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Design, Synthesis, and Evaluation of Potential GAR and AICAR Transformylase Inhibitors

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Abstract—The synthesis and evaluation of **1**–**4** as potential inhibitors of GAR Tfase and AICAR Tfase are detailed.

Introduction

In preceding studies,[1] we detailed the preparation and evaluation of potent inhibitors of glycinamide ribonucleotide transformylase (GAR Tfase) and aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase), folate dependent enzymes responsible for the transfer of a formyl group to GAR and AICAR in the de novo synthesis of purines, based on the 5,8,10-trideazafolate core. The most effective of the agents incorporated a reactive, electrophilic group including a nontransferable formyl group capable of reaction with the nucleophilic amine of the enzymatic substrates GAR and AICAR or active site residues of the enzymes themselves. Herein, we detail the extension of these studies to the preparation and evaluation of **1**–**4** as potential antineoplastic agents and inhibitors of GAR Tfase and AICAR Tfase (Figure 1).

Inhibitor Design

Key to the inhibitor design was the introduction of a nontransferable formyl group potentially capable of enzyme-assembled[2] imine formation with the enzymatic substrates.[2,3] With the prospect of formation of an enzyme-assembled tight binding inhibitor by virtue of imine formation with GAR or AICAR, the objective was to maintain the key elements of the folate–enzyme interaction at the active site but dispense with the entire benzoyl glutamate side chain, providing much smaller potential inhibitors and avoiding the unpredictable issues of active transport and polyglutamylation critical to the intracellular accumulation of cofactor analogues detailed to date. Moreover, in the absence of imine formation, ineffective competitive inhibition with the endogenous folate cofactors was expected potentially to convey selectivity for GAR and AICAR Tfase over other folate dependent enzymes not directly engaged in a formyl transfer reaction. The design of the GAR Tfase inhibitors was carried out based on the X-ray structures of phosphate bound GAR Tfase and GAR Tfase bound with the inhibitor 1476U89.[4] Model building of potential bound inhibitors employed MacroModel (AMBER and OPLSA force fields) with an emphasis on optimization of topological fit within the binding site as well as incorporation of complementary inhibitor–enzyme interactions. Based on initial modeling studies within the tetrahydrofolate cofactor binding site, four inhibitors, **1**–**4**, were pursued (Figure 2). Each inhibitor retains the key hydrogen bond donor–acceptor–donor array across the upper face of the pyrimidine ring which is suggested to represent a key factor in cofactor binding and orientation through the formation of hydrogen bonds with residues Arg-90 and Leu-92.[4,5] An additional key feature is the replacement of the cofactor formamide functionality by an aldehyde, which renders the inhibitors unable to undergo formyl

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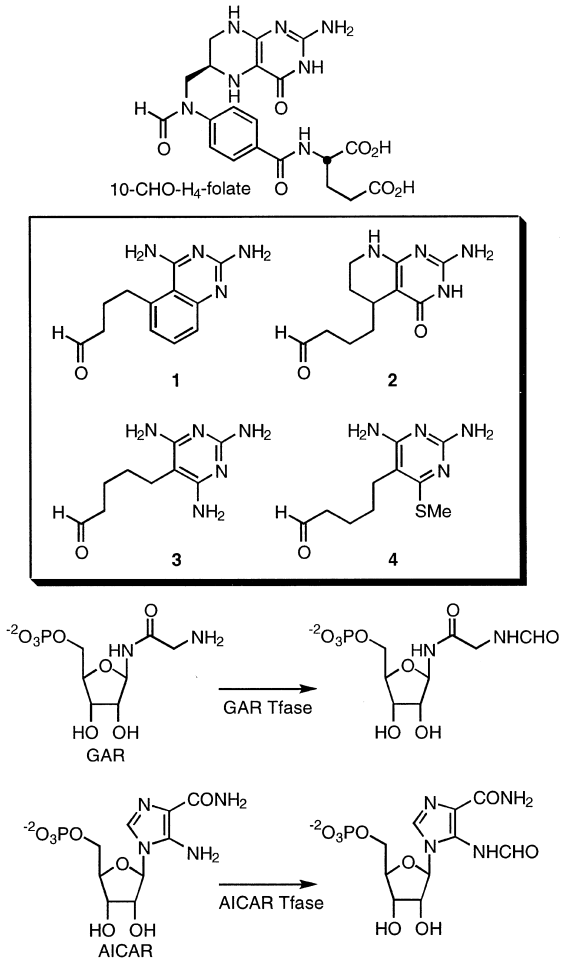


Figure 1.

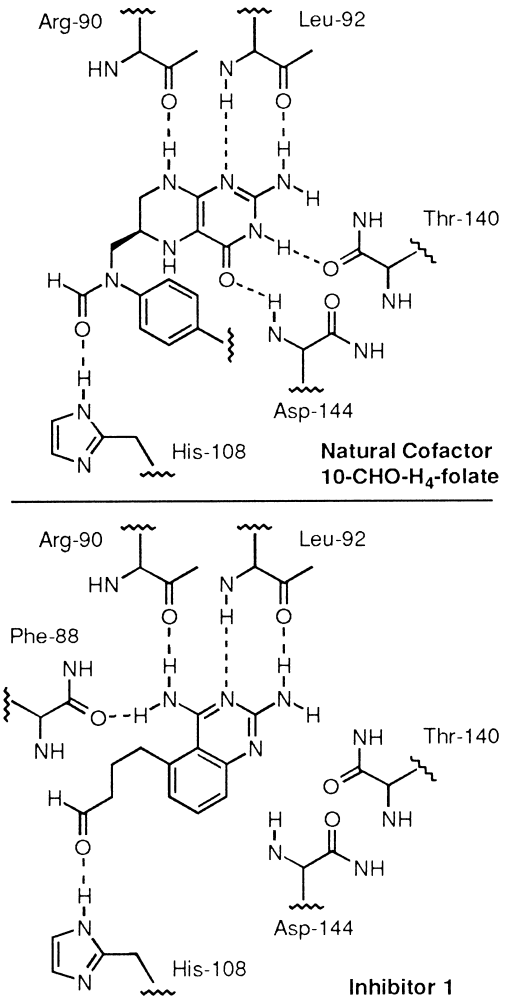


Figure 2.

transfer. These compounds were designed to form a tight binding ternary complex through enzyme-assisted imine formation with the substrate GAR. In addition to the pyrimidine hydrogen bonding array, inhibitor **1** incorporates the second ring across the bond adjacent to that of the natural cofactor. This design fills an additional portion of the available hydrophobic space within the binding pocket. The positioning of the side chain at C-5 was found in modeling studies to overlap well with the corresponding portion of the inhibitor 1476U89.[4]

Inhibitor **2** incorporates the 5-deazapteridine ring system, which is known to bind efficiently within the cofactor binding site (Figure 3).[6] This compound incorporates the side chain at C-5 rather than at the C-6 position found in the natural cofactor and the classical inhibitors including the clinical candidate DDATHF.[6] Such folate analogues based on 5-deazapteridine substituted at C-5 have not previously been examined as GAR Tfase inhibitors.

The two inhibitors **3** and **4** represent even more simplified analogues of which **4** possesses the additional capability of accommodating the Asp-144 backbone NH hydrogen bond provided to the folate cofactor C4 carbonyl.

Chemistry

The initial stages of the synthesis of 2,4-diamino-5-(4-oxobut-1-yl)quinazoline (**1**) followed well established methodology for the construction of 2,4-diaminoquinazolines (Scheme 1).[7] Commercially available 2-methyl-6-nitroaniline (**5**) was converted to nitrile **6** via diazotization and a subsequent Sandmeyer reaction. Reduction of the nitro group with Fe–HOAc afforded the aniline **7**, which was protected as the dibenzylamine **8**. The side chain was introduced by benzylic deprotonation and

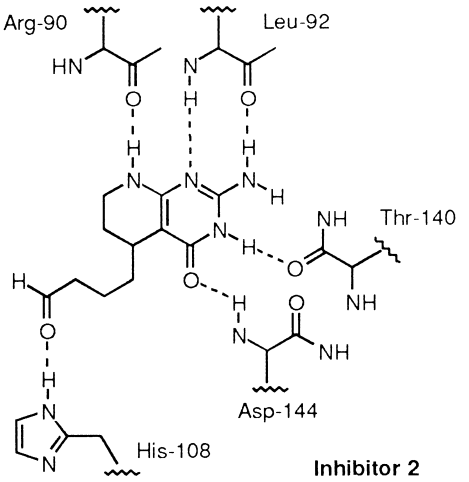
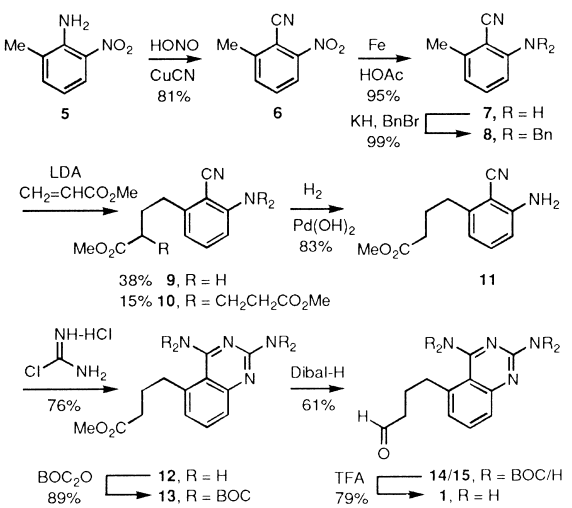


Figure 3.

Michael addition to methyl acrylate. This reaction provided a mixture of the desired ester **9** and **10** resulting from second reaction of **9** with methyl acrylate, as well as recovered starting material (21%). Hydrogenolysis of the benzyl protecting groups using Pearlman's catalyst regenerated the free amine **11**. Cyclization of **11** with chloroformamidine hydrochloride[8] produced the diaminoquinazoline **12**. Treatment of **12** with di-*t*-butyl dicarbonate and a catalytic amount of DMAP in the presence of Et_3N afforded the tetra-(*t*-butyloxycarbonyl) derivative **13** which was found to be readily soluble in the nonpolar media required for reduction to the aldehyde. Reduction of **13** (2 equiv. DIBAL-H) afforded a mixture of aldehydes **14** (41%) and **15** (20%) in which one of the BOC groups had been cleaved, as well as unreacted starting material (25%). Aldehydes **14** and **15** were converted to **1** by cleavage of the BOC groups with trifluoroacetic acid in chloroform.

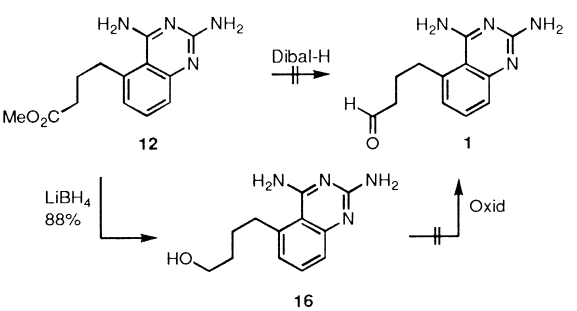


Scheme 1.

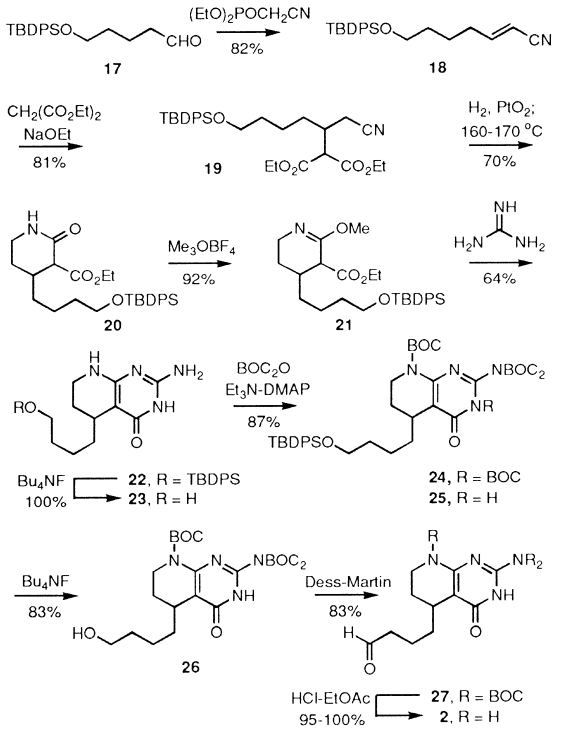
The direct reduction of **12** to the aldehyde **1** (DIBAL-H) was unsuccessful due to the insolubility of **1** in the conventional reaction medium (toluene). However, the ester could be reduced to alcohol **16** with $LiBH_4$ generated in situ from $NaBH_4$ and LiCl but **16** proved to be an exceptionally insoluble solid (Scheme 2). As a consequence of this insolubility, all attempts to effect its oxidation were not as successful providing only trace amounts of the desired aldehyde **1**.

The successful approach to the synthesis of **2** utilized the 3-ethoxycarbonyl lactam **20** (Scheme 3). The precursor to **20** was readily assembled by Michael addition of diethyl malonate to the α,β-unsaturated nitrile **18** derived from a Horner–Emmons condensation of diethyl cyanomethylphosphonate with the aldehyde **17**. The lactam was prepared from diester **19** by hydrogenation reduction of the nitrile followed by cyclization. Thus, hydrogenation of the nitrile **19** in acetic acid afforded the acetate salt of the product amine. Following removal of the catalyst and solvent, heating the residue under vacuum (0.1 mm Hg) at 160–170 °C resulted in cyclization to the lactam **20** with evolution of ethanol and acetic acid. In contrast to related published procedures which employ PtO_2 and 60 psi H_2[9] or Raney Nickel at 100 atm H_2,[10] the reduction of **19** using PtO_2 was found to proceed efficiently at atmospheric pressure. Treatment of the lactam with Me_3OBF_4 afforded the *O*-methyl lactam **21**.

Neat condensation of lactam **21** with guanidine free base,[11] prepared from the hydrochloride and sodium 2-ethoxyethoxide, resulted in formation of the tetrahydropyridopyrimidinone **22**, completing the construction of the carbon framework for inhibitor **2**.[12] Cleavage of the silyl protecting group afforded alcohol **23** and an important comparison sample for examination alongside **2**. The alcohol **23** was insoluble in most organic solvents and this property precluded its direct oxidation to **2**. While the ^1H NMR spectrum of **23** in CD_3OD was



Scheme 2.



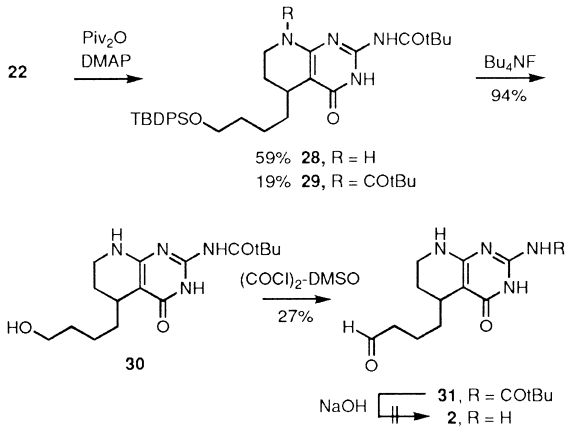
Scheme 3.

clean, the ^{1}H NMR spectrum in DMSO-d_6 was complex and the ^{13}C NMR spectrum showed doubling of five of the 11 carbon resonances. Presumably, the pyrimidone ring exists exclusively as the 4-keto tautomer in CD_3OD, while a mixture of the 4-keto and 4-hydroxy tautomers is present in DMSO-d_6.

In order to increase the solubility of the alcohol prior to oxidation, exhaustive carbamate formation with BOC_2O provided **24** of which one of the *t*-butyloxycarbonyl groups proved especially labile and could be lost on simple storage to provide **25**. Treatment of either **24** or **25** with Bu_4NF served to deprotect the primary alcohol cleanly providing **26** incorporating three *t*-butyloxycarbonyl groups, two of which were spectroscopically identical. Dess–Martin oxidation[13] of the primary alcohol (3 equiv. *o*-Ph(CO$_2$)I(OAc)$_3$, CH_2Cl_2, 25 °C, 2 h, 83%) followed by acid-catalyzed deprotection of **27** provided **2**.

The latter stages of the preparation of **2** detailed above proved superior to initial attempts employing the pivaloylamide **28** where both the oxidation of **30** and the final deprotection of **31** proved more problematic (Scheme 4).

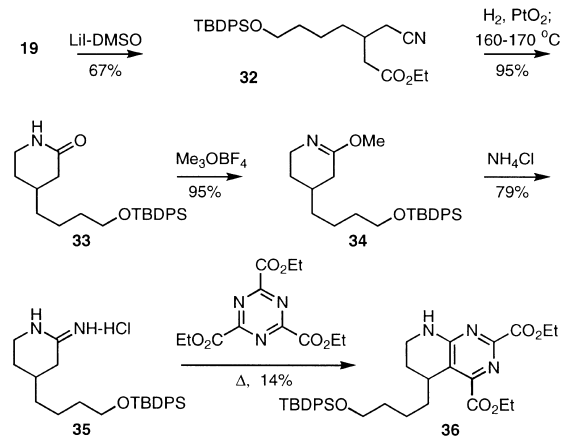
An interesting albeit less successful approach to **2** was also examined based on the inverse electron demand



Scheme 4.

Diels–Alder reaction of amidines with 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (Scheme 5).[14] Dealkylative decarboxylation of **19** followed by closure of **32** to the 4-substituted piperidone **33** effected by hydrogenation (1 atm) reduction of the nitrile and lactamization provided the key precursor to the requisite amidine **35**. Thermal reaction of the amidine with 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (DMF, 90–110 °C, 24–72 h) provided only low conversions to the desired pyrimidine **36** and these results contrast the effective conversions (75%) observed with the unsubstituted 2-iminopiperidine hydrochloride itself.[14]

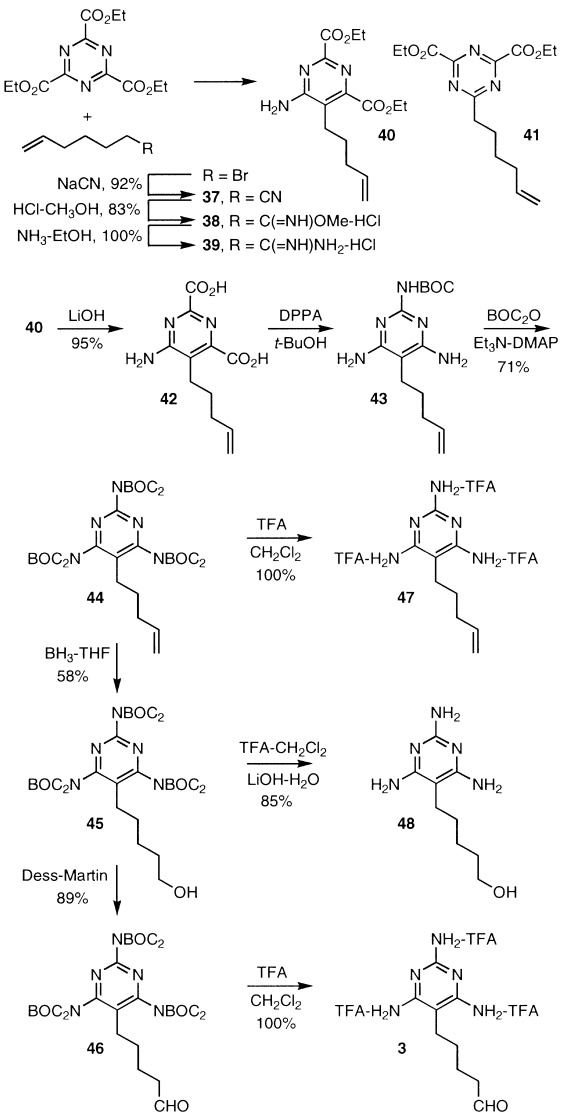
The synthesis of the inhibitors **3** and **4** features more successful applications of the inverse electron demand Diels–Alder reaction of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine and amidine hydrochlorides for the construction of the substituted pyrimidines. Commercially available 6-bromo-1-hexene was converted to the amidine **39** via the nitrile **37** and intermediate imidate **38**.



Scheme 5.

Diels–Alder reaction of **39** affected by treatment with 2,4,6-*tris*(ethoxycarbonyl)-1,3,5-triazine provided the pyrimidine **40** together with significant amount of the isomeric triazine **41** (Scheme 6). This latter unexpected product represents the reaction of the C=NH double bond of the amidine in the Diels–Alder reaction competitive with the in situ amidine tautomerization to the more reactive 1,1-diaminoethene. The best conversion to **40** was observed when 2 equiv. of the triazine was used and the amidine hydrochloride salt was added to the reaction mixture every 4–6 h over a period of 24 h. Direct ester hydrolysis of **40** (4 equiv. LiOH, THF–CH_3OH–H_2O, 25 °C, 2 h) followed by acidification (pH 0–1) led to precipitation of the desired amino diacid **42** (95%). Curtius rearrangement on **42** followed by exhaustive acylation with $(BOC)_2O$ provided **44**. Hydroboration of **44** effected with BH_3·THF provided **45** (58%) and Dess–Martin oxidation afforded **46** (89%). Finally, acid-catalyzed BOC deprotection of **44**–**46** effected by trifluoroacetic acid (TFA) cleanly provided **3** and two comparison analogues **47** and **48**.

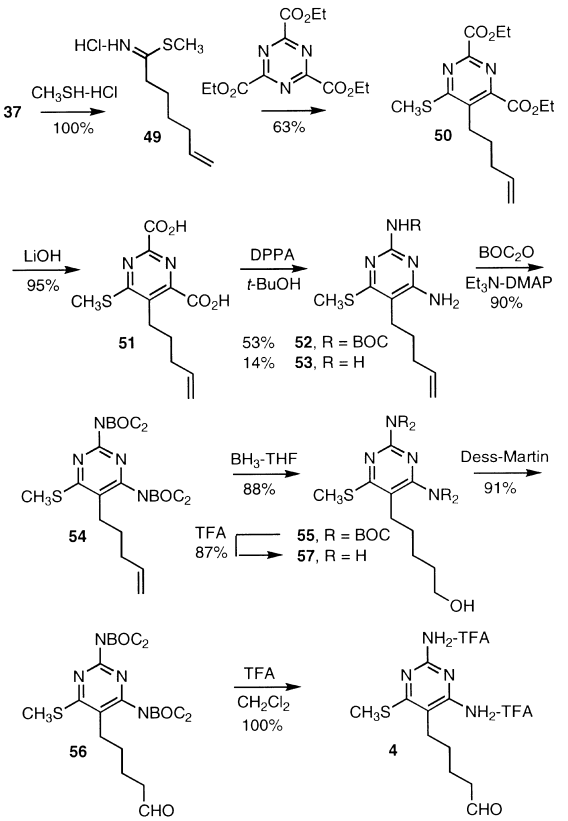
In an analogous, but more effective sequence, the thioimidate **49** was converted to the potential inhibitor **4** and its close comparison analogue **57** (Scheme 7). Inverse electron demand Diels–Alder reaction of **49**, derived from acid-catalyzed addition of methanethiol to nitrile **37**, with 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine cleanly provided the pyrimidine **50** (63%) requiring preferential loss of ammonia versus methanethiol from the [4+2] cycloadduct. Ester hydrolysis (4 equiv. LiOH, THF–CH_3OH–H_2O, 2 h, 25 °C, 95%), Curtius rearrangement (2.4 equiv. DPPA, 2.4 equiv. Et_3N, 24 h, 85–95 °C, 53–67%), and exhaustive acylation with $(BOC)_2O$ provided **54**. Hydroboration of **54** followed by Dess–Martin oxidation of the resulting alcohol **55** provided the aldehyde **56**. Acid-catalyzed deprotection of **56** and **55** provided the key aldehyde **4** and a close comparison analogue **57**.



Scheme 6.

Inhibitor Studies

The results of screening the various agents as enzyme inhibitors against GAR Tfase and AICAR Tfase are grouped in Tables 1–3. Thus, three of the four aldehydes proved to be effective inhibitors of GAR Tfase (K_i 15–40 μM) despite their simplified structures and one, **3**, was also an inhibitor of AICAR Tfase. This is significant given the simplified nature of the inhibitors which lack the entire benzoyl–glutamate side chain of the natural cofactor and the potent enzyme inhibitors disclosed to date. In the case of **1**, the corresponding alcohol and ester were ineffective in inhibiting GAR Tfase indicating that the non-transferable formyl group is contributing significantly to its properites (Table 1). Interestingly, only **2** among the four aldehydes was ineffective against GART even though it most closely embodies a cofactor analogue core characteristic of the potent inhibitors disclosed to date. None of the agents exhibited time-dependent inhibition consistent with GAR imine formation and enzyme-catalyzed multisubstrate adduct generation and inhibition. Thus, although the inhibitor potency of **4** increased with time, this time dependence was insensitive to preincubation with or without GAR. Since preincubation with 10-fDDAF partially protects inhibition with **4**, this suggests **4** acts as competitive inhibitor of the cofactor and may slowly form a tighter binding hemiacetal enzyme adduct.



Scheme 7.

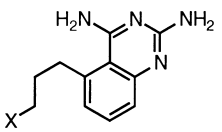
In Vitro Cytotoxic Activity

The aldehydes **1** and **2** along with the comparison analogues **12** and **16** or **22** and **23** were tested for cytotoxic activity in the CCRF–CEM and L1210 cell lines both in the absence (–) and presence (+) of hypoxanthine, Tables 4 and 5. Thus, the cells were cultured in RPMI 1640 medium using dialyzed FBS for assessment of the activity in the absence of purines and similarly cultured with added 50 μM hypoxanthine for its assessment in the presence of purines. The aldehyde **1** and its comparison analogues **12** and **16** all exhibited comparable cytotoxic activities and potencies in the presence or absence of hypoxanthine (Table 4) illustrating that the aldehyde of **1** did not enhance the potency or confer selective GAR or AICAR Tfase inhibiting activity to the agent. In addition, the potent activity of **1** does not appear to be derived from selective inhibition of the de novo synthesis of purines including its inhibition of GAR or AICAR Tfase. This is perhaps not surprising since the concentrations of **12** and **16** required for inhibition of cell growth are well below that required for detection of the inhibition of GAR Tfase and AICAR Tfase and that of **1** is at least an order of magnitude below the enzymatic K_i's.

Examination of **1**, **12**, and **16** in an even broader range of cell lines (A-549, BT-549, CAPAN-1, HT-29, MOLT-4, NHDF, OVCAR-3, UCLA-P3, SIHA, SK-N-SH, 786-0, SK-MEL-28, RPMI-7666) revealed comparable cytotoxic potencies to those listed in Table 4 (1–80 μM) and did not reveal selected cytotoxic activity or a sensitivity in the absence of purines or hypoxanthine.

The aldehyde **2** and its comparison analogue **23** proved to be less effective. Although **2** proved to be 5–10 times

Table 1. GAR and AICAR Tfase inhibition[a]



Agent	K_i GAR Tfase	K_i AICAR Tfase	X
1	39.5 ± 0.5 μM	> 100 μM	CHO
12	> 100 μM	> 100 μM	CH_2Me
16	> 100 μM	> 100 μM	CH_2OH

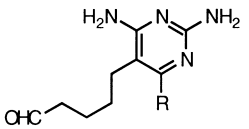
[a]*pur*N GAR Tfase, avian AICAR Tfase.

Table 2. GAR and AICAR Tfase inhibition[a]

Agent	K_i GAR Tfase	K_i AICAR Tfase	X
2	> 100 μM	> 100 μM	CHO
23	> 100 μM	> 100 μM	CH_2OH
22	97 ± 5 μM	> 100 μM	$CH_2OTBDPS$

[a]*pur*N GAR Tfase, avian AICAR Tfase.

Table 3. GAR and AICAR Tfase inhibition[a]



Agent	K_i GAR Tfase	K_i AICAR Tfase	R
3	35 ± 2 μM	> 100 μM	NH_2
4	15 ± 2 μM	> 100 μM	SMe

[a]*pur*N GAR Tfase, avian AICAR Tfase.

more potent than **23**, both were insensitive to the absence or presence of purines. Thus, the activity observed with **2** and **23** does not appear to be related to the inhibition of de novo purine synthesis including the inhibition of GAR or AICAR Tfase. In contrast to expectations, the TBDPS ether **22** was found to be a potent cytotoxic agent 10–100 times more potent than **2**, albeit insensitive to the presence or absence of medium hypoxanthine.

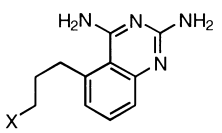
The results of the evaluation of **3** and **4** along with the comparison agents **47**–**48** and **53**/**57** are provided in Table 6. Although **3** consistently provided a three- to fourfold cytotoxicity sensitivity when tested in the absence of medium purines, it proved to be a relative nonpotent agent. Both **48** and especially **47**, which lack the aldehyde, were more potent. Although the aldehyde **4** was found to be 5–10 times more potent than **3**, it exhibited no cytotoxicity sensitivity to the presence or absence of medium purines, and proved to be only slightly more potent or comparable to **53** and **57**.

Experimental

2-Cyano-3-methyl-nitrobenzene (6). A solution of **5** (10 g, 65.7 mmol) in glacial HOAc (25 mL) was treated with 6 M HCl (24 mL). The resulting suspension was cooled to −10 °C and treated with a solution of $NaNO_2$ (5.55 g, 80.4 mmol) in H_2O (18 mL) and the orange solution was stirred for 30 min. A suspension of CuCl (8.07 g, 8.15 mmol) in H_2O (50 mL) was treated with NaCN (10.5 g, 214.2 mmol) forming a homogeneous solution, followed by the addition of C_6H_6 (80 mL). The solution containing the diazonium salt was added to the CuCN solution and the resulting mixture was stirred at 25 °C for 1 h and warmed at 70–75 °C for 1 h. The mixture was filtered and the layers separated. The aqueous layer was extracted with C_6H_6 (2×40 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated. Crystallization (EtOH–H_2O) afforded **6** (5.60 g, 53%) as yellow needles. The mother liquor was purified by flash chromatography (30% EtOAc–hexane) to afford a further 2.95 g (28%, 81% total) of **6**: mp 108–110 °C (lit.[7] mp 108–109 °C); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.12 (m, 1H, C6-H), 7.65–7.69 (m, 2H), 2.69 (s, 3H, CH_3); IR (film) ν_{max} 2221, 1531, 1457, 1344, 1300, 1196, 911, 808, 739 cm^{-1}; FABHRMS (NBA–NaI) *m*/*z* 185.0321 ($M^+ + Na$, $C_8H_6N_2O_2$ requires 185.0327).

2-Cyano-3-methylaniline (7). A suspension of **6** (8.0 g, 49 mmol) in 50% aqueous HOAc (300 mL) was heated to 55–60 °C until complete dissolution occurred. Iron powder (27.2 g, 487 mmol) was added in portions at such a rate as to just maintain reflux (ca. 30 min). After the addition was complete, the mixture was warmed at reflux for a further 15 min, then allowed to cool. The suspension was filtered, the filtrate was added to H_2O (1000 mL) and extracted with EtOAc (3×500 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ to neutrality, dried (Na_2SO_4) and concentrated. Chromatography (30% EtOAc–hexane) afforded

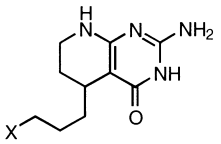
Table 4. Cytotoxic activity (IC_{50}, μM)[a]



Agent	L1210	CCRF-CEM
1 X = CHO	2,3	2,3
12 X = CO_2Me	2,4	23,18
16 X = CH_2OH	1,1	9,10

[a]Dialyzed FBS: − hypoxanthine, + hypoxanthine.

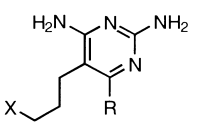
Table 5. Cytotoxic activity (IC_{50}, μM)[a]



Agent	L1210	CCRF-CEM
2 X = CHO	80,85	140,140
23 X = CH_2OH	520,840	> 100, > 100
22 X = $CH_2OTBDPS$	2,3	4,4

[a]Dialyzed FBS: − hypoxanthine, + hypoxanthine.

Table 6. Cytotoxic activity (IC_{50}, μM)[a]



Agent	L1210	CCRF-CEM
R = NH_2		
3 X = CH_2CHO	220, 930	450, 1500
48 X = CH_2CH_2OH	120, 200	210, 240
47 X = CH = CH_2	7,13	20, 45
R = SMe		
4 X = CH_2CHO	40, 25	25, 25
57 X = CH_2CH_2OH	90, 150	140, 170
53 X = CH = CH_2	50, 70	100, 110

[a]Dialyzed FBS: − hypoxanthine, + hypoxanthine.

7 (6.17 g, 6.52 g theoretical, 95%) as a yellow solid: mp 24–126 °C (EtOAc–hexane, yellow crystals) (lit.[7] mp 127–128 °C); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.19 (t, J = 7.9 Hz, 1H, C5-H), 6.54–6.62 (m, 2H, C4-H, C6-H), 4.37 (br, 2H, NH_2), 2.44 (s, 3H, CH_3); IR (film) ν_{max} 3395, 3333, 3231, 2205, 1646, 1595, 1574, 1472, 1297, 780 cm^{-1}; FABHRMS (NBA–NaI) m/z 133.0760 ($M^+ + H$, $C_8H_8N_2$ requires 133.0766).

***N,N*-Dibenzyl-2-cyano-3-methylaniline (8).** Potassium hydride (35 wt%, 5.0 g, 43.6 mmol) was suspended in anhydrous THF (40 mL) at 25 °C. A solution of **7** (2.5 g, 18.9 mmol) in THF (20 mL) was added and the resulting thick yellow precipitate was cooled to 0 °C. Benzyl bromide (10.6 mL, 89.1 mmol) was added over 10 min. The suspension was warmed to 25 °C and stirred for 5 h. The mixture was quenched by the addition of saturated aqueous NH_4Cl (75 mL) and H_2O (75 mL). The mixture was extracted with CH_2Cl_2 (3×100 mL) and the combined organic layers dried ($MgSO_4$) and concentrated. Chromatography (2–10% EtOAc–hexane, gradient elution) afforded **8** (5.87 g, 5.91 g theoretical, 99%) as a yellow solid: mp 77–78 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.18–7.27 (m, 11H, phenyl, C5-H), 6.85 (d, J = 7.4 Hz, 1H, C4-H), 6.76 (d, J = 8.3 Hz, 1H, C6-H), 4.34 (s, 4H, $PhCH_2$), 2.52 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 154.8, 143.9, 137.3, 132.4, 128.3, 127.2, 123.4, 119.0, 117.7, 107.8, 56.7, 21.0; IR (film) ν_{max} 3395, 3333, 3231, 2205, 1646, 1595, 1574, 1472, 1297, 780 cm^{-1}; FABHRMS (NBA–NaI) m/z 335.1540 ($M^+ + Na$, $C_{22}H_{20}N_2$ requires 335.1524). Anal. calcd for $C_{22}H_{20}N_2$: C, 84.58; H, 6.45; N, 8.97. Found: C, 84.60; H, 6.29; N, 8.86.

Methyl 4-(3-dibenzylamino-2-cyanophenyl)butanoate (9) and dimethyl 2-(2-(3-dibenzylamino-2-cyanophenyl)ethyl) pentanedioate (10). A solution of diisopropylamine (2.0 mL, 14.3 mmol) in anhydrous THF (20 mL) cooled to −20 °C was treated with *n*-BuLi (6.2 mL, 2.3 M in hexane, 14.3 mmol) and the resulting solution was stirred for 20 min before being cooled to −78 °C. A solution of **8** (4.0 g, 12.8 mmol) in THF (12 mL) was added and the deep red solution was stirred for 45 min at −78 °C. Methyl acrylate (1.3 mL, 14.4 mmol) was added and the mixture was stirred for 15 min before being quenched by the addition of saturated aqueous NH_4Cl (25 mL). The mixture was diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Chromatography (20% EtOAc–hexane) afforded **9** (1.95 g, 38%) and **10** (929 mg, 15%), as well as recovered **8** (840 mg, 21%).

For **9**: yellow oil; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.13–7.25 (m, 11H), 6.82 (d, J = 7.5 Hz, 1H, C6-H), 6.77 (d, J = 8.2 Hz, 1H, C4-H), 4.30 (s, 4H, $PhCH_2$), 3.61 (s, 3H, CH_3), 2.83 (t, J = 7.7 Hz, 2H, C4-H), 2.34 (t, J = 7.4 Hz, 2H, C2-H), 1.98 (p, J = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 173.2, 154.9, 147.1, 137.0, 132.5, 128.2, 127.1, 122.6, 119.5, 117.3, 107.4, 56.6, 51.4, 33.8, 25.5; IR (film) ν_{max} 3061, 3021, 2941, 2842, 2214, 1734, 1585, 1575, 1361, 738, 699 cm^{-1}; FABHRMS (NBA) m/z 399.2076 ($M^+ + H$, $C_{26}H_{26}N_2O_2$ requires 399.2073). Anal. calcd for $C_{26}H_{26}N_2O_2$: C, 78.36; H, 6.58; N, 7.03. Found: C, 78.20; H, 6.46; N, 7.21.

For **10**: yellow oil; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.18–7.30 (m, 11H), 6.84 (d, J = 7.5 Hz, 1H, C6-H), 6.79 (d, J = 8.2 Hz, 1H, C4-H), 4.33 (s, 4H, $PhCH_2$), 3.68 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 2.81 (t, J = 8.0 Hz, 2H), 2.42–2.53 (m, 1H), 2.34 (dt, J = 7.6, 2.7 Hz, 2H), 1.78–2.08 (m, 4H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 175.2, 173.2, 155.0, 147.1, 137.1, 132.6, 128.3 (2C), 127.2, 122.6, 119.6, 117.3, 107.3, 56.7, 51.7, 51.5, 44.1, 32.8, 32.5, 26.9; IR (film) ν_{max} 3061, 3031, 2941, 2852, 2214, 1729, 1585, 1575, 1495, 1470, 1450, 1241, 1201, 1162, 743, 699 cm^{-1}; FABHRMS (NBA–NaI) m/z 507.2241 ($M^+ + Na$, $C_{30}H_{32}N_2O_4$ requires 507.2260).

Methyl 4-(3-amino-2-cyanophenyl)butanoate (11). A solution of **9** (1.51 g, 3.79 mmol) in glacial HOAc (65 mL) was hydrogenated over a catalytic amount of moist $Pd(OH)_2$-C at 25 °C under 1 atm H_2. After 22 h, the mixture was filtered through Celite, and rinsed with H_2O (200 mL) and EtOAc (300 mL). The layers were separated and the aqueous layer was further extracted with EtOAc (200 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ to neutrality, then with H_2O (250 mL), dried (Na_2SO_4) and concentrated to afford **11** (683 mg, 827 mg theoretical, 83%) as an orange oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.12 (t, J = 7.9 Hz, 1H, Ar C5-H), 6.54 (d, J = 8.3 Hz, 1H, Ar C6-H), 6.48 (d, J = 7.5 Hz, 1H, Ar C4-H), 4.59 (br s, 2H, NH_2), 3.59 (s, 3H, CH_3), 2.67 (t, J = 7.6 Hz, 2H, C4-H), 2.28 (t, J = 7.4 Hz, 2H, C2-H), 1.89 (p, J = 7.5 Hz, 2H, C3-H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 173.3, 150.3, 145.0, 133.1, 117.6, 116.6, 112.5, 95.8, 51.2, 33.5, 32.8, 25.2; IR (film) ν_{max} 3459, 3369, 3240, 2941, 2862, 2204, 1729, 1629, 1595, 1580, 1475, 1435, 788, 738 cm^{-1}; FABHRMS (NBA–NaI) m/z 241.0949 ($M^+ + Na$, $C_{12}H_{14}N_2O_2$ requires 241.0953).

2,4-Diamino-5-(3-methoxycarbonylprop-1-yl)quinazoline (12). A mixture of **11** (303.8 mg, 1.39 mmol) and chloroformamidine hydrochloride[8] (292 mg, 2.54 mmol) in freshly distilled diglyme (7 mL) was warmed at 135 °C for 19 h. After cooling to 25 °C, the solvent was removed in vacuo. The residue was dissolved in H_2O (15 mL) and saturated aqueous $NaHCO_3$ was added to neutrality. The precipitated product was extracted into EtOAc (2×15 mL) and the organic phase dried (Na_2SO_4) and concentrated. Chromatography (85:15:1 $CHCl_3$–EtOH–Et_3N) afforded **12** (274 mg, 362 mg theoretical, 76%) as

a pale-yellow solid: mp 161–164 °C (EtOAc–hexane, white powder); ^{1}H NMR (CF_3CO_2D, 400 MHz) δ 7.87 (t, J = 8.0 Hz, 1H, C7-H), 7.42 (t, J = 7.2 Hz, 2H, C6 and C8-H), 3.75 (s, 3H, CH_3), 3.09–3.14 (m, 2H, C1′-H), 2.64 (t, J = 5.9 Hz, 2H, C3′-H), 1.91–1.98 (m, 2H, C2′-H); ^{13}C NMR (CF_3CO_2D, 100 MHz) δ 180.6, 160.2, 150.6, 146.1, 142.1, 141.5, 133.4, 119.4, 108.7, 54.6, 36.4, 33.7, 26.9; IR (KBr) ν_{max} 3461, 3324, 3162, 2945, 1719, 1637, 1603, 1573, 1554, 1507, 1473, 1393, 1369, 1265, 1224, 1199, 1151, 1042, 985, 814 cm^{-1}; FABHRMS (NBA–CsI) m/z 393.0328 (M^+ + Cs, $C_{13}H_{16}N_4O_2$ requires 393.0328). Anal. calcd for $C_{13}H_{16}N_4O_2$: C, 59.99; H, 6.20; N, 21.52. Found: C, 59.77; H, 6.01; N, 21.06.

***N*,*N*,*N*′,*N*′-Tetra(*t*-butyloxycarbonyl)-2,4-diamino-5-(3-methoxycarbonyl-prop-1-yl)quinazoline (13).** A solution of **12** (20 mg, 0.077 mmol) in THF (0.5 mL) was treated with di-*t*-butyl dicarbonate (88 μL, 0.39 mmol), Et_3N (54 μL, 0.39 mmol), and 4-dimethylaminopyridine (DMAP, 2 mg, 0.016 mmol). The resulting solution was stirred at 25 °C for 1 h before it was concentrated. Chromatography (50% EtOAc–hexane) afforded **13** (45 mg, 51 mg theoretical, 89%) as a white solid: mp 132–133.5 °C (EtOAc–hexane, white crystals); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.91 (dd, J = 8.4, 0.9 Hz, 1H), 7.78 (dd, J = 8.3, 7.3 Hz, 1H, C7-H), 7.48 (d, J = 7.2 Hz, 1H), 3.66 (s, 3H, CH_3), 3.10 (t, J = 7.9 Hz, 2H, C1′-H), 2.36 (t, J = 7.5 Hz, 2H, C3′-H), 2.00 (p, J = 7.7 Hz, 2H, C2′-H), 1.41 (s, 18H, *t*-Bu), 1.30 (s, 18H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 173.3, 160.1, 153.4, 150.7, 150.0, 138.4, 133.7, 129.9, 127.6, 119.8, 84.1, 83.3, 51.6, 33.7, 33.6, 27.8, 27.6, 26.0; IR (film) ν_{max} 2960, 2947, 1790, 1751, 1734, 1564, 1364, 1277, 1251, 1154, 1118, 1097, 851, 780, 728 cm^{-1}; FABHRMS (NBA–NaI) m/z 661.3450 (M^+ + H, $C_{33}H_{48}N_4O_{10}$ requires 661.3449). Anal. calcd for $C_{33}H_{48}N_4O_{10}$: C, 59.99; H, 7.32; N, 8.48. Found: C, 59.89; H, 7.53; N, 8.29.

***N*,*N*,*N*′,*N*′-Tetra(*t*-butyloxycarbonyl)-2,4-diamino-5-(4-oxobut-1-yl)quinazoline (14) and *N*,*N*,*N*′-tris(*t*-butyloxycarbonyl)-2,4-diamino-5-(4-oxobut-1-yl)quinazoline (15).** A solution of **13** (25 mg, 0.038 mmol) in anhydrous toluene (750 μL) at −78 °C was treated with a solution of DIBAL–H (40 μL, 1.0 M in hexanes, 0.040 mmol). After 15 min, an additional 50 μL (0.050 mmol) of DIBAL–H solution was added. After 15 min, the reaction was quenched by addition of CH_3OH (500 μL) and allowed to warm to 25 °C. The mixture was added to H_2O (10 mL) and extracted with EtOAc (3×10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. Chromatography (30% EtOAc–hexane) afforded **14** (9.7 mg, 41%) and **15** (4.0 mg, 20%), as well as recovered **13** (6.3 mg, 25%). For **14**: colorless oil; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.78 (s, 1H, CHO), 7.95 (d, J = 8.4 Hz, 1H), 7.81 (t, J = 7.8 Hz, 1H, C7-H), 7.50 (d, J = 7.0 Hz, 1H), 3.12 (t, J = 7.9 Hz, 2H, C1′-H), 2.54 (t, J = 7.3 Hz, 2H, C3′-H), 2.01 (p, J = 7.6 Hz, 2H, C2′-H), 1.44 (s, 18H, *t*-Bu), 1.32 (s, 18H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 201.4, 160.1, 155.2, 153.5, 150.7, 150.1, 138.4, 133.7, 130.0, 127.8, 119.8, 84.2, 83.3, 43.6, 33.7, 27.9, 27.6, 23.3; IR (film) ν_{max} 2980, 2933, 1793, 1762, 1718, 1560, 1369, 1276, 1251, 1156, 1119, 853 cm^{-1}; FABHRMS (NBA–CsI) m/z 763.2314 (M^+ + Cs, $C_{32}H_{46}N_4O_9$ requires 763.2319). For **15**: colorless oil; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.78 (t, J = 1.2 Hz, 1H, CHO), 7.92 (dd, J = 8.4, 0.9 Hz, 1H), 7.71 (dd, J = 8.4, 7.2 Hz, 1H), 7.44 (br s, 1H, NH), 7.32 (d, J = 7.2 Hz, 1H), 3.04 (t, J = 7.9 Hz, 2H, C1′-H), 2.53 (dt, J = 7.3, 1.0 Hz, 2H, C3′-H), 2.00 (p, J = 7.6 Hz, 2H, C2′-H), 1.57 (s, 9H, *t*-Bu), 1.32 (s, 18H, *t*-Bu); IR (film) ν_{max} 2979, 2923, 1793, 1762, 1718, 1560, 1369, 1275, 1251, 1162, 1105, 854 cm^{-1}; FABHRMS (NBA) m/z 531.2837 (M^+ + H, $C_{27}H_{38}N_4O_7$ requires 531.2819).

2,4-Diamino-5-(4-oxobut-1-yl)quinazoline (1). A solution of **14** (6.2 mg, 0.0098 mmol) in $CHCl_3$ (90 μL) was treated with CF_3CO_2H (30 μL), and the resulting solution stirred at 25 °C for 2 h. The solvents were evaporated and the residue triturated with EtOAc to afford **1** (2.6 mg, 4.5 mg theoretical, 57%) as a white solid: ^{1}H NMR (DMF–d_7, 400 MHz) δ 9.80 (s, 1H, CHO), 7.76 (t, J = 7.8 Hz, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 3.22 (t, J = 8.1 Hz, 2H, C1′-H), 1.98 (p, J = 8.1 Hz, 2H, C2′-H); ^{1}H NMR (CD_3OD, 400 MHz, hemiacetal) δ 7.65 (t, J = 7.9 Hz, 1H, C7-H), 7.25 (d, J = 8.0 Hz, 2H, C5-H, C6-H), 4.60 (t, J = 5.2 Hz, 1H, C4′-H), 3.14 (t, J = 8.0 Hz, 2H, C1′-H), 1.98 (p, J = 8.1 Hz, 2H, C2′-H); ^{1}H NMR (CF_3COOD, 400 MHz) δ 9.84 (s, 1H), 7.98 (t, J = 8 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.51 (d, J = 7.4 Hz, 1H), 3.16 (t, J = 8.8 Hz, 2H), 3.04 (t, J = 6.0 Hz, 2H), 2.06 (m, 2H); ^{13}C NMR (CF_3COOD, 100 MHz) δ 211.9, 160.3, 151.0, 146.3, 142.2, 141.6, 133.5, 119.5, 108.7, 44.1, 36.1, 24.6; IR (KBr) ν_{max} 3412, 1676, 1599, 1512, 1200, 1128, 915, 820 cm^{-1}; FABHRMS (NBA) m/z 231.1245 (M^+ + H, $C_{12}H_{14}N_4O$ requires 231.1246). Similarly, **15** (2.2 mg, 0.0066 mmol) in 48 μL of 3:1 v/v $CHCl_3$–CF_3CO_2H afforded **1** (1.5 mg, 1.9 mg theoretical, 79%).

2,4-Diamino-5-(4-hydroxybut-1-yl)quinazoline (16). A solution of **12** (50 mg, 0.19 mmol) in 3:2 v/v EtOH–THF (3.0 mL) was treated with $NaBH_4$ (36 mg, 0.95 mmol) and LiCl (40 mg, 0.94 mmol) and the mixture was warmed at 55 °C for 18 h. The reaction was quenched by addition of acetone (10 mL) and the solvents removed in vacuo. The residue was suspended in EtOH and filtered through Celite. The EtOH was removed in vacuo and the residue purified by flash chromatography (8:2:0.5 EtOAc:CH_3OH:Et_3N) to afford **16** (39 mg, 45 mg theoretical, 88%) as a white solid: mp > 210 °C (decomp.); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.45 (dd, J = 8.3, 7.3 Hz, 1H, C7-H), 7.18 (dd, J = 8.4, 1.1 Hz, 1H), 7.00

(d, $J=7.2$ Hz, 1H), 3.61 (t, $J=6.3$ Hz, 2H, C4′-H), 3.09 (br t, $J=7.9$ Hz, 2H, C1′-H), 1.75–1.83 (m, 2H, C2′-H), 1.64 (p, $J=6.8$ Hz, 2H, C3′-H); ^{13}C NMR (CD_3OD, 100 MHz) δ 164.7, 160.9, 155.1, 141.2, 133.7, 125.2, 123.8, 111.0, 62.2, 36.8, 32.9, 28.7; IR (KBr) ν_{max} 3476, 3351, 3139, 2925, 1663, 1618, 1603, 1577, 1550, 1477, 1400, 1269, 1059, 975, 750 cm^{-1}; FABHRMS (NBA) m/z 233.1409 ($M^+ + H$, $C_{12}H_{16}N_4O$ requires 233.1402).

(*E*,*Z*)-7-(*t*-Butyldiphenylsilyloxy)-2-heptenenitrile (18). A suspension of NaH (60% dispersion, 675 mg, 16.9 mmol) in anhydrous THF (75 mL) at 0 °C was treated with diethyl cyanomethylphosphonate (2.85 mL, 17.6 mmol). The resulting solution was stirred for 30 min and was followed by the addition of **17** (5.0 g, 14.7 mmol) in THF (25 mL). The mixture was stirred at 0 °C for 1 h before being added to H_2O (300 mL) and extracted with Et_2O (2×150 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (10% EtOAc–hexane) afforded **18** (4.40 g, 5.34 g theoretical, 82%) as a colorless oil judged to be a mixture of *E* and *Z* isomers (ca. 1:1 ratio by ^{1}H NMR): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62–7.65 (m, 8H, Ar), 7.34–7.44 (m, 12H, Ar), 6.66 (dt, $J=16.3$, 6.9 Hz, 1H, C3-H *E*-isomer), 6.43 (dt, $J=10.9$, 7.7 Hz, 1H, C3-H, *Z*-isomer), 5.28 (dt, $J=10.9$, 1.4 Hz, 1H, C2-H, *Z*-isomer), 5.26 (dt, $J=16.3$, 1.7 Hz, 1H, C2-H, *E*-isomer), 3.63–3.67 (m, 4H, C7-H), 2.37–2.42 (m, 2H, C4-H, *Z*-isomer), 2.15–2.20 (m, 2H, C4-H, *E*-isomer), 1.50–1.58 (m, 8H, C5 and C6-H), 1.04 (s, 18H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.8, 154.9, 135.4, 133.74, 133.68, 129.6, 129.5, 127.6, 117.5, 115.9, 99.7, 99.5, 63.2, 63.1, 32.8, 31.54, 31.48, 26.8, 24.5, 23.8, 19.1; IR (neat) ν_{max} 2935, 2859, 2218, 1628, 1105, 822, 738, 700, 686 cm^{-1}; FABHRMS (NBA–NaI) m/z 386.1902 ($M^+ + Na$, $C_{23}H_{29}NO_2Si$ requires 386.1916). Anal. calcd for $C_{23}H_{29}NO_2Si$: C, 75.98; H, 8.04; N, 3.85. Found: C, 76.06; H, 7.72; N, 4.03.

Ethyl 7-(*t*-butyldiphenylsilyloxy)-3-cyanomethyl-2-(ethoxycarbonyl)heptanoate (19). A neat mixture of diethyl malonate (3.5 mL, 23.0 mmol) and **18** (4.20 g, 11.6 mmol) was treated with 1.62 M NaOEt in EtOH (720 μL, 1.17 mmol) and the mixture was warmed at 65 °C for 25 h. The mixture was neutralized by addition of HOAc (67 μL), diluted with CH_2Cl_2 (80 mL) and washed with H_2O (80 mL). The aqueous layer was back extracted with CH_2Cl_2 (2×40 mL) and the combined organic layers were washed with saturated aqueous NaCl (80 mL), dried ($MgSO_4$) and concentrated. Chromatography (15% EtOAc–hexane) afforded **19** (4.90 g, 6.05 g theoretical, 81%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62–7.65 (m, 4H, Ar), 7.34–7.43 (m, 6H, Ar), 4.19 (q, $J=7.1$ Hz, 4H, $CO_2CH_2CH_3$), 3.64 (t, $J=6.2$ Hz, 2H, C7-H), 3.49 (d, $J=7.0$ Hz, 1H, C2-H), 2.54–2.65 (m, 2H, CH_2CN), 2.38–2.45 (m, 1H, C3-H), 1.34–1.57 (m, 6H), 1.25 (t, $J=7.1$ Hz, 3H, $CO_2CH_2CH_3$), 1.03 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 168.0, 167.7, 135.5, 133.9, 129.6, 127.6, 118.0, 63.4, 61.8, 61.7, 53.9, 34.9, 32.2, 30.9, 26.8, 23.0, 19.3, 19.2, 14.0; IR (neat) ν_{max} 2933, 2859, 2246, 1744, 1732, 1472, 1428, 1390, 1370, 1304, 1177, 1154, 1112, 1030, 823, 743, 704 cm^{-1}; FABHRMS (NBA–NaI) m/z 546.2637 ($M^+ + Na$, $C_{30}H_{41}NO_5Si$ requires 546.2652). Anal. calcd for $C_{30}H_{41}NO_5Si$: C, 68.80; H, 7.89; N, 2.67. Found: C, 68.89; H, 7.49; N, 2.86.

4-(4-(*t*-Butyldiphenylsilyloxy)but-1-yl)-3-ethoxycarbonyl-2-piperidone (20). A solution of **19** (718 mg, 1.37 mmol) in glacial HOAc (85 mL) was hydrogenated over PtO_2 (31 mg, 0.14 mmol) at 25 °C under 1 atm H_2 for 24 h. The reaction mixture was filtered through Celite and the solvent removed in vacuo. The residual yellow oil was heated at 160 °C under vacuum (0.1 mm Hg) for 1 h, then allowed to cool. The brown oil was purified by chromatography (80% EtOAc–hexane) to afford **20** (463 mg, 660 mg theoretical, 70%) as a pale-yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.61–7.65 (m, 4H, Ar), 7.32–7.41 (m, 6H, Ar), 6.07 (br s, 1H, NH), 4.20 (q, $J=7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.63 (t, $J=6.1$ Hz, 2H, C4′-H), 3.29–3.34 (m, 2H, C6-H), 3.03 (d, $J=9.6$ Hz, 1H, C3-H), 1.30–2.18 (m, 9H), 1.25 (t, $J=7.1$ Hz, 3H, $CO_2CH_2CH_3$), 1.02 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 170.5, 168.5, 135.5, 133.9, 129.5, 127.5, 63.5, 61.2, 55.4, 40.7, 35.9, 33.7, 32.4, 26.8, 25.8, 22.5, 19.1, 14.1; IR (neat) ν_{max} 3210, 2933, 2851, 1733, 1672, 1487, 1467, 1421, 1251, 1153, 1108, 1036, 821, 703 cm^{-1}; FABHRMS (NBA–CsI) m/z 614.1715 ($M^+ + Cs$, $C_{28}H_{39}NO_4Si$ requires 614.1703). Anal. calcd for $C_{28}H_{39}NO_4Si$: C, 69.82; H, 8.16; N, 2.91. Found: C, 69.69; H, 7.79; N, 3.05.

4-(4-(*t*-Butyldiphenylsilyloxy)but-1-yl)-3-ethoxycarbonyl-2-methoxy-3,4,5,6-tetrahydropyridine (21). A solution of **20** (169 mg, 0.35 mmol) in anhydrous CH_2Cl_2 (13 mL) at 4 °C was treated with Me_3OBF_4 (205 mg, 1.39 mmol) and the resulting suspension was stirred at 4 °C for 90 min, during which most of the suspended material dissolved. The reaction was warmed to 25 °C and stirred for 1 h, then added to CH_2Cl_2 (25 mL). The solution was washed with saturated aqueous $NaHCO_3$ (25 mL) and the aqueous phase back extracted with CH_2Cl_2 (2×20 mL). The combined organic layers were washed with H_2O (25 mL) and saturated aqueous NaCl (25 mL), dried ($MgSO_4$) and concentrated to afford **21** (581 mg, 628 mg theoretical, 92%) as a colorless oil which was used in the subsequent reaction without further purification: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.62–7.66 (m, 4H, Ar), 7.32–7.43 (m, 6H, Ar), 4.17 (q, $J=7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.61–3.66 (m, 5H, C4′-H, OCH_3), 3.56

(m, 2H, C6-H), 2.88 (d, $J=8.8$ Hz, 1H, C3-H), 1.11–1.77 (m, 9H), 1.24 (t, $J=7.1$ Hz, 3H, $CO_2CH_2CH_3$), 1.03 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 171.4, 158.7, 135.5, 133.9, 129.5, 127.5, 63.6, 61.0, 52.7, 50.6, 45.6, 35.9, 33.9, 32.5, 26.8, 26.2, 22.6, 19.1, 14.1; IR (neat) ν_{max} 2935, 2852, 1732, 1685, 1473, 1457, 1426, 1307, 1229, 1151, 1105, 1033, 820, 737, 701, 613 cm^{-1}; FABHRMS (NBA–NaI) *m/z* 496.2889 ($M^+ + H$, $C_{29}H_{41}NO_4Si$ requires 496.2883).

2-Amino-5-(4-(*t*-butyldiphenylsilyloxy)but-1-yl)-4-hydroxy-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (22). Sodium metal (48 mg, 2.1 mmol) was dissolved in 600 μL of distilled 2-ethoxyethanol and the resulting solution was added to a solution of guanidine hydrochloride (200 mg, 2.1 mmol) in 600 μL 2-ethoxyethanol using an additional 400 μL solvent to complete transfer and providing a 1.3 M solution of guanidine free base. The guanidine solution (1.17 mL, 1.52 mmol) was added to **21** (355 mg, 0.72 mmol) and the 2-ethoxyethanol removed in vacuo. The mixture was warmed at 90 °C for 16 h, during which solidification occurred. The solid was taken up in CH_2Cl_2 (20 mL) and H_2O (20 mL) and the aqueous layer adjusted to neutrality with dilute aqueous HCl. The layers were separated and the aqueous layer extracted with CH_2Cl_2 (2×10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Chromatography (5% CH_3OH–CH_2Cl_2) afforded **22** (219.1 mg, 341 mg theoretical, 64%) as a white solid: mp 184–185 °C (EtOAc–hexane, white powder); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62–7.65 (m, 4H, Ar), 7.32–7.41 (m, 6H, Ar), 5.70 (br s, 2H, N2-H), 5.04 (br s, 1H, N8-H), 3.63 (t, $J=6.2$ Hz, 2H, C4′-H), 3.25–3.30 (m, 2H, C7-H), 2.71 (m, 1H, C5-H), 1.17–1.81 (m, 8H), 1.01 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 163.0, 159.6, 153.4, 135.6, 134.1, 129.5, 127.6, 89.6, 63.9, 36.9, 34.1, 32.7, 28.7, 26.9, 24.0, 23.3, 19.2; IR (film) ν_{max} 3333, 3190, 2933, 2862, 1615, 1544, 1462, 1426, 1349, 1108, 703 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 609.1640 ($M^+ + Cs$, $C_{27}H_{36}N_4O_2Si$ requires 609.1662). Anal. calcd for $C_{27}H_{36}N_4O_2Si$: C, 68.03; H, 7.61; N, 11.75. Found: C, 67.87; H, 7.49; N, 12.00.

2-Amino-4-hydroxy-5-(4-hydroxybut-1-yl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (23). A solution of **22** (20 mg, 0.042 mmol) in THF (330 μL) was treated with Bu_4NF (1 M in THF, 85 μL, 0.085 mmol) and the mixture stirred at 25 °C for 18 h. The solvent was evaporated and the residual solid dissolved in 20% EtOH–$CHCl_3$. Chromatography (20% EtOH–$CHCl_3$) afforded pure **23** (10.0 mg, 10.0 mg theoretical, 100%) as a white solid: mp 228–231 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ 3.55 (t, $J=6.4$ Hz, 2H, C4′-H), 2.79 (m, 1H, C5-H), 1.24–1.89 (m, 8H, C7-H obscured by solvent at 3.30); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.1, 158.9 and 158.8, 153.1, 87.5 and 87.4, 60.8 and 60.7, 35.9 and 35.8, 34.3, 32.9 and 32.8, 28.3, 24.0, 23.0; IR (KBr) ν_{max} 3415, 3345, 2933, 2851, 1628, 1541, 1457, 1349, 1316, 1039, 782 cm^{-1}; FABHRMS (NBA) *m/z* 239.1512 ($M^+ + H$, $C_{11}H_{18}N_4O_2$ requires 239.1508).

2-*N*,*N*-Bis(*t*-butyloxycarbonyl)amino-5-(4-((*t*-butyldiphenylsilyl)oxy)but-1-yl)-3,8-di(*t*-butyloxycarbonyl)-4-hydroxy-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (24) and 2-*N*,*N*-bis(*t*-butyloxycarbonyl)amino-5-(4-((*t*-butyldiphenylsilyl)oxy)but-1-yl)-4-hydroxy-3,8-di(*t*-butyloxycarbonyl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (25). A solution of **22** (7.0 mg, 15 μmol) in THF (500 μL) was treated sequentially with DMAP (0.2 mg, 1.6 μmol), BOC_2O (17.2 μL, 75 μmol) and Et_3N (10.5 μL, 75 μmol) and the mixture was stirred at 25 °C for 2 h. The solvent was evaporated and the residual solid was purified by preparative centrifugal TLC (SiO_2, 0.5 mm plate, 10–50% EtOAc–hexane gradient elution) to afford **24** (6.8 mg, 11.4 mg theoretical, 60%) and **25** (2.7 mg, 10.1 mg theoretical, 27%). For **24**: colorless film; R_f 0.45 (SiO_2, 20% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (m, 4H), 7.38 (m, 6H), 3.96 (m, 1H), 3.64 (t, $J=6.4$ Hz, 2H), 3.51 (m, 1H), 1.93 (m, 1H), 1.79 (m, 1H), 1.60–1.30 (m, 6H), 1.51 (s, 9H), 1.49 (s, 9H), 1.42 (s, 18H), 1.03 (s, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 160.7, 154.3, 152.9, 150.6, 149.1, 139.9, 135.5, 129.6, 127.6, 111.3, 84.6, 83.1, 82.6, 63.6, 41.4, 32.7, 32.3, 30.0, 28.1, 27.8, 27.5, 26.9, 24.8, 23.2, 19.2; IR (KBr) ν_{max} 2922, 1732, 1634, 1562, 1366, 1306, 1153, 1111, 850, 702 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 1009.3715 ($M^+ + Cs$, $C_{47}H_{68}N_4O_{10}Si$ requires 1009.3759).

For **25**: colorless film; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (m, 4H), 7.38 (m, 6H), 3.70 (m, 1H), 3.63 (t, $J=6.4$ Hz, 2H), 3.52 (m, 1H), 2.85 (m, 1H), 1.78–1.60 (m, 8H), 1.55 (s, 18H), 1.46 (s, 9H), 1.02 (s, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.4, 154.7, 151.8, 149.2, 145.5, 135.6, 134.1, 129.5, 127.6, 108.9, 86.1, 81.3, 63.9, 42.8, 32.6, 32.3, 30.1, 28.2, 27.7, 26.9, 26.5, 23.3, 19.2.

2-*N*,*N*-Bis(*t*-butyloxycarbonyl)amino-8-(*t*-butyloxycarbonyl)-4-hydroxy-5-(4-hydroxybut-1-yl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (26). A solution of **24** (6.8 mg, 7.8 μmol) in THF (500 μL) was treated with Bu_4NF (16 μL, 1.0 M in THF, 16 μmol) and the mixture was stirred at 25 °C for 3 h. The solvent was evaporated and the residual solid was purified by flash chromatography (SiO_2, 2–5% CH_3OH–CH_2Cl_2 gradient elution) to afford **26** (3.5 mg, 4.2 mg theoretical, 83%) as a colorless thin film: R_f 0.3 (SiO_2, 5% CH_3OH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.70 (m, 1H), 3.56 (m, 2H), 3.48 (m, 1H), 2.82 (m, 1H), 2.03–1.50 (m, 8H), 1.47 (s, 18H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.7, 154.9, 151.8, 149.1, 145.5, 108.7, 86.0, 81.4, 62.8, 42.8, 32.5, 32.3, 29.8, 28.1, 27.7, 26.7, 23.2; IR (KBr) ν_{max} 2932, 1800, 1723, 1641, 1564, 1469, 1366, 1314, 1254,

1154, 1109, 1060 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 671.2026 ($M^+ + Cs$, $C_{26}H_{42}N_4O_8$ requires 671.2057).

2-*N*,*N*-Bis(*t*-butyloxycarbonyl)amino-8-(*t*-butyloxycarbonyl)-4-hydroxy-5-(4-oxobut-1-yl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (27). A solution of **26** (3.5 mg, 6.5 μmol) in CH_2Cl_2 (200 μL) was treated with the Dess–Martin periodinane[13] (*o*-$Ph(CO_2)I(OAc)_3$, 7.0 mg, 17 μmol) and the mixture was stirred at 25 °C for 2 h. The solvent was evaporated and the residual solid was purified by flash chromatography (SiO_2, 50% EtOAc–hexane) to afford **27** (2.9 mg, 3.5 mg theoretical, 83%) as a colorless film: R_f 0.35 (SiO_2, 50% EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.75 (t, $J = 1.5$ Hz, 1H), 3.74 (m, 1H), 3.52 (m, 1H), 2.87 (m, 1H), 2.48 (m, 2H), 1.78 (m, 4H), 1.24 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.7, 161.4, 154.9, 151.7, 149.1, 145.7, 108.1, 86.2, 81.4, 43.8, 42.8, 36.2, 29.9, 28.2, 27.7, 26.6, 19.5; IR (KBr) ν_{max} 2975, 2928, 1806, 1774, 1722, 1637, 1562, 1365, 1308, 1276, 1252, 1153, 1121, 979, 853 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 699.1870 ($M^+ + Cs$, $C_{26}H_{40}N_4O_8$ requires 669.1900).

2-Amino-4-hydroxy-5-(4-oxobut-1-yl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (2). A solution of **27** (2.4 mg, 4.5 μmol) in CH_2Cl_2 (200 μL) was treated with 4 N HCl–EtOAc (1.0 mL) and the mixture was stirred at 25 °C for 2 h. The solvent was evaporated to afford **2** (1.1 mg, 1.06 mg theoretical, 100%) as a light-yellow oil: ^1H NMR (CF_3COOD, 400 MHz) δ 9.73 (br s, 1H), 3.57 (m, 1H), 3.48 (dd, $J = 13.2$, 10.4 Hz, 1H), 3.00 (br s, 1H), 1.85–1.47 (m, 8H); ^{13}C NMR (CF_3COOD, 100 MHz) δ 213.0, 162.5, 154.1, 151.9, 91.8, 39.2, 34.5, 31.4, 29.7, 23.9, 20.5; IR (KBr) ν_{max} 3397, 2937, 1688, 1643, 1438, 1353, 1203, 1138, 843, 803, 718 cm^{-1}; FABHRMS (NBA) *m/z* 237.1352 ($M^+ + H$, $C_{11}H_{16}N_4O_2$ requires 237.1359).

2-(*t*-Butylcarbonyl)amino-5-(4-(*t*-butyldiphenylsilyloxy)but-1-yl)-4-hydroxy-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (28) and 8-(*t*-butylcarbonyl)-2-(*t*-butylcarbonyl)amino-5-(4-(*t*-butyldiphenylsilyloxy)but-1-yl)-4-hydroxy-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (29). 4-Dimethylaminopyridine (2.5 mg, 0.020 mmol) and **22** (46.8 mg, 0.098 mmol) were combined in trimethylacetic anhydride (330 μL) and the suspension was warmed at at 90 °C (complete solution occurred) for 6 h before being allowed to stand at 25 °C for 12 h. The mixture was added to H_2O (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Chromatography (30–50% EtOAc–hexane gradient elution) afforded **28** (32.5 mg, 59%) and **29** (12.6 mg, 19%). For **28**: white powder, mp 187–189 °C (EtOAc–hexane); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.62–7.66 (m, 4H, Ar), 7.31–7.42 (m, 6H, Ar), 4.70 (br s, 1H, N8-H), 3.64 (t, $J = 6.2$ Hz, 2H, C4′-H), 3.26–3.29 (m, 2H, C7-H), 2.89 (m, 1H, C5-H), 1.18–1.85 (m, 8H), 1.26 (s, 9H, *t*-Bu), 1.01 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 179.5, 160.0, 157.2, 147.9, 135.5, 134.1, 129.4, 127.4, 95.1, 64.0, 40.1, 37.0, 33.8, 32.7, 28.7, 27.0, 26.8, 23.6, 23.1, 19.2; IR (film) ν_{max} 3374, 3262, 2931, 2857, 1640, 1610, 1573, 1482, 1461, 1388, 1308, 1205, 1154, 1111, 822, 779, 738, 701 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 693.2258 ($M^+ + Cs$, $C_{32}H_{44}N_4O_3Si$ requires 693.2237).

For **29**: colorless oil; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.51 (br s, 1H, N3-H), 7.88 (br s, 1H, C2-NH), 7.62–7.65 (m, 4H, Ar), 7.33–7.41 (m, 6H, Ar), 3.67 (dd, $J = 8.7$, 4.0 Hz, 1H, C7-H), 3.63 (t, $J = 6.4$ Hz, 2H, C4′-H), 3.42 (m, 1H, C7-H), 2.86 (m, 1H, C5-H), 1.17–1.89 (m, 8H), 1.29 (s, 9H, *t*-Bu), 1.27 (s, 9H, *t*-Bu), 1.01 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 185.2, 179.6, 160.8, 155.9, 146.6, 135.5, 134.1, 129.4, 127.5, 106.6, 63.8, 44.1, 42.9, 40.2, 32.9, 32.6, 30.5, 28.8, 27.7, 26.9, 26.8, 23.2, 19.2; IR (film) ν_{max} 3204, 2948, 2931, 2858, 1635, 1609, 1568, 1480, 1403, 1316, 1261, 1188, 1144, 1111, 822, 796, 740 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 777.2847 ($M^+ + Cs$, $C_{37}H_{52}N_4O_4Si$ requires 777.2812).

2-(*t*-Butylcarbonyl)amino-4-hydroxy-5-(4-hydroxybut-1-yl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (30). A solution of **28** (32.5 mg, 0.058 mmol) in THF (460 μL) was treated with Bu_4NF (1 M in THF, 120 μL, 0.12 mmol) and the mixture was stirred at 25 °C for 16 h. The solvent was evaporated and the residual solid dissolved in CH_2Cl_2 (10 mL) and washed with H_2O (10 mL). The organic layer was dried ($MgSO_4$) and concentrated. Chromatography (5% CH_3OH–CH_2Cl_2) afforded **30** (17.6 mg, 18.7 mg theoretical, 94%) as a white powder: mp 213–215 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 8.03 (br s, 1H, NH), 4.83 (br s, 1H, N8-H), 3.58–3.69 (m, 2H, C4′-H), 3.30–3.37 (m, 2H, C7-H), 2.94 (m, 1H, C5-H), 1.30–1.84 (m, 8H), 1.26 (s, 9H, *t*-Bu); ^{13}C NMR (CD_3OD, 100 MHz) δ 182.7, 162.2, 160.7, 150.3, 94.5, 62.9, 41.3, 37.5, 35.0, 33.8, 30.1, 27.0, 24.5, 24.2; IR (film) ν_{max} 3374, 3265, 2934, 2862, 1653, 1576, 1559, 1540, 1457, 1349, 1313, 1200, 1149, 780, 728 cm^{-1}; FABHRMS (NBA) *m/z* 323.2075 ($M^+ + H$, $C_{16}H_{26}N_4O_3$ requires 323.2083).

2-(*t*-Butylcarbonyl)amino-4-hydroxy-5-(4-oxobut-1-yl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (31). A solution of oxalyl chloride (38 μL, 2.0 M in CH_2Cl_2, 0.076 mmol) in CH_2Cl_2 (100 μL) at −60 °C was treated with a solution of DMSO (11 μL, 0.155 mmol) in CH_2Cl_2 (35 μL) and the resulting milky-white solution was stirred for 10 min. A solution of **30** (7.9 mg, 0.025 mmol) in 1:1 v/v CH_2Cl_2–DMSO (150 μL) was cooled to −23 °C and added to the oxidant solution. The mixture was warmed to −23 °C and stirred for 15 min. Et_3N (55 μL, 0.395 mmol) was added and the

mixture stirred for 5 min, then allowed to warm to 25 °C. The mixture was added to H_2O (5 mL) and extracted with CH_2Cl_2 (3×5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Chromatography (5% CH_3OH–CH_2Cl_2) afforded **31** (2.1 mg, 7.8 mg theoretical, 27%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.75 (t, J = 1.7 Hz, 1H, CHO), 4.83 (br s, 1H, N8-H), 3.29–3.32 (m, 2H, C7-H), 2.91 (m, 1H, C5-H), 2.48 (m, 2H, C3′-H), 1.18–1.90 (m, 6H), 1.26 (s, 9H, *t*-Bu); IR (film) ν_{max} 3251, 2934, 2862, 1646, 1636, 1559, 1200, 1150 cm^{-1}; FABHRMS (NBA–NaI) m/z 343.1731 (M^+ + Na, $C_{16}H_{24}N_4O_3$ requires 343.1746).

Ethyl 7-(*t*-butyldiphenylsilyloxy)-3-cyanomethylheptanoate (32). A solution of **19** (5.3 g, 10.1 mmol) in anhydrous DMSO (50 mL) was treated with anhydrous LiI (10.6 g, 79.0 mmol) and the solution was warmed at 170 °C for 2 h. The DMSO was removed under vacuum and the residual oil was added to H_2O (100 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined extracts were washed with saturated aqueous NaCl (50 mL), dried (Na_2SO_4) and concentrated. Chromatography (15% EtOAc–hexane) afforded **32** (3.06 g, 4.57 g theoretical, 67%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.64–7.68 (m, 4H, Ar), 7.34–7.42 (m, 6H, Ar), 4.14 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 3.67 (t, J = 6.1 Hz, 2H, C7-H), 2.46 (m, 2H, CH_2CN), 2.40 (t, J = 6.4 Hz, 2H, C2-H), 2.17 (septet, J = 6.2 Hz, 1H, C3-H), 1.40–1.58 (m, 6H), 1.26 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 1.05 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 171.6, 135.5, 133.8, 129.5, 127.5, 118.0, 63.3, 60.6, 37.6, 33.0, 32.1, 31.8, 26.8, 22.8, 21.5, 19.1, 14.1; IR (neat) ν_{max} 2931, 2851, 2236, 1734, 1458, 1427, 1374, 1256, 1174, 1111, 1026, 822, 702 cm^{-1}; FABHRMS (NBA–NaI) m/z 474.2459 (M^+ + Na, $C_{27}H_{37}NO_3Si$ requires 474.2440). Anal. calcd for $C_{27}H_{37}NO_3Si$: C, 71.80; H, 8.26; N, 3.10. Found: C, 71.65; H, 8.51; N, 3.28.

4-(4-(*t*-Butyldiphenylsilyloxy)but-1-yl)-2-piperidone (33). A solution of **32** (1.03 g, 2.28 mmol) in glacial HOAc (145 mL) was hydrogenated over PtO_2 (52 mg, 0.23 mmol) at 25 °C under 1 atm H_2 for 20 h. The reaction mixture was filtered through Celite and the solvent removed in vacuo. The residual yellow oil was heated at 170 °C under vacuum (0.1 mm Hg) for 1 h, then allowed to cool. The brown oil was purified by flash chromatography (5% EtOH–EtOAc) to afford **33** (890 mg, 934 mg theoretical, 95%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.62–7.66 (m, 4H, Ar), 7.32–7.44 (m, 6H, Ar), 5.85 (br s, 1H, NH), 3.64 (t, J = 6.2 Hz, 2H, C4′-H), 3.24–3.32 (m, 2H, C6-H), 2.40 (ddd, J = 17.3, 4.8, 1.8 Hz, 1H, C3-H), 1.21–1.98 (m, 10H), 1.03 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 172.2, 135.5, 133.9, 129.5, 127.5, 63.5, 41.1, 37.9, 35.3, 32.4, 28.1, 26.8, 22.7, 19.1; IR (neat) ν_{max} 3221, 2924, 2858, 1663, 1495, 1468, 1428, 1337, 1107, 820, 739, 700 cm^{-1}; FABHRMS (NBA) m/z 410.2527 (M^+ + H, $C_{25}H_{36}NO_2Si$ requires 410.2512).

4-(4-(*t*-Butyldiphenylsilyloxy)but-1-yl)-2-methoxy-3,4,5,6-tetrahydropyridine (34). A solution of **33** (203 mg, 0.49 mmol) in anhydrous CH_2Cl_2 (4.8 mL) at 4 °C was treated with Me_3OBF_4 (81 mg, 0.55 mmol) and the resulting suspension was stirred at 4 °C for 2 h, during which most of the suspended material dissolved. The reaction was warmed to 25 °C and stirred for 1 h before being poured into saturated aqueous $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with H_2O (20 mL) and saturated aqueous NaCl (20 mL), dried (Na_2SO_4) and concentrated to afford **34** (200 mg, 210 mg theoretical, 95%) as a colorless oil which was used in the subsequent reaction without further purification: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.63–7.67 (m, 4H, Ar), 7.32–7.41 (m, 6H, Ar), 3.65 (t, J = 6.4 Hz, 2H, C4′-H), 3.61 (s, 3H, OCH_3), 3.29–3.42 (m, 2H, C6-H), 2.24 (dd, J = 15.9, 3.8 Hz, 1H, C3-H), 1.06–1.68 (m, 10H), 1.04 (s, 9H, *t*-Bu); IR (neat) ν_{max} 2931, 2857, 1683, 1472, 1428, 1216, 1111, 1008, 823, 740, 702, 614 cm^{-1}; FABHRMS (NBA) m/z 424.2660 (M^+ + H, $C_{26}H_{38}NO_2Si$ requires 424.2672).

4-(4-(*t*-Butyldiphenylsilyloxy)but-1-yl)-2-iminopiperidine hydrochloride (35). A solution of **34** (189.6 mg, 0.45 mmol) in absolute EtOH (1 mL) was treated with anhydrous NH_4Cl (24.7 mg, 0.46 mmol) at 25 °C for 24 h. The solvent was evaporated and the residue dissolved in Et_2O (10 mL). Hexane (10 mL) was added and the resulting precipitate collected by filtration to afford **35** (163 mg, 206 mg theoretical, 79%) as a hygroscopic white solid: mp 121–123 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.01 (br s, 1H, NH), 8.94 (br s, 1H, NH), 7.61–7.65 (m, 4H, Ar), 7.29–7.44 (m, 6H, Ar), 3.64 (t, J = 6.1 Hz, 2H, C4′-H), 3.47 (m, 1H, C6-H), 3.30 (dt, J = 11.9, 4.2 Hz, 1H, C6-H), 2.74 (dd, J = 18.1, 3.9 Hz, 1H, C3-H), 2.16 (dd, J = 18.1, 10.5 Hz, 1H, C3-H), 1.12–1.92 (m, 9H), 1.02 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 166.8, 135.5, 133.9, 129.6, 127.6, 63.4, 41.0, 34.7, 32.5, 32.3, 29.8, 26.8, 26.6, 22.5, 19.2; IR (film) ν_{max} 3436, 3354, 3190, 3138, 3067, 2933, 2851, 1687, 1646, 1462, 1420, 1344, 1185, 1092, 820, 692 cm^{-1}; FABHRMS (NBA) m/z 409.2668 (M^+ + H, $C_{25}H_{36}N_2OSi$ requires 409.2675).

2,4-Bis(ethoxycarbonyl)-5-(4-(*t*-butyldiphenylsilyloxy)but-1-yl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (36). A suspension of **35** (9.5 mg, 0.021 mmol) and 2,4,6-tris (ethoxycarbonyl)-1,3,5-triazine[14] (63 mg, 0.21 mmol) in anhydrous DMF (50 μL) was warmed at 120 °C for 36 h. The solvent was removed in vacuo and the residue purified by flash chromatography (1% CH_3OH–CH_2Cl_2) followed by PTLC (1% CH_3OH–CH_2Cl_2) to afford **36**

(1.8 mg, 12.6 mg theoretical, 14%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.61–7.64 (m, 4H, Ar), 7.32–7.43 (m, 6H, Ar), 6.12 (br s, 1H, NH), 4.44 (q, J = 7.1 Hz, 2H, C2-$CO_2CH_2CH_3$), 4.37 and 4.38 (two q, J = 7.1 Hz, 2H, C4-$CO_2CH_2CH_3$), 3.64 (t, J = 5.6 Hz, 2H, C4′-H), 3.42–3.46 (m, 3H, C5 and C7-H), 1.11–1.98 (m, 8H), 1.40 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 1.36 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 1.02 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.8, 163.1, 160.1, 153.0, 151.4, 135.5, 134.7, 133.9, 129.6, 128.9, 127.5, 120.1, 63.5, 62.8, 62.3, 36.7, 33.3, 32.2, 30.9, 26.8, 22.7, 21.8, 19.2, 14.2, 14.1; IR (film) ν_{max} 3259, 2931, 2858, 1735, 1596, 1334, 1246, 1210, 1188, 1111, 1023, 703 cm^{-1}; FABHRMS (NBA–NaI) m/z 612.2842 (M^+ + Na, $C_{33}H_{43}N_3O_5Si$ requires 612.2870).

Methyl hept-6-enimidate hydrochloride (38). A solution of **37** (1.24 g, 11.4 mmol) in anhydrous CH_3OH (0.37 g, 11.4 mmol) was treated with excess dry HCl gas at −75 °C. The reaction mixture was slowly warmed to 25 °C and stirred for 5 h. Anhydrous Et_2O (20 mL) was added at −40 °C and the precipitate was collected by filtration under N_2 and dried in vacuo to provide **38** (1.67 g, 83%) as white crystals: mp 79–80 °C (sealed tube); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.54 (br s, 1H), 11.59 (br s, 1H), 5.71 (ddt, J = 17.1, 10.2, 6.7 Hz, 1H), 5.01 (ddt, J = 17.1, 1.7, 1.6 Hz, 1H), 4.93 (ddt, J = 10.3, 2.0, 1.1 Hz, 1H), 4.27 (s, 1H), 2.74 (t, J = 7.7 Hz, 2H), 2.08 (dt, J = 7.2, 7.2 Hz, 2H), 1.71 (tt, J = 7.6, 7.8 Hz, 2H), 1.45 (tt, J = 7.6, 7.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.1, 137.5, 115.2, 60.6, 32.8, 32.6, 27.7, 24.9; IR (neat) ν_{max} 3402, 2924, 1661, 1641, 1472, 1402, 1198, 1098, 994, 909, 849, 815 cm^{-1}; FABHRMS (NBA) m/z 142.1238 (M^+ + H, $C_8H_{15}NO$ requires 142.1232).

Methyl hept-6-enethioimidate hydrochloride (49). Treatment of **37** with HCl and CH_3SH following the same procedure for **38** provided **49** (100%) as a thick yellow oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.81–5.74 (m, 1H), 5.04–4.94 (m, 2H), 2.83 (t, J = 7.6 Hz, 2H), 2.03 (dt, J = 6.8, 7.1 Hz, 2H), 1.68 (tt, J = 7.7, 7.4 Hz, 2H), 1.39 (tt, J = 7.6, 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.9, 136.7, 113.9, 36.1, 31.8, 27.1, 26.6, 15.1; IR (neat) ν_{max} 3405, 3067, 2852, 1831, 1636, 1544, 1462, 1421, 966, 909, 735 cm^{-1}; FABHRMS (NBA–CsI) m/z 158.1006 (M^+ + H, $C_8H_{15}NS$ requires 158.1003).

Hept-6-enamidine hydrochloride (39). A solution of **38** (0.80 g, 4.51 mmol) in anhydrous Et_2O (4 mL) at 0 °C was treated with NH_3 (8% in EtOH, 1.94 g, 9.13 mmol). The reaction mixture was allowed to warm to 25 °C and stirred under Ar for 8 h. The solvent was then removed under a mild stream of N_2 to provide **39** (0.73 g, 100%) as a white semisolid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.90 (br s, 2H), 8.52 (br s, 2H), 5.82 (ddt, J = 17.0, 10.4, 6.6 Hz, 1H), 5.02 (ddt, J = 17.0, 1.7, 1.7 Hz, 2H), 4.96 (ddt, J = 10.4, 1.9, 1.3 Hz, 2H), 2.35 (t, J = 7.7 Hz, 2H), 2.03 (dt, J = 7.3, 6.9 Hz, 2H), 1.59 (tt, J = 8.0, 7.4 Hz, 2H), 1.35 (tt, J = 7.7, 7.3 Hz, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.2, 139.2, 115.6, 34.2, 33.3, 29.2, 27.6; IR (neat) ν_{max} 3037, 2923, 1682, 1638, 1515, 1441, 1410, 1125, 994, 911 cm^{-1}; FABHRMS (NBA) m/z 127.1238 (M^+ + H, $C_7H_{14}N_2$ requires 127.1235).

6-Amino-2,4-bis(ethoxycarbonyl)-5-(pent-4-en-1-yl)pyrimidine (40). A solution of 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (2.56 g, 8.6 mmol) in anhydrous DMF (4 mL) was warmed to 105 °C and stirred under Ar. Samples of **39** (0.70 g, 4.3 mmol) was added in four portions every 5 h. After the final addition, the solution was allowed to stir at 105 °C for 48 h before the solvent was removed in vacuo. Flash chromatography (SiO_2, 20–50% EtOAc–hexane) followed by recrystallization from EtOAc–hexane provided **40** (230 mg, 35%) as bright-yellow crystals: R_f 0.28 (SiO_2, 50% EtOAc–hexane); mp 171–172 °C (yellow plates, EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.08 (br s, 2H), 5.82 (ddt, J = 17.1, 10.3, 6.7 Hz, 1H), 5.08 (ddt, J = 17.1, 1.7, 1.6 Hz, 1H), 5.04 (ddt, J = 10.3, 1.8, 1.4 Hz, 1H), 4.46 (q, J = 7.2 Hz, 2H), 4.42 (q, J = 7.2 Hz, 2H), 2.65–2.61 (m, 2H), 2.15 (dt, J = 7.1, 6.9 Hz, 2H), 1.69 (tt, J = 8.0, 7.3 Hz, 2H), 1.42 (t, J = 7.1 Hz, 3H), 1.41 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.3, 163.6, 163.3, 154.4, 153.9, 137.4, 118.6, 116.2, 62.7, 62.3, 33.4, 26.6, 25.7, 14.2, 14.1; IR (neat) ν_{max} 3342, 3204, 2976, 2935, 1734, 1627, 1571, 1444, 1235, 1018 cm^{-1}; FABHRMS (NBA–CsI) m/z 440.0569 (M^+ + Cs, $C_{15}H_{21}N_3O_4$ requires 440.0586).

2,4-Bis(ethoxycarbonyl)-6-methylthio-5-(pent-4-en-1-yl) pyrimidine (50). Warming **49** for 24 h at 105 °C following the procedure detailed for **40** provided **50** (63%) as a colorless oil: R_f 0.65 (SiO_2, 50% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.72 (ddt, J = 10.2, 17.0, 6.6 Hz, 1H), 4.96 (ddt, J = 1.6, 1.8, 17.0 Hz, 1H), 4.91 (ddt, J = 2.1, 1.0, 9.1 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H), 4.35 (q, J = 7.2 Hz, 2H), 2.73–2.69 (m, 2H), 2.58 (s, 3H), 2.07 (dt, J = 7.2, 6.9 Hz, 2H), 1.67–1.59 (m, 2H), 1.34 (t, J = 7.2 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 170.9, 164.7, 163.1, 153.4, 152.8, 137.3, 132.7, 115.4, 62.3, 60.2, 33.7, 27.9, 27.0, 13.9, 13.0; IR (neat) ν_{max} 3077, 2980, 2933, 1733, 1641, 1540, 1463, 1381, 1306, 1239, 1184, 1132, 1020, 914, 892, 865, 833, 737 cm^{-1}; FABHRMS (NBA) m/z 339.1369 (M^+ + H, $C_{16}H_{22}N_2O_4S$ requires 339.1379).

6-Amino-5-(pent-4-en-1-yl)pyrimidine-2,4-dicarboxylic acid hydrochloride (42). A solution of **40** (154 mg, 0.5 mmol) in THF:CH_3OH:H_2O (3:1:1, 20 mL) was treated with $LiOH·H_2O$ (84 mg, 2 mmol) at 25 °C for 2 h. The solvent was removed in vacuo and redissolved in H_2O (1.5 mL). Aqueous 10% HCl was added drop-

wise until the pH reached 0–1. The white precipitate was collected by filtration, washed with H_2O (2×5 mL), dried ($MgSO_4$), and concentrated to provide **42** (120 mg, 95%) as an off-white powder: mp 197–198 °C (sealed tube); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.40 (br s, 2H), 5.80 (ddt, J = 17.2, 10.4, 6.4 Hz, 1H), 5.00 (ddt, J = 17.2, 2.0, 1.4 Hz, 1H), 4.94 (ddt, J = 10.4, 2.1, 1.2 Hz, 1H), 2.55 (t, J = 8.0 Hz, 2H), 2.07 (dt, J = 7.4, 7.0 Hz, 2H), 1.51 (tt, J = 7.5, 7.5 Hz, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 176.5, 173.7, 165.5, 163.5, 161.2, 141.6, 117.4, 114.8, 35.4, 28.8, 27.9; IR (KBr) ν_{max} 3374, 3292, 3221, 2933, 1774, 1641, 1618, 1569, 1523, 1534, 1354, 1226, 1036, 918, 764 cm^{-1}; FABHRMS (NBA) m/z 252.0973 (M^+ + H, $C_{11}H_{13}N_3O_4$ requires 252.0984).

6-Methylthio-5-(pent-4-en-1-yl)pyrimidine-2,4-dicarboxylic acid (51). Following the procedure for the preparation of **42**, **51** was prepared from **50**. The white precipitate that appeared upon acidification was dissolved into EtOAc (2×20 mL). The organic layers were combined, washed with H_2O (2×5 mL), dried ($MgSO_4$), and concentrated to provide **51** (95%) as a white powder: ^{1}H NMR (CD_3OD, 400 MHz) δ 5.82 (ddt, J = 10.0, 17.2, 6.4 Hz, 1H), 5.03 (ddt, J = 1.5, 1.8, 16.8 Hz, 1H), 4.97 (ddt, J = 2.1, 1.0, 9.5 Hz, 1H), 2.90–2.86 (m, 2H), 2.67 (s, 3H), 2.16 (dt, J = 7.0, 6.8 Hz, 2H), 1.74–1.66 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.5, 167.3, 166.1, 154.2, 153.6, 138.8, 134.4, 115.7, 35.0, 29.1, 28.2, 13.5; IR (neat) ν_{max} 3455, 2928, 1733, 1640, 1547, 1390, 1331, 1303, 1258, 1182, 1103, 991, 916 cm^{-1}; FABHRMS (NBA) m/z 283.0760 (M^+ + H, $C_{12}H_{14}N_2O_4S$ requires 283.0753).

2-*N*-(*t*-Butyloxycarbonyl)amino-4,6-diamino-5-(pent-4-en-1-yl)pyrimidine (43). A mixture of **42** (300 mg, 1.2 mmol), powdered 4 Å molecular sieves (20 mg), and Et_3N (500 μL, 3.6 mmol) in anhydrous *t*-BuOH (100 mL), was treated dropwise with diphenylphosphoryl azide (DPPA, 773 μL, 3.6 mmol). The reaction mixture was allowed to stir at 100 °C for 72 h before the solvent was removed in vacuo. Flash chromatography (SiO_2, 1–10% CH_3OH–CH_2Cl_2) provided **43** (92 mg, 28%): R_f 0.28 (SiO_2, 5% CH_3OH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.80 (ddt, J = 17.4, 10.4, 6.5 Hz, 1H), 5.09–5.00 (m, 2H), 2.30 (t, J = 6.0 Hz, 2H), 2.13 (dt, J = 5.6, 5.6 Hz, 2H), 1.60 (tt, J = 6.0, 6.3 Hz, 2H), 1.52 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.2, 152.7, 151.6, 138.2, 115.7, 90.4, 82.1, 33.0, 28.3, 25.9, 23.0; IR (neat) ν_{max} 3334, 3195, 2981, 2927, 2857, 1726, 1638, 1601, 1582, 1434, 1364, 1244, 1146, 1044, 915 cm^{-1}; FABHRMS (NBA) m/z 294.1935 (M^+ + H, $C_{14}H_{23}N_5O_2$ requires 294.1930).

4-Amino-2-*N*-(*t*-butyloxycarbonyl)amino-6-methylthio-5-(pent-4-en-1-yl) pyrimidine (52). Following the procedure detailed for **43**, **51** provided **52** (53%) and **53** (14%). For **52**: colorless film; R_f 0.25 (SiO_2, 20% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (s, 1H), 5.78 (ddt, J = 17.2, 10.3, 6.6 Hz, 1H), 5.05 (ddt, J = 15.6, 1.5, 1.8 Hz, 1H), 4.98–4.94 (m, 1H), 2.47 (s, 3H), 2.38 (t, J = 5.7 Hz, 2H), 2.08 (dt, J = 7.0, 6.8 Hz, 2H), 1.56 (tt, J = 7.6, 7.6 Hz, 2H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.5, 160.5, 154.3, 151.2, 138.0, 115.3, 107.4, 80.9, 33.4, 28.2, 25.8, 25.4, 12.9; IR (neat) ν_{max} 3326, 3221, 2972, 2924, 1735, 1670, 1625, 1577, 1549, 1496, 1424, 1362, 1219, 1152 cm^{-1}; FABHRMS (NBA) m/z 325.1689 (M^+ + H, $C_{15}H_{24}N_4O_2S$ requires 325.1698).

For **53** (2,4-diamino-6-methylthio-5-(pent-4-en-1-yl) pyrimidine): colorless film; R_f 0.22 (SiO_2, 50% EtOAc–hexane); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.85 (ddt, J = 17.1, 10.3, 6.6 Hz, 1H), 5.02 (ddt, J = 17.1, 1.8, 1.6 Hz, 1H), 4.93 (ddt, J = 10.2, 2.1, 1.1 Hz, 1H), 2.41 (s, 3H), 2.40 (t, J = 6.5 Hz, 2H), 2.11 (dt, J = 7.0, 6.8 Hz, 2H), 1.56–1.52 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ 167.5, 162.3, 161.9, 139.8, 115.1, 103.5, 34.8, 28.0, 26.2, 12.9; IR (neat) ν_{max} 3460, 3319, 3178, 2925, 2850, 1605, 1544, 1422, 1347, 1258, 1103, 892 cm^{-1}; FABHRMS (NBA) m/z 225.1169 (M^+ + H, $C_{10}H_{16}N_4S$ requires 225.1174).

2,4,6-Tris[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-5-(pent-4-en-1-yl)pyrimidine (44). A solution of **43** (54 mg, 0.19 mmol) in THF (5 mL) was treated with di-*t*-butyl dicarbonate (255 μL, 1.11 mmol), DMAP, and Et_3N (154 μL, 1.11 mmol). The reaction mixture was stirred at 25 °C for 2 h followed by removal of the solvent in vacuo. Flash chromatography (SiO_2, 10% EtOAc–hexane) provided **44** (104 mg, 71%) as a colorless film: R_f 0.35 (SiO_2, 10% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.75 (ddt, J = 17.0, 10.3, 6.4 Hz, 1H), 5.02 (ddt, J = 17.0, 1.7, 1.6 Hz, 1H), 5.01–4.98 (m, 1H), 2.48–2.41 (m, 2H), 2.08 (dt, J = 7.2, 6.8 Hz, 2H), 1.62 (tt, J = 7.8, 7.9 Hz, 2H), 1.43 (s, 18H), 1.42 (s, 36H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.6, 155.9, 150.6, 150.0, 137.6, 128.2, 115.4, 83.9, 83.3, 33.7, 27.8, 27.7, 26.7, 26.0; IR (neat) ν_{max} 2979, 2932, 1795, 1762, 1725, 1551, 1457, 1450, 1392, 1369, 1275, 1251, 1158, 1115, 1097, 848, 778 cm^{-1}; FABHRMS (NBA–CsI) m/z 926.3550 (M^+ + Cs, $C_{39}H_{63}N_5O_{12}$ requires 926.3528).

2,4-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-6-methylthio-5-(pent-4-en-1-yl)pyrimidine (54). Following the procedure for **44**, **52** provided **54** (90%) as a colorless film: R_f 0.35 (SiO_2, 10% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.78 (ddt, J = 17.1, 10.3, 6.6 Hz, 1H), 5.02 (ddt, J = 17.1, 1.8, 1.6 Hz, 1H), 5.00–4.97 (m, 1H), 2.54 (s, 3H), 2.54–2.50 (m, 2H), 2.11 (dt, J = 7.2, 7.0 Hz, 2H), 1.66–1.62 (m, 2H), 1.43 (s, 18H), 1.41 (s, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.7, 156.6, 155.2, 150.8, 149.9, 137.6, 127.1, 115.3, 83.6, 83.1, 33.7, 28.0, 27.7,

26.7, 26.0, 13.4; IR (neat) ν_{max} 2979, 1797, 1762, 1730, 1560, 1530, 1458, 1394, 1368, 1275, 1253, 1156, 1117, 854, 777 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 757.2218 ($M^+ + Cs$, $C_{30}H_{48}N_4O_8S$ requires 757.2247).

5-(Pent-4-en-1-yl)-2,4,6-triaminopyrimidine trifluoroacetic acid salt (47). A solution of **44** (19.2 mg, 0.024 mmol) in CH_2Cl_2 (0.5 mL) was treated with trifluoroacetic acid (0.2 mL) at 25 °C for 2 h. The solvent was removed in vacuo to provide **47** (12.8 mg, 100%) as a light yellow film: ^1H NMR (CF_3COOD, 400 MHz) δ 5.75 (ddt, *J* = 17.0, 10.4, 6.6 Hz, 1H), 5.05–5.00 (m, 2H), 2.39 (t, *J* = 7.9 Hz, 2H), 2.12 (dt, *J* = 6.9, 6.8 Hz, 2H), 1.59 (tt, *J* = 7.7, 7.3 Hz, 2H); ^{13}C NMR (CF_3COOD, 100 MHz) δ 155.2, 151.4, 139.0, 117.8, 87.7, 34.1, 27.0, 22.6; IR (neat) ν_{max} 3349, 3204, 2941, 1681, 1640, 1550, 1445, 1382, 1196, 1137, 1055 cm^{-1}; FABHRMS (NBA) *m/z* 194.1408 ($M^+ + H$, $C_9H_{15}N_5$ requires 194.1406).

2,4,6-Tris[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-5-(5-hydroxypent-1-yl)pyrimidine (45). A solution of **44** (91 mg, 0.12 mmol) in anhydrous THF (0.3 mL) at 0 °C was treated with BH_3·THF (1.0 M, 230 μL, 0.46 mmol) and the reaction mixture was allowed to stir for 1 h. H_2O (25 μL) was added dropwise followed by aqueous 1.3 M NaOH (177 μL) and aqueous 50% H_2O_2 (16 μL). The mixture was stirred at 25 °C for 1 h and extracted with EtOAc (3×5 mL). The organic layers were combined, washed with H_2O (2×2 mL), and dried ($MgSO_4$). Flash chromatography (SiO_2, 20–30% EtOAc–hexane) provided **45** (54 mg, 58%) as a white film: R_f 0.30 (SiO_2, 30% EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.62 (t, *J* = 5.4 Hz, 2H), 2.44 (t, *J* = 6.4 Hz, 2H), 1.59–1.55 (m, 4H), 1.42 (s, 54H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.3, 156.1, 151.6, 150.5, 127.2, 84.4, 84.0, 62.2, 33.8, 27.8, 27.7, 27.5, 27.3, 26.7; IR (neat) ν_{max} 3407, 2979, 2931, 1796, 1762, 1729, 1549, 1458, 1368, 1273, 1254, 1154, 1121, 850 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 944.3670 ($M^+ + Cs$, $C_{39}H_{65}N_5O_{13}$ requires 944.3633).

2,4-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-5-(5-hydroxypent-1-yl)-6-(methylthio)pyrimidine (55). Following the procedure for the preparation of **45**, **54** provided **55** (88%) as a colorless film: R_f 0.30 (SiO_2, 50% EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.64 (t, *J* = 6.5 Hz, 2H), 2.55 (s, 3H), 2.55–2.49 (m, 2H), 1.60–1.52 (m, 6H), 1.44 (s, 18H), 1.40 (s, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.7, 156.6, 155.2, 150.8, 150.0, 127.1, 83.7, 83.1, 62.7, 32.4, 27.9, 27.8, 27.2, 26.8, 25.9, 13.4; IR (neat) ν_{max} 3486, 2978, 2928, 2868, 1792, 1757, 1732, 1553, 1528, 1458, 1393, 1364, 1274, 1254, 1154, 1115, 851, 776 cm^{-1}; FABHRMS (NBA) *m/z* 643.3344 ($M^+ + H$, $C_{30}H_{50}N_4O_9S$ requires 643.3377).

2,4,6-Triamino-5-(5-hydroxypent-1-yl)pyrimidine (48). A solution of **45** (16 mg, 20 μmol) in CH_2Cl_2 (0.5 mL) was treated with trifluoroacetic aicd (0.2 mL) at 25 °C for 2 h. The solvent was removed in vacuo. The residue was redissolved in THF:CH_3OH:H_2O (3:1:1, 0.2 mL) and treated with LiOH·H_2O (4.2 mg, 0.1 mmol) for 1 h. The solvent was removed in vacuo and the reaction mixture was extracted with EtOAc (3×5 mL). The organic layers were combined, washed with saturated aqueous NaCl (1 mL), H_2O (1 mL), dried ($MgSO_4$) and concentrated to provide **48** (3.6 mg, 85%) as an off-white film: R_f 0.25 (SiO_2, 10% CH_3OH–CH_2Cl_2); ^1H NMR (CD_3OD, 400 MHz) δ 3.55 (t, *J* = 6.5 Hz, 2H), 2.28 (t, *J* = 7.3 Hz, 2H), 1.59–1.54 (m, 2H), 1.45–1.41 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 161.5, 158.1, 87.2, 62.9, 33.6, 28.6, 26.7, 24.0; IR (neat) ν_{max} 3367, 3240, 2922, 2850, 1677, 1650, 1441, 1201, 1137, 842, 797 cm^{-1}; FABHRMS (NBA) *m/z* 212.1503 ($M^+ + H$, $C_9H_{17}N_5O$ requires 212.1511).

2,4-Diamino-5-(5-hydroxypent-1-yl)-6-methylthiopyrimidine (57). Following the procedure for the preparation of **48**, **55** provided **57** (87%) as a white film: R_f 0.33 (SiO_2, 10% CH_3OH–CH_2Cl_2); ^1H NMR (CD_3OD, 400 MHz) δ 3.55 (t, *J* = 6.5 Hz, 2H), 2.42 (s, 3H), 2.42–2.39 (m, 2H), 1.56 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.52–1.38 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 167.4, 162.2, 161.7, 104.6, 63.0, 33.5, 28.5, 26.9, 26.6, 12.9; IR (neat) ν_{max} 3354, 3220, 2925, 2853, 1616, 1549, 1428, 1355, 1261, 1045 cm^{-1}; FABHRMS (NBA) *m/z* 243.1288 ($M^+ + H$, $C_{10}H_{18}N_4OS$ requires 243.1280).

2,4,6-Tris[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-5-(5-oxopent-1-yl)pyrimidine (46). A solution of **45** (18 mg, 22 μmol) in CH_2Cl_2 (250 μL) was treated with Dess–Martin periodinane[13] (*o*-Ph(CO_2)I(OAc)$_3$, 18 mg, 44 μmol) and the white suspension was stirred at 25 °C for 1 h. The mixture was diluted with Et_2O (2 mL), treated with aqueous 1.3 M NaOH (300 μL), and extracted with Et_2O (3×2 mL). The organic layers were combined, washed with H_2O (1 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (SiO_2, 20% EtOAc–hexane) provided **46** (16 mg, 89%) as a colorless film: R_f 0.35 (SiO_2, 30% EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.74 (t, *J* = 1.6 Hz, 1H), 2.45 (t, *J* = 7.7 Hz, 2H), 2.41 (td, *J* = 7.0, 1.6 Hz, 2H), 1.63–1.59 (m, 4H), 1.42 (s, 54H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.6, 161.6, 156.0, 150.5, 150.0, 127.5, 84.1, 83.4, 43.5, 27.8, 27.7, 27.1, 26.3, 22.1; IR (neat) ν_{max} 2980, 2920, 2850, 1793, 1763, 1723, 1554, 1365, 1275, 1256, 1151, 1116, 852, 778 cm^{-1}; FABHRMS (NBA–CsI) *m/z* 942.3448 ($M^+ + Cs$, $C_{39}H_{63}N_5O_{13}$ requires 942.3477).

2,4-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-6-methylthio-5-(5-oxopent-1-yl)pyrimidine (56). Following the procedure for the preparation of **46**, **55** provided **56** (91%) as

a colorless film: R_f 0.30 (SiO_2, 20% EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.76 (t, J = 1.6 Hz, 1H), 2.55 (s, 3H), 2.56–2.50 (m, 2H), 2.45 (td, J = 7.1, 1.6 Hz, 2H), 1.74–1.66 (m, 2H), 1.64–1.58 (m, 2H), 1.44 (s, 18H), 1.40 (s, 18H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.9, 172.7, 157.0, 155.3, 150.7, 150.0, 126.6, 83.7, 83.2, 43.5, 27.9, 27.8, 26.9, 26.3, 22.0, 13.4; IR (neat) ν_{max} 2974, 2936, 1793, 1760, 1726, 1559, 1530, 1459, 1392, 1368, 1272, 1253, 1152, 1109, 851 cm^{-1}; FABHRMS (NBA–CsI) m/z 773.2210 ($M^+ + Cs$, $C_{30}H_{48}N_4O_9S$ requires 773.2196).

5-(5-Oxopent-1-yl)-2,4,6-triaminopyrimidine trifluoroacetic acid salt (3). A solution of **46** (16 mg, 20 μmol) in CH_2Cl_2 (0.5 mL) was treated with trifluoroacetic acid (0.2 mL) at 25 °C for 2 h. The solvent was removed in vacuo to provide **3** (11.0 mg, 100%) as a light-yellow film: ^1H NMR (CF_3COOD, 400 MHz) δ 9.83 (br s, 1H), 2.69 (t, J = 7.8 Hz, 2H), 2.43 (t, J = 7.4 Hz, 2H), 1.83–1.76 (m, 4H); ^{13}C NMR (CF_3COOD, 100 MHz) δ 212.5, 155.3, 151.5, 87.2, 27.5, 25.6, 23.6, 22.7; IR (neat) ν_{max} 3367, 2920, 1683, 1445, 1206, 1140, 804, 805, 719 cm^{-1}; FABHRMS (NBA) m/z 210.1350 ($M^+ + H$, $C_9H_{15}N_5O$ requires 210.1355).

2,4-Diamino-6-methylthio-5-(5-oxopent-1-yl)pyrimidine trifluoroacetic acid salt (4). Following the procedure for **3**, **56** provided **4** (100%) as a light-yellow film: ^1H NMR (CF_3COOD, 400 MHz) δ 9.68 (br s, 1H), 2.74 (s, 3H), 2.73–2.62 (m, 2H), 2.64 (t, J = 2.1 Hz, 2H), 1.79–1.75 (m, 2H), 1.58–1.50 (m, 2H); ^{13}C NMR (CF_3COOD, 100 MHz) δ 212.6, 161.7, 156.5, 154.5, 107.6, 34.9, 27.4, 26.7, 22.7, 14.6; IR (neat) ν_{max} 3426, 1679, 1443, 1206, 1138, 844, 800, 728 cm^{-1}; FABHRMS (NBA) m/z 241.1130 ($M^+ + H$, $C_{10}H_{16}N_4OS$ requires 241.1123).

GAR and AICAR Tfase inhibition studies

Characterization of the inhibitor properties was conducted as previously described.[1]

Cytotoxicity testing

The cytotoxic activity of the agents was established following protocols described in detail.[1]

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