Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 1075–1086

BIOORGANIC & MEDICINAL CHEMISTRY

Conformationally Restricted Analogues Designed for Selective Inhibition of GAR Tfase Versus Thymidylate Synthase or Dihydrofolate Reductase

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Received 29 September 1999; accepted 10 January 2000

Abstract—The synthesis and evaluation of a series of conformationally restricted analogues of 10-formyl-tetrahydrofolate as potential inhibitors of glycinamide ribonucleotide transformylase (GAR Tfase) or aminoimidazole carboxamide transformylase (AICAR Tfase) are reported.

Glycinamide ribonucleotide transformylase (GAR Tfase) is an enzyme central to de novo purine biosynthesis.[1–12] Since purines play a crucial role as required components of DNA and RNA, inhibition of enzymes in the purine biosynthetic pathway has been pursued as an effective approach for antineoplastic intervention.[13] The disclosure that (6*R*)-5,10-dideazatetrahydrofolate (Lometrexol, DDATHF) is an efficacious antitumor agent that acts as an effective inhibitor of GAR Tfase ($K_i = 0.1\ \mu M$) established inhibition of purine biosynthesis and GAR Tfase as viable targets for antineoplastic intervention.[14–23] GAR Tfase uses (6*R*)-10-formyl-tetrahydrofolate (**1**) to transfer a formyl group to the primary amine of its substrate, glycinamide ribonucleotide (**2a**, GAR; Fig. 1). This one carbon transfer constitutes the incorporation of the C-8 carbon of the purines and is the first of two formyl transfer reactions. The second formyl transfer reaction is catalyzed by the enzyme aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase) which also employs (6*R*)-10-formyl-tetrahydrofolate (**1**) to transfer a formyl group to the C-5 amine of its substrate, aminoimidazole carboxamide ribonucleotide (**2b**, AICAR; Fig. 1).[1,24–27]

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Inhibitor design

The X-ray crystal structure[28] of 5-DATHF (5-deazatetrahydrofolic acid) complexed with GAR Tfase indicated that it adopts a bound L-shaped conformation with an axial C6–C9 bond that is perpendicular to the bicyclic ring with an extended or *trans* (169°) versus *gauche* (50 to 69°) C5–C6–C9–N10 dihedral angle characteristic of bound TS or DHFR cofactor and inhibitors (Fig. 2).[29] An identical conformation was observed with the GAR Tfase bound inhibitor 1476U89[29] and the analogous dihedral angles of the two bound molecules in the unit cell were found to be 166 and 174°. Thus, a folate inhibitor designed to lock this trans conformation has the potential for specificity against GAR Tfase.[28]

Based on these observations, two classes of conformationally restricted inhibitors that embody these characteristics were designed for examination. These classes of conformationally restricted inhibitors are represented by **3** and **4** incorporating an acyclic folate core (Fig. 3). Mimics of the more stable *trans* versus *cis* amide[29b] are constrained to the GAR Tfase bound trans orientation (180°) by their incorporation into an aromatic pyrrole or pyridine ring where the basic nitrogen may serve to accept an active site H-bond (protonation) from the active site His-108.[29c] The atoms corresponding to the X5–C6–C9–X10 dihedral angle are locked in the key trans orientation (180°) by virtue of incorporation into the pyrrole or pyridine ring.

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PII: S0968-0896(00)00051-1

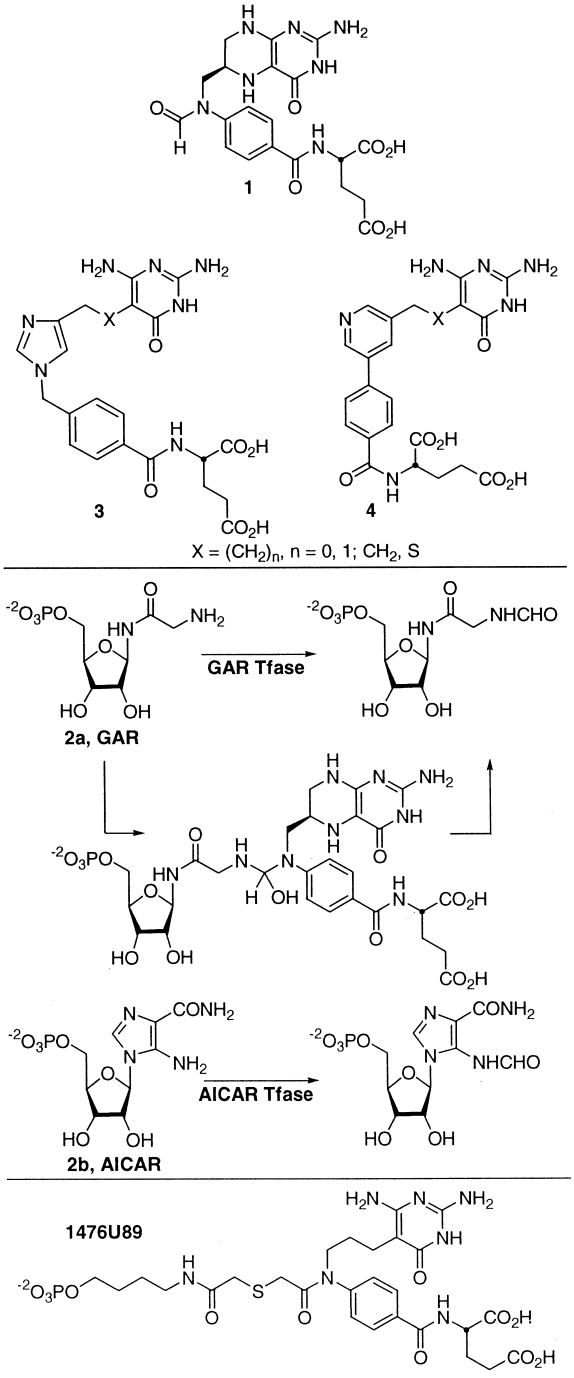


Figure 1.

Syntheses of conformationally restricted analogues 3a and 4a (X = CH_2, $n = 0$)

The synthesis of **3a** is depicted in Scheme 1. Starting from commercially available reagents, *N*-alkylation of 4-(hydroxymethyl)imidazole hydrochloride (**5**) with methyl (4-bromomethyl)benzoate (**6**, K_2CO_3, DMF, 70 °C, 1 h, 49%) provided the desired alkylated imidazole **7** in a 1.6:1 ratio of N^1-aryl-4-(hydroxymethyl)imidazole and N^1-aryl-5-(hydroxymethyl)imidazole. In addition to being the predicted major product, **7** exhibited ^{1}H NMR chemical shifts for the benzylic protons consistent with this assignment. The *N*-benzyl methlyene protons were observed at δ 5.08 and 5.28 for **7** and its isomer, respectively, in agreement with those observed for *N*-benzyl 4- and 5-hydroxymethylimidazole (δ 5.13 and 5.30, respectively).[30] The hydroxyl group of **7** was cleanly converted to the chloride **8** on warming with thionyl chloride (10.0 equiv, CH_2Cl_2, 40 °C, 3 h, 89%). Alkylation of the potassium salt of ethyl cyanoacetate (**9**) with **8** (K_2CO_3, DMF, 70 °C, 3 h, 76%) afforded **10**. Cyclization with the free base of guanidine (1.0 equiv, EtOH, 25 °C, 3 h, 62%) gave the desired pyrimidine **11**. Treatment of **11** with LiOH (5.0 equiv, 3:1 MeOH–H_2O, 60 °C, 84%) cleanly provided the carboxylic acid **12** which was coupled with di-*tert*-butyl-L-glutamate hydrochloride (**13**, EDCI, $NaHCO_3$, DMF, 54%) to provide **14**. Deprotection of **14** was accomplished by treatment with trifluoroacetic acid (10.0 equiv, CH_2Cl_2, 82%) to provide the desired conformationally restricted analogue **3a**.

The synthesis of **4a** was accomplished in a similar manner (Scheme 2). The preparation of **15** was accomplished in three steps starting from commercially available 5-bromonicotinic acid according to published procedure.[31] Palladium(0)-catalyzed Suzuki coupling of **15** with commercially available methyl 5-bromobenzoate **16** (diboron pinacol ester, KOAc, DMF, 80 °C, 2 h, 54%) with in situ generation of the boronic ester provided the desired diphenyl derivative **17**.[32] The preferential mixed versus homo coupling of the reactants was controlled by first forming the boronic ester derived from **16** in situ, prior to the addition of **15**. Deprotection of **17** was accomplished by treatment with Bu_4NF (3.0 equiv, THF, 90%) and was followed by conversion of the alcohol **18** to the chloride **19** (5.0 equiv $SOCl_2$, CH_2Cl_2, 3 h, 92%). Alkylation of the potassium salt of ethyl cyanoacetate (**9**) with **19** (K_2CO_3, DMF, 70 °C, 3 h, 79%) afforded **20**. Cyclization with the free base of guanidine (1.0 equiv, EtOH, 45 °C, 12 h, 61%) gave the desired pyrimidine **21**. Hydrolysis of the ester **21** with LiOH (5.0 equiv, 3:1 MeOH–H_2O, 25 °C, 88%), followed by glutamate coupling of the resulting carboxylic acid **22** (EDCI, $NaHCO_3$, DMF, 45%) provided **23**. Deprotection of **23** was accomplished using trifluoroacetic acid (10.0 equiv, CH_2Cl_2, 89%) to provide the desired conformationally restricted analogue **4a**.

Syntheses of conformationally restricted analogues 3b and 4b (X=CH_2, $n = 1$)

The synthesis of **3b** was accomplished in a manner similar to the synthesis of **3a** (Scheme 3). Commercially available tryptamine dihydrochloride was treated with $NaNO_2$ in 6 N HCl to provide the corresponding chloride **24**.[33] *N*-alkylation of 4-(chloroethyl)imidazole hydrochloride (**24**) with methyl (4-bromomethyl)benzoate (**6**, K_2CO_3, DMF, 70 °C, 1 h, 80%) provided the desired alkylated imidazole **25**. In addition to being the expected major product, **25** exhibited ^{1}H NMR chemical shifts for the *N*-benzyl protons at δ 5.12 (versus δ 5.30) consistent with this assignment. Alkylation of the potassium salt of ethyl cyanoacetate (**9**) with **23** (K_2CO_3, DMF, 70 °C, 3 h, 56%) afforded **26** and its cyclization with the free base of guanidine (1.0 equiv,

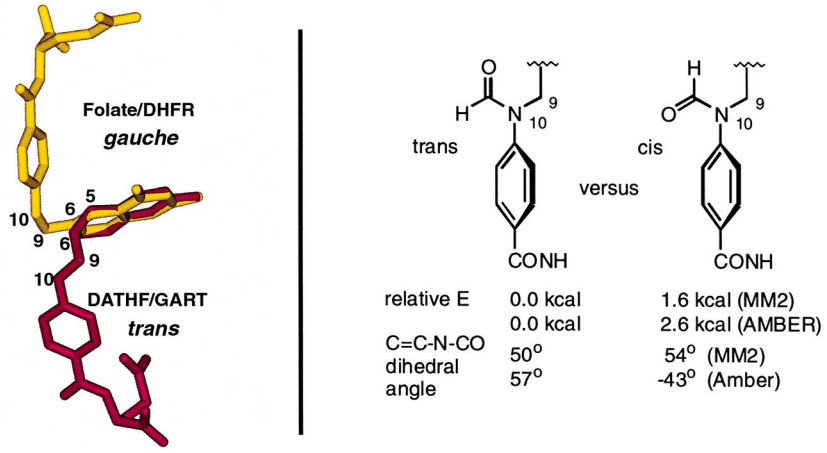


Figure 2. Left: structure overlay of tetrahydrofolate bound to dihydrofolate reductase (taken from an X-ray structure) and 5,10-dideazatetrahydrofolate bound to GAR Tfase (taken from an X-ray structure) illustrating the *gauche* (50–69°) versus the extended (*trans*, 166–174°) bound conformations that may be used to distinguish conformationally restricted folate analogues.[29] Right: comparison of the relative stability of the *trans* and *cis* amide conformations of 10-formyl-tetrahydrofolate.

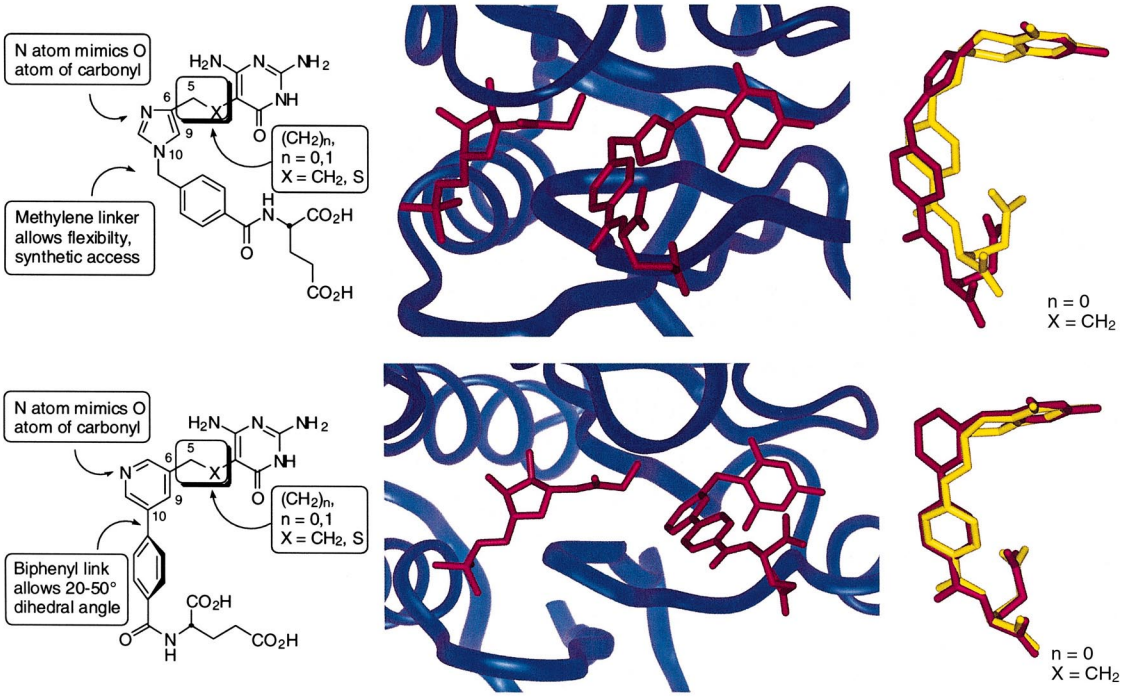
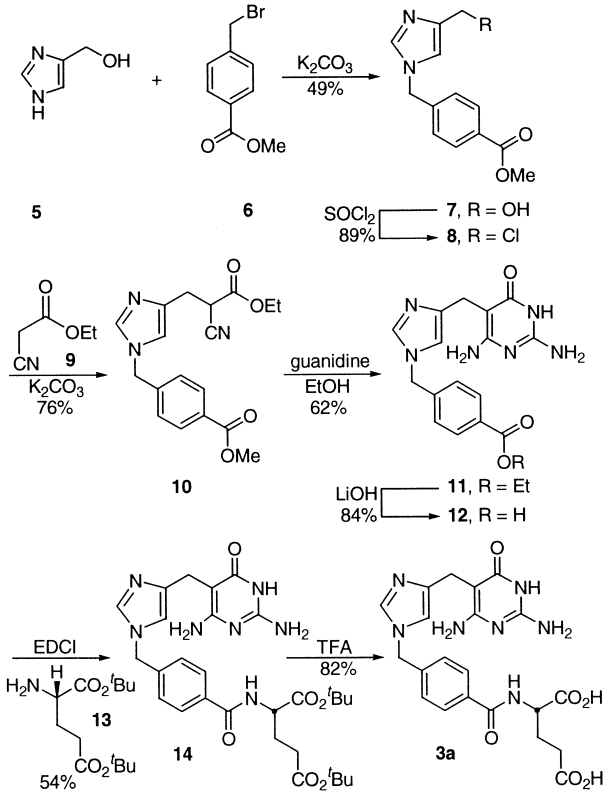


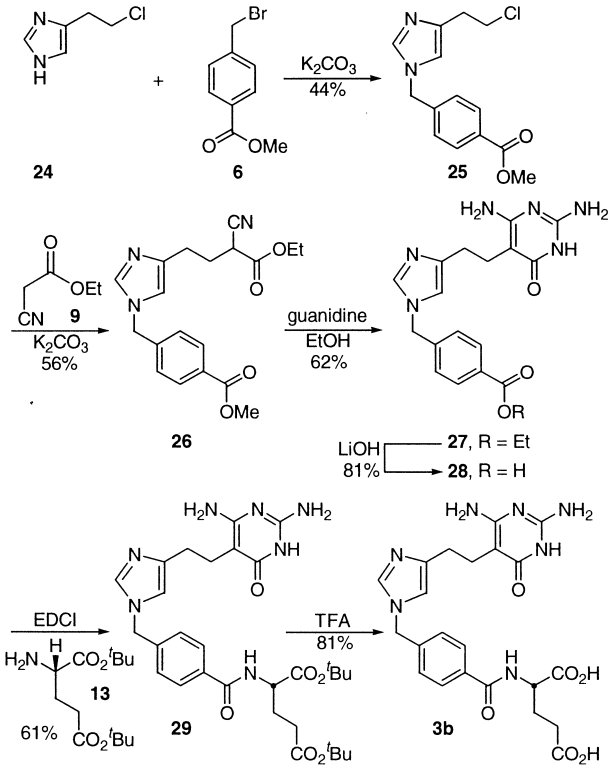
Figure 3. Models of **3** (brown, top; $n=0$, X=CH_2) and **4** (brown, bottom; $n=0$, X=CH_2) bound in the GAR Tfase active site and structural overlays with the structure of 5,10-dideazatetrahydrofolate (yellow) taken from the X-ray crystal structure bound to GAR Tfase..[28]

EtOH, 25 °C, 3 h, 62%) gave the desired pyrimidine **27**. Hydrolysis of the ester **27** with LiOH–H_2O (5.0 equiv, 3:1 MeOH–H_2O, 60 °C, 81%), followed by glutamate coupling with the resulting carboxylic acid **28** (EDCI, $NaHCO_3$, DMF, 61%) provided **29**. Deprotection of **29** was accomplished using trifluoroacetic acid (10.0 equiv, CH_2Cl_2, 81%) to provide **3b**.

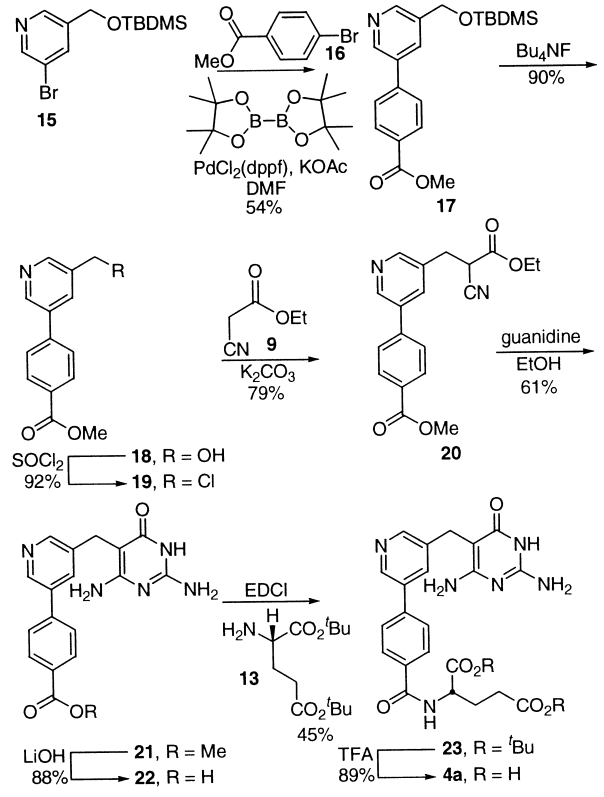
The synthesis of **4b** was accomplished in a manner similar to **3b** (Scheme 4). Palladium(0)-catalyzed Suzuki coupling of **30**, derived in three steps from commercially available 5-bromo-3-pyridine acetic acid,[31] with commercially available methyl 5-bromobenzoate (**16**, diboron pinacol ester, KOAc, DMF, 80 °C, 2 h, 63%) provided the desired diphenyl derivative **31**. Deprotection of **31** (2.0 equiv Bu_4NF, THF, 91%), followed by chlorination of the terminal hydroxyl group of **32** on warming with thionyl chloride (10.0 equiv, CH_2Cl_2, 3 h, 87%) provided **33**. Alkylation of the potassium salt of ethyl cyanoacetate (**9**) with **33** (K_2CO_3, DMF, 70 °C, 3 h, 63%) afforded **34** and cyclization of **34** with the free base of guanidine (1.0 equiv, EtOH, 25 °C, 3 h, 56%) gave the desired pyrimidine **35**. Hydrolysis of the ester **35** with LiOH–H_2O (5.0 equiv, 3:1 MeOH:H_2O, 60 °C, 91%), followed by glutamate coupling with the resulting carboxylic acid **36** (EDCI, $NaHCO_3$, DMF, 48%) provided **37**. Deprotection of **37** (10.0 equiv TFA, CH_2Cl_2, 83%) provided the desired conformationally restricted analogue **4b**.



Scheme 1.



Scheme 3.



Scheme 2.

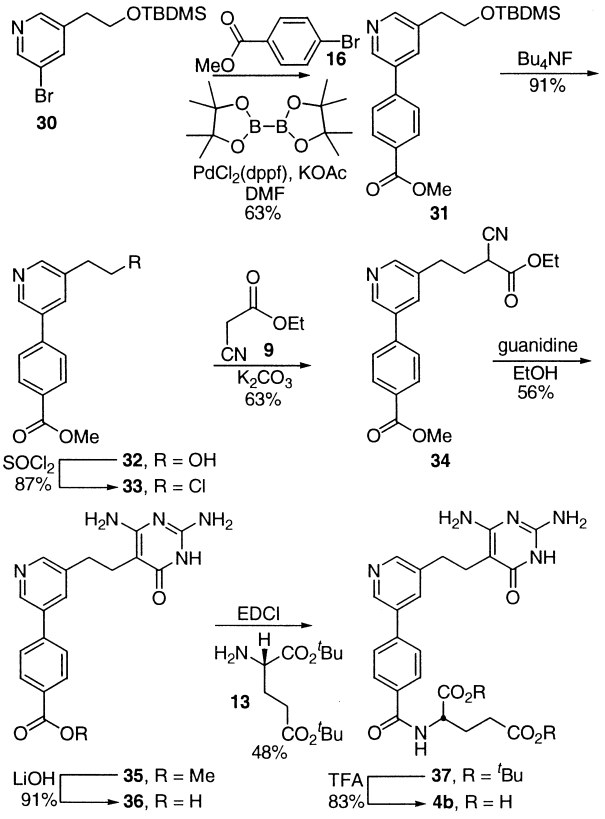
Syntheses of conformationally restricted analogues 3c and 4c (X = S)

The syntheses of conformationally restricted analogues **3c** and **4c** were accomplished by an alternative pathway. The syntheses of **3c** and **4c** were envisioned to proceed via a base-catalyzed coupling of a thiol to 5-bromo-2,6-diamino-4(*3H*)-pyrimidinone (**40**) using diisopropylethylamine in dimethylformamide.[34] The syntheses of the thiols **39** and **45** were accomplished by displacement of the chlorides **8** and **19** with potassium thioacetate (acetone, 50 °C, 2 h) followed by acid hydrolysis of the thiol esters **38** and **44** to provide the corresponding thiols **39** and **45**, respectively (Schemes 5 and 6). Several attempts to couple the thiol in the presence of base (iPr$_2$NEt, Et$_3$N) led exclusively to the dimerized thiol. Thus, coupling was accomplished without the use of base (DMF, 85 °C, 3 h) to provide the pyrimidines **42** and **46**. Hydrolysis, coupling and deprotection to provide **3c**, **4c** was accomplished in a manner similar to that previously reported for **3a,b** and **4a,b**.

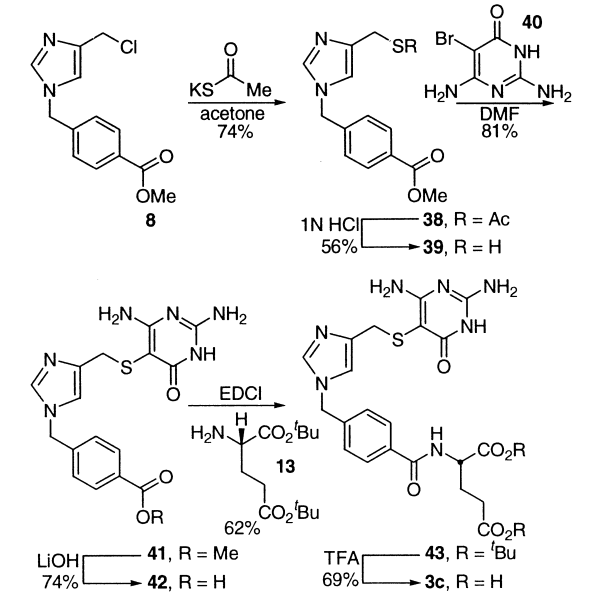
GAR Tfase inhibition

The agents **3** and **4** and their respective precursors were tested for inhibition of GAR Tfase and AICAR Tfase and the results are presented in Table 1. Compounds **3a**–**3c**, **4a** and **4c** all demonstrate similar binding to GAR Tfase, with a K_i range of 11–24 μM. Compound **4b** inhibited GAR Tfase with a K_i of 61 μM. The intermediates in the preparation of **4b**, compounds **36** and **37**, showed the best GAR Tfase inhibition with K_i's of

10 and 8 μM, respectively. Compounds **3b**, **3c**, **4b**, and **4c** did not inhibit AICAR Tfase and with the exception of **4a**, all were less active against AICAR versus GAR Tfase demonstrating a selectivity for GAR Tfase over AICAR Tfase. However, these results would appear to demonstrate that the conformationally restricted inhibitors do not have a positive effect on binding. The compounds are several orders of magnitude less potent than DDATHF, but bind to GAR Tfase with affinities roughly equivalent to a large series of TDAF inhibitors.[23] Given this weak GAR Tfase inhibition, the compounds were not examined as inhibitors of TS or DHFR.



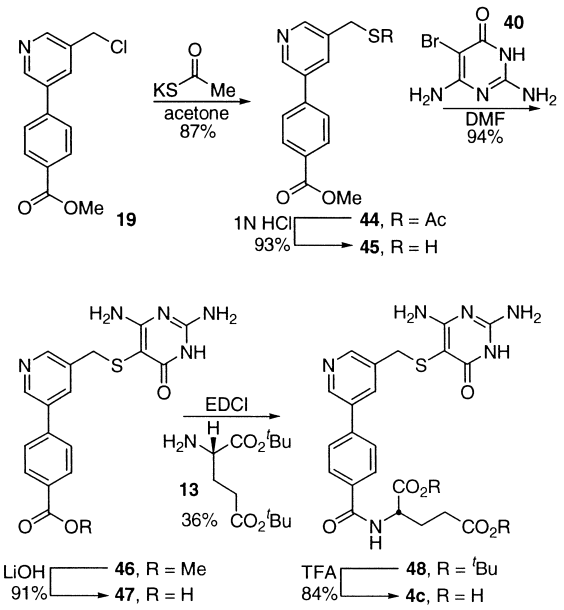
Scheme 4.



Scheme 5.

Cytotoxic activity

The agents **3** and **4** and their precursors were examined for cytotoxic activity both in the presence (+) and



Scheme 6.

Table 1. GAR and AICAR Tfase inhibition (K_i, μM)

Compound	K_i GAR Tfase	K_i AICAR Tfase
3a	11	150
4a	20	11
3b	24	ni[a]
4b	61	ni
3c	17	ni
4c	18	ni
11	70	120
12	ni	50
14	ni	ni
21	ni	ni
22	ni	50
23	35	ni
27	40	ni
28	52	ni
29	24	ni
35	28	ni
36	10	ni
37	8	ni
41	22	ni
42	37	ni
43	16	ni
46	40	ni
47	58	ni
48	54	ni
Lometrexol	0.1	Not tested

[a]ni, no inhibition.

absence (−) of added hypoxanthine and/or thymidine against the CCRF-CEM cell line in purine and pyrimidine free medium (Table 2). Consistent with their weak enzyme inhibition properties, the agents exhibited no or only modest cytotoxic activity. None exhibited a sensitivity to the medium absence of either purines or pyrimidines indicating that the modest activity is not due to inhibition of either purine or pyrimidine biosynthesis.

Experimental

Methyl 4-[(4-hydroxymethyl-1*H*-imidazol-1-yl)methyl]-benzoate (7). A stirred solution of 4-(hydroxymethyl) imidazole (**5**, 1.0 g, 7.4 mmol), methyl 4-(bromomethyl) benzoate (**6**, 2.04 g, 8.9 mmol, 1.2 equiv), and K_2CO_3 (3.08 g, 22.3 mmol, 3.0 equiv) in DMF (37 mL) was warmed at 100 °C for 3 h. The solution was diluted with H_2O (150 mL) and extracted with EtOAc (3×25 mL). The organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 5×15 cm, 10–40% MeOH–$CHCl_3$ gradient elution) afforded **7** (0.90 g, 49%) as a white solid: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.96 (d, J = 8.3 Hz, 2H), 7.46 (s, 1H), 7.16 (d, J = 8.2 Hz, 2H), 6.81 (s, 1H), 5.08 (s, 2H), 4.67 (br s, 1H), 4.53 (s, 2H), 3.86 (s, 3H); FABHRMS (NBA/NaI) m/z 247.1079 ($M+H^+$, $C_{13}H_{14}N_2O_3$ requires 247.1083). The undesired regioisomer methyl 4-[(5-hydroxymethyl-1*H*-imidazol-1-yl) methyl]benzoate was obtained as a higher R_f compound in 24% yield: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.95 (d, J = 8.3 Hz, 2H), 7.45 (s, 1H), 7.14 (d, J = 8.2 Hz, 2H), 6.92 (s, 1H), 5.28 (s, 2H), 4.46 (s, 2H), 3.88 (s, 3H).

Table 2. In vitro cytotoxic activity

Compd	CCRF-CEM (IC_{50}, μM)			
	(+) T, (+)H[a]	(−)T, (+)H[a]	(+)T, (−)H[a]	(−)T, (−)H[a]
3a	60	35	50	65
4a	60	40	70	70
3b	80	120	90	130
4b	50	65	40	60
3c	>200	>200	>200	>200
4c	60	150	140	>200
11	>250	>250	>250	>250
12	>250	>250	>250	>250
14	100	100	100	100
21	90	90	140	170
22	>250	>250	>250	>250
23	40	40	30	40
27	90	80	70	130
28	200	>250	>250	>250
29	55	100	75	60
35	90	90	90	80
36	>250	>250	>250	225
37	50	30	45	35
41	>250	145	>250	>250
42	>250	>250	>250	>250
43	>250	175	>250	>250
46	0.9	1.1	1.5	0.5
47	>250	200	>250	>250
48	60	75	>250	>250
Lometrexol	>250	>250	0.70	1.1

[a]T, thymidine, H, hypoxanthine.

Methyl 4-[(4-chloromethyl-1*H*-imidazol-1-yl)methyl]-benzoate (8). A stirred solution of **7** (0.50 g, 2.03 mmol) and thionyl chloride (1.48 mL, 20.3 mmol, 10.0 equiv) in CH_2Cl_2 (20 mL) was warmed at 40 °C for 3 h. After cooling to room temperature, the excess thionyl choride was quenched by the addition of saturated aqueous $NaHCO_3$ (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 4×15 cm, 10–20% MeOH–$CHCl_3$ gradient elution) afforded **8** (0.48 g, 89%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (d, J = 8.4 Hz, 2H), 7.52 (s, 1H), 7.21 (d, J = 8.1 Hz, 2H), 6.92 (s, 1H), 5.13 (s, 2H), 4.57 (s, 2H), 3.91 (s, 3H); FABHRMS (NBA/NaI) m/z 265.0749 ($M+H^+$, $C_{13}H_{13}ClN_2O_2$ requires 265.0744).

Methyl 4-[4-(3-carboethoxy-3-cyanoprop-1-yl)-1*H*-imidazol-1-yl]methyl]benzoate (10). A stirred solution of **8** (0.40 g, 1.51 mmol), ethyl cyanoacetate **9** (0.32 mL, 3.02 mmol, 2.0 equiv), and K_2CO_3 (1.04 g, 7.55 mmol, 5.0 equiv) in DMF (15 mL) was warmed at 70 °C for 3 h. The solution was cooled to 25 °C and diluted with H_2O (75 mL). The aqueous layer was extracted with EtOAc (4×20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 4×15 cm, 10–20% MeOH–$CHCl_3$ gradient elution) afforded **10** (0.39 g, 76%) as a white solid: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.00 (d, J = 8.3 Hz, 2H), 7.48 (s, 1H), 7.18 (d, J = 8.3 Hz, 2H), 6.83 (s, 1H), 5.13 (s, 2H), 4.23 (dd, J = 14.3, 7.1 Hz, 2H), 3.96 (dd, J = 8.7, 5.9 Hz, 1H), 3.90 (s, 3H), 3.22 (dd, J = 14.5, 5.8 Hz, 1H), 3.11 (dd, J = 14.5, 8.7 Hz, 2H), 1.27 (t, J = 7.2 Hz, 3H); FABHRMS (NBA/NaI) m/z 328.1288 ($M+H^+$, $C_{17}H_{17}N_3O_4$ requires 328.1297).

Ethyl 4-[4-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-1*H*-imidazol-1-yl]methyl]benzoate (11). A solution of NaOEt (0.051 g, 0.75 mmol, 1.0 equiv) in absolute EtOH (1.3 mL) was treated successively with a solution of **10** (0.20 g, 0.76 mmol) in EtOH (2.5 mL) and a solution of guanidine hydrochloride (0.072 g, 0.75 mmol, 1.0 equiv) and NaOEt (0.051 g, 0.75 mmol, 1.0 equiv) in EtOH (2.5 mL). The solution was stirred at 25 °C for 3 h. The solution was acidified to pH = 1 with the addition of trifluoracetic acid followed by concentration under reduced pressure. Chromatography (SiO_2, 2×15 cm, 10–80% MeOH–$CHCl_3$ gradient elution) afforded **11** (0.165 g, 62%) as a white solid: ^{1}H NMR (CD_3OD, 250 MHz) δ 7.99–7.96 (m, 3H), 7.32 (d, J = 8.2 Hz, 2H), 7.10 (s, 1H), 5.29 (s, 2H), 4.34 (dd, J = 14.3, 7.1 Hz, 2H), 3.16 (d, J = 9.1 Hz, 1H), 3.08 (d, J = 9.3 Hz, 1H), 1.36 (t, J = 7.1 Hz, 3H); FABHRMS (NBA/NaI) m/z 369.1675 ($M+H^+$, $C_{18}H_{20}N_6O_3$ requires 369.1675).

4-[4-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-1*H*-imidazol-1-yl]methyl]benzoic acid (12). A solution of **11** (0.010 g, 0.027 mmol), LiOH–H_2O (0.0057 g, 0.135 mmol, 5.0 equiv) in 3:1 MeOH–H_2O (0.27 mL) was warmed at 60 °C for 5 h. The mixture was cooled to 25 °C and diluted with H_2O (10 mL) and the aqueous layer was washed with EtOAc (3×3 mL) and acidified to pH = 1 with the addition of 1 M aqueous HCl. The solution was concentrated under reduced pressure and the residue was azeotropically treated with toluene (3×5 mL) to remove traces of glacial acetic acid and H_2O, dried

(Na_2SO_4), and concentrated under reduced pressure to provide **12** (0.008 g, 84%). The compound was used without further purification: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.81 (d, J = 7.8 Hz, 2H), 7.61 (s, 1H), 7.14 (d, J = 8.2 Hz, 2H), 6.63 (s, 1H), 6.47 (br s, 2H), 5.89 (br s, 2H), 5.05 (s, 2H), 3.16 (s, 3H); FABHRMS (NBA/NaI) m/z 363.1167 ($M + Na^+$, $C_{16}H_{16}N_6O_3$ requires 363.1182).

di-*tert*-Butyl *N*-{4-[4-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-1*H*-imidazol-1-yl]methyl]benzoyl}-L-glutamate (14). A solution of **12** (9.2 mg, 0.027 mmol) and di-*tert*-butyl L-glutamate hydrochloride (**13**, 11.2 mg, 0.041 mmol, 1.5 equiv) in DMF (2.7 mL) was treated succesively with $NaHCO_3$ (6.8 mg, 0.081 mmol, 3.0 equiv) and EDCI (15.5 mg, 0.081 mmol, 3.0 equiv). The solution was stirred at 25 °C for 12 h and concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (5 mL) and extracted with saturated aqueous $NaHCO_3$ (2 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography (SiO_2, 1×15 cm, 10–20% MeOH–$CHCl_3$ gradient elution) afforded **14** (8.5 mg, 54%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.69 (d, J = 7.6 Hz, 2H), 7.54 (br s, 1H), 7.34 (s, 1H), 7.04 (d, J = 7.6 Hz, 2H), 6.67 (s, 1H), 5.76 (br s, 2H), 5.66 (br s, 1H), 4.93 (s, 2H), 4.62 (dd, J = 12.7, 8.1 Hz, 2H), 3.54 (s, 2H), 2.45–2.30 (m, 2H), 2.20–2.17 (m, 1H), 2.09–2.01 (m, 1H), 1.46 (s, 9H), 1.39 (s, 1H); FABHRMS (NBA/NaI) m/z 714.2040 ($M + Cs^+$, $C_{29}H_{39}N_7O_6$ requires 714.2016).

***N*-{4-[4-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-1*H*-imidazol-1-yl]methyl]benzoyl}-L-glutamic acid (3a).** A solution of **14** (5.0 mg, 0.009 mmol) in CH_2Cl_2 (0.1 mL) cooled to 0 °C was treated with trifluoracetic acid (0.03 mL). The solution was stirred at 0 °C for 2 h and warmed to 25 °C and stirred for 12 h. Et_2O (1 mL) was added to the reaction mixture and a white precipitate formed. The precipitate was triturated with Et_2O (3×1 mL) and dried in vacuo to give **3a**–CF_3CO_2H (3.4 mg, 82%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.10 (s, 1H), 8.65 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 7.8 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.31 (s, 1H), 7.06 (br s, 2H), 6.49 (br s, 2H), 5.40 (s, 2H), 4.42–4.36 (m, 1H), 2.36–2.33 (m, 2H), 2.11–2.07 (m, 1H), 1.98–1.91 (m, 1H); MALDIFTMS (DHB) m/z 470.1791 ($M + H^+$, $C_{21}H_{23}N_7O_6$ requires 470.1788).

Methyl 4-[5-[[(1,1-dimethylethyl)silyl]oxy]methyl]-3-pyridinyl]benzoate (17). A solution of methyl 4-bromobenzoate (**16**, 0.81 g, 3.77 mmol, 1.0 equiv), diboron pinacol ester (1.05 g, 4.17 mmol, 1.1 equiv), KOAc (0.93 g, 11.3 mmol, 3.0 equiv), and $PdCl_2$(dppf) (0.09 g, 3 mol%) in DMF (22.5 mL) was warmed at 80 °C for 2 h. The solution was cooled to 25 °C and was treated successively with **15** (2.19 g, 7.55 mmol, 2.0 equiv), $PdCl_2$(dppf) (0.09 g, 3 mol%), and 2 M aqueous Na_2CO_3 (9.40 mL, 5.0 equiv). The solution was heated at 80 °C for 12 h, cooled to 25 °C and diluted with Et_2O (100 mL). The organic layer was washed with H_2O (50 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography (SiO_2, 5×15 cm, 15% hexanes–EtOAc) afforded **17** (0.72 g, 54%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.77 (br s, 1H), 8.59 (br s, 1H), 8.14 (d, J = 8.4 Hz, 2H), 7.89 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 4.83 (s, 2H), 3.94 (s, 3H), 0.93 (s, 9H), 0.13 (s, 6H); MALDIFTMS (DHB) m/z 358.1832 ($M + H^+$, $C_{20}H_{27}NO_3Si$ requires 358.1838).

Methyl 4-[5-hydroxymethyl-3-pyridinyl]benzoate (18). A solution of **17** (0.32 g, 0.89 mmol) in THF (10 mL) at 25 °C was treated with Bu_4NF (2.67 mL, 2.67 mmol, 3.0 equiv) as a 1 M solution in THF. The solution was stirred at 25 °C for 3 h and quenched by the addition of saturated aqueous NH_4Cl (50 mL). The product was extracted into EtOAc (3×10 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography (SiO_2, 4×15 cm, 8% MeOH–$CHCl_3$) provided **18** (0.20 g, 90%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.80 (s, 1H), 8.62 (s, 1H), 8.15 (d, J = 8.1 Hz, 2H), 7.97 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 4.84 (s, 2H), 3.95 (s, 3H); MALDIFTMS (DHB) m/z 244.0972 ($M + H^+$, $C_{14}H_{13}NO_3$ requires 244.0974).

Methyl 4-[5-chloromethyl-3-pyridinyl]benzoate (19). A stirred solution of **18** (0.197 g, 0.810 mmol) and thionyl chloride (0.29 mL, 4.1 mmol, 5.0 equiv) in CH_2Cl_2 (8.2 mL) was warmed at 50 °C for 3 h. After cooling to room temperature, the excess thionyl chloride was quenched by the addition of saturated aqueous $NaHCO_3$ (25 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 4×15 cm, 50% hexanes–EtOAc) afforded **19** (0.198 g, 92%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.84 (s, 1H), 8.65 (s, 1H), 8.16 (d, J = 7.9 Hz, 2H), 7.99 (s, 1H), 7.67 (d, J = 7.9 Hz, 2H), 4.68 (s, 2H), 3.96 (s, 3H); MALDIFTMS (DHB) m/z 262.0644 ($M + H^+$, $C_{14}H_{12}ClNO_2$ requires 244.0635).

Methyl 4-[5-[3-carboethoxy-3-cyanoprop-1-yl]-3-pyridinyl]benzoate (20). A stirred solution of **19** (0.21 g, 0.81 mmol), ethyl cyanoacetate (**9**, 0.26 mL, 2.43 mmol, 3.0 equiv), and K_2CO_3 (0.45 g, 3.24 mmol, 4.0 equiv) in DMF (8.1 mL) was warmed at 70 °C for 3 h. The solution was cooled to 25 °C and diluted with H_2O (50 mL). The aqueous layer was extracted with EtOAc (3×20 mL), dried (Na_2SO_4) and, concentrated under reduced pressure. Chromatography (SiO_2, 3×15 cm, 25% hexanes–EtOAc) afforded **20** (0.22 g, 79%): ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.82 (br s, 1H), 8.56 (br s, 1H), 8.14 (d, J = 8.5 Hz, 2H), 7.89 (s, 1H), 7.65 (d, J = 8.6 Hz, 2H), 4.27 (q, J = 14.3, 7.2 Hz, 2H), 3.95 (s, 3H), 3.82 (dd, J = 7.6, 6.0 Hz, 1H), 3.36–3.32 (m, 2H), 1.27 (t, J = 7.2 Hz, 3H); MALDIFTMS (DHB) m/z 339.1336 ($M + H^+$, $C_{19}H_{18}N_2O_2$ requires 339.1345).

Methyl 4-[5-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-3-pyridinyl]benzoate (21). A solution of NaOEt (5.1 mg, 0.74 mmol, 1.0 equiv) in absolute EtOH (0.13 mL) was treated successively with a solution of **20** (0.025 g, 0.074 mmol) in EtOH (0.26 mL) and a solution of guanidine hydrochloride (7.8 mg, 0.074 mmol, 1.0 equiv) and NaOEt (5.1 mg, 0.74 mmol 1.0 equiv) in EtOH (0.13 mL). The solution was warmed at 45 °C for 12 h. The solution was acidified with the addition of glacial acetic acid (pH = 1) and concentrated under reduced pressure. Chromatography (SiO_2, 1×15 cm,

15% MeOH–CH_2Cl_2) afforded **21** (0.016 g, 61%): ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.88 (s, 1H), 8.69 (s, 1H), 8.53 (s, 1H), 8.07 (d, J = 8.4 Hz, 2H), 7.99 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 6.01 (s, 2H), 5.93 (s, 2H), 3.88 (s, 3H), 3.60 (s, 2H); MALDIFTMS (DHB) m/z 352.1427 ($M+H^+$, $C_{18}H_{17}N_5O_3$ requires 352.1410).

4-[5-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-3-pyridinyl]benzoic Acid (22). A solution of **21** (0.036 g, 0.087 mmol) in 3:1 CH_3OH:H_2O (0.87 mL) was treated with LiOH–H_2O (0.011 g, 0.261 mmol, 3.0 equiv) and the mixture was stirred at 25 °C for 12 h. The mixture was diluted with H_2O (5 mL) and the aqueous layer was washed with EtOAc (3×2 mL) and acidified to pH = 1 with the addition of 1 M aqueous HCl. The solution was concentrated under reduced pressure and the residue was azeotropically treated with toluene (3×5 mL) to remove traces of H_2O to provide **22** (0.053 g, 88%): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.04 (s, 1H), 8.71 (s, 1H), 8.62 (s, 1H), 8.44 (s, 2H), 8.08 (d, J = 8.5 Hz, 2H), 7.94 (d, J = 8.2 Hz, 2H), 7.46 (br s, 2H), 3.49 (s, 2H); MALDIFTMS (DHB) m/z 338.1240 ($M+H^+$, $C_{17}H_{15}N_5O_3$ requires 338.1240).

di-*tert*-Butyl *N*-{4-[5-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-3-pyridinyl]benzoyl}-L-glutamate (23). A solution of **22** (0.026 g, 0.076 mmol) and **13** (0.033 g, 0.114 mmol, 1.5 equiv) in DMF (0.76 mL) was treated with $NaHCO_3$ (0.019 g, 0.228 mmol, 3.0 equiv) followed by EDCI (0.044 g, 0.228 mmol, 3.0 equiv). The reaction mixture was stirred at 25 °C for 12 h before the solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (5 mL) and extracted with saturated aqueous $NaHCO_3$ (2 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography (SiO_2, 1×15 cm, 10% MeOH–$CHCl_3$) provided **23** (0.019 g, 45%) as a white solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.88 (s, 1H), 8.31 (s, 1H), 7.98 (d, J = 8.3 Hz, 2H), 7.85 (s, 1H), 7.73 (d, J = 8.3 Hz, 2H), 6.83 (s, 1H), 5.13 (s, 2H), 4.54–4.49 (m, 1H), 3.73 (s, 1H), 3.58 (s, 1H), 2.42 (t, J = 7.2 Hz, 2H), 2.26–2.17 (m, 1H), 2.08–2.01 (m, 1H), 1.46 (s, 9H), 1.41 (s, 9H); MALDIFTMS (DHB) m/z 601.2739 ($M+Na^+$, $C_{30}H_{38}N_6O_6$ requires 601.2751).

***N*-{4-[5-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)methyl]-3-pyridinyl]benzoyl}-L-glutamic acid (4a).** A solution of **23** (3.2 mg, 0.0055 mmol) in CH_2Cl_2 (0.20 mL) cooled to 0 °C was treated with trifluoroacetic acid (0.04 mL). The solution was stirred at 0 °C for 2 h and 25 °C for 12 h. Et_2O (1 mL) was added to the reaction mixture and a white precipitate formed. The precipitate was triturated with Et_2O (3×1 mL) and dried in vacuo to give **4a**–CF_3CO_2H (2.1 mg, 89%) as a white solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.86 (s, 1H), 8.42 (s, 1H), 8.02 (s, 1H), 8.01 (d, J = 8.1 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 6.83 (s, 1H), 5.13 (s, 2H), 4.71–4.65 (m, 1H), 3.82 (s, 1H), 3.70 (s, 1H), 2.53–2.46 (m, 2H), 2.32–2.28 (m, 1H), 2.20–2.12 (m, 1H); MALDIFTMS (DHB) m/z 467.1679 ($M+Na^+$, $C_{22}H_{22}N_6O_6$ requires 467.1683).

Methyl 4-[(4-chloromethyl-1*H*-imidazol-1-yl)ethyl]benzoate (25). Obtained from **24** (1.10 g, 6.58 mmol) using the procedure described for **7**. Chromatography (SiO_2, 5×15 cm, 10% MeOH–EtOAc) afforded **25** (0.81 g, 44%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (d, J = 8.4 Hz, 2H), 7.48 (s, 1H), 7.13 (d, J = 8.4 Hz, 2H), 6.73 (s, 1H), 5.12 (s, 2H), 3.91 (s, 3H), 3.76 (t, J = 7.1 Hz, 2H), 3.00 (t, J = 7.1 Hz, 2H); FABHRMS (NBA/NaI) m/z 279.0904 ($M+H^+$, $C_{14}H_{15}ClN_2O_2$ requires 279.0900).

Methyl 4-[4-(3-carboethoxy-3-cyanobut-1-yl)-1*H*-imidazol-1-yl]ethyl]benzoate (26). Obtained from **25** (0.191 g, 0.683 mmol) using the procedure described for **10**. Chromatography (SiO_2, 3×15 cm, 100% EtOAc) afforded **26** (0.14 g, 56%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (d, J = 6.1 Hz, 2H), 7.52 (s, 1H), 7.19 (d, J = 6.1 Hz, 2H), 6.69 (s, 1H), 5.09 (s, 2H), 4.21 (dd, J = 14.3, 7.1 Hz, 2H), 3.88 (s, 3H), 3.56 (dd, J = 8.9, 6.0 Hz, 1H), 2.81–2.71 (m, 2H), 2.41–2.34 (m, 1H), 2.26–2.19 (m, 1H), 1.26 (t, J = 14.0 Hz, 3H); FABHRMS (NBA/NaI) m/z 356.1619 ($M+H^+$, $C_{19}H_{21}N_3O_4$ requires 356.1610).

Ethyl 4-[4-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)ethyl]-1*H*-imidazol-1-yl]methyl]benzoate (27). Obtained from **26** (0.042 g, 0.117 mmol) using the procedure described for **11**. Chromatography (SiO_2, 1×15 cm, 10–80% MeOH–$CHCl_3$ gradient elution) afforded **27** (0.165 g, 62%) as a tan solid: ^{1}H NMR (DMF-d_7, 400 MHz) δ 9.99 (br s, 1H), 8.00 (d, J = 8.1 Hz, 2H), 7.74 (br s, 1H), 7.43 (d, J = 8.1 Hz, 2H), 6.95 (s, 1H), 6.21 (br s, 1H), 5.73 (br s, 1H), 5.34 (s, 2H), 4.35 (dd, J = 14.2, 7.3 Hz, 2H), 2.61 (m, 4H), 1.35 (t, J = 7.0 Hz, 3H); FABHRMS (NBA/NaI) m/z 383.1829 ($M+H^+$, $C_{19}H_{22}N_6O_3$ requires 383.1832).

4-[4-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)ethyl]-1*H*-imidazol-1-yl]methyl]benzoic acid (28). Obtained from **27** (0.107 g, 0.300 mmol) using the procedure described for **12**. The solution was concentrated under reduced pressure and the residue was treated with toluene (3×5 mL) to remove traces of H_2O to provide **28** (0.083 g, 81%). The compound was used without further purification: ^{1}H NMR (DMF-d_7, 400 MHz) δ 9.22 (s, 1H), 7.97 (d, J = 8.1 Hz, 2H), 7.55 (s, 1H), 7.45 (d, J = 8.1 Hz, 2H), 5.48 (s, 2H), 2.66–2.62 (m, 2H), 2.57–2.50 (m, 2H); FABHRMS (NBA/NaI) m/z 355.1512 ($M+H^+$, $C_{17}H_{18}N_6O_3$ requires 355.1519).

di-*tert*-Butyl *N*-{4-[4-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)ethyl]-1*H*-imidazol-1-yl]methyl]benzoyl}-L-glutamate (29). Obtained from **28** (0.052 g, 0.152 mmol) using the procedure described for **14**. Chromatography (SiO_2, 1×15 cm, 50% hexanes–EtOAc) afforded **29** (0.055 g, 61%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, J = 7.3 Hz, 2H), 7.48 (s, 1H), 7.44 (s, 1H), 7.08 (d, J = 7.6 Hz, 2H), 6.55 (s, 1H), 5.04 (br s, 2H), 5.04 (s, 2H), 4.65–4.60 (m, 1H), 2.65–2.60 (m, 4H), 2.43–2.30 (m, 2H), 2.24–2.18 (m, 1H), 2.09–1.99 (m, 1H), 1.46 (s, 9H), 1.40 (s, 9H); FABHRMS (NBA/NaI) m/z 596.3214 ($M+H^+$, $C_{30}H_{41}N_7O_6$ requires 596.3197).

***N*-{4-[4-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)ethyl]-1*H*-imidazol-1-yl]methyl]benzoyl}-L-glutamic acid (3b).** Obtained from **29** (5.1 mg, 0.009 mmol) using the procedure described for **3a**. The precipitate was triturated

with Et_2O (3×1 mL) and dried in vacuo to give **3b**–CF_3CO_2H (3.6 mg, 81%) as a white solid: ^1H NMR (DMSO-d_6, 400 MHz) δ 9.24 (s, 1H), 7.78 (d, J = 7.3 Hz, 2H), 7.48 (s, 1H), 7.44 (s, 1H), 7.08 (d, J = 7.6 Hz, 2H), 6.55 (s, 1H), 5.04 (br s, 2H), 5.04 (s, 2H), 4.65–4.60 (m, 1H), 2.65–2.60 (m, 4H), 2.43–2.30 (m, 2H), 2.24–2.18 (m, 1H), 2.09–1.99 (m, 1H); FABHRMS (NBA/NaI) m/z 484.1928 ($M+H^+$, $C_{22}H_{25}N_7O_6$ requires 484.1945).

Methyl 4-[5-[[(1,1-dimethylethyl)silyl]oxy]ethyl]-3-pyridinyl]benzoate (31). Obtained from **30** (1.62 g, 5.12 mmol) using the procedure described for **17**. Chromatography (SiO_2, 5×15 cm, 25% hexanes–EtOAc) afforded **31** (0.598 g, 63%) as a white solid: ^1H NMR ($CDCl_3$, 500 MHz) δ 8.72 (d, J = 1.8 Hz, 1H), 8.49 (d, J = 1.8 Hz, 1H), 8.13 (d, J = 8.4 Hz, 2H), 7.77 (t, J = 1.8 Hz, 1H), 7.64 (d, J = 8.5 Hz, 2H), 3.94 (s, 3H), 3.87 (t, J = 6.6 Hz, 2H), 2.88 (t, J = 6.3 Hz, 2H), 0.84 (s, 9H), −0.03 (s, 6H); MALDIFTMS (DHB) m/z 372.1998 ($M+H^+$, $C_{21}H_{29}NO_3Si$ requires 372.1995).

Methyl 4-[5-hydroxyethyl-3-pyridinyl]benzoate (32). Obtained from **31** (0.579 g, 1.56 mmol) using the procedure described for **18**. Chromatography (SiO_2, 4×15 cm, 25% hexanes–EtOAc) afforded **32** (0.365 g, 91%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.57 (s, 1H), 8.41 (s, 1H), 8.05 (d, J = 8.1 Hz, 2H), 7.76 (s, 1H), 7.55 (d, J = 8.4 Hz, 2H), 3.91 (s, 3H), 2.90 (t, J = 5.0 Hz, 2H), 2.84–2.81 (m, 2H); MALDIFTMS (DHB) m/z 258.1121 ($M+H^+$, $C_{15}H_{15}NO_3$ requires 258.1130).

Methyl 4-[5-chloroethyl-3-pyridinyl]benzoate (33). Obtained from **32** (0.150 g, 0.581 mmol) using the procedure described for **19**. Chromatography (SiO_2, 3×15 cm, 40% hexanes–EtOAc) afforded **33** (0.14 g, 87%) as a white solid: ^1H NMR ($CDCl_3$, 500 MHz) δ 8.78 (s, 1H), 8.53 (s, 1H), 8.15 (d, J = 8.4 Hz, 2H), 7.79 (s, 1H), 7.66 (d, J = 8.4 Hz, 2H), 3.95 (s, 3H), 3.79 (t, J = 7.0 Hz, 2H), 3.17 (t, J = 7.0 Hz, 2H); MALDIFTMS (DHB) m/z 276.0785 ($M+H^+$, $C_{15}H_{14}ClNO_2$ requires 276.0791).

Methyl 4-[5-[3-carboethoxy-3-cyanobut-1-yl]-3-pyridinyl]-benzoate (34). Obtained from **33** (0.161 g, 0.583 mmol) using the procedure described for **20**. Chromatography (SiO_2, 3×15 cm, 50% EtOAc) afforded **34** (0.13 g, 63%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.74 (s, 1H), 8.50 (s, 1H), 8.11 (d, J = 8.1 Hz, 2H), 7.74 (s, 1H), 7.62 (d, J = 8.1 Hz, 2H), 4.22 (dd, J = 14.3, 7.2 Hz, 2H), 3.92 (s, 3H), 3.54 (t, J = 7.0 Hz, 1H), 2.30–2.87 (m, 2H), 2.34–2.29 (m, 2H), 1.29 (t, J = 7.0 Hz, 3H); FABHRMS (NBA/NaI) m/z 353.1508 ($M+H^+$, $C_{20}H_{20}N_2O_4$ requires 353.1501).

Methyl 4-[5-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)ethyl]-3-pyridinyl]benzoate (35). Obtained from **34** (0.149 g, 0.423 mmol) using the procedure described for **21**. Chromatography (SiO_2, 3×15 cm, 10–30% MeOH–$CHCl_3$ gradient elution) afforded **35** (0.086 g, 56%) as a white solid: ^1H NMR (DMSO-d_6, 500 MHz) δ 9.80 (br s, 1H), 8.74 (d, J = 1.8 Hz, 1H), 8.45 (d, J = 1.8 Hz, 1H), 8.07 (d, J = 8.4 Hz, 2H), 8.03 (t, J = 1.8 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 5.92 (s, 2H), 5.81 (s, 2H), 3.89 (s, 3H), 2.70 (t, J = 7.0 Hz, 2H), 2.53 (t, J = 7.0 Hz, 2H); MALDIFTMS (DHB) m/z 366.1576 (M + H +, $C_{19}H_{19}N_5O_3$ requires 366.1566).

4-[5-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)ethyl]-3-pyridinyl]benzoic acid (36). Obtained from **35** (0.020 g, 0.055 mmol) using the procedure described for **22**. The solution was concentrated under reduced pressure and the residue was treated with toluene (3×5 mL) to remove traces of H_2O to provide **36** (0.017 g, 91%): ^1H NMR (DMSO-d_6, 500 MHz) 9.05 (s, 1H), 8.76 (s, 1H), 8.66 (s, 1H), 8.32 (br s, 2H), 8.08 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H), 7.29 (br s, 2H), 2.86 (t, J = 7.0 Hz, 2H), 2.64 (t, J = 7.4 Hz, 2H); MALDIFTMS (DHB) m/z 352.1424 ($M+H^+$, $C_{18}H_{17}N_5O_3$ requires 352.1410).

di-*tert*-Butyl *N*-{4-[5-[(2,4-diamino-6(1*H*)-pyrimidino-5-yl) ethyl] - 3 - pyridinyl]benzoyl} - L - glutamate (37). Obtained from **36** (0.010 g, 0.028 mmol) using the procedure described for **23**. Chromatography (SiO_2, 1×15 cm, 20% MeOH–$CHCl_3$ gradient elution) afforded **37** (0.008 g, 48%) as a white solid: ^1H NMR ($CDCl_3$, 500 MHz) δ 8.60 (s, 1H), 8.41 (s, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.69 (s, 1H), 7.45 (d, J = 6.6 Hz, 1H), 5.47 (br s, 2H), 4.71–4.65 (m, 3H), 2.81 (s, 2H), 2.60 (s, 2H), 2.48–2.42 (m, 1H), 2.40–2.34 (m, 1H), 2.27–2.20 (m, 1H), 2.12–2.05 (m, 1H), 1.49 (s, 9H), 1.42 (s, 9H); MALDIFTMS (DHB) m/z 593.3089 ($M+H^+$, $C_{31}H_{40}N_6O_6$ requires 593.3087).

***N*-{4-[5-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)ethyl]-3-pyridinyl]benzoyl}-L-glutamic acid (4b).** Obtained from **37** (4.1 mg, 0.007 mmol) using the procedure described for **4a** to provide **4b** (2.8 mg, 83%) as a white solid: ^1H NMR (DMSO-d_6, 400 MHz) δ 8.90 (s, 1H), 8.75 (d, J = 7.6 Hz, 2H), 8.56 (s, 1H), 8.32 (s, 1H), 8.04 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 8.2 Hz, 2H), 7.51 (br s, 1H), 6.77 (br s, 2H), 4.45–4.41 (m, 1H), 2.78 (t, J = 6.8 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H), 2.38 (t, J = 7.3 Hz, 2H), 2.16–2.08 (m, 1H), 2.02–1.92 (m, 1H), FABHRMS (NBA/NaI) m/z 481.1825 ($M+H^+$, $C_{23}H_{24}N_6O_6$ requires 481.1836).

Methyl 4-[(4-(acetylthio)methyl-1*H*-imidazol-1-yl)methyl]benzoate (39). A solution of **8** (1.31 g, 4.91 mmol) and potassium thioacetate (1.68 g, 14.7 mmol, 3.0 equiv) in acetone (20 mL) was warmed at reflux for 30 min. The solution was cooled to 25 °C, diluted with H_2O (100 mL) and extracted with EtOAc (3×20 mL). The EtOAc was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 5×15 cm, 100% EtOAc) afforded **39** (1.11 g, 74%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.99 (d, J = 8.4 Hz, 2H), 7.44 (s, 1H), 7.17 (d, J = 8.1 Hz, 2H), 6.81 (s, 1H), 5.08 (s, 2H), 4.05 (s, 2H), 3.90 (s, 3H), 2.31 (s, 3H); MALDIFTMS (DHB) m/z 305.0975 ($M+H^+$, $C_{15}H_{16}N_2O_3S$ requires 305.0960).

Methyl 4-[(4-mercaptomethyl-1*H*-imidazol-1-yl)methyl]-benzoate (40). A solution of **39** (0.050 g, 0.164 mmol) in 1 N HCl–EtOH (0.75 mL) was warmed at 40 °C for 2 h. The solution was cooled to 25 °C and the solvent was removed under reduced pressure and the residue was dissolved in H_2O (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3×3 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 1×15 cm, 16% MeOH–$CHCl_3$) afforded **40** (0.024 g, 56%) as a white solid: ^1H NMR ($CDCl_3$, 500 MHz) δ 8.00 (d, J = 8.1 Hz, 2H), 7.58 (s, 1H), 7.20

(d, $J=8.1$ Hz, 2H), 6.78 (s, 1H), 5.12 (s, 2H), 3.90 (s, 3H), 3.69 (s, 2H); FABHRMS (NBA/NaI) m/z 263.0853 ($M+H^+$, $C_{13}H_{14}N_2O_2S$ requires 263.0854).

Methyl 4-[4-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)thio]-methyl]-1*H*-imidazol-1-yl]methyl]benzoate (41). Argon was bubbled through a solution of **40** (0.107 g, 0.408 mmol) and 5-bromo-2,6-diamino-4(3*H*)-pyrimidinone (**38**, 0.084 g, 0.408 mmol, 1.0 equiv) in DMF (2 mL) for 0.5 h. The reaction mixture was warmed at 80 °C for 2 h, cooled to 25 °C and concentrated under reduced pressure. Chromatography (SiO_2, 2×15 cm, 8% MeOH–$CHCl_3$) afforded **41** (0.13 g, 81%) as a white solid: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.93 (s, 1H), 7.95 (d, $J=8.2$ Hz, 2H), 7.67 (s, 1H), 7.28 (d, $J=8.2$ Hz, 2H), 6.97 (s, 1H), 6.26 (br s, 4H), 5.23 (s, 2H), 3.84 (s, 3H), 3.53 (s, 2H); FABHRMS (NBA/NaI) m/z 387.1236 ($M+H^+$, $C_{17}H_{18}N_6O_3S$ requires 387.1239).

4-[4-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)thio]methyl]-1*H*-imidazol-1-yl]methyl]benzoic acid (42). Obtained from **41** (0.082 g, 0.212 mmol) using the procedure described for **11** to provide **42** (0.058 g, 74%): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.30 (br s, 1H), 9.05 (s, 1H), 7.97 (d, $J=$ 8.4 Hz, 2H), 7.47 (s, 1H), 7.40 (d, $J=8.4$ Hz, 2H), 6.56 (br s, 4H), 5.44 (s, 2H), 3.68 (s, 2H); MALDIHRMS (DHB) m/z 395.0917 ($M+Na^+$, $C_{16}H_{16}N_6O_3S$ requires 395.0902).

di-*tert*-Butyl *N*-4-[4-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)thio]methyl]-1*H*-imidazol-1-yl]methyl]-L-glutamate (43). Obtained from **42** (0.014 g, 0.038 mmol) using the procedure described for **12**. Chromatography (SiO_2, 1×15 cm, 10–40% MeOH–CH_2Cl_2) afforded **43** (0.014 g, 62%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (d, $J=8.1$ Hz, 2H), 7.53 (d, $J=7.56$, 1H), 7.46 (s, 1H), 7.06 (d, $J=8.1$ Hz, 2H), 6.76 (br s, 2H), 6.58 (s, 1H), 5.60 (br s, 2H), 5.01 (s, 2H), 4.66–4.61 (m, 1H), 3.66 (d, $J=12.3$ Hz, 1H), 3.60 (d, $J=13.2$ Hz, 1H), 2.46–2.29 (m, 2H), 2.24–2.17 (m, 1H), 2.10–2.01 (m, 1H), 1.46 (s, 9H), 1.39 (s, 9H); FABHRMS (NBA/CsI) m/z 746.1719 ($M+Cs^+$, $C_{29}H_{39}N_7O_6S$ requires 746.1737).

***N*-4-[4-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)thio]methyl]-1*H*-imidazol-1-yl]methyl]-L-glutamic acid (3c).** Obtained from **43** (5.1 mg, 0.008 mmol) using the procedure described for **3a** to provide **3c**–CF_3CO_2H (2.8 mg, 69%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.40 (br s, 1H), 9.19 (s, 1H), 8.63 (d, $J=7.8$ Hz, 2H), 7.92 (d, $J=8.1$ Hz, 2H), 7.51 (s, 1H), 7.42 (d, $J=8.1$ Hz, 2H), 6.63 (br s, 4H), 5.43 (s, 2H), 4.41–4.38 (m, 1H), 3.69 (s, 3H), 2.34 (t, $J=7.3$ Hz, 2H), 2.14–2.07 (m, 1H), 1.99–1.90 (m, 1H); FABHRMS (NBA/CsI) m/z 634.0468 ($M+Cs^+$, $C_{21}H_{23}N_7O_6S$ requires 634.0485).

Methyl 4-[5-(acetylthio)methyl-3-pyridinyl]benzoate (44). Obtained from **19** (0.200 g, 0.764 mmol) using the procedure described for **39**. Chromatography (SiO_2, 3×15 cm, 5% MeOH–$CHCl_3$ afforded **44** (0.20 g, 87%) as a white solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.75 (s, 1H), 8.57 (s, 1H), 8.14 (d, $J=8.1$ Hz, 2H), 7.85 (s, 1H), 7.64 (d, $J=8.1$ Hz, 2H), 4.17 (s, 2H), 3.95 (s, 3H), 2.38 (s, 3H); FABHRMS (NBA/NaI) m/z 302.0854 ($M+H^+$, $C_{16}H_{15}NO_3S$ requires 302.0851).

Methyl 4-[5-mercaptomethyl-3-pyridinyl]benzoate (45). Obtained from **44** (0.040 g, 0.133 mmol) using the procedure described for **40**. Chromatography (SiO_2, 1×15 cm, 40% hexanes–EtOAc) afforded **45** (0.032 g, 93%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.75 (d, $J=2.2$ Hz, 1H), 8.58 (d, $J=2.2$ Hz, 1H), 8.15 (d, $J=8.4$ Hz, 2H), 7.91 (t, $J=2.2$ Hz, 1H), 7.66 (d, $J=8.4$ Hz, 2H), 3.95 (s, 3H), 3.82 (d, $J=7.8$ Hz, 2H), 1.87 (t, $J=7.8$ Hz, 1H); MALDIHRMS (DHB) m/z 260.0735 ($M+H^+$, $C_{14}H_{13}NO_2S$ requires 260.0745).

Methyl 4-[5-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)thio]-methyl]-3-pyridinyl]benzoate (46). Obtained from **45** (0.032 g, 0.123 mmol) using the procedure described for **41**. Chromatography (SiO_2, 1×15 cm, 20% MeOH–$CHCl_3$) afforded **46** (0.044 g, 94%) as a white solid: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.05 (s, 1H), 8.74 (s, 1H), 8.40 (s, 1H), 8.06 (d, $J=8.2$ Hz, 2H), 7.95 (s, 1H), 7.83 (d, $J=8.2$ Hz, 2H), 6.36 (br s, 2H), 6.04 (br s, 2H), 3.88 (s, 3H), 3.78 (s, 2H); FABHRMS (NBA/NaI) m/z 384.1137 ($M+H^+$, $C_{18}H_{17}N_5O_3S$ requires 384.1130).

4-[5-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)thio]methyl]-3-pyridinyl]benzoic acid (47). Obtained from **46** (0.062 g, 0.163 mmol) using the procedure described for **21**. The solution was concentrated under reduced pressure and the residue was treated with toluene (3×5 mL) to remove traces of H_2O to provide **46** (0.050 g, 91%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.70 (s, 1H), 8.37 (s, 1H), 8.01 (d, $J=8.1$ Hz, 2H), 7.80 (s, 1H), 7.69 (d, $J=8.4$ Hz, 2H), 6.55 (br s, 2H), 6.12 (br s, 2H), 3.78 (s, 2H); MALDIHRMS (DHB) m/z 370.0978 ($M+H^+$, $C_{17}H_{15}N_5O_3S$ requires 370.0974).

di-*tert*-Butyl *N*-{4-[5-[(2,4-diamino-6(1*H*)-pyrimidinon-5-yl)thio]methyl]-3-pyridinyl]benzoyl}-L-glutamate (48). Obtained from **47** (0.052 g, 0.141 mmol) using the procedure described for **23**. Chromatography (SiO_2, 1×15 cm, 20% MeOH–$CHCl_3$) afforded **48** (0.031 g, 36%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.95 (br s, 1H), 8.60 (s, 1H), 8.40 (s, 1H), 7.83 (d, $J=8.1$ Hz, 2H), 7.64 (s, 1H), 7.51 (d, $J=8.4$ Hz, 2H), 7.47 (d, $J=7.6$ Hz, 2H), 6.12 (br s, 2H), 5.38 (br s, 2H), 4.70–4.64 (m, 1H), 3.75 (s, 2H), 2.49–2.34 (m, 1H), 2.12–2.03 (m, 1H), 1.50 (s, 9H), 1.42 (s, 9H); FABHRMS (NBA/CsI) m/z 743.1605 ($M+Cs^+$, $C_{30}H_{38}N_6O_6S$ requires 743.1605).

***N*-{4-[5-[(2,4-Diamino-6(1*H*)-pyrimidinon-5-yl)thio]methyl]-3-pyridinyl]benzoyl}-L-glutamic acid (4c).** Obtained from **48** (3.0 mg, 0.006 mmol) using the procedure described for **4a** to provide **4c**–CF_3CO_2H (2.5 mg, 84%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.92 (s, 1H), 8.75 (d, $J=7.8$ Hz, 1H), 8.55 (s, 1H), 8.19 (s, 1H), 8.03 (d, $J=$ 8.4 Hz, 2H), 7.84 (d, $J=8.4$ Hz, 2H), 6.66 (br s, 2H), 6.42 (br s, 2H), 4.44–4.41 (m, 1H), 2.38 (t, $J=7.6$ Hz, 2H), 2.16–2.08 (m, 1H), 2.02–1.92 (m, 1H); FABHRMS (NBA/NaI) m/z 499.1388 ($M+H^+$, $C_{22}H_{22}N_6O_6S$ requires 499.1400).

GAR and AICAR Tfase inhibition. This was conducted as previously detailed[23] with the exception that the AICAR Tfase inhibition was conducted in the absence of 5 μM β-mercaptoethanol and screened with 10 μM enzyme, 25 μM inhibitor and 22.5 μM of cofactor.

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA 63536) and the Skaggs Institute for Chemical Biology.

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