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Tetrahedron 60 (2004) 943–959
Tetrahedron

The synthesis of 7-deazaguanines as potential inhibitors of guanosine triphosphate cyclohydrolase I

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Received 4 September 2003; revised 20 October 2003; accepted 13 November 2003

Abstract—Variously substituted 7-deazaguanines are of interest as inhibitors of GTP cyclohydrolase I, the first enzyme in the biosynthetic pathway leading to dihydrofolate and tetrahydrobiopterin. Methods are described for the synthesis of 7-deazaguanines substituted at positions 2, 6 and 9 (purine numbering) such that a wide diversity of compounds can be prepared. These methods supplement our previous work that established routes for the synthesis of 7- and 8-substituted 7-deazaguanines. Emphasis is placed on the properties of 2-thioalkyl pyrimidines as intermediates because they provide the basis for a traceless solid-state synthesis of purines, pteridines, and their analogues. Compounds prepared have been assessed in a primary screen for their ability to inhibit GTPCH I and 8-methyldeazaguanine has been shown to be significantly more potent than any inhibitor yet described. Several compounds appeared to undergo transformation by GTPCH I; with the aid of a model reaction, their behaviour can be interpreted in the context of the mechanism of the hydrolytic phase of GTPCH I.

Guanosine triphosphate cyclohydrolase I (GTPCH I) is the first enzyme in the biosynthetic pathway leading to dihydrofolate and the pathway leading to tetrahydrobiopterin, two essential metabolic cofactors.[1] Recent clarification of the crystal structure of GTPCH I[2] has demonstrated the presence of a zinc cation at the active site and assigned it a role in acid–base catalysis of the hydrolytic opening of the purine ring. This information has strengthened the information for the design of inhibitors of GTPCH I. Such inhibitors may have value in antibacterial therapy or in agrochemistry.[3] It is notable that several antibiotics containing deazaguanines as aglycones have been discovered.[4] In a previous paper in this series[5] we described versatile syntheses of 7-deazaguanines with substituent diversity at positions 7 and 8. Here we describe extensions of these methods to provide diversity at positions 2, 6 and 9 (purine numbering). Taken together, the two papers provide a large part of the basis for a versatile solid phase synthesis methodology for purines, pteridines, and their analogues.[6] Without ability to tolerate a wide range of substituents, these methods would be severely limited. We have therefore studied the capacity of these synthetic methods to prepare a range of highly substituted deazaguanines. The compounds prepared have been assayed against GTP-cyclohydrolase 1. New inhibitors have been identified and unexpected reactivity of several compounds sheds light on the mechanism of action of the enzyme.

1. Synthesis of 9-substituted 7-deazaguanines

In our previous work,[5] N(9)-alkyl groups, alternatives to ribose, were introduced by substitution of 2-amino-6-chloro-4(3*H*)-pyrimidinone **1** (Scheme 1a). It is important for obtaining significant biological activity to be able to include polyfunctional substituents, especially hydroxyl groups that might be phosphorylated in vivo. Consequently, the substitution strategy was extended in this work to include 3-aminopropane-1,2-diol and 4-aminobutan-1-ol as substituents; the corresponding pyrimidines **2b** and **2c** underwent smooth cyclisation with chlorocyanoacetaldehyde to afford the 7-cyano-7-deazaguanines **3b** and **3c** in 60–70% yield. However, this route was shown to be limited to primary amines lacking α-substituents; none of ribosylamine, cyclopentylamine, cyclohexylamine or 1,2-dihydroxy-3-amino-5-hydroxymethylcyclopentane reacted with the 6-chloropyrimidine. We also showed in our previous work[5] that a range of 8-substituted 9-alkyl deazaguanines **4** could be prepared from alkyldiaminopyrimidines such as **2** and the oximes of α-haloketones in the presence of base via intermediate 5-substituted oximes.

Keywords: Purine analogues; Synthesis; Deazaguanines; GTP cyclohydrolase 1; Inhibition; Mechanism.
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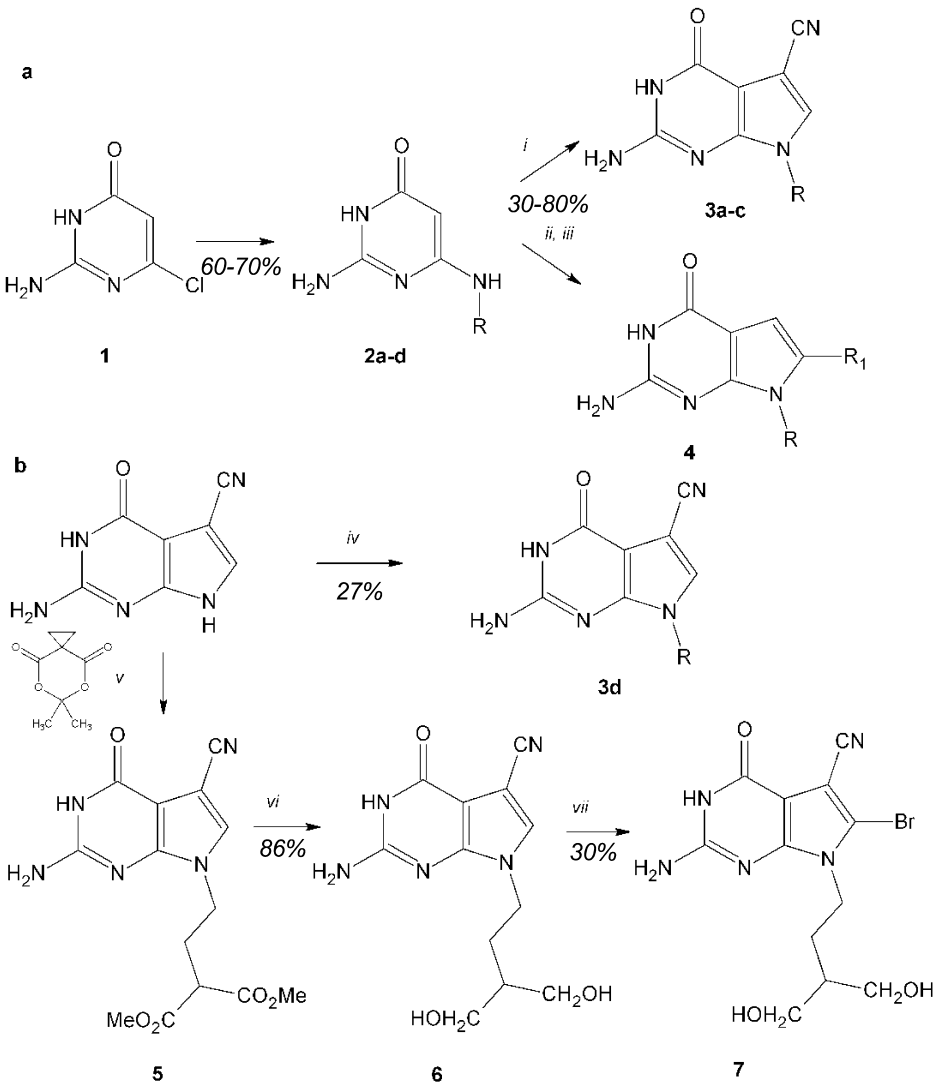
0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.11.030

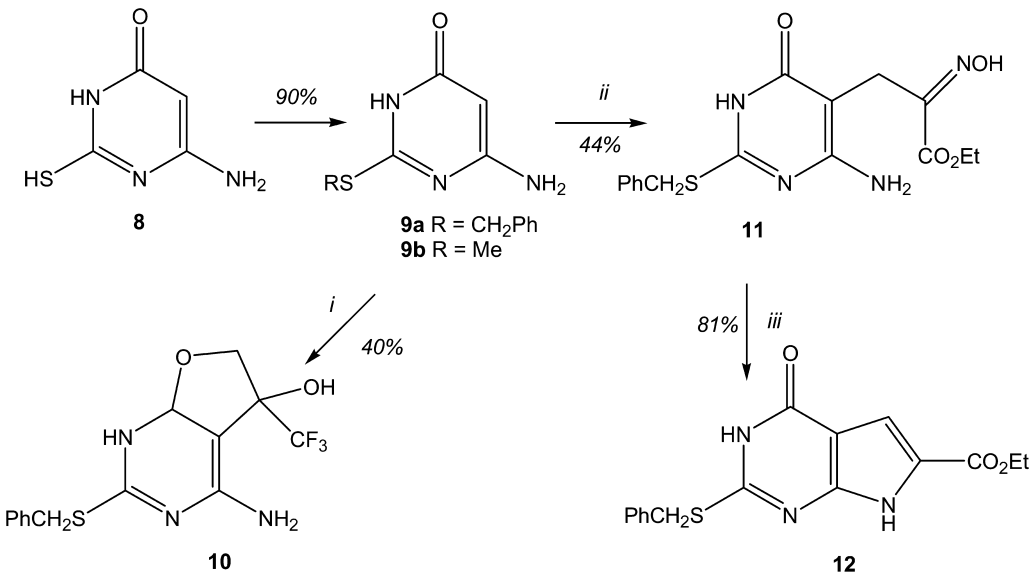


Scheme 1. R=**a** H, **b** $CH_2CH(OH)CH_2OH$, **c** $(CH_2)_4OH$, **d** $CH_2CH{=}CH_2$. *Reagents*: (i) CHCl(CN)CHO; (ii) $R_1C(=NOH)CH_2Br$, Et_3N; (iii) H^+, PhCHO; (iv0 $CH_2{=}CHCH_2Br$, K_2CO_3; (v) 6,6-dimethyl-5,7-dioxaspiro[2.5]octane-4,8-dione, K_2CO_3; (vi) $NaBH_4$, *t*-BuOH; (vii) NBS.

In order to insert substituents more similar to ribose, it was necessary, therefore, to investigate direct alkylation of N-9 as an alternative strategy (Scheme 1b). Whilst this strategy is effective with guanosines,[7] we have found that with deazaguanines there are difficulties associated with the several possible sites of alkylation on nitrogen and oxygen.[4] It was therefore prudent to establish that selective alkylation at N9 was indeed possible. This was done by alkylating the 7-cyano-7-deazaguanine **3a** with allyl bromide in DMF in the presence of potassium carbonate, which afforded a single compound, **3d**, in 68% yield after purification. That alkylation had taken place exclusively at N9 was confirmed by synthesis of **3d** from chloropyrimidine **1** by reaction of the latter with allylamine and subsequent cyclisation. The material thus obtained was identical in all respects to that prepared by direct alkylation. Alkylation with highly functionalised reagents to provide closer analogues to ribose was therefore attempted, based upon published syntheses of the drugs penciclovir and famciclovir.[8] For clean alkylation at N9, a reactive electrophile was required. Triethyl 3-bromopropane-1,1,1-tricarboxylate failed to react on extended reaction times but 6,6-dimethyl-5,7-dioxaspiro[2.5]octane-4,8-dione alkylated 7-cyano-7-deazaguanine **3a** to afford the derivative **5** in 65% yield. Reduction of the diester was accomplished with sodium borohydride affording the corresponding deazaguanine diol, **6**, substituted by an acyclic ribose analogue, in 86% yield. This compound was brominated at C8 with *N*-bromosuccinimide to give **7** and access to the range of sulfur-containing derivatives described by us previously.[5] It is probable that the 7-cyano group is important in controlling the selectivity in these reactions by delocalisation of an anion formed at N9. Similar reactions attempted with 8-ethoxycarbonyl-7-deazaguanine (**4**, R=H, $R_1{=}CO_2Et$) led to complex mixtures; in that case, it seems that introduction of the alkyl group at the pyrimidine stage is essential.

2. Substitution at C2

It is well known that the amino–oxo substitution pattern of the pyrimidine ring of pteridines and guanosines leads not only to ambident reactivity but also to experimental



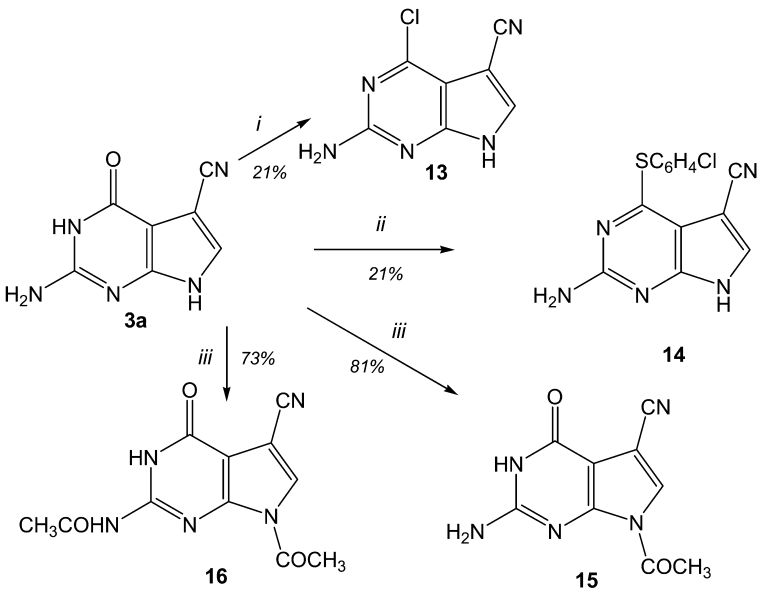
Scheme 2. R=**a** CH_2Ph, **b** Me. *Reagents*: (i) CF_3COCH_2Br, EtOH, 60 °C, 17 h; (ii) $BrCH_2C(=NOH)CO_2Et$, Et_3N, DMF, room temperature, 5 h; (iii) PhCHO, H^+, EtOH, reflux, ~12 h.

problems of low solubility. With a view to extension to solid phase methodologies, the cyclisation chemistry of pyrimidine thioethers was investigated (Scheme 2). 2-Benzylsulfanyl- and 2-methylsulfanyl-6-amino-4(3*H*)-pyrimidinones (**9a**,**b**) were prepared by direct alkylation of the corresponding thiol **8**. Surprisingly, compounds **9a** and **9b** failed to give purines or pyridopyrimidines when treated with C-electrophiles such as chloroacetaldehyde, bromoacetaldehyde diethyl acetal, chlorocyanoacetaldehyde, bromomalonate, ethyl acrylate, acetylacetone, diethyl oxalate, ethyl glyoxylate, and ethyl 2,4-dioxopentanoate, in DMF or ethanol in the presence of base (triethylamine) or acid (4-toluenesulfonic acid) under reflux for periods of up to 4 days.

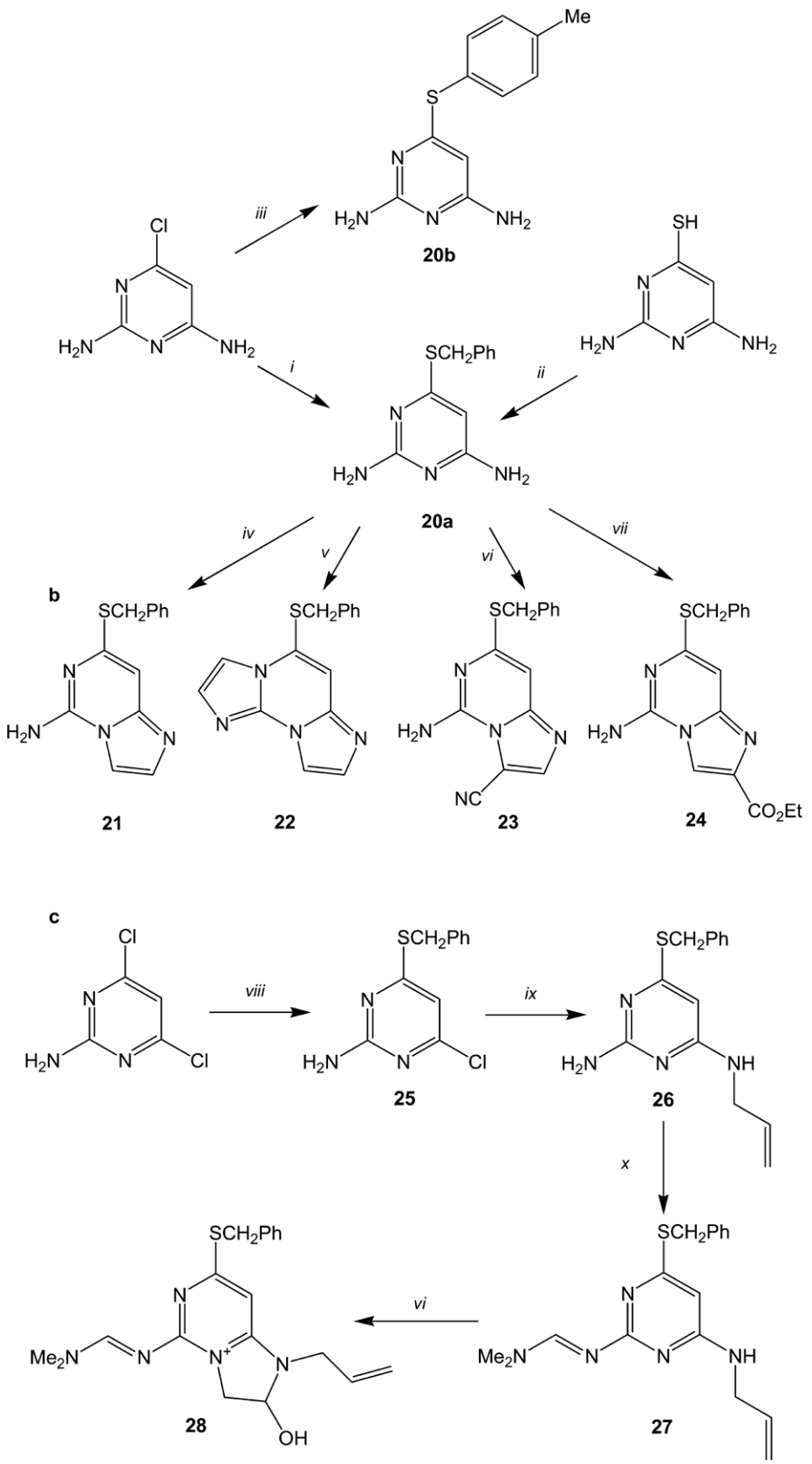
Only two reagents were found to react. Bromotrifluoroacetone reacted with **9a** to afford the furo[2,3-*d*]pyrimidine **10** in 40% yield, a reaction course observed previously with this reagent.[9] Ethyl bromopyruvate oxime, on the other hand, did give the desired C-5 alkylated pyrimidine **11** when reacted with **9a**, and the product **11** was cyclised under our normal conditions by transoximation with benzaldehyde and acid catalysis to afford the 8-ethoxycarbonyl deazaguanine **12** in 81% yield. The influence of the C2 substituent in the reactivity of pyrimidines in this series is striking. Although carbon electrophiles show very limited reactivity, we have shown[5,10] that nitrosation occurs readily leading to effective syntheses of sulfanyl pteridines and purines.

3. Substitution at C6 (C4 in pyrrolo[2,3-*d*] numbering)

Derivatisation of the 4-oxo function in pyrimidines has been found to be a versatile method of controlling reactivity. This



Scheme 3. *Reagents*: (i) $POCl_3$, pyridine, reflux, overnight; (ii) TFAA, pyridine, ClC_6H_4SH, NH_4OH, H_2O_2, 0 °C; (iii) Ac_2O, DMAP, reflux, 6 h.



Scheme 4. *Reagents*: (a) (i) $PhCH_2SH$, NaOH, aq. EtOH, 80 °C, overnight; (ii) $PhCH_2Cl$; (iii) $CH_3C_6H_4SH$, NaOH, aq. EtOH; (b) (iv) $ClCH_2CHO$, 1 equiv. 50 °C, overnight; (v) $ClCH_2CHO$, 2 equiv., 50 °C, 22 h; (vi) Cl(CN)CHCHO, 50 °C, overnight; (vii) bromopyruvate oxime, Et_3N, 80 °C, 10 h, then H^+, PhCHO; (c) (viii) $PhCH_2SH$, NaOH, room temperature, 10 min, (ix) $CH_2{=}CHCH_2Br$, K_2CO_3, 100 °C, 5 days; (x) Bredereck's reagent, 60 °C, 10 min.

modification was applied both to 7-deazaguanines themselves and to the precursor pyrimidines but led to a variety of undesired cyclisations (Schemes 3 and 4) as described below. Conversion of 7-cyano-7-deazaguanine **3a** into more soluble, lipophilic derivatives was approached by chlorination with phosphorus oxychloride to afford **13** in modest yield. Preferably, therefore, **13** was treated sequentially with trifluoroacetic anhydride and pyridine, 4-chlorothiophenol, ammonium hydroxide, and hydrogen peroxide to give the (4-chlorophenylsulfanyl)-deazaguanine **14** in 52% overall yield.[8] Other reagents investigated included acetic anhydride/DMAP; with one equivalent, monosubstitution at N9 giving **15** was observed but used in excess, 3,9-diacetyl-7-deazaguanine **16** was obtained.

In view of the problems associated with C6 substitution described above, it was important to extend the investigation of sulfanyl ethers to C4 in pyrimidines (Scheme 4a). 2,4-Diamino-6-chloropyrimidine readily underwent substitution with benzyl mercaptan to afford the benzyl sulfanyl ether **20a** in 85% yield and the same compound was obtained by alkylation of 2,4-diaminopyrimidine-6-thiol with benzyl chloride. With extension to solid phase synthesis in mind and the evidence that thiophenyl ethers are easier to cleave than thiobenzyl ethers,[11] the (4-methylphenyl)sulfanyl ether **20b** was also prepared. Disappointingly, the 4-sulfanyl ethers introduced yet another reactivity pattern into the pyrimidine ring. Once again, substitution at C5 was not observed. Instead, cyclisation between N3 and 4-NH_2 occurred (Scheme 4b). This was unambiguously demonstrated using chloroacetaldehyde (1 equiv.) which afforded the imidazo[1,2-*c*]-pyrimidine **21** from **20a** in low yield (25%), the structure being confirmed by NMR and X-ray crystallography. Excess chloroacetaldehyde led to dicyclisation and **22**. Chlorocyanoacetaldehyde gave the cyanoimidazo[1,2-*c*]-pyrimidine **23**. Even the highly reactive bromopyruvate oxime in the presence of triethylamine, which had given C-substitution in the 2-sulfanyl series, reacted on nitrogen to form **24**.

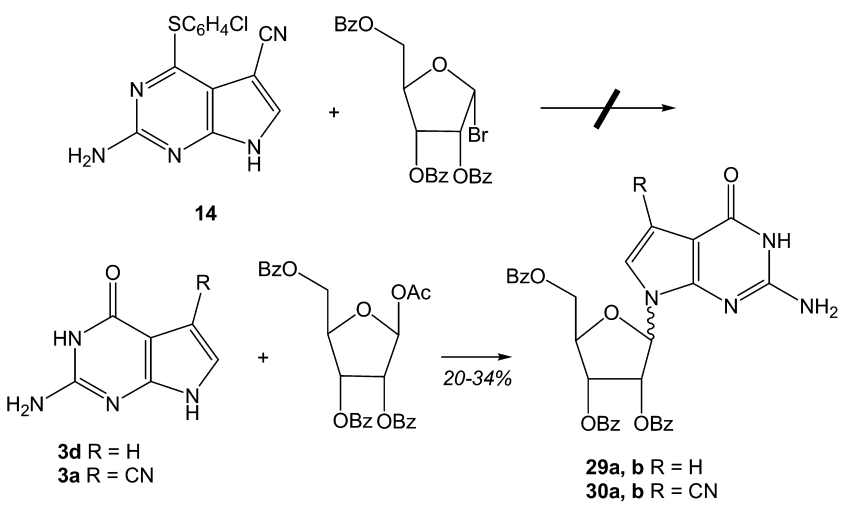
Arguing that this greatly diminished reactivity at C5 was due to a pronounced decrease in electron density and to high nucleophilic reactivity of the 4-amino group, an attempt was made to modify the reactivity to obviate these problems (Scheme 4c). Accordingly 2-amino-4,6-dichloropyrimidine was converted into the benzylsulfanyl ether **25** (96%) and a sterically hindered amino group introduced by substitution of the second chloride with allylamine to give **26** (45%). The remaining amino group was protected with a strongly electron donating dimethylaminoformimino group using Bredereck's reagent to give the required modified pyrimidine **27** (87%). Disappointingly, C5 substitution was not observed with chloracetaldehyde; cyclisation on N3 and the allylamine occurred to give **28**, analogous to the previous reactions but without dehydration. It must therefore be concluded that the synthesis of deazaguanines using 2- and 4-pyrimidine sulfanyl ethers is severely limited to 2-sulfanyl systems with electrophiles derived from α-halo-oximes.

4. Ribosyl derivatives of 7-deazaguanines

A second potential benefit of sulfanyl ethers is the simplification of alkylation chemistry. The synthesis of deazaguanine glycosides in particular might be facilitated by the reduced nucleophilicity of the sulfanyl derivatives compared with their amino and oxo analogues. α-1-Bromoribose-2,3,5-tribenzoate[12] was reacted with the 4-chlorophenyl sulfanyl ether **14** under phase transfer conditions but no riboside formation was observed. However riboside formation was successful using 7-deazaguanine **3d** and the 7-cyano analogue **3a** using 1-acetoxy-ribose-2,3,5-tribenzoate with Lewis acid catalysis[13] affording **29** or **30**, respectively as a mixture of anomers in each case (Scheme 5). It was not found possible to separate the anomers and, since interesting biological activity and structural possibilities were found with simpler compounds, no development work was carried out on glycosylation reactions.

5. Leads from molecular modelling

As part of our international collaboration, Professor Kyuji



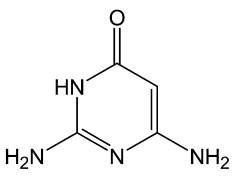
Scheme 5. Ribosyl derivatives of deazaguanines. *Reagents*: hexamethyldisilazane, TMSCl, $SnCl_4$, MeCN, 21 h room temperature.

Ohta in Japan has been investigating the potential binding of deazaguanines and other compounds to the active site of GTPCH I. His studies included the zinc ion and led to the suggestion that deazaguanines with carboxyl or thiol substituents might be very tight binding inhibitors thereby obviating the need for phosphorylated derivatives.[14] Accordingly, target molecules represented by the deazaguanine carboxylates with simplified ribose analogues **31**, and **32** were identified and their synthesis developed (Scheme 6). The precursor pyrimidine **33** was prepared by the conventional substitution methods and cyclised with each of the two established reagents, chlorocyanoacetaldehyde and ethyl bromomalonate oxime. In the former case, a mixture of acetal **34a** and deprotected deazaguanine **34b** in a ratio of 2.5:1 was obtained. Hydrolysis of the cyano group with potassium hydroxide afforded the mixture of carboxylic acids **35a** and **35b** and esterification with thionyl chloride and methanol gave one of the target compounds **31**. In the latter case, C5 substitution occurred without loss of the protecting group to give **36** but we were unable to find conditions under which cyclisation to the deazaguanine **32** could be achieved without cleavage of the protecting group. However, the ester **32** was obtained in 62% yield on cyclisation of **36** thereby confirming the practical utility of the nitroso alkene route for the synthesis of polyfunctional 8,9-substituted deazaguanines.[17]

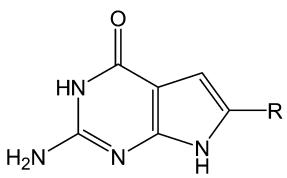
6. 7-Deazaguanines as inhibitors of GTPCH I

We have undertaken assays of the activity of compounds prepared in our studies as inhibitors of GTPCH I using screens designed for rapid assay using small quantities of scarce enzyme. Known inhibitors include guanine, 8-hydroxy-, 8-methyl-, 8-mercapto-, and 8-bromoguanine,[15] 8-azaguanine, and what is probably the most widely used inhibitor, 2,6-diamino-4(3*H*)-pyrimidinone (DAP).[16] GTPCH I is also inhibited naturally by tetrahydrobiopterin mediated by GTPCH I feedback regulatory protein.[15] We have developed two screening assays, one based on UV and the other on HPLC, both of them being suitable for a high throughput of samples and a total of 52 compounds were screened. The compound set included a wide variety of substituted pyrimidines, purines, pteridines, and 7-deazapurines that were synthesised in the present work and in previous studies.[17] The UV assay was carried out by adding a solution of the test compound dissolved in DMSO to a solution of GTPCH I in KCl/Tris buffer, pH 8.5, initiating the reaction by the addition of enzyme. Reaction progress was monitored over 3 min at 1 s intervals by following the absorption at 330 nm. At this wavelength, the product 7,8-dihydroneopterin triphosphate absorbs but the substrate GTP does not. Control experiments were carried out under the same conditions in the absence of test compound and to demonstrate that DMSO had no effect on the reaction in the concentrations used. The HPLC assay monitored the formation of 7,8-dihydroneopterin triphosphate by separation of it on the HPLC column from other reaction components and integration of the product peak. For reasons of availability and activity of GTPCH I, it was not possible to undertake full kinetic assays of the compounds prepared. The available assays, however, functioned together as an effective screen to identify compounds that were significant inhibitors and compounds that underwent reaction with GTPCH I.

The assays showed that 7-deazapurines, substituted at position 8 but unsubstituted at positions 7 and 9, had behaviour consistent with some form of inhibition on GTPCH I. In particular, the HPLC assay showed that in the presence of 8-methyl-7-deazaguanine **37b** (2.9 mM), the amount of 7,8-dihydroneopterin formed from GTP was only 6% of the amount formed in the absence of **37b**, under otherwise identical conditions. The most widely discussed inhibitor, DAP, by comparison showed only 58% inhibition under these conditions. Other related compounds showed similar but smaller decreases: 8-trifluoromethyl-7-deazaguanine **37c** 17%, 8-azaguanine 43%, and 8-ethoxycarbonyl-7-deazaguanine 78%. This general behaviour has been confirmed independently and using a different method (Dr Christian Hesslinger, Frankfurt[18]). 8-Methyl-7-deazaguanine **37b** thus appears to be the most potent inhibitor of GTPCH 1 to have been described so far.



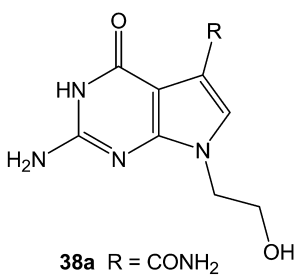
DAP



37a R = H

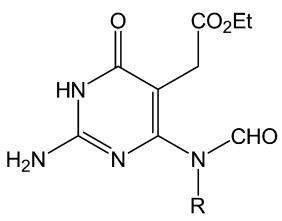
37b R = Me

37c R = CF_3



38a R = $CONH_2$

38b R = CO_2Et



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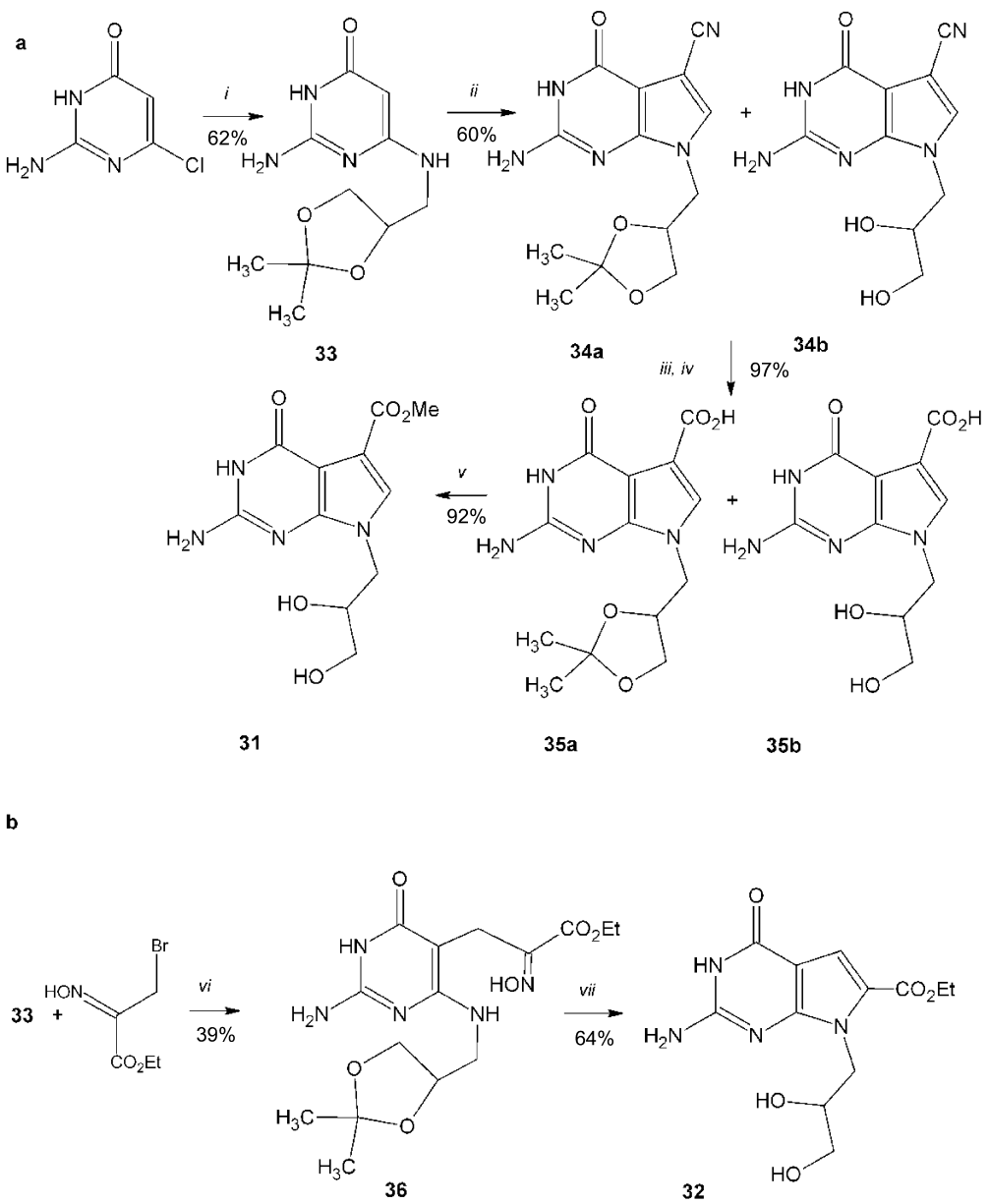
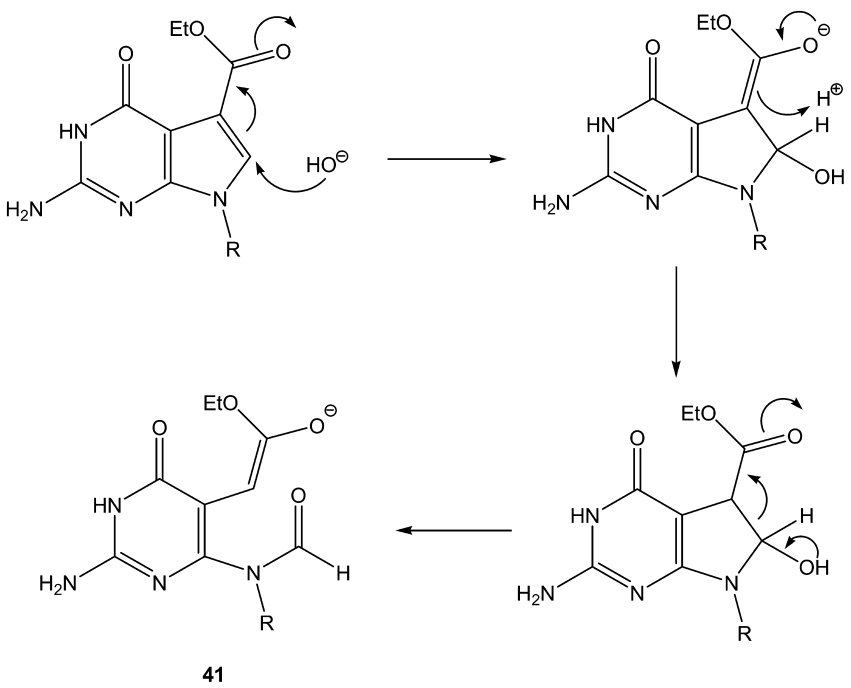


Scheme 6. *Reagents*: (i) RNH_2, Et_3N, EtOH, reflux, 2 days; (ii) Cl(CN)CHCHO, aq. NaOAc, 50 °C, 20 h; (iii) aq. KOH, reflux, 5 h; (iv) AcOH; (v) $SOCl_2$, MeOH, 50 °C, 3 h; (vi) Et_3N, DMF, room temperature, 6 h; (vii) HCl, aq. EtOH, PhCHO, reflux, 2 days.

Observations in the UV assay suggested that **37b** and related compounds may react with the GTPCH I to form a new product, other than 7,8-dihydroneopterin triphosphate. Even in the absence of GTP, the absorption at 330 nm increased over the first minute of assay before slowly decreasing during the remainder of the observation period. Compounds **37a**, **37b**, **37c**, **38a**, and **38b** all showed this effect in the UV assay. A possible explanation of the apparent reaction of these compound with GTPCH I is that, through its normal mechanism, GTPCH I is causing hydrolytic opening of the pyrazole ring. In the case of **38b** this would lead to compound **39**. To determine whether this proposed pathway had a reasonable basis in laboratory chemistry, the ester **38b** was incubated in d_6-DMSO in the presence of sodium ethoxide and the NMR spectrum monitored over many days; special precautions to dry the solvent and reagents were not taken. The use of sodium ethoxide under these conditions provided for the presence of a low concentration of hydroxide thereby avoiding more extensive hydrolytic decomposition. A slow reaction was clearly observed, most notably from the downfield region of the spectrum, where a new peak at δ 8.51 was consistent with the presence of a formamide and a peak at δ 7.05 indicative of the presence of a deshielded alkene (Fig. 1). After 100 days, integration suggested that about 20% conversion into this compound **39** had occurred. A reasonable interpretation of these results (Scheme 7) would be in terms of formation of the ring opened formamide **39**. These preliminary observations encourage further work on the properties and reactivity of deazaguanines with GTPCH I.

7. Conclusions

In this work and in our previous study[5] we have defined the limitations inherent in the ring synthesis of deazaguanines



Scheme 7. Hypothesis for reaction of deazaguanines with GTPCH I.

from substituted pyrimidines and have shown that within these limitations, a wide range of structural diversity can be accessed. We have developed chemistry potentially applicable to solid phase synthesis of a range of related bicyclic heterocyclic compounds.[5] Importantly, we have adduced preliminary evidence that deazaguanines do indeed inhibit and react with GTPCH I in ways that are of interest in the context of the mechanism of action of the enzyme. Moreover our most recent results indicate that deazaguanines are effective in modulating the activity of pteridine biosynthesis in cellular as well as purified enzyme systems.[18]

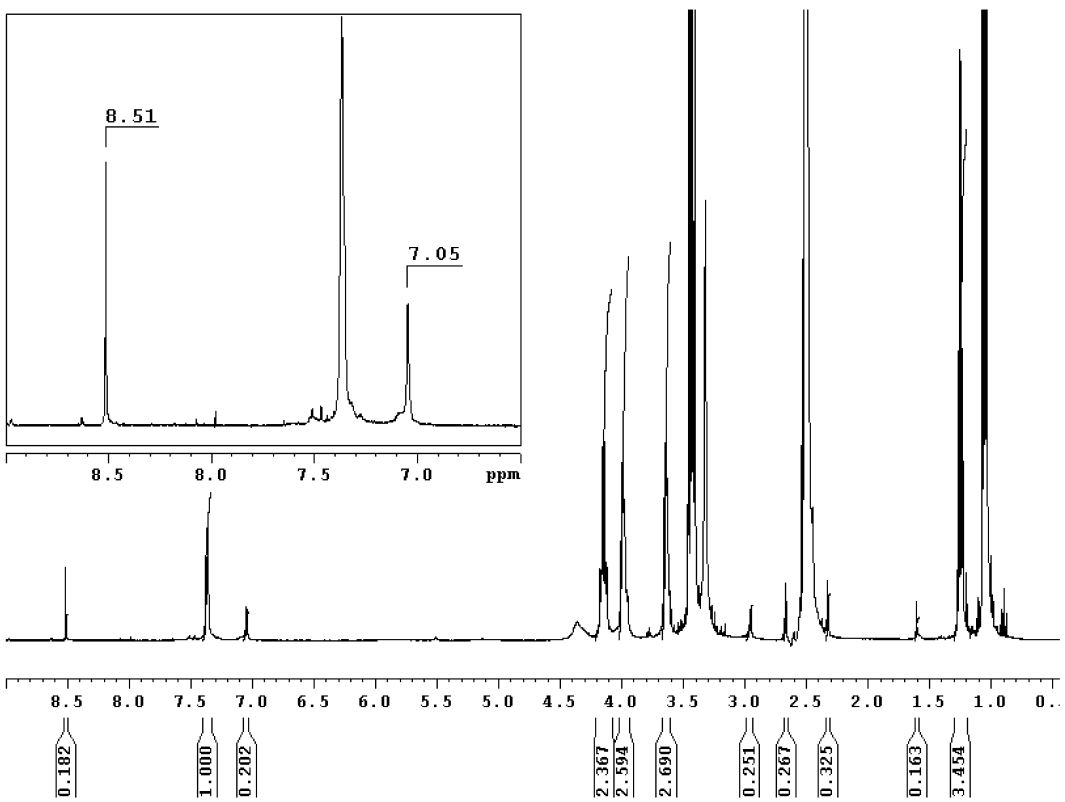


Figure 1. 400 MHz spectrum of **40b** after reacting for 100 days with NaOEt in DMSO solution.

8. Experimental

8.1. Instrumentation and general materials

NMR spectra were determined on a Bruker Spectrospin spectrometer operating at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C spectra. Chemical shifts are reported as ppm relative to TMS measured from the solvent resonance. IR spectra were determined using a Mattson 1000 FT spectrometer or a Nicolet Impact 400D FT spectrometer. Mass spectra were measured on a Jeol JMS AX505 spectrometer. Microanalyses were carried out using a Perkin–Elmer Series II instrument at the University of Strathclyde. UV spectra were determined using a Perkin–Elmer Lambda 2 spectrometer. Melting points, when measurable, were determined on a Reichert hot stage apparatus and are uncorrected. TLC was carried out on silica (Merck 0.25 mm 60 F_{254}). Column chromatography was carried out using silica gel (230–400 mesh; 40–60 μm).

Reaagents were bought from Aldrich (Gillingham, Dorset, UK).

8.1.1. 2-Amino-6-[(2,3-dihydroxypropyl)amino]-4(3*H*)-pyrimidinone 2b. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one **1** (1.130 g, 6.93 mmol) in water (2.8 mL), was added ethylene glycol dimethyl ether (10 mL) and 1-amino-2,3-propandiol (1.7 g, 18.7 mmol). The mixture was heated to reflux for 6 h and then the solvent was evaporated under reduced pressure to give the required pyrimidinone **2b** as a colourless solid (0.927 g, 4.63 mmol, 68%). After washing successively with water, ethanol and diethyl ether and drying under reduced pressure, the product had mp 204–206 °C. Found: HRMS (FAB) 201.0980; $C_7H_{13}N_4O_3$ (M+1) requires 201.0988. δ_H (DMSO) 2.96 (1H, m, C(9)H), 3.16 (2H, m, C(10)H_2), 3.52 (2H, m, C(8)H_2), 4.47 (1H, s, C(5)H), 4.55 (1H, s, C(10)OH), 4.77 (1H, s, C(9)OH), 6.14 (3H, br s, N(7)H_2, N(6)H), 9.66 (1H, br s, N(3)H). δ_C (DMSO) 44.77 (C-8), 64.23 (C-10), 70.65 (C-9), 75.71 (C-5), 155.33 (C-2), 163.42 (C-4), 164.77 (C-6). ν_{max} (KBr) 3425, 1670, 1537, 1455, 1256, 984, 783 cm^{-1}.

8.1.2. 2-Amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3b. To a suspension of 2-amino-6-[(2,3-dihydroxypropyl)-amino]-4(3*H*)-pyrimidinone **3b** (0.912 g, 4.56 mmol) and sodium acetate (0.94 g, 10 mmol) in water (30 mL), was added freshly prepared chloro(formyl)-acetonitrile[19] (0.54 g, 5.24 mmol). The mixture was left stirring for 20 h at 50 °C. A precipitate was collected by filtration, washed with water, methanol and diethyl ether to give the required pyrrolopyridine **3b** as a dark solid (0.32 g, 1.3 mmol, 30%; mp >260 °C (lit.[20] 276–278 °C)). Found: HRMS (EI) found 215.0807 (M−2×OH); $C_{10}H_{11}N_5O_3$ requires 249.0862. δ_H (DMSO) 3.26 (2H, m, C(12)H_2), 3.75 (2H, m, C(10)H_2), 4.10 (1H, m, C(11)H), 4.74 (1H, s, C(12)H_2O*H*), 4.99 (1H, s, C(11)HO*H*), 6.50 (2H, s, N(8)H_2), 7.59 (1H, s, C(6)H), 10.73 (1H, s, N(3)H).

8.1.3. 2-Amino-6-[(4-hydroxybutyl)amino]-4(3*H*)-pyrimidinone 2c. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one **1** (1.13 g, 6.93 mmol) in water (3 mL), was added ethanol (20 mL), triethylamine (2 mL) and 1-amino-4-butanol (1.6 g, 18.4 mmol). The mixture was heated to reflux for 2 days, then the solvent was evaporated under reduced pressure to give a colourless solid that was washed with water, ethanol and diethyl ether, dried under reduced pressure, to afford the title compound **2c** (0.95 g, 4.9 mmol, 70%; 206–208 °C (lit.[21] 206 °C)). Found: HRMS (FAB) 199.1194; $C_8H_{15}N_4O_2$ (M+1) requires 199.1195. δ_H (DMSO) 1.38–1.47 (4H, m, C(10)H_2, C(11)H_2), 3.01 (2H, br s, C(12)H_2), 3.34–3.40 (2H, m, C(9)H_2), 4.40 (1H, s, C(5)H), 4.41 (1H, br s, OH), 6.12 (2H, br s, NH_2), 6.33 (1H, br s, N(8)H), 9.66 (1H, br s, N(3)H).

8.1.4. 2-Amino-7-(4-hydroxybutyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3c. To a suspension of 2-amino-6-[(4-hydroxybutyl)amino]-4(3*H*)-pyrimidinone **2c** (1.10 g, 5.5 mmol) and sodium acetate (1.03 g) in water (20 mL) was added chloro(formyl)acetonitrile[16] (2 g, 19.4 mmol). Upon addition, the solution became green, turning deep blue immediately. The reaction was stirred at 50 °C for 2 days. The solution was filtered and the colourless solid obtained was washed with water, ethanol, and diethyl ether and dried under reduced pressure to afford the title compound **3c** (1.14 g, 4.7 mmol, 84%; mp >260 °C). Found: HRMS (FAB) 248.1138, $C_{11}H_{14}N_5O_2$ (M+1) requires 248.1147. δ_H (DMSO) 1.30–1.37 (2H, m, C(11)H_2), 1.68–1.76 (2H, m, C(12)H_2), 3.31–3.39 (2H, m, C(13)H_2), 3.92–3.96 (2H, m, C(10)H_2), 4.40–4.43 (1H, m, OH), 6.49 (2H, br s, NH_2), 10.70 (1H, s, N(3)H) δ_C (DMSO) 26.66 (C-11), 29.81 (C-12), 44.85 (C-13), 60.56 (C-10), 85.06 (C-5), 99.21 (C-4a), 116.03 (CN), 130.68 (C-6), 151.07, 154.12, 157.80 (C-4, C-7a, C-2). ν_{max} (KBr) 3343 (NH_2), 3182, 2228 (CN), 1681, 1645, 1599, 1548, 1423, 1343, 1101, 785 cm^{-1}.

8.1.5. 6-(Allylamino)-2-amino-4(3*H*)-pyrimidinone 2d. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one **56** (1.13 g, 6.93 mmol) in water (3 mL), was added ethylene glycol dimethyl ether (10 mL) and allyl amine (1.04 g, 18.4 mmol). The mixture was heated to reflux for 6 h, then the solvent was evaporated under reduced pressure to give a colourless solid that was washed with water, ethanol and diethyl ether and dried under reduced pressure to afford the title compound **2d** (0.68 g, 4.1 mmol, 60%; mp 196–198 °C). Found: HRMS (EI) 166.0860: $C_7H_{10}N_4O$ requires 166.0855. δ_H ($CDCl_3$) 3.68 (2H, s, C(9)H_2), 4.41 (1H, s, C(5)H), 5.04 (1H, d, *J*=9.2 Hz, 1×C(11)H_2), 5.13 (1H, d, *J*=17.2 Hz, 1×C(11)H_2), 5.75–5.84 (1H, m, C(10)H), 6.15 (2H, br s, N(7)H_2), 6.52 (1H, br s, N(8)H), 9.75 (1H, br s, N(3)H). δ_C (DMSO) 45.45 (C-9), 77.89 (C-5), 117.66 (C-11), 134.11 (C-10), 156.23 (C-2), 162.59 (C-4), 165.07 (C-6).

8.1.6. 7-Allyl-2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 3d. (a) *Using allyl bromide*. To 7-cyano-7-deazaguanine **3a** (0.5 g, 2.8 mmol) in anhydrous DMF (40 mL) was added allyl bromide (0.35 g, 2.9 mmol) and potassium carbonate (0.45 g, 3.2 mmol). The reaction was left stirring for 4 days at 100 °C, then filtered and evaporated under reduced pressure. The residue was dissolved in water and extracted with dichloromethane, dried over anhydrous sodium sulphate,

and evaporated to give the product **3d** as pale a yellow solid (0.15 g, 0.8 mmol, 27%; mp >260 °C). Found: HRMS (EI) 215.0792, $C_{10}H_9N_5O$ requires 215.0807. δ_H (DMSO) 4.58 (2H, d, J=4.8 Hz, C(10)H_2), 4.95 (1H, d, J=17.2 Hz, 1×C(12)H_2), 5.17 (1H, d, J=10.4 Hz, 1×C(12)H_2), 5.92–6.01 (1H, m, C(11)H), 6.52 (2H, br s, N(8)H_2), 10.76 (1H, s, N(3)H). δ_C (DMSO) 46.85 (C-10), 85.52 (C-5), 99.13 (C-4a), 115.87 (C-9), 117.63 (C-12), 130.49 (C-6), 133.73 (C-11), 151.07, 154.27, 157.82 (C-4, C-7a, C-2). ν_{max} (KBr) 3416, 3325, 2224, 1673, 1626, 1176, 929 cm^{-1}.

(b) *Using allyl amine*. To a suspension of 6-(allylamino)-2-amino-4(3*H*)-pyrimidinone **2d** (0.68 g, 4 mmol) and sodium acetate (0.9 g) in water (20 mL) was added chloro(formyl)-acetonitrile (1.2 g, 11.6 mmol). This was left stirring at 50 °C overnight. After the solution was filtered and the solid obtained was washed with water, ethanol, and diethyl ether to afford the product that was found to be identical with the product prepared by the method (a) above (0.4 g, 1.86 mmol, 45%).

8.1.7. Dimethyl 2-[2-(2-amino-5-cyano-4-oxo-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl] malonate 5. A mixture of 7-cyano-7-deazaguanine **3a** (0.5 g, 2.8 mmol), 2,2-dimethyl-1,3-dioxaspiro[5.2]octane-4,6-dione (0.65 g, 3.8 mmol), and potassium carbonate (0.75 g) in dry DMF (20 mL) was stirred at 55 °C under an atmosphere of N_2 for 4 days. The resulting mixture was filtered and evaporated under reduced pressure to give a brown solid (the potassium salt). This was dissolved in methanol (35 mL) saturated with HCl and a precipitate formed. A further portion of methanol (35 mL) was added and the mixture was left stirring at room temperature overnight. The solution was then filtered, evaporated under reduced pressure and the residue was dissolved in acetone. Silica gel column chromatography eluting with ethyl acetate/methanol 5/1, gave the product **5** as a colourless solid (0.3 g, 0.83 mmol, 30%; mp >260 °C). Found: HRMS (FAB) 334.1147, $C_{14}H_{16}N_5O_5$ (M+1) requires 334.1151. δ_H (DMSO) 2.26 (2H, q, J=6.9 Hz, C(11)H_2), 3.43 (1H, t, J=6.9 Hz, C(12)H), 3.62 (6H, s, 2×C(16)H_3), 4.01 (2H, t, J=6.9 Hz, C(10)H_2), 6.47 (2H, br s, N(8)H_2), 7.65 (1H, s, C(6)H), 10.73 (1H, s, N(3)H). δ_C (DMSO) 28.81 (C-11), 42.72 (C-10), 48.70 (C-12), 52.92, 52.75 (C-16, C-14), 85.45 (C-5), 99.21 (C-4a), 115.87 (C-9), 130.51 (C-6), 151.22, 154.14, 157.74 (C-4, C-7a, C-2), 169.13 (C-15, C-13). IR ν_{max} (KBr) 3432 (NH_2), 2232 (CN), 1720 (C═O), 1685, 1630, 1427, 787 cm^{-1}.

8.1.8. 2-Amino-7-[4-hydroxy-3-(hydroxymethyl)butyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 6. Dimethyl 2-[2-(2-amino-5-cyano-4-oxo-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]malonate **5** (0.83 g, 2.4 mmol) was partially dissolved in *t*-butanol (30 mL) at 60 °C under an atmosphere of dry nitrogen. Sodium borohydride (0.456 g, 12 mmol) was added and the mixture was heated under reflux while methanol (4 mL) was added dropwise over 1.75 h. The mixture was then cooled, methanol (40 mL) was added, and after the effervescence had ceased, the solvents were evaporated. The residue was dissolved in water (30 mL) and the solution was neutralised with 1 M HCl. Evaporation afforded a colourless solid, which was purified by column chromatography on silica gel eluting with ethyl acetate/methanol 100/0 up to 0/100. The product **6** (0.556 g, 2.0 mmol, 86%) was collected from the last fractions as a pale yellow solid, mp >260 °C. Found: HRMS (FAB) 278.1247, $C_{12}H_{16}N_5O_3$ (M+1) requires 278.1253. δ_H (DMSO) 1.39–1.44 (1H, m, C(12)H), 1.66 (2H, q, J=7.2 Hz, C(11)H_2), 3.30–3.43 (4H, m, C(14)H_2, C(13)H_2), 4.00 (2H, t, J=7.2 Hz, C(10)H_2), 4.50 (2H, s, 2×OH), 6.86 (2H, s, N(8)H_2), 7.71 (1H, s, C(6)H), 11.04 (1H, br s, N(3)H). δ_C (DMSO) 29.05 (C-11), 41.10 (C-12), 43.29 (C-10), 61.57 (C-13, C-14), 85.05 (C-5), 97.14 (C-4a), 116.12 (C-9), 130.49 (C-6), 151.62, 154.41, 157.60 (C-2, C-7a, C-4). ν_{max} (KBr) 3417 (NH_2), 3350, 3233 (NH), 2940 (CH), 2227 (CN), 1683 (C═O), 1637, 1597, 1428, 1031, 778 cm^{-1}.

8.1.9. 2-Amino-6-bromo-7-[4-hydroxy-3-(hydroxymethyl)butyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 7. To a suspension of 2-amino-7-[4-hydroxy-3-(hydroxymethyl)butyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **6** (0.556 g, 2.07 mmol) in dry DMF (20 mL), was added *N*-bromosuccinimide (0.398 g, 2.24 mmol). The mixture was stirred at room temperature for 6 h, then evaporated to dryness under reduced pressure and the residue was triturated with water (10 mL). The resulting solid was filtered off, washed with water, ethanol, and then diethyl ether and dried under reduced pressure to afford the product **7** as a microcrystalline off-white solid (0.22 g, 0.61 mmol, 30%; mp 195–199 °C). Found: HRMS (EI) 357.0258, $C_{12}H_{14}N_5O_3^{81}Br$ requires 357.0259. δ_H (DMSO) 1.46–1.51 (1H, m, C(12)H), 1.64 (2H, q, J=7.6 Hz, C(11)H_2), 3.35–3.42 (4H, m, C(13)H_2, C(14)H_2), 4.08 (2H, t, J=7.6 Hz, C(10)H_2), 4.41 (2H, s, 2×OH), 6.61 (2H, br s, N(8)H_2), 10.88 (1H, s, N(3)H). δ_C (DMSO) 28.60 (C-11), 41.10 (C-12), 41.92 (C-10), 61.74 (C-13, C-14), 79.71 (C-5), 107.38 (C-9), 113.95 (C-4a), 125.83 (C-6), 154.46 (C-2), 156.88 (C-7a). ν_{max} (nujol) 3342 (NH_2), 3222, 2942 (CH), 2229 (CN), 1680, 1640, 1562, 1400, 1039, 780 cm^{-1}.

8.1.10. 6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone 9a. 6-Amino-2-sulfanyl-4(3*H*)-pyrimidinone monohydrate **8** (8 g, 50 mmol) was suspended in a mixture of water (30 mL) and ethanol (50 mL). Triethylamine (10 g, 0.1 mol) was added and the solution became clear. Benzyl chloride (7 g, 55 mmol) was added to the stirring solution. Within a few minutes, an exothermic reaction started with formation of a colourless precipitate. Stirring was continued for 30 min, the mixture was cooled to 4 °C and the precipitate was filtered and washed with water and diethyl ether to afford the title compound **8a** (10.5 g, 45 mmol, 90%; 248–253 °C (lit.[22] 250–252 °C)). Found: HRMS (EI) 233.0614, $C_{11}H_{11}N_3OS$ requires 233.0623. δ_H (DMSO) 4.33 (2H, s, C(7)H_2), 4.96 (1H, s, C(5)H), 6.54 (2H, br s, N(12)H_2), 7.21–7.32 (3H, m, 2×C(10)H, C(11)H), 7.41–7.43 (2H, m, 2×C(9)H), 11.48 (1H, br s, N(3)H).

8.1.11. 4-Amino-2-(benzylsulfanyl)-5-(trifluoromethyl)-5,6-dihydrofuro[2,3-*d*]pyrimidin-5-ol 10. 6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **9a** (1 g, 4.3 mmol) and bromotrifluoroacetone **224** (1 g, 5.2 mmol) were suspended in ethanol (30 mL) and stirred under nitrogen at 60 °C for 17 h. The solvent was evaporated under reduced

pressure and the residue was dissolved in ethyl acetate, and purified by silica gel column chromatography (1:2 solution of ethyl acetate/*n*-hexane) and then recrystallised from diethyl ether/*n*-hexane. The product **10** was obtained as a white crystalline solid (0.6 g, 1.7 mmol, 40%; mp 145–147 °C). Found: HRMS (EI) 343.0615, $C_{14}H_{12}F_3N_3O_2S$ requires 343.0602. δ_H (DMSO) 4.31 (2H, s, C(8)H_2), 4.33–4.39 (1H, m, 1×C(6)H_2), 4.74–4.77 (1H, m, 1×C(6)H_2), 6.35 (1H, br s, OH), 7.18–7.46 (5H, m, 2×C(10)H, 2×C(11)H, C(12)H). δ_C (DMSO) 34.35 (C-8), 76.12 (C-6), 79.2 (C-13, J=48.2 Hz), 88.42 (C-5), 127.33 (C-4a), 127.35 (C-12), 128.74 (2×C-10), 129.37 (2×C-11), 138.62 (C-9), 160.28 (C-4), 172.46 (C-7a), 174.28 (C-2). ν_{max} (KBr) 3508 and 3305 (NH_2), 3159, 1645, 1606, 1481, 1183, 1172, 713 cm^{-1}.

8.1.12. Ethyl (2*Z*)-3-[4-amino-2-(benzylsulfanyl)-6-oxo-1,6-dihydro-5-pyrimidinyl]-2-(hydroxyimino)-propanoate 11. 6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **9a** (1 g, 4.31 mmol) was dissolved in dry DMF (15 mL). Triethylamine (0.43 g, 4.31 mmol) was added and the mixture was stirred under nitrogen at room temperature. A solution of the ethyl 3-bromopyruvate oxime (1 g, 4.76 mmol) in dry DMF (15 mL) was added over a period of 5 h to the stirred solution with the aid of a syringe pump. Stirring was continued for a further hour after the addition. The solution was evaporated under reduced pressure and the residue was dissolved in ethyl acetate/methanol (4:1 solution, 10 mL). The resulting solution was absorbed on top of a silica gel chromatography column and it was eluted with 100% ethyl acetate, increasing the polarity up to 4:1 ethyl acetate/methanol solution. The product **11** was obtained as a pale yellow solid (0.69 g, 1.90 mmol, 44%; mp 184–186 °C (dec)). Found: HRMS (FAB) 363.1131, $C_{16}H_{19}N_4O_4S$ (M+1) requires 363.1127. δ_H (DMSO) 1.17 (3H, t, J=7.1 Hz, C(18)H_3), 3.44 (2H, s, C(13)H_2), 4.10 (2H, q, J=7.1 Hz, C(17)H_2), 4.33 (2H, s, C(8)H_2), 6.25 (2H, br s, NH_2), 7.22–7.32 (3H, m, 2×C(11)H, C(12)H), 7.42–7.43 (2H, m, 2×C(10)H), 11.66 (1H, br s, NOH), 12.16 (1H, br s, N(1)H). δ_C (DMSO) 14.28 (C-18), 19.67 (C-13), 33.55 (C-8), 60.92 (C-17), 88.56 (C-5), 127.55 (C-12), 128.78 (2×C-11), 129.51 (2×C-10), 138.23 (C-9), 150.66 (C-14), 160.24, 163.05, 163.13, 164.10 (C-2, C-4, C-6, C-15). ν_{max} (KBr) 3498 (NH_2), 3360 (NH), 1731 (C=O), 1575, 1422, 1235, 1128, 770, 705 cm^{-1}.

8.1.13. Ethyl 2-(benzylsulfanyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate 12. Ethyl (2*Z*)-3-[4-amino-2-(benzylsulfanyl)-6-oxo-1,6-dihydro-5-pyrimidinyl]-2-(hydroxyimino)propanoate **11** (0.5 g, 1.38 mmol) was suspended in a mixture of ethanol (25 mL), water (25 mL) and conc. hydrochloric acid (5 drops). Benzaldehyde (4 mL) was added and the reaction mixture was heated to reflux under nitrogen until reaction was complete as shown by TLC. The solvent was removed under reduced pressure using co-evaporation with toluene/ethanol to remove residual water. The residue was suspended in ethyl acetate/diethyl ether (1:1 solution, 20 mL) and filtered to afford the title compound **12** as an off-white microcrystalline solid (0.37 g, 1.12 mmol, 81%; mp 185–188 °C). Found: HRMS (EI) 329.0849, $C_{16}H_{15}N_3O_3S$ requires 329.0834. δ_H (DMSO) 1.31 (3H, t, J=7.1 Hz, C(15)H_3), 4.29 (2H, q, J=7.1 Hz, C(14)H_2), 4.43 (2H, s, C(8)H_2), 7.04 (1H, s, C(5)H), 7.19–7.51 (5H, m, 2×C(10)H, 2×C(11)H, C(12)H), 12.29 (1H, s, N(7)H), 12.66 (1H, s, N(3)H). δ_C (DMSO) 14.63 (C-15), 34.01 (C-8), 60.71 (C-14), 105.95 (C-4a), 109.53 (C-5), 122.85 (C-6), 127.68 (C-12), 128.79 (2×C-10), 129.71 (2×C-11), 137.71 (C-9), 149.83 (C-7a), 157.29 (C-4), 159.05 (C-2), 160.68 (C-13). ν_{max} (KBr) 3486 (NH), 1717 (C=O), 1624, 1495, 1241, 698 cm^{-1}.

8.1.14. 2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 13. To 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **136** (1.0 g, 5.7 mmol) was added phosphoryl chloride (10 mL, 70 mmol) and *N*,*N*-diisopropylamine (1.0 g, 10 mmol) and the solution was heated to reflux overnight. At the conclusion of this period the mixture was poured in cold-ice water (100 mL) and stirred for 1 h, whereupon, dichloromethane (50 mL) was added. This mixture was filtered and the filtrate was washed with methanol, and ether to afford the crude product. Purification by column chromatography on silica gel (ethyl acetate/methanol—4/1) afforded the title compound **13** as an amorphous solid (0.3 g, 1.55 mmol, 27%; mp >260 °C (lit.[20] >300 °C)). Found: HRMS (EI) found 193.0139, 195.0124. $C_7H_4N_5^{35-37}Cl$ requires 193.0155, 195.0126. δ_H (DMSO) 6.91 (2H, br s, N(8)H_2), 8.10 (1H, s, C(6)H), 12.5 (1H, s, N(3)H). δ_C (DMSO) 83.5 (C-5), 106.5 (C-4a), 115.4 (C-9), 134.5 (C-6), 151.5, 155, 160.8 (C-2, C-7a, C-4).

8.1.15. 2-Amino-4-[(4-chlorophenyl)sulfanyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 14. Trifluoroacetic anhydride (2.42 mL, 17 mmol) was added dropwise over a period of 15 min to a stirred suspension of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3a** (1 g, 5.7 mmol) in dry pyridine (15 mL) at 0 °C (ice-water bath) under a nitrogen atmosphere. After 20 min, solid (4-chloro)thiophenol (2.061 g, 14.2 mmol) was added, and the stirred reactants were allowed to warm up to room temperature. After a further period of 2 h, conc. aqueous ammonia (*d* 0.88, 5.7 mL) was added dropwise over a period of 10 min, followed by 27% aqueous hydrogen peroxide (0.57 mL). After the reaction mixture had been stirred for a further period of 3 h, the products were evaporated to dryness under reduced pressure. The residue was re-evaporated with toluene (20 mL) under reduced pressure, and was then shaken with toluene (15 mL) and water (15 mL). The resulting mixture was filtered and the residue washed first with toluene and then with water. Column chromatography of the residue on silica gel with ethyl acetate/acetone 1/1 gave the title compound **14** as an amorphous, nearly colourless solid (0.90 g, 3 mmol, 52%; mp >260 °C). Found: HRMS (EI) 301.0189, 303.0179 $C_{13}H_8^{35-37}ClN_5S$ requires 301.0189, 303.0159. δ_H (DMSO) 6.39 (2H, s, N(8)H_2), 7.50–7.53 (2H, m, 2×C(11)H), 7.60–7.64 (2H, m, 2×C(12)H), 8.00 (1H, s, C(6)H), 12.27 (1H, s, N(7)H). δ_C (DMSO) 83.08 (C-5), 106.12 (C-4a), 116.43 (C-14), 126.92 (C-13), 129.61 (2×C-11), 133.11 (C-6), 134.44 (C-10), 136.76 (2×C-12), 152.98, 159.76, 160.55 (C-2, C-7a, C-4).

8.1.16. Synthesis of *N*-(7-acetyl-5-cyano-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)acetamide 15. A mixture of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile **3a** (2 g, 11.4 mmol), acetic

anhydride (30 mL) and a catalytic amount of DMAP were heated to reflux for 6 h. The mixture was then evaporated under reduced pressure to dryness and the residue was washed with acetone and diethyl ether, to yield the title compound **15** (2.406 g, 9.26 mmol, 81%; mp >240 °C). Found: HRMS (FAB) 260.0765, $C_{11}H_{10}N_5O_3$ (M+1) requires 260.0784. δ_H (DMSO) 2.21 (3H, s, C(10)H_3), 2.84 (3H, s, C(13)H_3), 8.34 (1H, s, C(6)H), 11.71 (1H, br s, N(8)H), 12.11 (1H, br s, N(3)H). δ_C (DMSO) 24.33 (C-13), 25.79 (C-10), 90.40 (C-5), 105.10 (C-4a), 113.98 (C-11), 129.51 (C-6), 148.70, 149.17, 155.46 (C-2, C-4, C-7a), 168.31, 174.27 (C-9, C-12). ν_{max} (KBr) 3450, 3136, 2230 (CN), 1729 (C=O), 1707, 1658, 1371, 782 cm^{-1}.

8.1.17. 7-Acetyl-2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 16. A mixture of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3a** (0.2 g, 1.13 mmol), acetic anhydride (0.159 g, 1.2 mmol) and DMAP (5 mg, catalytic quantity) was gently heated for 5 min with a heating gun. The mixture was then evaporated under reduced pressure, filtered and washed with acetone and diethyl ether and dried under reduced pressure to give the title compound **16** as a colourless solid (0.18 g, 0.83 mmol, 73%; >240 °C). Found: HRMS (FAB) found 218.0669, $C_9H_8N_5O_2$ (M+1) requires 218.0678. δ_H (DMSO) 2.81 (3H, s, C(11)H_3), 6.78 (2H, br s, NH_2), 8.03 (1H, s, C(6)H), 11.11 (1H, s, NH). δ_C (DMSO) 25.77 (C-11), 90.51 (C-5), 100.26 (C-4a), 114.58 (C-9), 126.60 (C-6), 151.88, 154.75, 157.46 (C-2, C-4, C-7a), 168.72 (C-10). ν_{max} (KBr) 3418, 3324, 2228 (CN), 1742 (C=O), 1683, 1635, 1593, 1374, 1310, 780 cm^{-1}.

8.2. General method for the synthesis of benzylsulfanyl- and (4-methylphenyl)sulfanyl-pyrimidines from 6-chloro-2,4-pyrimidinediamine

To a suspension of 6-chloro-2,4-pyrimidinediamine (1 g, 6.94 mmol) and sodium hydroxide (0.35 g, 8 mmol) in ethanol (30 mL) and water (20 mL), the appropriate thiol (1.3 g, 10.46 mmol) was added. The reaction mixture was stirred at 80 °C overnight. The solution was concentrated by evaporation under reduced pressure, and water (20 mL) was added to the residue. The precipitate was collected by filtration, washed with water and diethyl ether, to afford the required product as a white solid.

8.2.1. 6-(benzylsulfanyl)-2,4-pyrimidinediamine 20a. Obtained using benzylthiol in 85% yield (1.37 g, 5.90 mmol), mp 144–146 °C (lit.[23] 146–148 °C). Found: HRMS (FAB) found: 233.0871, $C_{11}H_{13}N_4S$ (M+1) requires 233.0861. δ_H (DMSO) 4.26 (2H, s, C(7)H_2), 5.63 (1H, s, C(5)H), 6.01 (2H, s, NH_2), 6.20 (2H, s, NH_2), 7.20–7.40 (5H, m, 2×C(9)H, 2×C(10)H, C(11)H). δ_C (DMSO) 32.44 (C-7), 90.37 (C-5), 127.26 (C-11), 128.74 (2×C-9), 129.29 (2×C-10), 138.77 (C-8), 162.85 (C-4), 164.14 (C-2), 166.02 (C-6). ν_{max} (KBr) 3440 (NH_2), 3302, 1612, 1562, 1430, 1362, 787, 715 cm^{-1}.

8.2.2. 6-[(4-Methylphenyl)sulfanyl]-2,4-pyrimidinediamine 20b. Obtained using 4-methylbenzenethiol in 94% yield (1.51 g, 6.50 mmol; mp 240–242 °C). Found: HRMS (EI) found: 232.0788, $C_{11}H_{12}N_4S$ requires 232.0783. δ_H (DMSO) 2.35 (3H, s, C(11)H_3), 5.08 (1H, s, C(5)H), 5.97 (2H, br s, NH_2), 6.18 (2H, br s, NH_2), 7.29 (2H, d, *J*=7.8 Hz, 2×C(8)H), 7.44 (2H, d, *J*=7.8 Hz, 2×C(9)H). δ_C (DMSO) 21.71 (C-11), 90.05 (C-5), 126.49 (C-7), 131.26 (2×C-8), 136.36 (2×C-9), 140.16 (C-10), 163.28 (C-4), 164.96 (C-2), 170.12 (C-6) ν_{max} (KBr) 3485, 3371, 1642, 1619, 1558, 1363, 899, 506 cm^{-1}.

8.2.3. 7-(Benzylsulfanyl)imidazo[1,2-*c*]pyrimidin-5-ylamine 21. To a suspension of 6-(benzylsulfanyl)-2,4-pyrimidinediamine **20a** (1 g, 4.31 mmol) and sodium acetate (0.707 g, 8.62 mmol) in water (20 mL) was added chloroacetaldehyde (50% in water) (0.744 g, 4.74 mmol). This was left stirring at 50 °C overnight. After the solution was evaporated to dryness, the residue was suspended in 10 mL of a 4:1 solution of ethyl acetate/methanol and filtered. The solution was purified by silica gel chromatography, using 4:1 ethyl acetate/methanol as eluent. Evaporation of the relevant fractions afforded the title compound **21** as an off-white solid (0.276 g, 1.08 mmol, 25%; mp >240 °C). Found: HRMS (FAB) 257.0851, $C_{13}H_{13}N_4S$ (M+1) requires 257.0861. δ_H (DMSO) 4.32 (2H, s, C(10)H_2), 6.66 (1H, s, C(8)H), 7.21–7.24 (1H, m, C(14)H), 7.28–7.35 (2H, m, 2×C(13)H), 7.39–7.44 (2H, m, 2×C(12)H), 7.40 (1H, s, C(3)H), 7.73 (2H, br s, NH_2), 7.79 (1H, s, C(2)H). δ_C (DMSO) 34.47 (C-10), 95.96 (C-8), 108.37 (C-3), 127.43 (C-14), 128.84 (2×C-13), 129.24 (2×C-12), 133.39 (C-2), 138.10 (C-11), 146.34 (C-7), 147.33 (C-5), 150.93 (C-8a). ν_{max} (KBr) 3435 (NH_2), 3111, 3081, 1670, 1546, 1310, 1156, 931, 742, 704 cm^{-1}.

8.2.4. 5-(Benzylsulfanyl)diimidazo[1,2-*a*:1,2-*c*]pyrimidine 22. To a suspension of 6-(benzylsulfanyl)-2,4-pyrimidinediamine **20a** (0.5 g, 2.15 mmol) and sodium acetate (0.350 g, 4.26 mmol) in water (20 mL) was added chloroacetaldehyde (50% in water) (0.843 g, 5.37 mmol). This was left stirring at 50 °C for 22 h. After the solution was evaporated to dryness, the residue was suspended in 10 mL of a 5:1 solution of ethyl acetate/methanol and filtered. The solution was purified by silica gel chromatography, using 100% ethyl acetate as eluent, and then 4:1 ethyl acetate/methanol. The product was obtained from early fractions as a brown solid (0.144 g, 0.51 mmol, 24%; mp 135–137 °C). Found: HRMS (FAB) 281.0863, $C_{15}H_{13}N_4S$ (M+1) requires 281.0861. δ_H (DMSO) 4.48 (2H, s, C(12)H_2), 7.21 (1H, s, C(6)H), 7.22–7.32 (3H, m, C(16)H, 2×C(15)H), 7.32 (1H, s, C(3)H), 7.36–7.38 (2H, m, 2×C(14)H), 7.55 (1H, s, C(8)H), 7.81 (1H, s, C(2)H), 8.10 (1H, s, C(9)H). δ_C (DMSO) 37.30 (C-12), 104.78 (C-6), 111.90 (C-2), 113.14 (C-9), 128.03 (C-16), 128.48 (C-3), 128.96 (2×C-15), 129.40 (2×C-14), 132.15 (C-13), 132.92 (C-8), 136.15 (C-10a), 136.89 (C-6a), 141.39 (C-5). ν_{max} (KBr) 3125, 3041, 1628, 1568, 1311, 1135, 1030, 858, 707, 695 cm^{-1}.

8.2.5. 5-Amino-7-(benzylsulfanyl)imidazo[1,2-*c*]pyrimidine-3-carbonitrile 23. To a suspension of 6-(benzylsulfanyl)-2,4-pyrimidinediamine **20a** (0.3 g, 1.29 mmol) and sodium acetate (0.212 g, 2.60 mmol) in water (15 mL) was added freshly prepared chloro(formyl)acetonitrile (0.147 g, 4.74 mmol). This was left stirring at 50 °C overnight. After the solution was evaporated to dryness, the residue was suspended in 10 mL of a 4:1 solution of ethyl acetate/methanol and filtered. The solution was

purified by silica gel chromatography, using 4:1 ethyl acetate/methanol as eluent. The title compound **23** was obtained as a brown solid (0.105 g, 0.37 mmol, 29%; mp >240 °C). Found: HRMS (FAB) 282.0819, $C_{14}H_{12}N_5S$ (M+1) requires 282.0813. δ_H (DMSO) 4.45 (2H, s, C(10)H_2), 6.26 (1H, s, C(8)H), 7.22–7.44 (5H, m, 2×C(12)H_2, 2×C(13)H_2, C(14)H), 7.31 (2H, br s, NH_2), 8.37 (1H, s, C(2)H). δ_C (DMSO) 33.23 (C-10), 89.77 (C-8), 92.12 (C-3), 113.42 (C-9), 127.53 (C-14), 128.83 (2×C-12), 129.31 (2×C-13), 137.90 (C-11), 146.26 (C-2), 149.62 (C-8a), 151.70 (C-5), 163.96 (C-7). ν_{max} (KBr) 3340, 3122, 2225 (CN), 1630, 1315, 705 cm^{-1}.

8.2.6. Ethyl 5-amino-7-(benzylsulfanyl)imidazo[1,2-*c*]pyrimidine-2-carboxylate 24. 6-(Benzylsulfanyl)-2,4-pyrimidinediamine **20a** (0.3 g, 1.29 mmol) was dissolved in dry DMF (15 mL). Triethylamine (0.13 g, 1.29 mmol) was added and the mixture was stirred under nitrogen at room temperature. A solution of the ethyl 3-bromopyruvate oxime (0.3 g, 1.42 mmol) in dry DMF (10 mL) was added at room temperature over a period of 5 h to the stirred solution with the aid of a syringe pump. TLC showed no reaction after 2 h, so stirring was continued for a further 10 h at 80 °C. The solution was evaporated under reduced pressure and the residue was dissolved in ethyl acetate/methanol (4:1 solution, 10 mL). The resulting solution was absorbed on top of a silica gel chromatography column and it was eluted with 100% ethyl acetate, increasing the polarity up to 4:1 ethyl acetate/methanol solution. The product **24** was obtained as a brownish solid (0.07 g, 0.21 mmol, 16%; mp >240 °C). HRMS (FAB) found: 329.1064, $C_{16}H_{17}N_4O_2S$ (M+1) requires 329.1072. δ_H (DMSO) 1.30 (3H, t, *J*=7.1 Hz, C(12)H_3), 4.29 (2H, q, *J*=7.1 Hz, C(11)H_2), 4.33 (2H, s, C(14)H_2), 6.66 (1H, s, C(8)H), 7.22–7.25 (1H, m, C(18)H), 7.30–7.33 (2H, m, C(17)H_2), 7.43–7.45 (2H, m, C(16)H_2), 7.92 (2H, br s, NH_2, exchange with D_2O), 8.51 (1H, s, C(3)H). δ_C (DMSO) 14.62 (C-12), 34.41 (C-14), 60.67 (C-11), 95.53 (C-8), 113.99 (C-3), 127.46 (C-18), 128.83 (2×C-16), 129.23 (2×C-17), 136.05 (C-15), 137.84 (C-2), 146.41 (C-8a), 147.42 (C-5), 153.11 (C-9), 162.79 (C-7). ν_{max} (KBr) 3311, 3026, 1716 (C=O), 1626, 1530, 1341, 1014, 787 cm^{-1}.

8.2.7. 4-(Benzylsulfanyl)-6-chloro-2-pyrimidinamine 25. To a suspension of 4,6-dichloro-2-pyrimidinamine (2 g, 12.2 mmol) in 20 mL of ethanol, was added sodium hydroxide (0.48 g, 12.2 mmol) in water (10 mL) with stirring. After 5 min, benzylthiol (1.51 g, 12.2 mmol) was added to the solution and almost immediately a white precipitate formed. Then, water (20 mL) was added to the solution and it was left stirring at room temperature for a further 10 min. The product was collected by filtration, washing extensively with water and diethyl ether, and dried under reduced pressure affording the title compound **25** as a white crystalline solid (2.8 g, 11.06 mmol, 91%; mp 118–120 °C). Found: HRMS (FAB) 252.0367, $C_{11}H_{11}N_3S^{35}Cl$ (M+1) requires 252.0362; found 254.0315, $C_{11}H_{11}N_3S^{37}Cl$ (M+1) requires 254.0333. δ_H (DMSO) 4.39 (2H, s, C(8)H_2), 6.60 (1H, s, C(5)H), 7.20 (2H, s, NH_2), 7.22–7.43 (5H, m, C(12)H, 2×C(11)H, 2×C(10)H). δ_C (DMSO) 32.80 (C-8), 105.18 (C-5), 127.56 (C-12), 128.84 (2×C-11), 129.42 (2×C-10), 137.77 (C-9), 159.36 (C-6), 162.63 (C-2), 171.45 (C-4). ν_{max} (KBr) 3478, 3296, 1633, 1521, 1411, 1215, 816, 785, 696 cm^{-1}.

8.2.8. N^4-Allyl-6-(benzylsulfanyl)-2,4-pyrimidinediamine 26. To a solution of 4-(benzylsulfanyl)-6-chloro-2-pyrimidinamine **25** (2.6 g, 10.3 mmol) in methoxyethanol (30 mL), was added allylamine (2.28 g, 3 mL, 40 mmol) at room temperature. The resulting solution was stirred and heated at 100 °C for 5 days. Then the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using as eluent a gradient of ethyl acetate/*n*-hexane (1:1)→100% ethyl acetate. The product **26** was obtained as a white solid (1.25 g, 4.6 mmol, 45%; mp 135–138 °C). Found: HRMS (FAB) found: 273.1185, $C_{14}H_{17}N_4S$ (M+1) requires 273.1174. δ_H (DMSO) 3.84 (2H, br s, C(14)H_2), 4.26 (2H, s, C(8)H_2), 5.05 (1H, dd, *J*=10.3, 1.6 Hz, 1×C(16)H_2), 5.14 (1H, dd, *J*=17.2, 1.6 Hz, 1×C(16)H_2), 5.66 (1H, s, C(5)H), 5.80–5.89 (1H, m, C(15)H), 6.05 (2H, br s, NH_2), 6.83 (1H, br s, N(13)H), 7.20–7.24 (1H, m, C(12)H), 7.27–7.31 (2H, m, C(10)H_2), 7.38–7.40 (2H, m, C(11)H_2). δ_C (DMSO) 32.48 (C-8), 42.61 (C-14), 90.30 (C-5), 115.38 (C-16), 127.23 (C-12), 128.71 (2×C-10), 129.27 (2×C-11), 136.21 (C-9), 138.82 (C-15), 162.62, 163.05, 165.47 (C-2, C-4, C-6). ν_{max} (KBr) 3458, 3136, 1629, 1594, 1559, 1435, 1232, 1166, 788 cm^{-1}.

8.2.9. *N*′-[4-(Allylamino)-6-(benzylsulfanyl)-2-pyrimidinyl]-*N*,*N*-dimethylimidoformamide 27. To a stirring suspension of N^4-allyl-6-(benzylsulfanyl)-2,4-pyrimidinediamine **26** (0.5 g, 1.84 mmol) in anhydrous DMF (5 mL) was added bis(dimethylamino)-*tert*-butoxymethane (Bredereck's reagent) (0.38 g, 2.2 mmol) and the mixture was heated to 60 °C under nitrogen for 20 min. The resulting solution was concentrated under reduced pressure and the residue was dissolved in ethyl acetate (5 mL). Upon addition of diethyl ether a white product crushed out of solution, which was filtered off and washed with diethyl ether and dried under reduced pressure to afford the title compound **27** as a white solid (0.526 g, 1.6 mmol, 87%; mp 133–135 °C). Found: HRMS (FAB) found: 328.1599, $C_{17}H_{22}N_5S$ (M+1) requires 328.1596. δ_H (DMSO) 2.96 (3H, s, C(10)H_3), 3.06 (3H, s, C(10a)H_3), 3.88 (2H, br s, C(12)H_2), 4.32 (2H, s, C(16)H_2), 5.06 (1H, dd, *J*=10.3, 1.6 Hz, 1×C(14)H_2), 5.15 (1H, dd, *J*=17.2, 1.6 Hz, 1×C(14)H_2), 5.86 (1H, m, C(13)H), 5.94 (1H, s, C(5)H), 7.04 (1H, t, *J*=5.7 Hz, N(11)H), 7.21–7.24 (1H, m, C(20)H), 7.28–7.32 (2H, m, 2×C(19)H), 7.38–7.39 (2H, m, 2×C(18)H), 8.53 (1H, s, C(8)H). δ_C (DMSO) 32.73 (C-16), 34.74 and 40.65 (C-10 and C-10a), 42.75 (C-12), 94.47 (C-5), 115.46 (C-14), 127.24 (C-20), 128.74 (2×C-18), 129.11 (2×C-19), 136.16 (C-13), 138.67 (C-17), 158.26 (C-8), 163.15 (C-2), 165.68 (C-4). ν_{max} (KBr) 3436, 3216, 1625, 1592, 1514, 1344, 1111, 796, 713 cm^{-1}.

8.2.10. *N*′-[1-Allyl-7-(benzylsulfanyl)-2-hydroxy-1*H*,2*H*,3*H*-imidazo[1,2-*c*]pyrimidin-4-ium-5-yl]-*N*,*N*-dimethylimidoformamide chloride 28. To a suspension of *N*′-[4-(allylamino)-6-(benzylsulfanyl)-2-pyrimidinyl]-*N*,*N*-dimethylimidoformamide **27** (0.25 g, 0.76 mmol) and sodium acetate (0.125 g, 1.53 mmol) in acetonitrile (10 mL) and water (3 mL) was added chloroacetaldehyde (50% in water) (0.144 g, 0.91 mmol). This was left stirring at 50 °C overnight. After the solution was evaporated to dryness, the residue was taken in 20 mL of dichloromethane, washed with water (10 mL) and purified by silica

gel column chromatography (100% dichloromethane→ dichloromethane/methanol 9:1). Evaporation of the relevant fractions afforded the title compound **28** as its chloride salt, which was recrystallised from acetone/*n*-hexane as a white crystalline solid (0.144 g, 0.35 mmol, 47%; mp 158–160 °C). Found: HRMS (FAB) found: 370.1706, $C_{19}H_{24}N_5OS$ (M+) requires 370.1702. δ_H (DMSO) 3.17 and 3.32 (2×3H, 2×s, C(15)H_3, C(16)H_3), 4.01–4.12 (2H, m, C(9)H_2), 4.18–4.23 (1H, m, 1×C(3)H_2), 4.34–4.39 (1H, m, 1×C(3)H_2), 4.52 (2H, s, C(17)H_2), 5.21 (1H, d, J=10.3, 1.1 Hz, 1×C(11)H_2), 5.38 (1H, dd, J=17.2, 1.1 Hz, 1×C(11)H_2), 5.52–5.57 (1H, m, C(2)H), 5.78–5.87 (1H, m, C(10)H), 6.52 (1H, s, C(8)H), 7.22–7.38 (5H, m, 2×C(19)H, 2×C(20)H, C(21)H), 7.49–7.51 (1H, m, OH), 8.89 (1H, s, C(13)H). δ_C (DMSO) 34.00 (C-17), 35.91 and 41.98 (C-15, C-16), 44.41 (C-9), 53.12 (C-3), 82.07 (C-2), 88.11 (C-8), 118.76 (C-11), 127.63 (C-21), 128.90 (2×C-19), 129.20 (2×C-20), 131.86 (C-10), 137.58 (C-18), 154.03 (C-8a), 154.68 (C-5), 160.02 (C-13), 173.59 (C-7). ν_{max} (KBr) 3435, 3025, 1626, 1541, 1507, 1262, 1132, 1100, 947 cm^{-1}.

8.2.11. 2-Amino-7-(2′,3′,5′-tri-*O*-benzoyl-α-D-ribofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 29a and 2-amino-7-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 29b. To 2-amino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **3d** (0.3 g, 1.98 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1 g, 1.98 mmol) in 50 mL acetonitrile were added hexamethyldisilazane (0.31 mL, 1.5 mmol), trimethylchlorosilane (0.2 mL, 1.5 mmol) and finally stannic chloride (0.3 mL, 2.5 mmol) in acetonitrile (10 mL) under nitrogen. After a short period of magnetic stirring everything had dissolved and the mixture was stirred for 21 h at room temperature, then dichloromethane (75 mL) was added and the mixture was extracted with aqueous satd $NaHCO_3$ solution. After re-extracting the aqueous phase with dichloromethane, the combined organic phase was washed with satd NaCl solution, dried (Na_2SO_4) and evaporated. The residue was dissolved in dichloromethane and absorbed into the top of a silica gel chromatography column and eluted with dichloromethane (200 mL), then with dichloromethane/methanol—98/2 to give a mixture of two inseparable anomers **29a** and **29b** (unknown ratio) as above (0.40 g, 0.673 mmol, 34%). Found: HRMS (FAB) found 595.1814, $C_{32}H_{27}N_4O_8$ (M+1) requires 595.1829. δ_H($CDCl_3$) 4.58–4.75 (3H, m, C(5′)H_2, C(4′)H), 5.90–6.12 (3H, m, C(3′)H, C(2′)H, C(1′)H), 6.35 (1H, br s, C(5)H), 6.67 (1H, br s, C(6)H), 7.17–7.54 (11H, m, 6×C(9′)H, 3×C(10′)H, NH_2), 7.73–8.14 (6H, m, 6×C(8′)H), 11.20 (1H, br s, N(3)H). δ_C($CDCl_3$) 64.34, 64.65 (C-5′), 70.11, 72.00, 72.91, 74.23, 79.13, 80.25, 81.42, 85.22 (C-1′, C-2′, C-3′, C-4′), 101.57, 101.77 (C-4a), 103.24 (C-5), 118.11, 118.20 (C-6), 128.54–128.80 (C-9′), 128.87–129.74 (C-7′), 129.86–130.26 (C-8′), 133.34–133.85 (C-10′), 150.13, 150.84 (C-7a), 150.57, 151.32 (C-2), 161.09, 161.89 (C-4), 165.31–166.44 (C-6′). ν_{max}(KBr) 3368 (NH_2), 2965 (CH), 1728, (C═O), 1671, 1607, 1580, 1455, 1269, 1125, 712 cm^{-1}.

8.2.12. 2-Amino-7-(2′,3′,5′-tri-*O*-benzoyl-α-D-ribofuranosyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 30a and 2-amino-7-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 30b. To 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **3a** (0.7 g, 3.97 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2 g, 3.97 mmol) in acetonitrile (50 mL) were added hexamethyldisilazane (0.62 mL, 3 mmol), trimethylchlorosilane (0.4 mL, 3 mmol) and finally $SnCl_4$ (0.6 mL, 5 mmol) in acetonitrile (10 mL). After a short period of magnetic stirring everything had dissolved and the mixture was stirred for 24 h at room temperature, then dichloromethane (75 mL) was added and the mixture was extracted with satd $NaHCO_3$ solution. After re-extracting the aqueous phase with dichloromethane, the combined organic phase was washed with satd NaCl solution, dried (Na_2SO_4) and evaporated. The residue was dissolved in dichloromethane and absorbed onto the top of a silica gel chromatography column and eluted with dichloromethane (200 mL), then with dichloromethane/methanol—98/2 to give a mixture of two inseparable anomers **30a** and **30b** as above (0.50 g, 0.81 mmol, 20%). Found: HRMS (FAB) found 620.1766, $C_{33}H_{26}N_5O_8$ (M+1) requires 620.1781. δ_H ($CDCl_3$) 4.48–4.87 (3H, m, C(5′)H_2, C(4′)H), 5.97–6.12 (3H, m, C(3′)H, C(2′)H, C(1′)H), 7.22–7.34 (9H, m, 6×C(9′)H, C(6)H, NH_2), 7.37–7.48 (3H, m, 3×C(10′)H), 7.84–7.95 (6H, m, 3×C(8′)H), 10.18 and 10.79 (1H, br s, N(3)H of **a** and **b**). δ_C($CDCl_3$) 64.15 (C-5′), 71.77, 74.31, 78.77 (C-2′, C-3′, C-4′), 86.18 (C-1′), 87.77 (C-5), 100.85 (C-4a), 115.46 (CN), 128.54–128.61 (C-9′), 128.97–129.43 (C-7′), 129.84–130.03 (C-8′, C-6), 133.44–133.71 (C-10′), 150.70, 151.09, 159.16 (C-2, C-7a, C-4), 165.27–166.80 (C-6′). ν_{max} (KBr) 3307 (NH_2), 2228 (CN), 1725 (C═O), 1681, 1601, 1492, 1271, 1123, 710 cm^{-1}.

8.2.13. 2-Amino-6-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-4(3*H*)-pyrimidinone 33. To a suspension of 2-amino-6-chloropyrimidin-4(3*H*)-one (4 g, 27 mmol) in methoxyethanol (80 mL), was added triethylamine (2.7 g, 27 mmol) and (2,2-dimethyl-1,3-dioxolan-4-yl)methylamine (5 g, 38 mmol) with stirring. The mixture was heated at reflux for 2 days, then the solvent was evaporated under reduced pressure and ethyl acetate (50 mL) was added. The resulting solution was left standing overnight at 4 °C and a pale yellow precipitate formed, which was filtered and washed with little ethyl acetate and diethyl ether and dried under reduced pressure to afford the title compound **33** as colourless microcrystals (4 g, 16 mmol, 62%; mp 134–136 °C). Found: HRMS (FAB) found: 241.1301, $C_{10}H_{17}N_4O_3$ (M+1) requires 241.1301. δ_H (DMSO) 1.24 and 1.32 (2×3H, s, 2×C(13)H_3), 3.17–3.40 (2H, m, C(10)H_2), 3.63 (1H, dd, J=8.2, 6.1 Hz, C(8)H), 3.95 (1H, dd, J=8.2, 6.1 Hz, C(8)H), 4.10–4.16 (1H, m, C(9)H), 4.49 (1H, s, C(5)H), 6.23 (2H, br s, NH_2), 6.41 (1H, br s, N(7)H), 9.77 (1H, br s, N(3)H). δ_C (DMSO) 25.71 and 27.21 (2×C-13), 41.93 (C-8), 67.17 (C-10), 72.52 (C-9), 74.6 (C-5), 108.73 (C-12), 155.47 (C-6), 163.18 (C-4), 164.53 (C-2). ν_{max} (KBr) 3351 (NH_2), 2931, 1639, 1213, 787 cm^{-1}.

8.2.14. 2-Amino-7-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*] pyrimidine-5-carbonitrile 34a and 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile 34b. To a suspension of 2-amino-6-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-4(3*H*)-pyrimidinone **33** (1.5 g, 6.25 mmol) and sodium acetate

(1.2 g, 14.6 mmol) in water (28 mL), was added freshly prepared chloro(formyl)acetonitrile (1.93 g, 18.75 mmol). The mixture was left stirring for 20 h at 50 °C. A precipitate was collected by filtration, washed with water, methanol and diethyl ether to give a 2.5:1 mixture (by NMR) of **34a** and **34b**, respectively (0.995 g, 3.7 mmol, 60%). Found:(FAB) **a**: 290.1263, $C_{13}H_{16}N_5O_3$ (M+1) requires 290.1253. δ_H (DMSO) 1.23 and 1.31 (2×3H, 2×s, 2×C(13)H_3 of **a**), 3.25–3.38 (2H, m, C(10)H_2 of **b**), 3.71–3.74 (2H, m, C(8)H_2 of **b**), 3.79–3.84 (2H, m, C(8)H_2 of **a**), 3.95–3.99 (2H, m, C(10)H_2 of **a**), 4.08–4.12 (1H, m, C(9)H of **b**), 4.35–4.41 (1H, m, C(9)H of **a**), 4.74 (1H, t, *J*=5.5 Hz, C(10)H_2O*H* of **b**), 5.00 (1H, d, *J*=5.2 Hz, C(9)HO*H* of **b**), 6.50 (2H, s, N(2)H_2 of **b**), 6.52 (2H, s, N(2)H_2 of **a**), 7.59 (1H, s, C(6)H of **b**), 7.65 (1H, s, C(6)H of **a**), 10.72 (1H, s, N(3)H of **b**), 10.75 (1H, s, N(3)H of **a**). δ_C (DMSO) 25.47 and 26.95 (2×C-13 of **a**), 47.37 (C-8 of **a**), 48.23 (C-8 of **b**), 63.89 (C-10 of **b**), 66.40 (C-10 of **a**), 70.16 (C-9 of **b**), 77.93 (C-9 of **a**), 84.77 (C-11 of **b**), 85.27 (C-11 of **a**), 99.48 (C-4a of **b**), 99.99 (C-4a of **a**), 109.36 (C-12 of **a**), 115.85 (C-5 of **a**), 116.05 (C-5 of **b**), 131.32 (C-6 of **a**), 131.77 (C-6 of **b**), 151.22 (C-7a of **b**), 151.32 (C-7a of **a**), 154.06 (C-2 of **b**), 154.23 (C-2 of **a**), 157.73 (C-4 of **a**), 157.76 (C-4 of **b**).

8.2.15. 2-Amino-7-[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid 35a and 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid 35b. The obtained 2.5:1 mixture of nitriles **203** and **204** (0.900 g, 3.3 mmol) was dissolved in 5 M aqueous potassium hydroxide (15 mL). The solution was heated to reflux for 5 h. After cooling, the mixture was neutralised with glacial acetic acid and cooled to 5 °C. The precipitate was filtered off, washed with water, ethanol, and diethyl ether, to give the required carboxylic acid as a 1:3 mixture (by NMR) of **35a** and **b**, respectively (0.920 g, 3.2 mmol, 97%). Found: HRMS (FAB) found for **b**: 269.0861, $C_{10}H_{13}N_4O_5$ (M+1) requires 269.0885; for **a**: 309.1217, $C_{13}H_{17}N_4O_5$ (M+1) requires 309.1199. δ_H (DMSO) 1.24 and 1.32 (2×3H, 2×s, 2×C(13)H_3 of **a**), 3.26–3.38 (2H, m, C(10)H_2 of **b**), 3.71–3.88 (2H, m, C(8)H_2 of **b**), 3.72–3.76 (2H, m, C(8)H_2 of **a**), 3.97–4.00 (2H, m, C(10)H_2 of **a**), 4.09–4.17 (1H, m, C(9)H of **b**), 4.40–4.43 (1H, m, C(9)H of **a**), 4.77 (1H, br s, C(10)H_2O*H* of **b**), 5.02 (1H, br s, C(9)HO*H* of **b**), 6.75 (2H, s, N(2)H_2 of **a** and **b**), 7.48 (1H, s, C(6)H of **b**), 7.52 (1H, s, C(6)H of **a**), 11.75 (1H, br s, N(3)H of **a** and **b**), 14.36 (1H, br s, C(14)OOH of **a** and C(11)OOH of **b**). δ_C (DMSO) 25.50 and 26.97 (2×C-13 of **a**), 47.15 (C-8 of **a**), 47.91 (C-8 of **b**), 63.89 (C-10 of **b**), 66.47 (C-10 of **a**), 70.34 (C-9 of **b**), 74.13 (C-9 of **a**), 96.69 (C-4a of **b** and **a**), 109.39 (C-12 of **a**), 109.71 (C-5 of **b**), 110.21 (C-5 of **a**), 129.39 (C-6 of **a**), 130.03 (C-6 of **b**), 152.09 (C-7a of **b**), 152.22 (C-7a of **a**), 154.26 (C-2 of **b**), 154.62 (C-2 of **a**),162.48 (C-4 of **b**), 162.63 (C-4 of **a**), 163.65 (C-14 of **a**, and C-11 of **b**). ν_{max} (KBr) 3436, 3349, 1688, 1646, 1423, 1069, 757 cm^{-1}.

8.2.16. Methyl 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylate 31. To a suspension of the obtained 7:3 mixture of **35a** and **35b** (0.5 g, 1.7 mmol) in methanol (20 mL) was added thionyl chloride (3 mL, 4 mmol). The solution was left stirring for 3 h at 50 °C and then the solvent was evaporated. The resulting residue was taken in water (10 mL), filtered, washed with ethanol and water, and dried under reduced pressure to afford the required ester **31** as an amorphous, off white solid (0.44 g, 1.56 mmol, 92%; mp >240 °C). Found: HRMS (FAB) found: 283.1045, $C_{11}H_{15}N_4O_5$ (M+1) requires 283.1042. δ_H (DMSO) 3.26–3.37 (2H, m, C(10)H_2), 3.69 (3H, s, C(13)H_3), 3.75 (1H, m, C(9)H), 3.86–3.91 (1H, m, C(8)H), 4.13–4.17 (1H, m, C(8)H), 7.05 (2H, br s, N(2)H_2), 7.48 (1H, s, C(6)H). δ_C (DMSO) 48.24 (C-8), 51.33 (C-13), 63.76 (C-10), 70.46 (C-9), 97.90 (C-4a), 109.36 (C-5), 129.89 (C-6), 149.08 (C-7a), 153.14 (C-2), 156.89 (C-4), 163.46 (C-11). ν_{max} (KBr) 3325 (NH_2), 1735 (C=O), 1667, 1311, 1107, 1063, 754 cm^{-1}.

8.2.17. Ethyl 3-(2-amino-4-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-6-oxo-1,6-dihydro-5-pyrimidinyl)-2-(hydroxyimino)propanoate 36. 2-Amino-6-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-4(3*H*)-pyrimidinone **33** (1 g, 4.16 mmol) was dissolved in dry DMF (15 mL). Triethylamine (0.5 g, 5 mmol) was added and the mixture was stirred under nitrogen at room temperature. A solution of ethyl 3-bromopyruvate oxime (1 g, 4.76 mmol) in dry DMF (8 mL) was added over a period of 5 h to the stirred solution with the aid of a syringe pump. Stirring was continued for a further hour after the addition. The solution was evaporated under reduced pressure and the residue was dissolved in water (20 mL). The resulting solution was cooled to 4 °C and filtered to give the title compound **36** as a white solid (0.6 g, 1.62 mmol, 39%; mp 233–237 °C). Found: HRMS (FAB) found: 370.1739, $C_{15}H_{24}N_5O_6$ (M+1) requires 370.1727. δ_H (DMSO) 1.17 (3H, t, *J*=7.1 Hz, C(19)H_3), 1.25 and 1.33 (2×3H, 2×s, 2×C(13)H_3), 3.24–3.54 (2H, m, C(10)H_2), 3.39 (2H, s, C(14)H_2), 3.63 (1H, dd, *J*=8.2, 6.1 Hz, C(8)H), 3.93 (1H, dd, *J*=8.2, 6.1 Hz, C(8)H), 4.01–4.15 (1H, m, C(9)H), 4.10 (2H, q, *J*=7.1 Hz, C(18)H_2), 5.87–5.90 (1H, m, N(7)H), 6.18 (2H, s, N(2)H_2), 9.93 (1H, br s, N(1)H), 12.16 (1H, br s, NOH). δ_C (DMSO) 14.28 (C-19), 19.08 (C-14), 25.75 and 27.19 (2×C-13), 43.63 (C-8), 60.92 (C-18), 67.18 (C-10), 74.89 (C-9), 82.47 (C-5), 108.66 (C-12), 151.71 (C-4), 153.87 (C-2), 161.0 (C-15), 162.13 (C-6), 164.37 (C-16). ν_{max} (KBr) 3425, 3335, 1702 (C=O), 1593, 1483, 1326, 827, 778, 701 cm^{-1}.

8.2.18. Ethyl 2-amino-7-(2,3-dihydroxypropyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate 32. Ethyl 3-(2-amino-4-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}-6-oxo-1,6-dihydro-5-pyrimidinyl)-2-(hydroxyimino)propanoate **36** (0.35 g, 0.95 mmol) was suspended in a mixture of ethanol (50 mL), water (15 mL) and conc. hydrochloric acid (6 drops). Benzaldehyde (4 mL) was added and the reaction mixture was heated under reflux under nitrogen for 2 days. The solvent was removed under reduced pressure using co-evaporation with toluene to remove residual water. The residue was recrystallised from ethanol–water to give the title compound **32** as a white solid (0.18 g, 0.61 mmol, 64%; mp >240 °C). Found: HRMS (FAB) found: 297.1202, $C_{12}H_{17}N_4O_5$ (M+1) requires 297.1199. δ_H (DMSO) 1.28 (3H, t, *J*=7.1 Hz, C(11)H_3), 3.24–3.28 (2H, m, C(14)H_2), 3.75–3.80 (1H, m, C(13)H), 4.21 (2H, q, *J*=7.1 Hz, C(10)H_2), 4.25–4.38 (2H, m, C(12)H_2), 4.53 (1H, t, *J*=5.7 Hz, C(14)H_2O*H*), 4.67 (1H, d, *J*=5.1 Hz, C(13)HO*H*), 6.58 (2H, br s, N(2)H_2), 7.04 (1H,

s, C(5)H), 10.57 (1H, br s, N(3)H). δ_C (DMSO) 15.08 (C-11), 46.58 (C-12), 60.57 (C-10), 64.82 (C-14), 71.46 (C-13), 101.05 (C-4a), 112.51 (C-5), 121.59 (C-6), 154.43 (C-2), 154.85 (C-7a), 159.54 (C-4), 161.47 (C-8). ν_{max} (KBr) 3346 (NH_2), 1698 (C═O), 1603, 1265, 1187, 1098, 752 cm^{-1}.

8.3. Screening for GTPCH inhibitory activity

GTPCH I was kindly supplied by Professor A. Bacher and Dr N. Schramek, of the Technical University, Munich. The enzyme was stored in vials in aqueous solution at −75 °C, at a concentration of 1.6 mg/mL (65 nM/mL of monomer). Individual vials were thawed before use, and centrifuged to remove insoluble material.

The HPLC screening assay was automated using a Shimadzu LC-10ADvp liquid chromatograph linked to a SIL-10ADvp auto-injector and a Shimadzu SPD-M10Avp diode array detector. The column used was a Develosil 5 μm RP Aqueous column, 250×2.0 ID, fitted with a C18 guard column, both from Phenomenex. The mobile phase comprised an aqueous solution containing triethylamine (1%), isopropanol (0.8%) and 85% phosphoric acid (0.3%), as described by Bacher et al.[24] For the assay, a series of vials in the HPLC autosampler contained the test compounds, each vial containing one of the test compounds (4 μL of a 100 mM DMSO solution), GTP (80 μL of a 0.5 mM solution), Tris buffer (20 μL of a 1 M solution, pH 8.5), KCl (20 μL of a 1 M solution) and triply distilled water (16 μL), making a total volume in each vial of 140 μL. The control vial used DMSO (4 μL) containing no test compound. To each of the test vials in turn at time zero, the autosampler added a solution of GTP cyclohydrolase I (60 μL) with mixing, making a total reaction mixture of 200 μL. The final concentration of enzyme in this reaction mixture was 20 μM of active site equivalent (one active site per monomer). The final concentration of GTP in the reaction mixture was 200 μM, thus giving a 10:1 molar ratio of GTP to enzyme active site. Samples of each reaction mixture were injected on to the column after 23, 38, 53 and 68 min reaction time, each chromatogram being run for 13.5 min. Integration of the peak centred at 5.4 min gave a measure of the amount of 7,8-dihydroneopterin triphosphate formed in the reaction.

The UV screening assay used a Shimadzu UV-2401PC ultraviolet–visible spectrophotometer with 400 μL quartz cells of path length 10 mm. For each test compound the following solution was prepared in a vial at 26 °C: GTP (20 μL of a 0.5 mM solution), KCl (20 μL of a 1 M solution), Tris buffer (20 μL of a 1 M solution, pH 8.5), triply distilled water (70 μL) and the test compound (4 μL of either a 50 or100 mM solution in DMSO). At time zero, a solution of GTP cyclohydrolase I at 26 °C (60 μL, 1.6 g/L) was added to the vial, the contents of which were mixed and transferred immediately to the UV cell and placed in the spectrophotometer. Absorption was measured at 330 nm at 1 second intervals over a period of 3 min. Control experiments were carried out using pure DMSO (4 μL) instead of the test compound solution. Data were processed using Microsoft Excel, and slopes were obtained by linear regression on the experimental data points. Percentage inhibition by the test compounds was calculated using the following formula:

$$\% \text{ inhibition} = 100(\text{Control} - \text{Test})/\text{Test},$$

where 'Control' is the rate of reaction in the absence of the test compound and 'Test' is the rate of reaction in presence of the test compound.

Acknowledgements

This research was supported by an EU TMR Network 'Pteridines' EUFMRXCT980204.

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