 3.20 (t, 2H, $C\mathit{H}_2$-I, *J*= 7.5 Hz); 3.94 (s, 3H, $OC\mathit{H}_3$); 5.25 (s, $1H_{olef}$); 5.44 (s, $1H_{olef}$); 7.48 (d, $2H_{arom}$, *J*= 9 Hz); 8.02 (d, $2H_{arom}$, *J*= 9 Hz). HRMS, found 330.0122 (M^+), calcd for $C_{13}H_{15}O_2I$ 330.0119.

Methyl 4-(5-Iodopent-2-en-2-yl)benzoate (15). A 500 mL round-bottomed flask equipped with a gas inlet was charged with 4.4 g (20 mmol) of **7** in 350 mL of anhydrous acetonitrile, 4.9 g (1.5 eq) of N,N'-

carbonyl diimidazole (CDI), and 6.2 mL (5.0 eq) of methyl iodide. After stirring for thirty min at rt, the solution was refluxed for three days under a nitrogen atmosphere. The reaction mixture was quenched with saturated ammonium chloride solution and extracted with diethyl ether (3x). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was passed over a silica pad, eluting with ethyl acetate/hexanes (1:3), to afford 6.04 g (91.5%) of pure **15** as a colorless oil; ^1H-NMR ($CDCl_3$, 300 MHz): δ 2.07 (s, 3H, $=CH-CH_3$); 2.85 (dt, 2H, CH_2-CH=, J= 7.5 Hz); 3.25 (t, 2H, CH_2-I, J= 7.5 Hz); 3.92 (s, 3H, OCH_3); 5.83 (t, $1H_{olef}$, J= 7.5 Hz); 7.47 (d, $2H_{arom}$, J= 9); 8.00 (d, $2H_{arom}$, J= 9 Hz).

Methyl 4-(6-Carboethoxy-6-cyanohex-2-en-2-yl)benzoate (16). A 250 mL three-neck round-bottomed flask, equipped with a reflux condenser, addition funnel, and gas inlet, was torched dry, cooled to rt, and charged with 1.37g (5.0 eq) of 80% sodium hydride in 50 mL of anhydrous THF. The mixture was brought to 0 °C (ice bath) and an anhydrous THF solution of 5.32 mL (5.5 eq) of ethyl cyanoacetate was added dropwise under a nitrogen atmosphere. The mixture was stirred vigorously while warming to rt until hydrogen evolution was no longer observed (30 min). A solution of 3.0 g (9.09 mmol, 1.0 eq) of **15** in anhydrous THF was added, and the reaction mixture was refluxed for 18 h. The reaction mixture was quenched with saturated ammonium chloride solution and extracted with diethyl ether (3x). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford 6 g of crude oil containing 2-3 eq of ethyl cyanoacetate. Flash chromatography, eluting with ethyl acetate/hexanes (3:5), yielded 5.33 g of product containing 2.1 eq of ethyl cyanoacetate. The latter was removed by Kugelrohr distillation (40 °C, 1.0 mm Hg), and 1.3 g (45.3%) of pure **16** was obtained as a colorless oil; ^1H-NMR (300 MHz, $CDCl_3$): δ 1.21 (t, 3H, CH_3-CH_2O, J= 7.5 Hz); 1.99 (s, 3H, $=CH-CH_3$); 2.04 (dt, 2H, CH-CH_2, J= 7.5 Hz); 2.38 (dt, 2H, CH_2-CH=, J= 7.5 Hz); 3.56 (t, 1H, CH-CN, J= 7 Hz); 3.80 (s, 3H, OCH_3); 4.16 (q, 2H, OCH_2-CH_3, J= 7.5 Hz); 5.75 (t, 1H, CH=, J= 7.5 Hz); 7.35 (d, $2H_{arom}$, J= 9 Hz); 7.87 (d, $2H_{arom}$, J= 9 Hz).

Methyl 4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)pent-2-en-2-yl]benzoate (17). A mixture of 0.221 g (9.61 mmol, 1.6 eq) of sodium in 25 mL of anhydrous methanol was stirred until the sodium metal was completely dissolved (~15 min), and 0.921 g (9.64 mmol, 1.6 eq) of dry guanidine hydrochloride was added. The reaction mixture was stirred at rt for an additional 30 min. The precipitated sodium chloride was filtered off and the resulting colorless solution was transferred to a 50 mL round-bottomed flask equipped with a reflux condenser and gas inlet. After addition of 1.86 g (5.90 mmol, 1.0 eq) of **16** in 10 mL of anhydrous methanol, the solution was refluxed for 16 h under a nitrogen atmosphere. Upon cooling to rt, a precipitate resulted which was filtered off and dried to yield 0.42 g of pure **17**. The mother liquor was then concentrated under reduced pressure and the resulting precipitate was collected by filtration and dried to yield an additional 0.29 g of pure **17** as a colorless solid; total yield 0.71 g (36.6%), mp 182 °C. ^1H-NMR (DMSO-d^6, 300 MHz): δ 1.94 (s, 3H, =C(Ar)-CH_3); 2.24 (t, 2H, CH_2-pyr, J = 7.5 Hz); 2.30 (dt, 2H, CH_2-CH=, J= 7.5 Hz); 3.82 (s, 3H, CH_3O); 5.75 (s (broad), 2H, NH_2); 5.93 (s (broad), 2H, NH_2); 6.02 (t, $1H_{olef}$, J= 7.5 Hz); 7.49 (d, $2H_{arom}$, J = 9Hz); 7.85 (d, $2H_{arom}$, J = 9 Hz); 9.81 (s (broad), 1H, NH). HRMS, found 328.1526 (M^+), calcd for $C_{17}H_{20}N_4O_3$ 328.1535.

4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-2-en-2-yl]benzoic Acid (18). A 50 mL round-bottomed flask was charged with 0.689 g (2.10 mmol, 1.0 eq) of **17** in 10 mL of 1 N aqueous NaOH (~5 eq). The mixture was stirred at rt overnight (after 30 min all of the starting material was dissolved, indicating complete hydrolysis). After removal of the solid impurities by filtration, the clear filtrate was acidified with 0.5 mL of glacial acetic acid (~10 mmol). The resulting precipitate was collected by centrifugation with distilled water several times, and was then transferred to a round-bottomed flask with methanol. The solution was concentrated under reduced pressure to afford 0.660 g (100%) of pure **18** as a colorless solid, mp 281 °C; ^1H-NMR (DMSO-d^6, 300 MHz): δ 1.92 (s, 3H, =C(Ar)-CH_3); 2.12 (t, 2H, CH_2-pyr, J= 7.5 Hz); 2.20 (dt, 2H, CH_2-CH=, J= 7.5 Hz); 3.82 (s, 3H, CH_3O); 5.63 (s (broad), 2H, NH_2); 5.84 (s (broad), 2H, NH_2); 5.92 (t, $1H_{olef}$, J= 7.5 Hz); 7.32 (d, $2H_{arom}$, J = 9 Hz); 7.77 (d, $2H_{arom}$, J = 9 Hz); 9.78 (s (broad), 1H, NH). HRMS, found 314.1378 (M^+), calcd for $C_{16}H_{18}N_4O_3$ 314.1379.

Dimethyl *N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-2-en-2-yl]benzoyl}-*L*-glutamate (19). A 25 mL round-bottomed flask equipped with a gas inlet was charged with 300 mg (0.954 mmol, 1.0 eq) of **18** and 116 mg (0.126 mL, 1.15 mmol, 1.2 eq) of *N*-methylmorpholine in 4.0 mL of anhydrous DMF. The mixture was stirred vigorously at rt for 5 min, and 184 mg (1.05 mmol, 1.1 eq) of 2,4-dimethoxy-6-

chloro-1,3,5-triazine was then added. After stirring the solution at rt for 40 min, an additional 116 mg (0.126 mL, 1.15 mmol, 1.2 eq) of *N*-methylmorpholine was introduced, followed by 263 mg (1.24 mmol, 1.3 eq) of dry dimethyl *L*-glutamate hydrochloride (the hydrochloride immediately dissolved; after 5 min, triazinone precipitated from solution). Stirring was continued overnight at rt under a nitrogen atmosphere. After removing the solvent under reduced pressure (water bath at 50°C, ~7 mm Hg), the crude oil was dissolved in chloroform and extracted with saturated aqueous sodium bicarbonate to remove unreacted acid, triazine and triazinone. The organic layer was dried over $MgSO_4$, filtered and evaporated to dryness. Purification was carried out by flash chromatography, eluting with methanol/chloroform (1:10), to afford 260 mg (57.8%) of pure **19** as a colorless solid, mp 97 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.80 (s, 3H, =C(Ar)-CH_3); 2.00-2.54 (m, 8H, 4 x CH_2); 3.57 (s, 3H, CH_3O); 3.68 (s, 3H, CH_3O); 4.67 (dt, 1H, CH_{glu}, *J*= 6, 7.5 Hz); 5.11(s (broad), 2H, NH_2); 5.75 (t, $1H_{olef}$, *J*= 7.5 Hz); 6.03 (s (broad), 2H, NH_2); 7.19 (d, $2H_{arom}$, *J*= 9 Hz); 7.68 (m (broad), 1H, NH_{glu}); 7.70 (d, $2H_{arom}$, *J*= 9 Hz); 11.24 (s (broad), 1H, NH_{pyr}). HRMS, found 471.2132 (M^+), calcd for $C_{23}H_{29}N_5O_6$ 471.2118.

***N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-2-en-2-yl]benzoyl}-*L*-glutamic Acid (20).** A 10 mL round-bottomed flask was charged with 100 mg (0.212 mmol) of **19** dissolved in 1.0 mL of 1N aqueous sodium hydroxide. The solution was stirred overnight at rt (after 1 h a clear solution was observed), and 0.12 mL (2 eq) of glacial acetic acid was then added dropwise (pH = 4.0) as the solution was stirred vigorously. The precipitate was filtered off and subsequently washed three times with 1 mL of cold water. The product was dried under high vacuum at rt to afford 77 mg (81.9%) of pure **20** as a white solid, mp 178 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.93 (s, 3H, =C(Ar)-CH_3); 2.02-2.16 (m, 4H, 2 x CH_2); 2.23 (m, 2H, CH_{2}pyr); 2.31 (m, 2H, CH_2-CH=); 4.28 (dt, 1H, CH_{glu}, *J*= 6, 7.5 Hz); 5.74 (s (broad), 2H, NH_2); 5.98 (t, $1H_{olef}$, *J*= 7.5 Hz); 6.04 (s (broad), 2H, NH_2); 7.44 (d, $2H_{arom}$, *J*= 9 Hz); 7.77 (d, $2H_{arom}$, *J*= 9 Hz); 8.21 (m (broad), 1H, NH_{glu}); 10.03 (s (broad), 1H, NH_{pyr}); 14.10 (s (broad), 2H, 2 x CO_2*H*). HRMS (FAB), found 444.1892 (M^+ + H), calcd for $C_{21}H_{26}N_5O_6$ 444.1883.

Dimethyl *N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-2-yl]benzoyl}-*L*-glutamate (21). A solution of 130 mg (0.276 mmol, 1.0 eq) of **19** in 10 mL of anhydrous methanol containing 5 mg of Pd/C (10%) was hydrogenated at 50 psi (4 atm) at rt for 7 days on a Parr hydrogenator. The mixture was filtered over Celite to remove the catalyst and concentrated under reduced pressure. Flash chromatography, eluting with methanol/chloroform (1:10), yielded 65 mg (49.8%) of pure **21** as a colorless solid. Alternatively, **21** was prepared in quantitative yield by hydrogenation of **12**: To a solution of 200 mg (0.424 mmol) of **12** in 10 mL of anhydrous methanol was added 50 mg of Pd/C (10%). The mixture was hydrogenated at 50 psi at rt for 24 h on a Parr hydrogenator. After filtration over Celite and evaporation of the solvent, 195 mg (97.1%) of pure **21** was obtained as a colorless solid. An analytical sample was prepared by passing the product in 10% methanolic chloroform over a silica gel pad. A white foamy solid was obtained, mp 95 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.06-1.40 (m, 2H, CH_2-CH_2-CH_2); 1.44-1.62 (m, 2H, CH_2-CH_2-CH_2); 2.02 (s, 3H, CH-CH_3); 2.10-2.40 (m, 4H, 2 x CH_2); 2.48 (dt, 2H, CH_2-CO_2Me, *J*= 7.5 Hz); 2.62-2.73 (m, 1H, C*H*-CH_3); 3.63 (s, 3H, CH_3O); 3.75 (s, 3H, CH_3O); 4.78 (dt, 1H, CH_{glu}, *J*= 6, 7.5 Hz); 5.37 (s (broad), 2H, NH_2); 6.64 (s (broad), 2H, NH_2); 7.15 (d, $2H_{arom}$, *J*= 9 Hz); 7.48 (m (broad), 1H, NH_{glu}); 7.70 (d, $2H_{arom}$, *J*= 9 Hz); 9.54 (s (broad), 1H, NH_{pyr}). HRMS, found 473.2274 (M^+), calcd for $C_{23}H_{31}N_5O_6$ 473.2274.

***N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-2-yl]benzoyl}-*L*-glutamic Acid (22).** A 10 mL round-bottomed flask was charged with 60 mg (0.127 mmol) of **21** dissolved in 1.0 mL of 1 N aqueous sodium hydroxide. The solution was stirred overnight at rt (after 1 h a clear solution was observed), and 0.12 mL (2.0 eq) of glacial acetic acid was then added dropwise (pH = 4.0) as the solution was stirred vigorously. The precipitate was filtered off and subsequently washed three times with 1 mL of cold water. After drying under high vacuum at rt, 28 mg (49.6%) of pure **22** was obtained as a white solid, mp 155 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 1.00-1.30 (m, 5H, CH_2-CH_2-CH_2 + CH-CH_3); 1.51 (dt, 2H, CH_2-CH_2-CH_2, *J*= 7.5 Hz); 1.85-2.00 (dt, 2H, CH_2-CH_{glu}, *J* = 7.5 Hz); 2.03-2.13 (dt, 2H, CH_2-CO_2Me, *J*= 7.5 Hz); 2.33 (t, 2H, CH_2-pyr, *J* = 7.5 Hz); 2.73 (m, 1H, C*H*-CH_3); 4.18 (m, 1H, NH_{glu}); 5.58 (s (broad), 2H, NH_2); 5.89 (s (broad), 2H, NH_2); 7.27 (d, $2H_{arom}$, *J* = 9.0 Hz); 7.77 (d, $2H_{arom}$, *J* = 9.0 Hz); 8.50 (d, 1H, NH_{glu}, *J* = 6 Hz); 9.74 (s (broad), 1H, NH_{pyr}); 12.40 (s (broad), 2H, 2 x CO_2*H*). HRMS (FAB), found 446.1987 (M^+ + H), calcd for $C_{21}H_{27}N_5O_6$ 446.2039.

Dimethyl *N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinone-5-yl)-pent-1-hydroxy-2-yl]benzoyl}-*L*-glutamate (24). A 50-mL round-bottomed flask equipped with a gas inlet was torched dry, cooled to rt, and charged with 500 mg (1.06 mmol, 1.0 eq) of **12** in 10 mL of anhydrous THF, followed by addition of 9.0 mL (9.0 eq) of 1.0 M $\mathrm{BH_3{\cdot}THF}$ over a period of 3 h (3 mL per h) at 55 °C under a nitrogen atmosphere. After vigorous stirring for an additional 3 h, the reaction mixture was cooled to rt and 200 mg (1.2 eq) of $\mathrm{NaBO_3{\cdot}4H_2O}$ in 10 mL of $\mathrm{H_2O}$ was added. The solution was stirred at rt for 12 h, concentrated under reduced pressure and the product was purified by flash chromatography, eluting with methanol/chloroform (1:4), to afford 260 mg (51%) of pure **24** as a white solid, mp 104 °C; ^{1}H-NMR ($\mathrm{CD_3OD}$, 300 MHz): δ 1.22-1.37 (m, 2H, $\mathrm{CH_2}$-$\mathrm{C}H_2$-$\mathrm{CH_2}$); 1.55-1.85 (m, 2H, $\mathrm{CH_2}$-$\mathrm{CH_2}$-$\mathrm{C}H_2$); 2.04-2.35 (m, 2H, $\mathrm{C}H_2$-$\mathrm{CH_{glu}}$); 2.25 (t, 2H, $\mathrm{C}H_2$-pyr, *J*=7.5 Hz); 2.48 (t, 2H, $\mathrm{C}H_2$-$\mathrm{CO_2Me}$, *J*=7.5 Hz); 2.75-2.87 (m, 1H, $\mathrm{C}H$-$\mathrm{CH_2OH}$); 3.63 (s, 3H, $\mathrm{C}H_3\mathrm{O}$); 3.66 (d, 2H, $\mathrm{C}H_2\mathrm{OH}$, *J*=7.5 Hz); 3.74 (s, 3H, $\mathrm{C}H_3\mathrm{O}$); 4.62 (dt, 1H, $\mathrm{C}H_{\mathrm{glu}}$, *J*= 6, 7.5 Hz); 7.30 (d, $\mathrm{2H_{arom}}$, *J* = 9 Hz); 7.76 (d, $\mathrm{2H_{arom}}$, *J* = 9 Hz). ^{13}C-NMR ($\mathrm{CD_3OD}$, 300 MHz): δ 23.05; 26.45; 26.72 ($\mathrm{C_{b(glu)}}$); 30.62 ($\mathrm{C_{g(glu)}}$); 32.27 ($C\mathrm{H_{2(pyr)}}$); 49.17 ($C\mathrm{H}$-$\mathrm{CH_2OH}$); 51.69 ($C\mathrm{H_3O}$); 52.32 ($C\mathrm{H_3O}$); 53.00 ($\mathrm{C_{a(glu)}}$); 66.90 ($C\mathrm{H_2}$-OH); 89.53 ($\mathrm{C^5_{pyr}}$); 127.88 ($C\mathrm{H_{ar}}$); 128.67 ($C\mathrm{H_{ar}}$); 132.06 (*C*-CONH); 148.73 ($\mathrm{C_{ar}}$); 154.08 ($\mathrm{C^2_{pyr}}$); 163.38 ($\mathrm{C^4_{pyr}}$); 164.60 ($\mathrm{C^6_{pyr}}$); 169.52 ($C\mathrm{ONH_{glu}}$); 173.17 ($C\mathrm{O_2Me}$); 174.26 ($C\mathrm{O_2Me}$). HRMS, found 489.2233 ($\mathrm{M^+}$), calcd for $\mathrm{C_{23}H_{31}N_5O_7}$ 489.2223.

Dimethyl *N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-1-dihydroxyboro-2-yl]-benzoyl}-*L*-glutamate (23). This compound was obtained as a stable intermediate from the hydroboration reaction with sodium perborate described above. If the oxidation is stopped after 1 h at rt and the sodium perborate is immediately removed, a less polar product can be isolated from the column in 18% yield as a colorless foamy solid, mp 89 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 0.96 (m, 2H, $\mathrm{C}H_2$-B); 1.18 (m, 2H, pyr-$\mathrm{CH_2}$-$\mathrm{C}H_2$); 1.55 (m, 2H, $\mathrm{C}H_2$-CH); 2.0-2.2 (m, 2H, $\mathrm{C}H_{2(\mathrm{glu})}$); 2.15 (t, 2H, pyr-$\mathrm{C}H_2$, *J* = 7.5 Hz); 2.49 (t, 2H, $\mathrm{C}H_2$-$\mathrm{CO_2Me}$, *J* = 7.5 Hz); 2.92 (tt, 1H, $\mathrm{C}H$-$\mathrm{CH_2B}$, *J* = 7.5 Hz); 3.62 (s, 3H, $\mathrm{C}H_3\mathrm{O}$); 3.68 (s, 3H, $\mathrm{C}H_3\mathrm{O}$); 4.47 (dt, 1H, $\mathrm{C}H_{\mathrm{glu}}$, *J* = 7.5 Hz); 5.61 (s, 2H, $\mathrm{N}H_2$); 5.94 (s, 2H, $\mathrm{N}H_2$); 7.26 (d, $\mathrm{2H_{arom.}}$, *J* = 8 Hz); 7.52 (s, 2H, B-O*H*); 7.77 (d, $\mathrm{2H_{arom.}}$, *J* = 8 Hz); 8.71 (d, 1H, $\mathrm{N}H_{\mathrm{glu}}$, *J* = 6 Hz); 9.78 (s, broad, 1H, $\mathrm{N}H_{\mathrm{pyr}}$). ^{13}C-NMR ($\mathrm{CD_3OD}$, 300 MHz): δ ~22 (broad, ^{11}B-coupling, $C\mathrm{H_2}$-B); 23.06; 26.45; 27.36 ($\mathrm{C_{b(glu)}}$); 30.91 ($\mathrm{C_{g(glu)}}$); 38.73 ($C\mathrm{H_{2(pyr)}}$); 41.68 ($C\mathrm{H}$-$\mathrm{CH_2B}$); 52.40 ($C\mathrm{H_3O}$); 52.85 ($\mathrm{C_{a(glu)}}$); 53.04 ($C\mathrm{H_3O}$); 90.34 ($\mathrm{C^5_{pyr}}$); 127.84 ($C\mathrm{H_{ar}}$); 127.97 ($C\mathrm{H_{ar}}$); 131.41 (*C*-CONH); 153.14 ($\mathrm{C_{ar}}$); 153.77 ($\mathrm{C^2_{pyr}}$); 162.62 ($\mathrm{C^4_{pyr}}$); 164.81 ($\mathrm{C^6_{pyr}}$); 168.84 ($C\mathrm{ONH_{glu}}$); 173.27 ($C\mathrm{O_2Me}$); 174.43 ($C\mathrm{O_2Me}$).

***N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-1-hydroxy-2-yl]benzoyl}-*L*-glutamic Acid (3).** A 10 mL round-bottomed flask was charged with 60 mg (0.122 mmol) of **24** dissolved in 0.5 mL of 1 N aqueous sodium hydroxide. The solution was stirred vigorously for 1 h at rt, and 0.06 mL (2.0 eq) of glacial acetic acid was then added dropwise to the solution (pH = 4.0). A faint precipitate was observed. After refrigerating for 30 min at -10 °C, additional precipitation resulted. The solid was filtered off, washed with 1 mL of ice water, and dried to afford 30 mg of **3** containing 1 eq of sodium acetate (44% yield of pure **3**) as a white solid, mp 164 °C; ^{1}H-NMR (DMSO-d_6, 270 MHz): δ 1.24-1.35 (m, 2H, $\mathrm{CH_2}$-$\mathrm{C}H_2$-$\mathrm{CH_2}$); 1.55-1.95 (m, 2H, $\mathrm{CH_2}$-$\mathrm{CH_2}$-$\mathrm{C}H_2$); 1.98-2.20 (m, 2H, $\mathrm{C}H_2$-$\mathrm{CH_{glu}}$); 2.25 (t, 2H, $\mathrm{C}H_2$-pyr, *J*= 7.5 Hz); 2.47 (dt, 2H, $\mathrm{C}H_2$-$\mathrm{CO_2H}$, *J*= 7.5 Hz); 2.80-2.90 (m, 1H, $\mathrm{C}H$-$\mathrm{CH_2OH}$, *J* = 7.5 Hz); 4.51 (dt, 1H, $\mathrm{C}H_{\mathrm{glu}}$, *J*= 6, 7.5 Hz); 5.70 (s (broad), 2H, $\mathrm{N}H_2$); 6.08 (s (broad), 2H, $\mathrm{N}H_2$); 7.42 (d, $\mathrm{2H_{arom}}$, *J*= 9 Hz); 7.90 (d, $\mathrm{2H_{arom}}$, *J*= 9 Hz); 8.50 (d, 1H, $\mathrm{N}H_{glu}$, *J*= 6 Hz); 9.95 (s (broad), 1H, $\mathrm{N}H_{pyr}$); 12.6 (s (broad), 2H, 2 x $\mathrm{CO_2}H$). Anal. Calcd for $\mathrm{C_{21}H_{27}N_5O_7}$ x 1 eq $\mathrm{C_2H_3O_2Na}$: C, 50.83; H, 5.56; N, 12.89. Found: C, 51.09; H, 5.27; N, 12.63.

***N*-{4-[5-(2,4-Diamino-6(1H)-pyrimidinon-5-yl)-pent-1-dihydroxyboro-2-yl]benzoyl}-*L*-glutamic Acid (25).** A 10 mL round-bottomed flask was charged with 150 mg (0.29 mmol) of **23** dissolved in 0.5 ml of 1 N aqueous sodium hydroxide, and the solution was stirred vigorously at rt for 8 h. A precipitate resulted upon acidification with 0.08 ml (2.6 eq) of glacial acetic acid and subsequent addition of 2 mL of cold water. After refrigerating for 10 min at -10 °C, the mixture was filtered through a small Büchner funnel. The product was then washed with ice water (3 x 1 mL) and dried overnight at rt under high vacuum to afford 56 mg (39.5%) of pure **25** as a white solid, mp 256 °C (dec.); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 0.93 (m, 2H, $\mathrm{C}H_2$-B); 1.07 (m, 2H, pyr-$\mathrm{CH_2}$-$\mathrm{C}H_2$); 1.45 (m, 2H, $\mathrm{C}H_2$-CH); 1.8-2.15 (m, 2H, $\mathrm{C}H_{2(\mathrm{glu})}$); 2.04 (t, 2H, pyr-$\mathrm{C}H_2$, *J* = 7.5 Hz); 2.30 (t, 2H, $\mathrm{C}H_2$-$\mathrm{CO_2Me}$, *J* = 7.5 Hz); 2.84 (tt, 1H, $\mathrm{C}H$-$\mathrm{CH_2B}$, *J* = 7.5 Hz); 4.33 (dt, 1H,

CH_{glu}, J = 7.5 Hz); 5.54 (s, 2H, NH_2); 5.87 (s, 2H, NH_2); 7.19 (d, $2H_{arom.}$, J = 8 Hz); 7.41 (s, 2H, B-O*H*); 7.69 (d, $2H_{arom.}$, J = 8 Hz); 8.42 (d, 1H, NH_{glu}, J = 7 Hz); 9.76 (s, broad, 1H, NH_{pyr}); 12.18 (s, broad, 2H, $2xCO_2H$). ^{11}B-NMR (D_2O, 250 MHz): +18.19 ppm ($NaBH_4$ as ext. ref. at -42.6 ppm). Anal. Calcd for $C_{12}H_{28}N_5O_8B$: C, 51.55; H, 5.77; N, 14.31. Found: C, 51.28; H, 5.77; N, 14.29.

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