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Design, Synthesis, and Biological Evaluation of Simplified α-Keto Heterocycle, Trifluoromethyl Ketone, and Formyl Substituted Folate Analogues as Potential Inhibitors of GAR Transformylase and AICAR Transformylase

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Abstract—A series of simplified α-keto heterocycle, trifluoromethyl ketone, and formyl substituted folate analogues lacking the benzoylglutamate subunit were prepared and examined as potential inhibitors of glycinamide ribonucleotide transformylase (GAR Tfase) and aminoimidazole carboxamide transformylase (AICAR Tfase).
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Glycinamide ribonucleotide transformylase (GAR Tfase) and aminoimidazole carboxamide ribonucleotide transformylase (AICAR Tfase) are folate-dependent enzymes central to the de novo purine biosynthetic pathway. GAR Tfase utilizes the cofactor (6*R*)-N^{10}-formyltetrahydrofolate (Fig. 1) to transfer a formyl group to the primary amine of its substrate, glycinamide ribonucleotide (GAR, Fig. 1). This one carbon transfer incorporates 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 AICAR Tfase which also employs (6*R*)-N^{10}-formyltetrahydrofolate to transfer a formyl group to the C-5 amine of its substrate, aminoimidazole carboxamide ribonucleotide (AICAR, Fig. 1).[1] The discovery that (6*R*)-5,10-dideazatetrahydrofolate [Lometrexol, (6*R*)-DDATHF, Fig. 2] achieves its potent anticancer activity by selective GAR Tfase inhibition established GAR Tfase and the purine de novo biosynthetic pathway as viable targets for antineoplastic intervention.[2–4] In addition to the ongoing clinical investigation of Lometrexol,[5] two related folate analogues, LY309887[6] and AG2034,[7] that selectively inhibit GAR Tfase have also advanced to clinical trials. Herein, we report the synthesis and evaluation of a novel series of α-keto heterocycle (**13–22**, **29**, **30**, **37**, **38**), trifluoromethyl ketone (**47**, **48**), and aldehyde (**65**, **66**) substituted simplified folate analogues lacking the benzoylglutamate subunit as potential GAR Tfase and AICAR Tfase inhibitors.

Inhibitor Design

The use of α-keto heterocycles as electrophilic, tight-binding reversible enzyme inhibitors was first disclosed by Edwards et al. in 1992.[8] Since then, a number of potent enzyme inhibitors have been disclosed based

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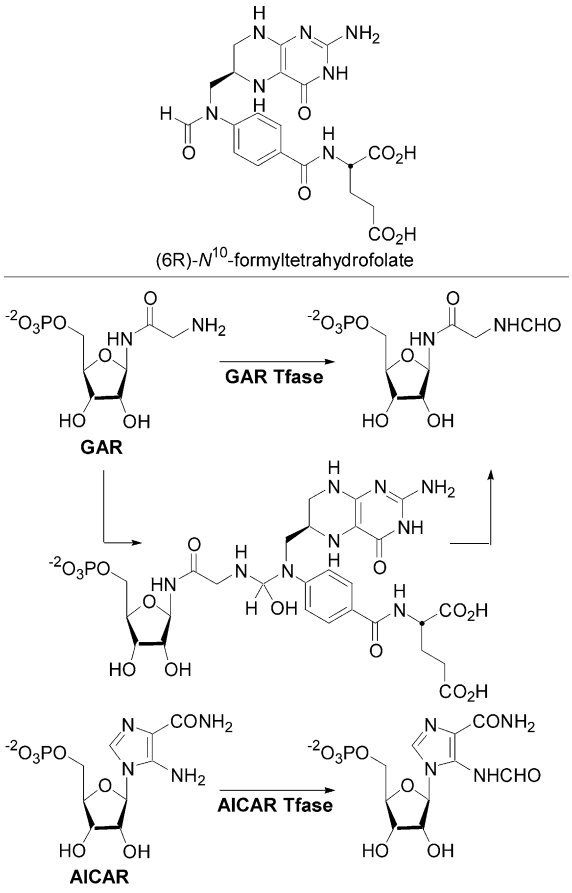


Figure 1.

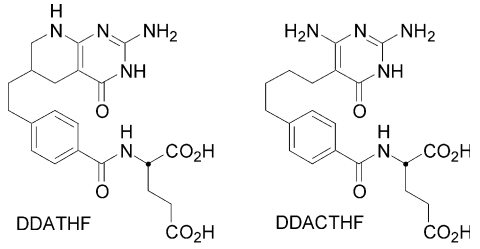


Figure 2.

upon analogous design principles,[9] including our own work on the development of fatty acid amide hydrolase inhibitors.[10] Likewise, the highly electrophilic trifluoromethyl ketone functional group has seen wide application in the field of inhibitor design, most notably in the field of serine protease inhibitors.[11]

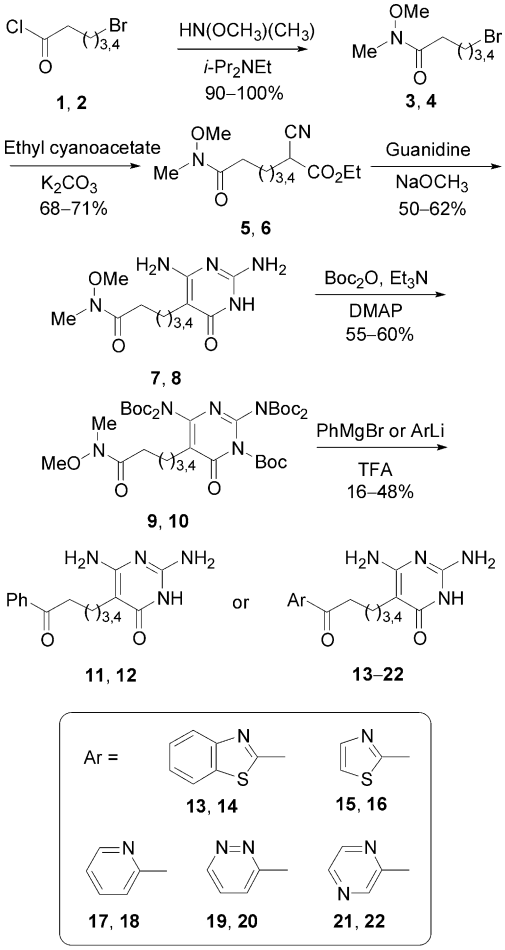
In previous studies, we examined folate-based inhibitors which incorporated electrophilic functional groups that could potentially interact either with active site nucleophiles or the GAR/AICAR substrate amines.[12] It was envisioned that the properly positioned electrophilic carbonyl of an α-keto heterocycle could potentially form an imine or a tetrahedral adduct with these same potential nucleophiles or serve to stabilize gem diol formation of the electrophilic carbonyl and promote active site binding by mimicking the tetrahedral intermediate of the formyl transfer reactions. This latter effect was observed with folate-based inhibitors bearing a nontransferable formyl group and has provided potent and efficacious GAR Tfase inhibitors.[12–14] In addition to the electrophilic carbonyl, an appropriately positioned nitrogen atom within the heterocyclic ring might form additional hydrogen bonds with an active site residue (e.g., protonated His-108), thereby further stabilizing the inhibitor complex. The large number of such heterocyclic systems would allow the generation of a wide variety of candidate inhibitors with significantly different physiochemical properties, thereby maximizing the ability to identify potent and specific inhibitors of either GAR or AICAR transyformylase. In previous studies of fluoronitrophenyl-based GAR and AICAR Tfase inhibitors, we observed that both enzyme active sites have the ability to accommodate the binding of aromatic ring systems in this position of folate-based agents.[15]

The initial set of 14 simplified α-keto heterocycle containing compounds **13**–**22**, **29**, **30**, **37**, **38** incorporated the benzothiazole (**13**, **14**), thiazole (**15**, **16**), pyridine (**17**, **18**), pyridazine (**19**, **20**), pyrazine (**21**, **22**), benzoxazole (**29**, **30**), and oxazole (**37**, **38**) heterocyclic ring systems. All seven heterocyclic ring systems were linked to the folate core of 5,10-dideaza-acyclic-5,6,7,8-tetrahydrofolic acid (DDACTHF, Fig. 2) with two different linker lengths in order to probe flexibility in the placement of the electrophilic carbonyl. A goal with this series of simplified analogues was the possible identification of potent, folate-based inhibitors of GAR Tfase or AICAR Tfase that lack the benzoylglutamate subunit. Such inhibitors would not be dependent on reduced-folate active transport or polyglutamation for functional or in vivo activity, a characteristic required in the design of classical folate-based inhibitors. Based upon a similar design and for comparison purposes, the analogous simplified trifluoromethyl ketone (**47**, **48**) and aldehyde (**65**, **66**) inhibitors were synthesized and evaluated. Just as significantly, these and the related α-keto heterocycle inhibitors serve as important comparison structures for the analogous benzoylglutamate-bearing folate analogues bearing a nontransferable aldehyde,[12–14] trifluoromethyl ketone,[16] or α-keto heterocycle.[17]

Numerous analogues that incorporate the DDACTHF scaffold have been shown to retain potent cytotoxic and enzyme inhibitory properties of the DDATHF ring system exemplified by Lometrexol.[13,16,18] Thus, the nonbenzoylglutamate DDACTHF scaffold was chosen in the present study in order to provide the inhibitors maximal flexibility to facilitate the active site placement of the electrophilic carbonyl or gem diol.

Chemistry

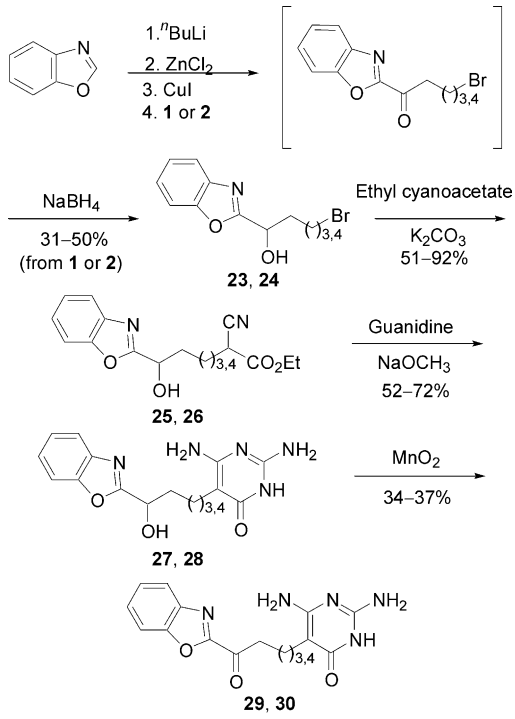
The syntheses of phenyl ketone-based compounds **11**, **12** and the α-keto heterocycle-based compounds **13**–**22** from the common intermediates **9** and **10** are shown in Scheme 1. Starting from the commercially available reagents 5-bromovaleryl chloride (**1**) or 6-bromohexanoyl chloride (**2**), Weinreb amides **3** and **4** were generated by reaction



Scheme 1.

of *N*,*O*-dimethylhydroxylamine (*i*-Pr_2NEt, anhydrous CH_2Cl_2, 25 °C, 12 h, 90–100%). Alkylation of the potassium salt of ethyl cyanoacetate with **3** or **4** (K_2CO_3, anhydrous DMF, 70 °C, 1 h, 68–71%) afforded **5** and **6**. Cyclization with the free base of guanidine under basic conditions (anhydrous CH_3OH, 25 °C, 1 h, 50–62%) gave the desired pyrimidinones **7** and **8**. Compounds **7** and **8** were converted into the corresponding Boc protected derivatives **9** and **10** by reaction with *tert*-butyl dicarbonate (anhydrous DMF, Et_3N, cat. DMAP, 40 °C, 2 h, 55–60%). Reaction of **9** or **10** with phenylmagnesium bromide (anhydrous THF, −30 °C, 1 h) followed by Boc deprotection with TFA (4:1 v/v $CHCl_3$/TFA, 25 °C, 2 h) generated phenyl ketones **11** and **12** (36–48% over two steps from **9** or **10**). Reaction of **9** or **10** with the indicated lithiated heterocycles[19,20] followed by Boc deprotection with TFA (4:1 v/v $CHCl_3$/TFA, 25 °C, 2 h) generated the α-keto heterocycles **13**–**22** (16–48% over two steps from **9** or **10**).

The syntheses of the benzoxazole-based α-keto heterocycles **29** and **30** are shown in Scheme 2 and required a route different from that shown in Scheme 1 because of the instability of 2-lithiobenzoxazole. Benzoxazole was lithiated with *n*-BuLi (anhydrous THF, −78 °C, 20 min) followed by transmetalation first with $ZnCl_2$ (0 °C, 45 min) followed by CuI (0 °C, 10 min) before acylation with commercially available **1** or **2** (0 °C, 30 min).[21]

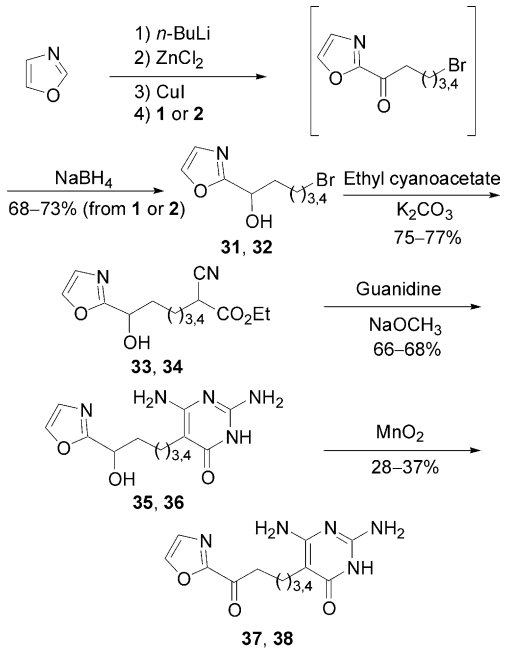


Scheme 2.

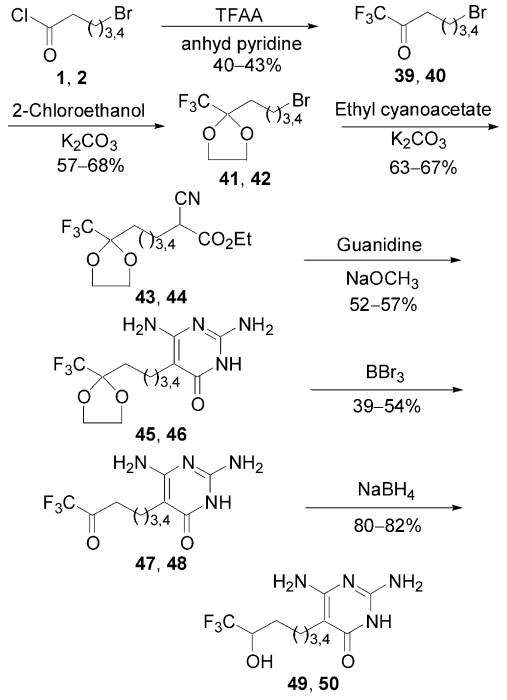
Following aqueous workup, the crude acylation products were reduced with $NaBH_4$ (anhydrous CH_3OH, −20 °C, 30 min) to generate compounds **23** and **24** (31–50% over two steps from **1** or **2**). Alkylation of the potassium salt of ethyl cyanoacetate with **23** or **24** (K_2CO_3, anhydrous DMF, 70 °C, 1 h, 51–92%) afforded **25** and **26**. Cyclization with the free base of guanidine under basic conditions (anhydrous CH_3OH, 25 °C, 1 h, 52–72%) gave the desired pyrimidinones **27** and **28**. Without optimization, oxidation of **27** or **28** with MnO_2 (4:1 v/v $CHCl_3$/DMF, 25 °C, 2 h, 34–37%) generated the benzoxazole-based α-keto heterocycles **29** and **30**.

The syntheses of oxazole-based α-keto heterocycles **37** and **38**, using the same sequence of reactions described in Scheme 2 replacing benzoxazole with oxazole,[22] are shown in Scheme 3.

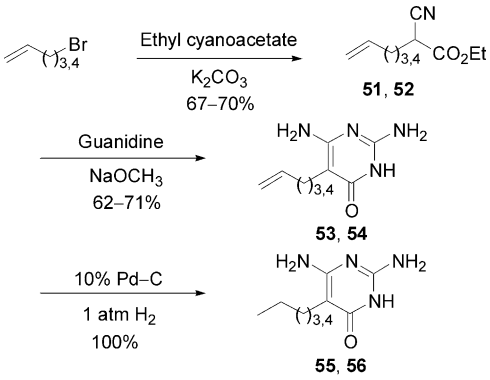
The syntheses of trifluoromethyl ketone-based compounds **47** and **48** are shown in Scheme 4. The commercially available acid chlorides **1** and **2** were transformed into the corresponding trifluoromethyl ketones **39** and **40** by reaction of trifluoroacetic anhydride (anhydrous pyridine, anhydrous CH_2Cl_2, 25 °C, 2 h) followed by aqueous quench (40–43%).[23] The carbonyl functionalities of **39** and **40** were protected as their corresponding 1,3-dioxolanes by base-catalyzed reaction with 2-chloroethanol (K_2CO_3, anhydrous DMF, 25 °C, 18 h, 57–68%)[24] generating **41** and **42**. Alkylation of the potassium salt of ethyl cyanoacetate with **41** or **42** (K_2CO_3, anhydrous DMF, 70 °C, 1 h, 63–67%) afforded **43** and **44**. Cyclization with the free base of guanidine under basic conditions (anhydrous CH_3OH, 25 °C, 1 h, 52–57%) gave the desired pyrimidinones **45** and **46**. Deprotection of **45** and **46** with BBr_3 (anhydrous CH_2Cl_2, 0 °C, 1 h, followed by aqu-



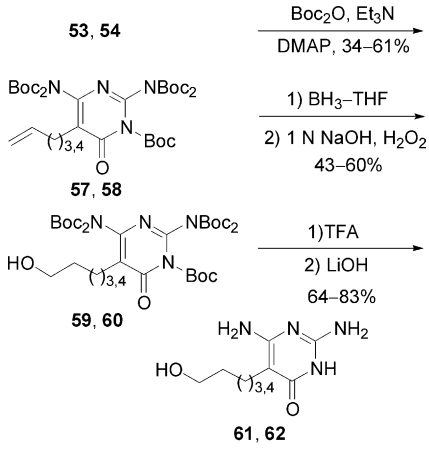
Scheme 3.



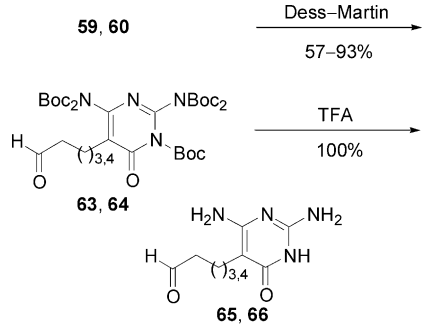
Scheme 4.



Scheme 5.



Scheme 6.



Scheme 7.

eous quench, 39–54%)[25] generated the trifluoromethyl ketones **47** and **48**. Reduction of **47** and **48** ($NaBH_4$, anhydrous CH_3OH, −20 °C, 30 min, 80–82%) generated the comparison alcohols **49** and **50**.

The syntheses of the aldehyde-based agents **65** and **66**, as well as the corresponding alkanes (**55**, **56**) and alcohols (**61**, **62**), are shown in Schemes 5–7. Alkylation of the potassium salt of ethyl cyanoacetate with commercially available 5-bromo-1-pentene or 6-bromo-1-hexene (K_2CO_3, anhydrous DMF, 70 °C, 5 h, 67–70%) afforded compounds **51** and **52**. Cyclization with the free base of guanidine under basic conditions (anhydrous CH_3OH, 25 °C, 1 h, 62–71%) gave the desired pyrimidinones **53** and **54**. Hydrogenation of **53** and **54** (10% Pd/C, 1 atm H_2, CH_3OH, 25 °C, 12 h, quantitative) generated the alkane-substituted pyrimidinones **55** and **56**. Compounds **53** and **54** were converted into the corresponding Boc protected derivatives **57** and **58** by reaction with *tert*-butyl dicarbonate (anhydrous DMF, Et_3N, cat. DMAP, 40 °C, 2 h, 34–61%). Hydroboration of **57** and **58** (BH_3–THF, 1 N NaOH–H_2O_2, anhydrous THF, 0 to 25 °C, 43–60%) generated the corresponding alcohols **59** and **60**. Boc deprotection with TFA (4:1 v/v $CHCl_3$/TFA, 25 °C, 2 h) followed by hydrolysis (0.5 M LiOH–H_2O, 3:1:1 THF/CH_3OH/H_2O, 25 °C, 2 h) produced the alcohol-substituted alkylpyrimidinones **61**

and **62** (64–83% over two steps from **59** or **60**). Oxidation of **59** and **60** (Dess–Martin periodinane, anhydrous CH_2Cl_2, 0 °C, 45 min, 57–93%) generated the corresponding aldehydes **63** and **64**. Boc deprotection with TFA (1:1 v/v $CHCl_3$/TFA, 25 °C, 4 h, quantitative) produced the aldehydes **65** and **66**.

GAR Tfase and AICAR Tfase Inhibition

Compounds **11**–**22**, **27**–**30**, **35**–**38**, **45**–**50**, **55**, **56**, **61**, **62**, **65**, and **66** were tested for inhibition of *Escherichia coli* and recombinant human GAR Tfase (rhGAR Tfase), as well as AICAR Tfase. The results are presented in Table 1. Twenty-one (**11**–**22**, **27**, **29**, **30**, **55**, **56**, **61**, **62**, **65**, **66**) of the 32 compounds tested demonstrated low to moderate inhibition of *E. coli* GAR Tfase, with a K_i range of 9–100 μM. Most of the simplified α-keto heterocycles exhibited potency in the range of 15–87 μM. The only simplified α-keto heterocycles which were inactive ($K_i > 100$ μM) were the oxazole-based agents **37** and **38**. In addition, the ability of the benzoxazole alcohol (**27**) to bind with similar potency to the analogous benzoxazole α-keto heterocycle (**29**) indicates that at least in some cases, additional binding affinity is not being achieved from the ketone carbonyl functionality. However, it is interesting to note that the one-methylene homologated benzoxazole α-keto heterocycle **30** ($K_i = 15$ μM against *E. coli* GAR Tfase) completely loses activity with the replacement of the ketone carbonyl with the corresponding hydroxyl group (**28**). In contrast to the simplified α-keto heterocycles, the corresponding simplified trifluoromethyl ketones (**47**, **48**), as well as the related 1,3-dioxolanes (**45**, **46**) and alcohols (**49**, **50**), were all inactive against GAR Tfase ($K_i > 100$ μM). This is especially surprising given that the analogous alkyl compounds (**55**, **56**) and hydroxy compounds (**61**, **62**) retained marginal activity against *E. coli* and rhGAR Tfase, indicating that most of the binding affinity within this series of compounds can be achieved by the DDACTHF ring system alone. The simplified analogues with the highest affinity against *E. coli* GAR Tfase are the aldehyde-based analogues **65** and **66**, which have K_i values of 11 and 9 μM, respectively. Both are more potent than the corresponding alcohols or alkanes indicating some potentiation by the non-transferable formyl groups of **65** and **66**. Fourteen compounds were tested against rhGAR Tfase including the more potent aldehydes **65** and **66** and none showed activity ($K_i > 100$ μM). In addition, all of the simplified analogues were found to be inactive against recombinant human AICAR Tfase ($K_i > 100$ μM) indicating selectivity between the two enzymes.

Table 1. *E. coli* GAR Tfase and rhGAR Tfase inhibition (K_i, μM)[a]

Compd	K_i *E. coli* GAR Tfase	K_i rhGAR Tfase	Compd	K_i *E. coli* GAR Tfase	K_i rhGAR Tfase
11	37	nd	**36**	> 100	> 100
12	37	nd	**37**	> 100	> 100
13	45	nd	**38**	> 100	> 100
14	61	nd	**45**	> 100	> 100
15	28	nd	**46**	> 100	> 100
16	87	nd	**47**	> 100	> 100
17	32	nd	**48**	> 100	> 100
18	22	nd	**49**	> 100	> 100
19	18	nd	**50**	> 100	> 100
20	31	nd	**55**	25	nd
21	23	nd	**56**	29	nd
22	24	nd	**61**	56	nd
27	14	nd	**62**	13	nd
28	> 100	nd	**65**	11	> 100
29	25	> 100	**66**	9	> 100
30	15	> 100	Lometrexol	0.1	0.06[b]
35	> 100	> 100			

[a]nd, not determined.
[b]Ref 26.

Cytotoxic Activity

Compounds **11**–**22**, **27**–**30**, **35**–**38**, **45**–**50**, **55**, **56**, **61**, **62**, **65**, and **66** were examined for cytotoxic activity both in the presence (+) and absence (−) of added hypoxanthine and thymidine against the CCRF–CEM cell line (Table 2). The cytotoxic activity of the simplified agents (**11**–**22**, **27**–**30**, **35**–**38**, **45**–**47**, **49**, **50**, **55**, **56**, **61**, **62**, **65**, **66**) was relatively nonpotent ($IC_{50} > 25$ μM) and uniform against the CCRF–CEM cell line regardless of whether the assay was conducted in the presence or absence of a media purine (hypoxanthine) or pyrimidine (thymidine). This indicates a lack of activity due to selective inhibition of the purine or pyrimidine biosynthetic pathways. Only compound **48** exhibited moderate cytotoxic activity ($IC_{50} = 7$–9 μM), but this activity was not sensitive to the presence of media purines or pyimidines.

Table 2. In vitro cytotoxic activity

Compd	CCRF–CEM (IC_{50}, μM)			
	(+) T, (+) H[a]	(−) T, (+) H	(+) T, (−) H	(−) T, (−) H
11	> 100	> 100	77	> 100
12	> 100	92	29	> 100
13	26	46	61	44
14	> 100	> 100	> 100	98
15	86	98	98	64
16	> 100	> 100	> 100	> 100
17	45	50	60	35
18	79	72	79	77
19	> 100	80	> 100	> 100
20	91	82	94	86
21	47	87	60	62
22	> 100	100	96	> 100
27	> 100	> 100	> 100	> 100
28	> 100	> 100	> 100	> 100
29	76	> 100	> 100	98
30	97	> 100	67	> 100
35	> 100	> 100	> 100	> 100
36	> 100	> 100	> 100	> 100
37	> 100	> 100	> 100	> 100
38	> 100	> 100	> 100	> 100
45	> 100	> 100	100	> 100
46	36	95	71	59
47	47	67	89	50
48	8	8	9	7
49	> 100	> 100	> 100	> 100
50	88	91	91	85
55	> 100	> 100	> 100	> 100
56	> 100	> 100	> 100	> 100
61	> 100	> 100	> 100	> 100
62	> 100	> 100	> 100	> 100
65	> 100	> 100	> 100	83
66	> 100	> 100	> 100	> 100
Lometrexol	> 100	> 100	0.52	0.23

[a]T, thymidine; H, hypoxanthine.

Conclusions

A novel series of simplified folate analogues incorporating electrophilic α-keto heterocycle, trifluoromethyl ketone and formyl groups have been synthesized and evaluated as potential inhibitors of GAR Tfase and AICAR Tfase. Although none of the candidate compounds inhibited AICAR transformylase, some of the simplified analogues show modest activity comparable to other GAR Tfase inhibitors reported in the literature. Most significantly, none of the modest GAR Tfase inhibitors exhibited functional cytotoxic activity in cellular assays attributable to the selective inhibition of the enzyme. When compared with the results of the examination of the corresponding aldehyde,[13] trifluoroacetyl,[16] or α-keto heterocycle[17] derivatives of DDACTHF, which are potent GAR Tfase inhibitors and efficacious cytotoxic compounds sensitive to the presence of purines, the study establishes the essential role of the benzoylglutamate for the compounds examined in these three inhibitor classes.

Experimental

5-Bromo-*N*-methoxy-*N*-methylpentanamide (3). *i*-Pr_2NEt (5.72 mL, 32.9 mmol, 2.20 equiv) was added to a stirred suspension of *O*,*N*-dimethylhydroxylamine hydrochloride (1.60 g, 16.4 mmol, 1.10 equiv) and 5-bromovaleryl chloride (**1**, 2.00 mL, 14.9 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C. The solution was allowed to warm to 25 °C and stirred 12 h. The reaction was diluted with CH_2Cl_2 (50 mL) and washed successively with 1 N aqueous HCl (3×50 mL) and saturated aqueous NaCl (1×20 mL) followed by concentration under reduced pressure affording **3** (3.34 g, quantitative) as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.70 (s, 3H), 3.58 (t, J = 6.2 Hz, 2H), 3.20 (s, 3H), 2.48 (t, J = 6.6 Hz, 2H), 1.94–1.76 (m, 4H); MALDIFTMS (DHB) m/z 224.0277 ($M+H^+$, $C_7H_{14}BrNO_2$ requires 224.0281).

6-Bromo-*N*-methoxy-*N*-methylhexanamide (4). Obtained from 6-bromohexanoyl chloride (**2**, 1.00 mL, 6.53 mmol) using the procedure described for **3** affording **4** (1.40 g, 90%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.69 (s, 3H), 3.56 (t, J = 6.6 Hz, 1H), 3.43 (t, J = 6.8 Hz, 1H), 3.19 (s, 3H), 2.45 (t, J = 7.5 Hz, 2H), 1.95–1.76 (m, 2H), 1.74–1.62 (m, 2H), 1.56–1.44 (m, 2H); MALDIFTMS (DHB) m/z 158.1180 ($M-Br+H^+$, $C_8H_{16}BrNO_2$ requires 158.1181).

Ethyl 2-cyano-7-(methoxymethylamino)-7-oxoheptanoate (5). A stirred solution of **3** (1.62 g, 7.23 mmol), ethyl cyanoacetate (2.31 mL, 21.7 mmol, 3.0 equiv), and K_2CO_3 (3.99 g, 28.9 mmol, 4.0 equiv) in anhydrous DMF (20 mL) was warmed at 70 °C for 1 h. The solution was cooled to 25 °C and concentrated under reduced pressure. The resulting reaction mixture was suspended in EtOAc (300 mL) and washed successively with H_2O (5×50 mL) and saturated aqueous NaCl (1×50 mL) followed by concentration under reduced pressure. Chromatography (SiO_2, 1:1 hexanes/EtOAc) afforded **5** (1.26 g, 68%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.23 (q, J = 7.0 Hz, 2H), 3.65 (s, 3H), 3.49 (t, J = 7.2 Hz, 1H), 3.15 (s, 3H), 2.43 (t, J = 7.2 Hz, 2H), 2.03–1.92 (m, 2H), 1.73–1.48 (m, 4H), 1.29 (t, J = 7.2 Hz, 3H); MALDIFTMS (DHB) m/z 257.1503 ($M+H^+$, $C_{12}H_{20}N_2O_4$ requires 257.1496).

Ethyl 2-cyano-8-(methoxymethylamino)-8-oxooctanoate (6). Obtained from **4** (1.32 g, 5.54 mmol) using the procedure described for **5**. Chromatography (SiO_2, 1:1 EtOAc/hexanes) afforded **6** (1.07 g, 71%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 4.27 (q, J = 7.3 Hz, 2H), 3.69 (s, 3H), 3.51 (t, J = 7.1 Hz, 1H), 3.19 (s, 3H), 2.44 (t, J = 7.3 Hz, 2H), 2.01–1.92 (m, 2H), 1.73–1.39 (m, 6H), 1.33 (t, J = 7.2 Hz, 3H); MALDIFTMS (DHB) m/z 293.1472 ($M+Na^+$, $C_{13}H_{22}N_2O_4$ requires 293.1472).

5-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-*N*-methoxy-*N*-methylpentanamide (7). Sodium metal (0.136 g, 5.93 mmol, 2.30 equiv) was added to anhydrous CH_3OH (1.6 mL) and stirred at 25 °C for 10 min to generate $NaOCH_3$. Guanidine hydrochloride (0.296 g, 3.09 mmol, 1.20 equiv) was added to this solution and stirred at 25 °C for 30 min. Separately, **5** (0.661 g, 2.58 mmol, 1.0 equiv) was dissolved in anhydrous CH_3OH (3.2 mL) and the resulting solution quickly added to the stirring reaction mixture. The solution was stirred at 25 °C for 1 h. The reaction mixture was applied directly to a SiO_2 plug. After complete air evaporation of the CH_3OH reaction solvent, impurities were removed by washing with 5:1 hexanes/EtOAc. The product was subsequently eluted by washing the SiO_2 with 10:1 $CHCl_3/CH_3OH$ to afford **7** (0.421 g, 62%) as a white solid: ^{1}H NMR (CD_3OD, 250 MHz) δ 3.70 (s, 3H), 3.17 (s, 3H), 2.48 (t, J = 7.4 Hz, 2H), 2.31 (t, J = 7.7 Hz, 2H), 1.71–1.58 (m, 2H), 1.52–1.37 (m, 2H); MALDIFTMS (DHB) m/z 270.1565 ($M+H^+$, $C_{11}H_{19}N_5O_3$ requires 270.1561).

6-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-*N*-methoxy-*N*-methylhexanamide (8). Obtained from **6** (0.777 g, 2.88 mmol) using the procedure described for **7** which afforded **8** (0.393 g, 50%) as a white solid: ^{1}H NMR (CD_3OD, 250 MHz) δ 3.70 (s, 3H), 3.16 (s, 3H), 2.44 (t, J = 6.5 Hz, 2H), 2.28 (t, J = 7.0 Hz, 2H), 1.68–1.56 (m, 2H), 1.48–1.33 (m, 4H); MALDIFTMS (DHB) m/z 284.1714 ($M+H^+$, $C_{12}H_{21}N_5O_3$ requires 284.1717).

5-{2,4-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl)-6-oxo-1,6-dihydropyrimidin-5-yl}-*N*-methoxy-*N*-methylpentanamide (9). A stirred solution of **7** (0.774 g, 2.96 mmol), di-*tert*-butyl dicarbonate (3.23 g, 14.8 mmol, 5.0 equiv), Et_3N (2.06 mL, 14.8 mmol, 5.0 equiv), and DMAP (0.072 g, 0.59 mmol, 0.2 equiv) in anhydrous DMF (10 mL) was warmed at 40 °C for 2 h. The solution was cooled to 25 °C and diluted with EtOAc (300 mL). This solution was washed with H_2O (1×50 mL), 1 N aqueous HCl (3×50 mL), saturated aqueous $NaHCO_3$ (3×50 mL) and saturated aqueous NaCl (1×50 mL) followed by concentration under reduced pressure. Chromatography (SiO_2, 1:2 hexanes/EtOAc) afforded **9** (1.26 g, 55%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.67 (s, 3H), 3.17 (s, 3H), 2.54 (t, J = 7.3 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 1.74–

1.36 (m, 49H); MALDIFTMS (DHB) m/z 792.3984 ($M+Na^+$, $C_{36}H_{59}N_5O_{13}$ requires 792.4001).

6-{2,4-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl)-6-oxo-1,6-dihydropyrimidin-5-yl}-*N*-methoxy-*N*-methylhexanamide (10). Obtained from **8** (0.339 g, 1.23 mmol) using the procedure described for **9**. Chromatography (SiO_2, 1:2 hexanes/EtOAc) afforded **10** (0.569 g, 60%) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 3.63 (s, 3H), 3.14 (s, 3H), 2.49 (t, $J=7.7$ Hz, 2H), 2.36 (t, $J=7.2$ Hz, 2H), 1.66–1.30 (m, 51H); MALDIFTMS (DHB) m/z 806.4176 ($M+Na^+$, $C_{37}H_{61}N_5O_{13}$ requires 806.4158).

2,6-Diamino-5-(5-oxo-5-phenylpentyl)-3*H*-pyrimidin-4-one trifluoroacetic acid salt (11). Compound **9** (0.040 g, 0.053 mmol) was dissolved in anhydrous THF (0.9 mL) and cooled to −35 °C. Phenylmagnesium bromide (3 M solution in Et_2O, 88 μL, 0.263 mmol, 5.0 equiv) was added dropwise and the resulting solution stirred at −30 °C for 1 h. The reaction mixture was quenched at −30 °C by the addition of cold H_2O dropwise to the stirring solution, followed by warming to 25 °C. The reaction mixture was partitioned between H_2O (20 mL) and EtOAc (50 mL). The organic layer was washed successively with H_2O (1×20 mL) and saturated aqueous NaCl (1×20 mL) followed by concentration under reduced pressure affording the crude product as a yellow oil. This crude product was dissolved in $CHCl_3$ (1.0 mL), cooled to 0 °C, and TFA (0.25 mL) was added dropwise. After this addition was complete, the reaction mixture was removed from the cooling bath and allowed to stir at 25 °C for 2 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sequential sonication and concentration from CH_3CN (1×5 mL) and Et_2O (1×5 mL). The resulting crude product was sonicated in Et_2O (10 mL) and the product precipitate collected by filtration affording **11**–CF_3CO_2H (0.013 g, 48% over two steps from **9**) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 7.98 (d, $J=7.0$ Hz, 2H), 7.58 (d, $J=7.3$ Hz, 1H), 7.48 (dd, $J=7.7$, 7.0 Hz, 2H), 3.06 (t, $J=7.2$ Hz, 2H), 2.36 (t, $J=7.4$ Hz, 2H), 1.80–1.68 (m, 2H), 1.57–1.45 (m, 2H); MALDIFTMS (DHB) m/z 287.1491 ($M+H^+$, $C_{15}H_{18}N_4O_2$ requires 287.1503).

2,6-Diamino-5-(6-oxo-6-phenylhexyl)-3*H*-pyrimidin-4-one trifluoroacetic acid salt (12). Obtained from **10** (0.055 g, 0.071 mmol) using the procedure described for **11** which afforded **12**–CF_3CO_2H (0.013 g, 36% over two steps from **10**) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 7.98 (d, $J=7.1$ Hz, 2H), 7.58 (d, $J=7.4$ Hz, 1H), 7.48 (dd, $J=7.4$, 7.1 Hz, 2H), 3.03 (t, $J=7.2$ Hz, 2H), 2.38–2.28 (m, 2H), 1.80–1.68 (m, 2H), 1.51–1.36 (m, 4H); MALDIFTMS (DHB) m/z 301.1649 ($M+H^+$, $C_{16}H_{20}N_4O_2$ requires 301.1659).

2,6-Diamino-5-[5-(benzothiazol-2-yl)-5-oxopentyl]-3*H*-pyrimidin-4-one trifluoroacetic acid salt (13). Benzothiazole (27 μL, 0.24 mmol, 5.0 equiv) was dissolved in anhydrous THF (0.4 mL) and cooled to −35 °C. *n*-BuLi (1.93 M in hexanes, 113 μL, 4.5 equiv) was added dropwise and the reaction stirred 10 min maintaining the temperature at −25 to −30 °C. Separately, compound **9** (0.037 g, 0.049 mmol) was dissolved in anhydrous THF (0.4 mL) and cooled to −35 °C. The pre-cooled solution of **9** was added quickly to the stirring benzothiazole anion solution. The reaction was stirred 15 min at −30 °C. The reaction mixture was quenched by addition of saturated aqueous NH_4Cl (2 mL) at −30 °C, followed by warming to 25 °C. The reaction mixture was diluted with EtOAc (50 mL). The organic layer was washed with saturated aqueous NH_4Cl (1×10 mL), saturated aqueous $NaHCO_3$ (1×10 mL) and saturated aqueous NaCl (1×10 mL) followed by concentration under reduced pressure affording the crude product as a yellow oil. This crude product was dissolved in $CHCl_3$ (1.0 mL), cooled to 0 °C, and TFA (0.25 mL) was added dropwise. After this addition was complete, the reaction mixture was removed from the cooling bath and allowed to stir at 25 °C for 2 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sequential sonication and concentration from CH_3CN (1×5 mL) and Et_2O (1×5 mL). The resulting crude product was sonicated in Et_2O (10 mL) and the product precipitate collected by filtration affording **13**–CF_3CO_2H (0.009 g, 32% over two steps from **9**) as a yellow solid: ^1H NMR (DMSO-d_6, 400 MHz) δ 9.73 (bs, 1H), 8.26 (d, $J=5.6$ Hz, 1H), 8.24 (d, $J=5.9$ Hz, 1H), 7.66 (dd, $J=5.6$, 6.5 Hz, 1H), 7.63 (dd, $J=6.5$, 7.0 Hz, 1H), 5.90 (bs, 2H), 5.68 (bs, 2H), 3.25 (t, $J=7.3$ Hz, 2H), 2.19 (t, $J=7.5$ Hz, 2H), 1.72–1.64 (m, 2H), 1.44–1.35 (m, 2H); MALDIFTMS (DHB) m/z 344.1174 ($M+H^+$, $C_{16}H_{17}N_5O_2S$ requires 344.1176).

2,6-Diamino-5-[6-(benzothiazol-2-yl)-6-oxohexyl]-3*H*-pyrimidin-4-one trifluoroacetic acid salt (14). Obtained from **10** (0.076 g, 0.098 mmol) using the procedure described for **13** which afforded **14**–CF_3CO_2H (0.011 g, 19% over two steps from **10**) as a yellow solid: ^1H NMR (CD_3OD, 300 MHz) δ 8.17 (d, $J=7.9$ Hz, 1H), 8.09 (d, $J=7.4$ Hz, 1H), 7.62 (dd, $J=1.8$, 5.7 Hz, 1H), 7.57 (dd, $J=5.7$, 1.3 Hz, 1H), 3.27 (t, $J=7.5$ Hz, 2H), 2.34 (t, $J=6.4$ Hz, 2H), 1.91–1.77 (m, 2H), 1.59–1.45 (m, 4H); MALDIFTMS (DHB) m/z 358.1340 ($M+H^+$, $C_{17}H_{19}N_5O_2S$ requires 358.1332).

2,6-Diamino-5-[5-oxo-5-(thiazol-2-yl)pentyl]-3*H*-pyrimidin-4-one trifluoroacetic acid salt (15). Thiazole (16 μL, 0.22 mmol, 5.0 equiv) was dissolved in anhydrous THF (0.4 mL) and cooled to −35 °C. *n*-BuLi (1.93 M in hexanes, 104 μL, 4.5 equiv) was added dropwise and the reaction stirred 10 min maintaining the temperature at −25 to −30 °C. Separately, compound **9** (0.034 g, 0.045 mmol) was dissolved in anhydrous THF (0.4 mL) and cooled to −35 °C. The pre-cooled solution of **9** was added quickly to the stirring thiazole anion solution. The reaction was stirred 15 min at −30 °C. The reaction mixture was quenched by addition of saturated aqueous NH_4Cl (2 mL) at −30 °C, followed by warming to 25 °C. The reaction mixture was diluted with EtOAc (50 mL). The organic layer was washed with saturated aqueous NH_4Cl (1×10 mL), saturated aqueous $NaHCO_3$ (1×10 mL) and saturated aqueous NaCl (1×10 mL) followed by concentration under reduced pressure affording the crude product as a yellow oil. This crude product was dissolved in $CHCl_3$ (1.0 mL),

cooled to 0 °C, and TFA (0.25 mL) was added dropwise. After this addition was complete, the reaction mixture was removed from the cooling bath and allowed to stir at 25 °C for 2 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sequential sonication and concentration from CH_3CN (1×5 mL) and Et_2O (1×5 mL). The resulting crude product was sonicated in Et_2O (10 mL) and the product precipitate collected by filtration affording **15**–CF_3CO_2H (0.009 g, 39% over two steps from **9**) as a off-white solid: ^1H NMR (CD_3OD, 250 MHz) δ 8.03 (d, J = 3.0 Hz, 1H), 7.95 (d, J = 3.0 Hz, 1H), 3.17 (t, J = 7.4 Hz, 2H), 2.37 (t, J = 7.6 Hz, 2H), 1.84–1.73 (m, 2H), 1.58–1.45 (m, 2H); MALDIFTMS (DHB) m/z 294.1028 ($M+H^+$, $C_{12}H_{15}N_5O_2S$ requires 294.1019).

2,6-Diamino-5-[6-oxo-6-(thiazol-2-yl)hexyl]-3*H*-pyrimidin-4-one trifluoroacetic acid salt (16). Obtained from **10** (0.050 g, 0.065 mmol) using the procedure described for **15** which afforded **16**–CF_3CO_2H (0.016 g, 47% over two steps from **10**) as a tan solid: ^1H NMR (CD_3OD, 250 MHz) δ 8.03 (d, J = 3.0 Hz, 1H), 7.93 (d, J = 3.0 Hz, 1H), 3.13 (t, J = 7.4 Hz, 2H), 2.29 (t, J = 6.9 Hz, 2H), 1.84–1.75 (m, 2H), 1.54–1.40 (m, 4H); MALDIFTMS (DHB) m/z 308.1170 ($M+H^+$, $C_{13}H_{17}N_5O_2S$ requires 308.1176).

2,6-Diamino-5-[5-oxo-5-(pyridin-2-yl)pentyl]-3*H*-pyrimidin-4-one trifluoroacetic acid salt (17). *n*-BuLi (1.93 M in hexanes, 220 μL, 5.7 equiv) was dissolved in anhydrous Et_2O (1.0 mL) at −20 °C. 2-Bromopyridine (42 μL, 0.44 mmol, 6.0 equiv) was added dropwise and the reaction mixture was stirred 10 min at −20 °C followed by cooling to −30 °C. Separately, compound **9** (0.056 g, 0.074 mmol) was dissolved in anhydrous Et_2O (0.5 mL) and this solution was added dropwise to the stirring pyridine anion solution. The reaction mixture was stirred 20 min at −30 °C. The reaction was quenched by addition of saturated aqueous NH_4Cl (2 mL) at −30 °C, followed by warming to 25 °C. The reaction mixture was diluted with EtOAc (50 mL). The organic layer was washed with H_2O (1×20 mL) and saturated aqueous NaCl (1×10 mL) followed by concentration under reduced pressure affording the crude product as a red oil. This crude product was dissolved in $CHCl_3$ (1.0 mL), cooled to 0 °C, and TFA (0.25 mL) was added dropwise. After this addition was complete, the reaction mixture was removed from the cooling bath and allowed to stir at 25 °C for 2 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sequential sonication and concentration from CH_3CN (1×5 mL) and Et_2O (1×5 mL). The resulting crude product was sonicated in Et_2O (10 mL) and the product precipitate collected by filtration affording **17**–CF_3CO_2H (0.011 g, 30% over two steps from **9**) as a gray solid: ^1H NMR (CD_3OD, 250 MHz) δ 8.66 (d, J = 5.0 Hz, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.95 (dd, J = 7.4, 8.0 Hz, 1H), 7.58 (dd, J = 4.9, 5.2 Hz, 1H), 3.22 (t, J = 7.3 Hz, 2H), 2.37 (t, J = 7.6 Hz, 2H), 1.80–1.69 (m, 2H), 1.57–1.45 (m, 2H); MALDIFTMS (DHB) m/z 288.1456 ($M+H^+$, $C_{14}H_{17}N_5O_2$ requires 288.1455).

2,6-Diamino-5-[6-oxo-6-(pyridin-2-yl)hexyl]-3*H*-pyrimidin-4-one trifluoroacetic acid salt (18). Obtained from **10** (0.054 g, 0.070 mmol) using the procedure described for **17** which afforded **18**–CF_3CO_2H (0.017 g, 47% over two steps from **10**) as a gray solid: ^1H NMR (CD_3OD, 250 MHz) δ 8.66 (d, J = 4.4 Hz, 1H), 8.01 (d, J = 7.7 Hz, 1H), 7.95 (dd, J = 7.0, 8.0 Hz, 1H), 7.58 (dd, J = 5.4, 6.4 Hz, 1H), 3.19 (t, J = 7.3 Hz, 2H), 2.32 (t, J = 6.9 Hz, 2H), 1.79–1.68 (m, 2H), 1.52–1.38 (m, 4H); MALDIFTMS (DHB) m/z 324.1431 ($M+Na^+$, $C_{15}H_{19}N_5O_2$ requires 324.1431).

2,6-Diamino-5-[5-oxo-5-(pyridazin-3-yl)pentyl]-3*H*-pyrimidin-4-one (19). 2,2,6,6-Tetramethylpiperidine (82 μL, 0.49 mmol, 5.0 equiv) was dissolved in anhydrous THF (2.5 mL) and cooled to −30 °C. *n*-BuLi (1.93 M in hexanes, 250 μL, 0.49 mmol, 5.0 equiv) was added dropwise. Following the *n*-BuLi addition, the reaction was warmed to 0 °C. After the reaction had stirred 30 min at 0 °C, it was cooled to −78 °C. Separately, pyridazine (35 μL, 0.49 mmol, 5.0 equiv) was dissolved in anhydrous THF (1.6 mL) and this solution was added dropwise to the stirring LiTMP solution. The reaction mixture was stirred 5 min at −78 °C. Separately, compound **9** (0.074 g, 0.097 mmol) was dissolved in anhydrous THF (0.35 mL) and was added dropwise to the stirring pyridazine anion solution. The reaction mixture was stirred 1.5 h, allowing the reaction to slowly warm up to −30 °C. The reaction was quenched by addition of saturated aqueous NH_4Cl (2 mL) at −30 °C, followed by warming to 25 °C. The reaction mixture was diluted with EtOAc (50 mL). The organic layer was washed with saturated aqueous NH_4Cl (1×10 mL), saturated aqueous $NaHCO_3$ (1×10 mL) and saturated aqueous NaCl (1×10 mL) followed by concentration under reduced pressure affording the crude product as a red oil. This crude product was dissolved in $CHCl_3$ (1.0 mL), cooled to 0 °C, and TFA (0.25 mL) was added dropwise. After this addition was complete, the reaction mixture was removed from the cooling bath and allowed to stir at 25 °C for 2 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sequential sonication and concentration from CH_3CN (1×5 mL) and Et_2O (1×5 mL). Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) afforded **19** (0.008 g, 16% over two steps from **9**) as a tan solid: ^1H NMR (CD_3OD, 250 MHz) δ 9.32 (d, J = 5.0 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.86 (dd, J = 5.0, 5.0 Hz, 1H), 3.37 (t, J = 7.3 Hz, 2H), 2.35 (t, J = 7.7 Hz, 2H), 1.86–1.76 (m, 2H), 1.59–1.48 (m, 2H); MALDIFTMS (DHB) m/z 311.1226 ($M+Na^+$, $C_{13}H_{16}N_6O_2$ requires 311.1227).

2,6-Diamino-5-[6-oxo-6-(pyridazin-3-yl)hexyl]-3*H*-pyrimidin-4-one (20). Obtained from **10** (0.043 g, 0.055 mmol) using the procedure described for **19** which afforded **20** (0.014 g, 48% over two steps from **10**) as a red solid: ^1H NMR (CD_3OD, 250 MHz) δ 9.33 (d, J = 5.0 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.87 (dd, J = 5.0, 5.0 Hz, 1H), 3.34 (t, J = 7.0 Hz, 2H), 2.37–2.27 (m, 2H), 1.85–1.73 (m, 2H), 1.56–1.38 (m, 4H); MALDIFTMS (DHB) m/z 303.1561 ($M+H^+$, $C_{14}H_{18}N_6O_2$ requires 303.1864).

2,6-Diamino-5-[5-oxo-5-(pyrazin-2-yl)pentyl]-3*H*-pyrimidin-4-one (21). 2,2,6,6-Tetramethylpiperidine (114 μL, 0.68 mmol, 5.0 equiv) was dissolved in anhydrous THF (4 mL) and cooled to −30 °C. *n*-BuLi (1.93 M in hexanes, 350 μL, 0.68 mmol, 5.0 equiv) was added dropwise. Following the *n*-BuLi addition, the reaction mixture was warmed to 0 °C. After the reaction had stirred 30 min at 0 °C, it was cooled to −78 °C. Separately, pyrazine (0.054 g, 0.68 mmol, 5.0 equiv) was dissolved in anhydrous THF (0.5 mL) and this solution was added dropwise to the stirring LiTMP solution. The reaction was stirred 5 min at −78 °C. Separately, compound **9** (0.10 g, 0.14 mmol) was dissolved in anhydrous THF (0.5 mL) and was added dropwise to the stirring pyrazine anion solution. The reaction was stirred 1.5 h, allowing the reaction to slowly warm up to −30 °C. The reaction was quenched by addition of saturated aqueous NH_4Cl (2 mL) at −30 °C, followed by warming to 25 °C. The reaction mixture was diluted with EtOAc (50 mL). The organic layer was washed successively with saturated aqueous NH_4Cl (1×10 mL), saturated aqueous $NaHCO_3$ (1×10 mL) and saturated aqueous NaCl (1×10 mL) followed by concentration under reduced pressure affording the crude product as a red oil. This crude product was dissolved in $CHCl_3$ (1.0 mL), cooled to 0 °C, and TFA (0.25 mL) was added dropwise. After this addition was complete, the reaction mixture was removed from the cooling bath and allowed to stir at 25 °C for 2 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sequential sonication and concentration from CH_3CN (1×5 mL) and Et_2O (1×5 mL). Chromatography (SiO_2, 10:1 $CHCl_3/CH_3OH$) afforded **21** (0.032 g, 46% over two steps from **9**) as a tan solid: ^1H NMR (CD_3OD, 250 MHz) δ 9.11 (d, $J=1.3$ Hz, 1H), 8.76 (d, $J=2.4$ Hz, 1H), 8.73–8.69 (m, 1H), 3.22 (t, $J=7.3$ Hz, 2H), 2.34 (t, $J=7.6$ Hz, 2H), 1.86–1.70 (m, 2H), 1.59–1.45 (m, 2H); MALDIFTMS (DHB) m/z 289.1415 ($M+H^+$, $C_{13}H_{16}N_6O_2$ requires 289.1407).

2,6-Diamino-5-[6-oxo-6-(pyrazin-2-yl)hexyl]-3*H*-pyrimidin-4-one (22). Obtained from **10** (0.039 g, 0.050 mmol) using the procedure described for **21** which afforded **22** (0.010 g, 38% over two steps from **10**) as a red solid: ^1H NMR (CD_3OD, 250 MHz) δ 9.11 (s, 1H), 8.77 (d, $J=2.4$ Hz, 1H), 8.72–8.69 (m, 1H), 3.19 (t, $J=7.2$ Hz, 2H), 2.35–2.25 (m, 2H), 1.84–1.69 (m, 2H), 1.48–1.37 (m, 4H); MALDIFTMS (DHB) m/z 303.1560 ($M+H^+$, $C_{14}H_{18}N_6O_2$ requires 303.1564).

1-(Benzoxazol-2-yl)-5-bromopentan-1-ol (23). Benzoxazole (0.500 g, 4.20 mmol) was dissolved in anhydrous THF (30 mL) and cooled to −78 °C. *n*-BuLi (1.93 M in hexanes, 2.40 mL, 4.62 mmol, 1.1 equiv) was added dropwise and the reaction mixture was stirred 20 min at −78 °C. $ZnCl_2$ (0.5 M in THF, 16.8 mL, 8.40 mmol, 2.0 equiv) was added to the reaction dropwise at −78 °C. After this addition was complete, the reaction was warmed to 0 °C and stirred for 45 min at 0 °C. CuI (0.80 g, 4.20 mmol, 1.0 equiv) was added and the reaction was stirred for an additional 10 min at 0 °C. 5-Bromovaleryl chloride (**1**, 1.12 mL, 8.40 mmol, 2.0 equiv) was added quickly to the reaction mixture and stirring was continued at 0 °C for 30 min. The reaction mixture was diluted with EtOAc (100 mL). The organic layer was washed with 1:1 v/v NH_4OH/H_2O (2×50 mL), H_2O (2×20 mL) and saturated aqueous NaCl (2×10 mL) followed by concentration under reduced pressure. The product was partially purified by chromatography (SiO_2, 1:1 EtOAc/hexanes) affording the crude product as a red oil. This crude product was dissolved in anhydrous CH_3OH (50 mL) and cooled to −20 °C. $NaBH_4$ (0.476 g, 12.6 mmol, 3.0 equiv) was added slowly and the reaction mixture was stirred at −20 °C for 30 min. The reaction was quenched by addition of H_2O (10 mL) at −20 °C, followed by warming to 25 °C. The reaction mixture was diluted with EtOAc (200 mL) and washed with H_2O (1×50 mL) and saturated aqueous NaCl (1×50 mL) followed by concentration under reduced pressure. Chromatography (SiO_2, 1:1 EtOAc/hexanes) afforded **23** (0.370 g, 31% over two steps from **1**) as a red oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.71–7.64 (m, 1H), 7.52–7.45 (m, 1H), 7.36–7.28 (m, 2H), 4.96 (dd, $J=5.5$, 5.5 Hz, 1H), 3.39 (d, $J=6.6$ Hz, 1H), 3.38 (t, $J=6.8$ Hz, 2H), 2.10–1.87 (m, 4H), 1.71–1.58 (m, 2H); MALDIFTMS (DHB) m/z 284.0280 ($M+H^+$, $C_{12}H_{14}BrNO_2$ requires 284.0281).

1-(Benzoxazol-2-yl)-6-bromohexan-1-ol (24). Obtained from benzoxazole (0.500 g, 4.20 mmol) and 6-bromohexanoyl chloride (**2**, 1.28 mL, 8.39 mmol, 2.0 equiv) using the procedure described for **23**. Chromatography (SiO_2, 1:1 hexanes/EtOAc) afforded **24** (0.630 g, 50% over two steps from **2**) as an off-white solid: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.71–7.66 (m, 1H), 7.53–7.47 (m, 1H), 7.36–7.29 (m, 2H), 4.95 (dd, $J=5.7$, 5.7 Hz, 1H), 3.38 (t, $J=6.8$ Hz, 2H), 3.29 (d, $J=5.7$ Hz, 1H), 2.12–1.78 (m, 4H), 1.60–1.44 (m, 4H); MALDIFTMS (DHB) m/z 298.0447 ($M+H^+$, $C_{13}H_{16}BrNO_2$ requires 298.0437).

Ethyl 7-(benzoxazol-2-yl)-2-cyano-7-hydroxyheptanoate (25). Obtained from **23** (0.279 g, 0.982 mmol) using the procedure described for **5**. Chromatography (SiO_2, 2:1 EtOAc/hexanes) afforded **25** (0.158 g, 51%) as a red oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.75–7.68 (m, 1H), 7.57–7.50 (m, 1H), 7.40–7.33 (m, 2H), 5.04–4.05 (m, 1H), 4.26 (q, $J=7.2$ Hz, 2H), 3.68 (bs, 1H), 3.51 (t, $J=7.0$, 1H), 2.18–1.92 (m, 4H), 1.69–1.53 (m, 4H), 1.32 (t, $J=7.2$ Hz, 3H); MALDIFTMS (DHB) m/z 317.1490 ($M+H^+$, $C_{17}H_{20}N_2O_4$ requires 317.1496).

Ethyl 8-(benzoxazol-2-yl)-2-cyano-8-hydroxyoctanoate (26). Obtained from **24** (0.300 g, 1.00 mmol) using the procedure described for **5**. Chromatography (SiO_2, 1:2 EtOAc/hexanes) afforded **26** (0.306 g, 92%) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.76–7.70 (m, 1H), 7.59–7.51 (m, 1H), 7.41–7.34 (m, 2H), 4.99 (dd, $J=5.5$, 5.5 Hz, 1H), 4.28 (q, $J=7.0$ Hz, 2H), 3.49 (t, $J=7.0$, 1H), 3.07 (d, $J=5.5$ Hz, 1H), 2.13–1.93 (m, 4H), 1.61–1.40 (m, 6H), 1.33 (t, $J=7.2$ Hz, 3H); MALDIFTMS (DHB) m/z 331.1654 ($M+H^+$, $C_{18}H_{22}N_2O_4$ requires 331.1652).

2,6-Diamino-5-[5-(benzoxazol-2-yl)-5-hydroxypentyl]-3*H*-pyrimidin-4-one (27). Obtained from **25** (0.150 g, 0.474 mmol) using the procedure described for **7**. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) followed by trituration with Et_2O afforded **27** (0.110 g, 72%) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 7.69–7.65 (m, 1H), 7.63–7.56 (m, 1H), 7.42–7.31 (m, 2H), 2.35–2.23 (m, 2H), 2.05–1.94 (m, 2H), 1.60–1.38 (m, 4H); MALDIFTMS (DHB) *m/z* 330.1556 ($M+H^+$, $C_{16}H_{19}N_5O_3$ requires 330.1561).

2,6-Diamino-5-[6-(benzoxazol-2-yl)-6-hydroxyhexyl]-3*H*-pyrimidin-4-one (28). Obtained from **26** (0.214 g, 0.648 mmol) using the procedure described for **7**. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) followed by trituration with CH_2Cl_2 afforded **28** (0.112 g, 52%) as an off-white solid: ^1H NMR (CD_3OD, 250 MHz) δ 7.70–7.65 (m, 1H), 7.63–7.57 (m, 1H), 7.42–7.32 (m, 2H), 2.35–2.23 (m, 2H), 2.08–1.90 (m, 2H), 1.58–1.38 (m, 6H); MALDIFTMS (DHB) *m/z* 344.1719 ($M+H^+$, $C_{17}H_{21}N_5O_3$ requires 344.1719).

2,6-Diamino-5-[5-(benzoxazol-2-yl)-5-oxopentyl]-3*H*-pyrimidin-4-one (29). MnO_2 (0.610 g, 10×w/w) was added to a solution of **27** (0.061 g, 0.190 mmol) in DMF (1.5 mL) and $CHCl_3$ (6.0 mL) and stirred at 25 °C for 1 h. The MnO_2 was removed by filtration though Celite and the filtrate was concentrated under reduced pressure. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) afforded **29** (0.012 g, 20%) as an off-white solid as well as recovered starting material **27** (0.023 g, 37%). For **29**: ^1H NMR (DMSO-d_6, 300 MHz) δ 9.73 (bs, 1H), 7.99 (d, $J=7.9$ Hz, 1H), 7.89 (d, $J=7.9$ Hz, 1H), 7.61 (dd, $J=7.0$, 7.5 Hz, 1H), 7.52 (dd, $J=8.3$, 7.0 Hz, 1H), 5.90 (bs, 2H), 5.68 (bs, 2H), 3.18 (t, $J=7.5$ Hz, 2H), 2.19 (t, $J=7.5$ Hz, 2H), 1.74–1.63 (m, 2H), 1.54–1.35 (m, 2H); MALDIFTMS (DHB) *m/z* 328.1404 ($M+H^+$, $C_{16}H_{17}N_5O_3$ requires 328.1404).

2,6-Diamino-5-[6-(benzoxazol-2-yl)-6-oxohexyl]-3*H*-pyrimidin-4-one (30). Obtained from **28** (0.050 g, 0.149 mmol) using the procedure described for **29**. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) afforded **30** (0.022 g, 44%) as an off-white solid as well as recovered starting material **28** (0.017 g, 34%). For **30**: ^1H NMR (DMSO-d_6, 250 MHz) δ 9.73 (bs, 1H), 7.99 (d, $J=8.0$ Hz, 1H), 7.89 (d, $J=8.0$ Hz, 1H), 7.62 (dd, $J=7.3$, 8.0 Hz, 1H), 7.52 (dd, $J=7.7$, 7.3 Hz, 1H), 5.88 (bs, 2H), 5.63 (bs, 2H), 3.16 (t, $J=7.2$ Hz, 2H), 2.20–2.08 (m, 2H), 1.75–1.62 (m, 2H), 1.41–1.25 (m, 4H); MALDIFTMS (DHB) *m/z* 342.1554 ($M+H^+$, $C_{17}H_{19}N_5O_3$ requires 342.1561).

5-Bromo-1-(oxazol-2-yl)pentan-1-ol (31). Obtained from oxazole (0.200 g, 2.89 mmol) and 5-bromovaleryl chloride (**1**, 1.16 g, 5.79 mmol, 2.0 equiv) using the procedure described for **23**. Chromatography (SiO_2, 1:2 hexanes/EtOAc) afforded **31** (0.496 g, 73% over two steps from **1**) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.56 (s, 1H), 6.98 (s, 1H), 4.95 (d, $J=5.3$ Hz, 1H), 4.80–4.72 (m, 1H), 3.34 (t, $J=6.8$ Hz, 2H), 1.94–1.79 (m, 4H), 1.62–1.45 (m, 2H); MALDIFTMS (DHB) *m/z* 234.0127 ($M+H^+$, $C_8H_{12}BrNO_2$ requires 234.0124).

6-Bromo-1-(oxazol-2-yl)hexan-1-ol (32). Obtained from oxazole (0.200 g, 2.89 mmol) and 6-bromohexanoyl chloride (**2**, 1.24 g, 5.79 mmol, 2.0 equiv) using the procedure described for **23**. Chromatography (SiO_2, 1:2 hexanes/EtOAc) afforded **32** (0.473 g, 68% over two steps from **2**) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.61 (s, 1H), 7.04 (s, 1H), 4.88–4.79 (m, 1H), 4.48 (d, $J=5.5$ Hz, 1H), 3.39 (t, $J=6.8$ Hz, 2H), 1.98–1.82 (m, 4H), 1.56–1.40 (m, 4H); MALDIFTMS (DHB) *m/z* 248.0288 ($M+H^+$, $C_9H_{14}BrNO_2$ requires 248.0281).

Ethyl 2-cyano-7-hydroxy-7-(oxazol-2-yl)heptanoate (33). Obtained from **31** (0.466 g, 1.991 mmol) using the procedure described for **5**. Chromatography (SiO_2, 2:1 EtOAc/hexanes) afforded **33** (0.388 g, 77%) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.88 (s, 1H), 7.30 (s, 1H), 5.14–5.02 (m, 1H), 4.96–4.88 (m, 1H), 4.51 (q, $J=7.3$ Hz, 2H), 3.78 (t, $J=6.9$ Hz, 1H), 2.28–2.10 (m, 4H), 1.90–1.69 (m, 4H), 1.57 (t, $J=7.1$ Hz, 3H); MALDIFTMS (DHB) *m/z* 267.1340 ($M+H^+$, $C_{13}H_{18}N_2O_4$ requires 267.1339).

Ethyl 2-cyano-8-hydroxy-8-(oxazol-2-yl)octanoate (34). Obtained from **32** (0.453 g, 1.83 mmol) using the procedure described for **5**. Chromatography (SiO_2, 2:1 EtOAc/hexanes) afforded **34** (0.385 g, 75%) as a clear oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.62 (s, 1H), 7.04 (s, 1H), 4.78 (q, $J=6.2$ Hz, 1H), 4.25 (q, $J=7.3$ Hz, 2H), 4.18 (d, $J=5.1$ Hz, 1H), 3.49 (t, $J=7.2$ Hz, 1H), 1.99–1.85 (m, 4H), 1.58–1.30 (m, 6H), 1.31 (t, $J=7.2$ Hz, 3H); MALDIFTMS (DHB) *m/z* 303.1308 ($M+Na^+$, $C_{14}H_{20}N_2O_4$ requires 303.1315).

2,6-Diamino-5-[5-hydroxy-5-(oxazol-2-yl)pentyl]-3*H*-pyrimidin-4-one (35). Obtained from **33** (0.360 g, 1.352 mmol) using the procedure described for **7**. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) followed by trituration with Et_2O afforded **35** (0.257 g, 68%) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 7.86 (s, 1H), 7.11 (s, 1H), 4.72 (t, $J=6.8$ Hz, 1H), 2.27 (t, $J=7.1$ Hz, 2H), 1.97–1.85 (m, 2H), 1.59–1.31 (m, 4H); MALDIFTMS (DHB) *m/z* 280.1400 ($M+H^+$, $C_{12}H_{17}N_5O_3$ requires 280.1404).

2,6-Diamino-5-[6-hydroxy-6-(oxazol-2-yl)hexyl]-3*H*-pyrimidin-4-one (36). Obtained from **34** (0.378 g, 1.348 mmol) using the procedure described for **7**. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) followed by trituration with Et_2O afforded **36** (0.262 g, 66%) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 7.86 (s, 1H), 7.11 (s, 1H), 4.71 (t, $J=6.9$ Hz, 1H), 2.27 (t, $J=6.0$ Hz, 2H), 1.94–1.82 (m, 2H), 1.53–1.25 (m, 6H); MALDIFTMS (DHB) *m/z* 294.1565 ($M+H^+$, $C_{13}H_{19}N_5O_3$ requires 294.1561).

2,6-Diamino-5-[5-(oxazol-2-yl)-5-oxopentyl]-3*H*-pyrimidin-4-one (37). Obtained from **35** (0.050 g, 0.179 mmol) using the procedure described for **29**. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) afforded **37** (0.014 g, 28%) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 8.09 (s, 1H), 7.39 (s, 1H), 3.09 (t, $J=7.3$ Hz, 2H), 2.33 (t, $J=7.7$ Hz, 2H), 1.80–1.71 (m, 2H), 1.54–1.45 (m, 2H); MAL-

DIFTMS (DHB) m/z 278.1254 ($M+H^+$, $C_{12}H_{15}N_5O_3$ requires 278.1248).

2,6-Diamino-5-[6-(oxazol-2-yl)-6-oxohexyl]-3*H*-pyrimidin-4-one (38). Obtained from **36** (0.050 g, 0.170 mmol) using the procedure described for **29**. Chromatography (SiO_2, 5:1 $CHCl_3/CH_3OH$) afforded **38** (0.018 g, 37%) as a tan solid: ^1H NMR (CD_3OD, 250 MHz) δ 8.09 (s, 1H), 7.40 (s, 1H), 3.05 (t, $J=7.4$ Hz, 2H), 2.29 (t, $J=7.0$ Hz, 2H), 1.81–1.69 (m, 2H), 1.56–1.42 (m, 4H); MALDIFTMS (DHB) m/z 292.1407 ($M+H^+$, $C_{13}H_{17}N_5O_3$ requires 292.1404).

6-Bromo-1,1,1-trifluorohexan-2-one (39). 5-Bromovaleryl chloride (**1**, 1.00 mL, 7.47 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL). Trifluoroacetic anhydride (6.63 mL, 44.8 mmol, 6.0 equiv) was added slowly to the stirring solution. Anhydrous pyridine (4.87 mL, 59.8 mmol, 8.0 equiv) was added dropwise and the resulting solution stirred at 25 °C for 2 h. The reaction was cooled to 0 °C and quenched by the dropwise addition of H_2O (20 mL) to the stirring solution, followed by warming to 25 °C. The reaction mixture was partitioned between H_2O (100 mL) and CH_2Cl_2 (300 mL). The organic layer was washed with 1 N aqueous HCl (1×100 mL) followed by concentration under reduced pressure. Chromatography (SiO_2, 1:2 EtOAc/hexanes) afforded **39** (0.748 g, 43%) as a red oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 3.39 (t, $J=6.1$ Hz, 2H), 2.74 (t, $J=6.6$ Hz, 2H), 1.98–1.79 (m, 4H); MALDIFTMS (DHB) m/z 232.9790 ($M+H^+$, $C_6H_8BrF_3O$ requires 232.9784).

7-Bromo-1,1,1-trifluoroheptan-2-one (40). Obtained from 6-bromohexanoyl chloride (**2**, 1.0 mL, 6.53 mmol) using the procedure described for **39**. Chromatography (SiO_2, 1:2 EtOAc/hexanes) afforded **40** (0.640 g, 40%) as a red oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 3.44 (t, $J=6.6$ Hz, 2H), 2.77 (t, $J=7.2$ Hz, 2H), 2.00–1.84 (m, 2H), 1.81–1.68 (m, 2H), 1.64–1.48 (m, 2H); MALDIFTMS (DHB) m/z 246.9937 ($M+H^+$, $C_7H_{10}BrF_3O$ requires 246.9940).

2-(4-Bromobutyl)-2-trifluoromethyl-[1,3]-dioxolane (41). 2-Chloroethanol (0.31 mL, 4.70 mmol, 3.0 equiv) was added to a solution of **39** (0.365 g, 1.57 mmol) in anhydrous DMF (7 mL). After this solution was stirred at 25 °C for 45 min, K_2CO_3 (0.649 g, 4.70 mmol, 3.0 equiv) was added and the resulting mixture was stirred for 18 h. The reaction mixture was concentrated under reduced pressure and the crude product partitioned between H_2O (50 mL) and EtOAc (300 mL). The organic layer was washed with H_2O (5×50 mL) and saturated aqueous NaCl (1×50 mL) followed by concentration under reduced pressure, yielding **41** (0.247 g, 57%) as a red oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 4.20–4.11 (m, 4H), 3.42 (t, $J=6.6$ Hz, 2H), 1.96–1.84 (m, 4H), 1.65–1.54 (m, 2H); MALDIFTMS (DHB) m/z 277.0048 ($M+H^+$, $C_8H_{12}BrF_3O_2$ requires 277.0046).

2-(5-Bromopentyl)-2-trifluoromethyl-[1,3]-dioxolane (42). Obtained from **40** (0.050 g, 0.20 mmol) using the procedure described for **41**, affording **42** (0.040 g, 68%) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 4.24–4.08 (m, 4H), 3.43 (t, $J=6.8$ Hz, 2H), 1.96–1.82 (m, 4H), 1.59–1.42 (m, 4H); MALDIFTMS (DHB) m/z 291.0199 ($M+H^+$, $C_9H_{14}BrF_3O_2$ requires 291.0202).

Ethyl 2-cyano-6-(2-trifluoromethyl-[1,3]-dioxolan-2-yl)-hexanoate (43). Obtained from **41** (0.185 g, 0.668 mmol) using the procedure described for **5**. Chromatography (SiO_2, 1:2 EtOAc/hexanes) afforded **43** (0.130 g, 63%) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 4.28 (q, $J=7.0$ Hz, 2H), 4.20–4.08 (m, 4H), 3.52 (t, $J=7.0$ Hz, 1H), 2.04–1.94 (m, 2H), 1.90–1.82 (m, 2H), 1.66–1.47 (m, 4H), 1.35 (t, $J=7.1$ Hz, 3H); MALDIFTMS (DHB) m/z 310.1262 ($M+H^+$, $C_{13}H_{18}F_3NO_4$ requires 310.1261).

Ethyl 2-cyano-7-(2-trifluoromethyl-[1,3]-dioxolan-2-yl)-heptanoate (44). Obtained from **42** (0.116 g, 0.399 mmol) using the procedure described for **5**. Chromatography (SiO_2, 1:2 EtOAc/hexanes) afforded **44** (0.086 g, 67%) as a yellow oil: ^1H NMR ($CDCl_3$, 250 MHz) δ 4.29 (q, $J=7.3$ Hz, 2H), 4.23–4.08 (m, 4H), 3.51 (t, $J=7.0$ Hz, 1H), 2.04–1.94 (m, 2H), 1.90–1.82 (m, 2H), 1.60–1.35 (m, 6H), 1.35 (t, $J=7.1$ Hz, 3H); MALDIFTMS (DHB) m/z 346.1249 ($M+Na^+$, $C_{14}H_{20}F_3NO_4$ requires 346.1237).

2,6-Diamino-5-[4-(2-trifluoromethyl-[1,3]-dioxolan-2-yl)-butyl]-3*H*-pyrimidin-4-one (45). Obtained from **43** (0.180 g, 0.582 mmol) using the procedure described for **7** which afforded **45** (0.095 g, 52%) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 4.11 (bs, 4H), 2.29 (t, $J=6.2$ Hz, 2H), 1.88–1.75 (m, 2H), 1.58–1.39 (m, 4H); MALDIFTMS (DHB) m/z 323.1313 ($M+H^+$, $C_{12}H_{17}F_3N_4O_3$ requires 323.1326).

2,6-Diamino-5-[5-(2-trifluoromethyl-[1,3]-dioxolan-2-yl)-pentyl]-3*H*-pyrimidin-4-one (46). Obtained from **44** (0.210 g, 0.650 mmol) using the procedure described for **7** which afforded **46** (0.121 g, 57%) as a white solid: ^1H NMR (CD_3OD, 250 MHz) δ 4.11 (bs, 4H), 2.28 (t, $J=6.4$ Hz, 2H), 1.84–1.72 (m, 2H), 1.55–1.34 (m, 6H); MALDIFTMS (DHB) m/z 337.1482 ($M+H^+$, $C_{13}H_{19}F_3N_4O_3$ requires 337.1482).

2,6-Diamino-5-(6,6,6-trifluoro-5-oxohexyl)-3*H*-pyrimidin-4-one (47). Compound **45** (0.030 g, 0.096 mmol) was suspended in anhydrous CH_2Cl_2 (3.0 mL) and cooled to 0 °C. BBr_3 (1 M in CH_2Cl_2, 0.69 mL, 0.668 mmol, 7.0 equiv) was added slowly to this suspension and the resulting solution was stirred for 1 h at 0 °C. The reaction was quenched at 0 °C by the dropwise addition of H_2O (3.0 mL), followed by warming to 25 °C. The reaction mixture was partitioned between H_2O (10 mL) and EtOAc (20 mL). The aqueous layer was concentrated under reduced pressure. PCTLC (SiO_2, 1 mm plate, 1:5 $CH_3OH/CHCl_3$) provided **47** (0.013 g, 39%) as a white solid: ^1H NMR (CD_3OD, 300 MHz) δ 2.29 (t, $J=7.0$ Hz, 2H), 1.88–1.73 (m, 2H), 1.58–1.40 (m, 4H); MALDIFTMS (DHB) m/z 279.1070 ($M+H^+$, $C_{10}H_{13}F_3N_4O_2$ requires 279.1063).

2,6-Diamino-5-(7,7,7-trifluoro-6-oxoheptyl)-3*H*-pyrimi-

din-4-one (48). Obtained from **46** (0.010 g, 0.031 mmol) using the procedure described for **47** which afforded **48** (0.006 g, 54%) as a yellow solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 2.28 (t, J = 7.3 Hz, 2H), 1.78–1.65 (m, 2H), 1.49–1.29 (m, 6H); MALDIFTMS (DHB) m/z 293.1215 ($M+H^+$, $C_{11}H_{15}F_3N_4O_2$ requires 293.1220).

2,6-Diamino-5-(6,6,6-trifluoro-5-hydroxyhexyl)-3*H*-pyrimidin-4-one (49). Compound **47** (0.011 g, 0.031 mmol) was dissolved in anhydrous CH_3OH (1.5 mL) and cooled to −20 °C. $NaBH_4$ (0.003 mg, 0.079 mmol, 2.6 equiv) was added slowly and the resulting solution was stirred for 30 min at −20 °C. The reaction was quenched at −20 °C by the dropwise addition of H_2O (1.5 mL), followed by warming to 25 °C. The reaction mixture was concentrated under reduced pressure. PCTLC (SiO_2, 1 mm plate, 1:5 $CH_3OH/CHCl_3$) provided **49** (0.009 g, 82%) as a white solid: ^{1}H NMR (CD_3OD, 300 MHz) δ 3.91–3.83 (m, 1H), 2.30 (t, J = 7.2 Hz, 2H), 1.74–1.41 (m, 6H); MALDIFTMS (DHB) m/z 281.1213 ($M+H^+$, $C_{10}H_{15}F_3N_4O_2$ requires 281.1220).

2,6-Diamino-5-(7,7,7-trifluoro-6-hydroxyheptyl)-3*H*-pyrimidin-4-one (50). Obtained from **48** (0.005 g, 0.014 mmol) using the procedure described for **49** which afforded **50** (0.004 g, 80%) as a white solid: ^{1}H NMR (CD_3OD, 300 MHz) δ 3.91–3.81 (m, 1H), 2.29 (t, J = 6.6 Hz, 2H), 1.70–1.30 (m, 8H); MALDIFTMS (DHB) m/z 295.1378 ($M+H^+$, $C_{11}H_{17}F_3N_4O_2$ requires 295.1376).

Ethyl 2-cyanohept-6-enoate (51). A stirred solution of 5-bromo-1-pentene (1.00 mL, 8.48 mmol), ethyl cyanoacetate (1.35 mL, 12.7 mmol, 1.5 equiv), and K_2CO_3 (3.52 g, 25.4 mmol, 3.0 equiv) in anhydrous DMF (20 mL) was warmed at 70 °C for 5 h. The solution was cooled to 25 °C and concentrated under reduced pressure. The resulting reaction mixture was suspended in EtOAc (300 mL) and washed with H_2O (5×50 mL) and saturated aqueous NaCl (1×50 mL) followed by concentration under reduced pressure. Chromatography (SiO_2, 5:1 hexanes/EtOAc) afforded **51** (1.07 g, 70%) as a clear oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.85–5.71 (m, 1H), 5.10–4.96 (m, 2H), 4.26 (q, J = 7.3 Hz, 2H), 3.51 (t, J = 7.3 Hz, 1H), 2.12 (dd, J = 7.0, 7.0 Hz, 2H), 1.96 (dd, J = 8.8, 7.0 Hz, 2H), 1.66–1.54 (m, 2H), 1.32 (t, J = 7.2 Hz, 3H); MALDIFTMS (DHB) m/z 182.1173 ($M+H^+$, $C_{10}H_{15}NO_2$ requires 182.1176).

Ethyl 2-cyanooct-7-enoate (52). Obtained from 6-bromo-1-hexene (1.00 g, 6.13 mmol) using the procedure described for **51**. Chromatography (SiO_2, 5:1 hexanes/EtOAc) afforded **52** (0.798 g, 67%) as a clear oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.85–5.68 (m, 1H), 5.09–4.91 (m, 2H), 4.23 (q, J = 7.3 Hz, 2H), 3.46 (t, J = 7.0 Hz, 1H), 2.06 (dd, J = 7.0, 6.2 Hz, 2H), 1.93 (dd, J = 7.3, 7.0 Hz, 2H), 1.59–1.39 (m, 4H), 1.30 (t, J = 7.1 Hz, 3H); MALDIFTMS (DHB) m/z 196.1334 ($M+H^+$, $C_{11}H_{17}NO_2$ requires 196.1332).

2,6-Diamino-5-(pent-4-en-1-yl)-3*H*-pyrimidin-4-one (53). Obtained from **51** (1.08 g, 5.68 mmol) using the procedure described for **7** which afforded **53** (0.680 g, 62%) as a white solid: ^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.76 (bs, 1H), 5.91 (bs, 2H), 5.89–5.69 (m, 1H), 5.63 (bs, 2H), 5.15–4.89 (m, 2H), 2.15 (t, J = 7.5 Hz, 2H), 2.00 (dd, J = 5.8, 7.7 Hz, 2H), 1.42–1.29 (m, 2H); MALDIFTMS (DHB) m/z 195.1241 ($M+H^+$, $C_9H_{14}N_4O$ requires 195.1240).

2,6-Diamino-5-(hex-5-en-1-yl)-3*H*-pyrimidin-4-one (54). Obtained from **52** (0.758 g, 3.88 mmol) using the procedure described for **7** which afforded **54** (0.553 g, 71%) as a white solid: ^{1}H NMR (CD_3OD, 250 MHz) δ 5.89–5.74 (m, 1H), 5.00–4.80 (m, 2H), 2.35–2.24 (m, 2H), 2.18–2.04 (m, 2H), 1.52–1.38 (m, 4H); MALDIFTMS (DHB) m/z 209.1391 ($M+H^+$, $C_{10}H_{16}N_4O$ requires 209.1397).

2,6-Diamino-5-pentyl-3*H*-pyrimidin-4-one (55). A solution of **53** (0.037 g, 0.19 mmol) and 10% Pd/C (0.0034 g, 10% w/w) in CH_3OH (2 mL) was stirred under 1 atm H_2 at 25 °C for 12 h. The catalyst was removed by filtration though Celite and the filtrate was concentrated under reduced pressure to afford **55** (0.037 g, quantitative) as a white solid: ^{1}H NMR (CD_3OD, 300 MHz) δ 2.27 (t, J = 7.3 Hz, 2H), 1.49–1.30 (m, 6H), 0.89 (t, J = 6.6 Hz, 3H); MALDIFTMS (DHB) m/z 197.1391 ($M+H^+$, $C_9H_{16}N_4O$ requires 197.1397).

2,6-Diamino-5-hexyl-3*H*-pyrimidin-4-one (56). Obtained from **54** (0.023 g, 0.115 mmol) using the procedure described for **55** which afforded **56** (0.023 g, quantitative) as a white solid: ^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.75 (bs, 1H), 5.90 (bs, 2H), 5.60 (bs, 2H), 2.18–2.09 (m, 2H), 1.36–1.20 (m, 8H), 0.93–0.81 (m, 3H); MALDIFTMS (DHB) m/z 211.1548 ($M+H^+$, $C_{10}H_{18}N_4O$ requires 211.1553).

2,6-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl) - 5 - (pent - 4 - en - 1 - yl)pyrimidin - 4 - one (57). Obtained from **53** (0.057 g, 0.29 mmol) using the procedure described for **9**. Chromatography (SiO_2, 4:1 hexanes/EtOAc) afforded **57** (0.125 g, 61%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.89–5.73 (m, 1H), 5.10–4.98 (m, 2H), 2.54 (t, J = 7.9 Hz, 2H), 2.17–2.05 (m, 2H), 1.59–1.40 (m, 47H); MALDIFTMS (DHB) m/z 617.3172 ($M+Na^+-Boc$, $C_{34}H_{54}N_4O_{11}$ requires 617.3157).

2,6-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl)-5-(hex-5-en-1-yl)pyrimidin-4-one (58). Obtained from **54** (0.519 g, 2.592 mmol) using the procedure described for **9**. Chromatography (SiO_2, 4:1 hexanes/EtOAc) afforded **58** (0.625 g, 34%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.87–5.68 (m, 1H), 5.07–4.90 (m, 2H), 2.51 (t, J = 7.5 Hz, 2H), 2.12–2.00 (m, 2H), 1.61–1.39 (m, 49H); MALDIFTMS (DHB) m/z 731.3869 ($M+Na^+$, $C_{35}H_{56}N_4O_{11}$ requires 731.3858).

2,6-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl)-5-(5-hydroxypentyl)pyrimidin-4-one (59). Compound **57** (0.170 g, 0.245 mmol) was dissolved in anhydrous THF (1.0 mL) and cooled to 0 °C. BH_3–THF (1 M solution, 0.57 mL, 0.57 mmol, 2.3 equiv) was added

dropwise and the resulting solution stirred at 0 °C for 1 h. H_2O (60 μL), 1 N aqueous NaOH (0.57 mL, 0.57 mmol, 2.3 equiv) and H_2O_2 (50% solution, 38 μL, 0.57 mmol, 2.3 equiv) were added dropwise sequentially at 0 °C. The cooling bath was removed and the solution was stirred at 25 °C for 1 h. The reaction solution was partitioned between EtOAc (100 mL) and H_2O (50 mL). The aqueous layer was extracted with EtOAc (2×50 mL) and the combined organic layers washed with H_2O (2×50 mL) and saturated aqueous NaCl (1×50 mL) followed by concentration under reduced pressure. Chromatography (SiO_2, 1:1 hexanes/EtOAc) afforded **59** (0.104 g, 60%) as a clear oil: ^{1}H NMR (CD_3OD, 250 MHz) δ 3.53 (t, J = 6.6 Hz, 2H), 2.55 (t, J = 7.7 Hz, 2H), 1.63–1.36 (m, 51H); MALDIFTMS (DHB) m/z 735.3800 ($M+Na^+$, $C_{34}H_{56}N_4O_{12}$ requires 735.3787).

2,6-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl) - 5 - (6 - hydroxyhexyl)pyrimidin - 4 - one (60). Obtained from **58** (0.249 g, 0.355 mmol) using the procedure described for **59**. Chromatography (SiO_2, 1:2 hexanes/EtOAc) afforded **60** (0.109 g, 43%) as a clear oil: ^{1}H NMR (CD_3OD, 250 MHz) δ 3.53 (t, J = 6.4 Hz, 2H), 2.54 (t, J = 7.7 Hz, 2H), 1.66–1.36 (m, 53H); MALDIFTMS (DHB) m/z 749.3969 ($M+Na^+$, $C_{35}H_{58}N_4O_{12}$ requires 749.3969).

2,6-Diamino-5-(5-hydroxypentyl)-3*H*-pyrimidin-4-one (61). A sample of **59** (0.004 g, 0.006 mmol) was dissolved in $CHCl_3$ (1 mL) at 0 °C and TFA (0.25 mL) was added dropwise. After this addition was complete, the cooling bath was removed and the solution was allowed to stir at 25 °C for 2 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sequential sonication and concentration from MeCN (1×5 mL) and Et_2O (1×5 mL). The resulting crude product was dissolved in THF (0.3 mL), CH_3OH (0.1 mL) and H_2O (0.1 mL). LiOH–H_2O (0.010 g, 0.238 mmol) was added and the solution stirred at 25 °C for 2 h. The solution was concentrated under reduced pressure. Chromatography (SiO_2, 5:1 $CHCl_3$/CH_3OH) followed by trituration with Et_2O afforded **61** (1.0 mg, 83%) as a white powder: ^{1}H NMR (CD_3OD, 300 MHz) δ 3.45 (t, J = 6.6 Hz, 2H), 2.20 (t, J = 7.0 Hz, 2H), 1.55–1.20 (m, 6H); MALDIFTMS (DHB) m/z 213.1353 ($M+H^+$, $C_9H_{16}N_4O_2$ requires 213.1346).

2,6-Diamino-5-(6-hydroxyhexyl)-3*H*-pyrimidin-4-one (62). Obtained from **60** (0.025 g, 0.035 mmol) using the procedure described for **61**. Chromatography (SiO_2, 5:1 $CHCl_3$/CH_3OH) afforded **62** (0.005 g, 64%) as a white solid: ^{1}H NMR (CD_3OD, 250 MHz) δ 3.53 (t, J = 6.6 Hz, 2H), 2.28 (t, J = 7.0 Hz, 2H), 1.57–1.28 (m, 8H); MALDIFTMS (DHB) m/z 227.1499 ($M+H^+$, $C_{10}H_{18}N_4O_2$ requires 227.1503).

5-{2,4-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl)-6-oxo-1,6-dihydropyrimidin-5-yl}pentanal (63). Dess–Martin periodinane (0.081 g, 0.190 mmol, 1.5 equiv) was added to a stirred solution of **59** (0.088 g, 0.127 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C. The solution was stirred at 0 °C for 45 min. The reaction was diluted with Et_2O (10 mL) and quenched by the addition of 1 N aqueous NaOH (2 mL). The resulting mixture was partitioned between Et_2O (50 mL) and H_2O (50 mL). The aqueous layer was extracted with Et_2O (2×50 mL). The combined organic layers were washed with H_2O (1×20 mL) and saturated aqueous NaCl (1×20 mL) followed by concentration under reduced pressure affording **63** (0.082 g, 93%) as a clear oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.74 (t, J = 1.5 Hz, 1H), 2.54 (t, J = 7.0 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 1.73–1.38 (m, 49H); MALDIFTMS (DHB) m/z 733.3647 ($M+Na^+$, $C_{34}H_{54}N_4O_{12}$ requires 733.3630).

6-{2,4-Bis[*N*,*N*-bis(*t*-butyloxycarbonyl)amino]-3-(*t*-butyloxycarbonyl)-6-oxo-1,6-dihydropyrimidin-5-yl}hexanal (64). Obtained from **60** (0.086 g, 0.12 mmol) using the procedure described for **63**. Chromatography (SiO_2, 1:2 EtOAc/hexanes) afforded **64** (0.049 g, 57%) as a clear oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.77 (s, 1H), 2.53 (t, J = 7.7 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 1.72–1.33 (m, 51H); MALDIFTMS (DHB) m/z 747.3771 ($M+Na^+$, $C_{35}H_{56}N_4O_{12}$ requires 747.3787).

5-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)pentanal trifluoroacetic acid salt (65). Compound **63** (0.030 g, 0.043 mmol) was dissolved in $CHCl_3$ (4 mL) at 0 °C and TFA (4 mL) was added dropwise. After this addition was complete, the cooling bath was removed and the solution was allowed to stir at 25 °C for 4 h. The solution was concentrated under reduced pressure. Residual TFA was removed via sonication and concentration from Et_2O (2×10 mL) affording **65**–CF_3CO_2H (0.019 g, quantitative) as a gray solid: ^{1}H NMR (CD_3OD, 250 MHz, CD_3OD hemiacetal) δ 4.48 (t, J = 5.4 Hz, 1H), 2.31 (t, J = 7.0 Hz, 2H), 1.63–1.41 (m, 6H); MALDIFTMS (DHB) m/z 211.1191 ($M+H^+$, $C_9H_{14}N_4O_2$ requires 211.1190).

6-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)hexanal trifluoroacetic acid salt (66). Obtained from **64** (0.020 g, 0.028 mmol) using the procedure described for **65** affording **66**–CF_3CO_2H (0.013 g, quantitative) as a white solid: ^{1}H NMR (CD_3OD, 250 MHz, CD_3OD hemiacetal) δ 4.47 (t, J = 5.4 Hz, 1H), 2.31 (t, J = 7.0 Hz, 2H), 1.65–1.32 (m, 8H); MALDIFTMS (DHB) m/z 225.1351 ($M+H^+$, $C_{10}H_{16}N_4O_2$ requires 225.1346).

Cytotoxicity, GAR Tfase and AICAR Tfase inhibition

Cytotoxicity, *E. coli*, and rhGAR[27] and rhAICAR Tfase[28] inhibition studies were conducted as previously detailed[12] with the exception that the AICAR Tfase inhibition was conducted in the absence of 5 μM β-mercaptoethanol and screened with 10 nM enzyme, 25 μM inhibitor and 22.5 μM of cofactor.

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