The syntheses and properties of tricyclic pyrrolo[2,3-*d*]pyrimidine analogues of S^6-methylthioguanine and O^6-methylguanine†

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The syntheses of novel tricyclic pyrrolo[2,3-*d*]pyrimidine analogues of S^6-methylthioguanine are described. The crystal structures and pK_a values of these and related O^6-methylguanine analogues are reported. All compounds display higher pK_a values than O^6-methylguanine with the sulfur-containing analogues being the more basic and exhibiting higher stability in aqueous solution. In a standard substrate assay with the human repair protein O^6-methylguanine-DNA methyltransferase (MGMT) only the oxygen-containing analogue displayed activity.

Introduction

O^6-Methylguanine (**1**) is one of a number of modified bases that can arise in DNA following exposure to alkylating agents. The high toxicity of this lesion derives from the ability of the analogue to mispair with thymine during DNA replication. The human repair protein O^6-methylguanine-DNA methyltransferase[1] (MGMT) repairs this lesion by transferring the alkyl group to an active site cysteine (Cys145) in a stoichiometric, irreversible reaction in which the protein becomes inactivated.[2] MGMT also protects tumour cells from the action of alkylating agents such as temozolomide and BCNU that are used in cancer therapy. This has generated considerable interest in compounds that inactivate MGMT and thereby sensitise tumour cells to killing by these agents.[3,4] Crystal structures of MGMT have been reported for the human protein[5] and an active, truncated human form,[6] and a mechanism for the repair reaction that is performed by MGMT has been suggested by Daniels *et al.*[5] In this mechanism, the nucleophilic thiolate anion of Cys145 reacts with the O6-alkyl group in an S_N2 reaction and Tyr114 is the proposed proton donor required to regenerate guanine within the damaged DNA.

The initial crystal structures of MGMT were obtained in the absence of substrate DNA and under such circumstances, the active site cysteine was observed to be buried in the centre of the protein, far removed from the damaged guanine base. This has led to the search for suitable oligonucleotide pseudosubstrates which might undergo mechanism-based covalent cross-linking to MGMT for subsequent structural investigation. Recently the crystal structure of such a complex between MGMT and an oligonucleotide containing N^1,O^6-ethanoxanthosine (**2**) (the cross-link is shown in Fig. 1) was reported.[7,8] This revealed a repair mechanism involving nucleotide flipping and recognition of the DNA *via* the minor groove using a HTH motif. The authors also report the structure of a C145S mutant of MGMT[8] in complex with O^6-methylguanine-containing DNA which reveals a hydrogen bond between the phenolic OH of Tyr114 and the N-3 position of the modified base.

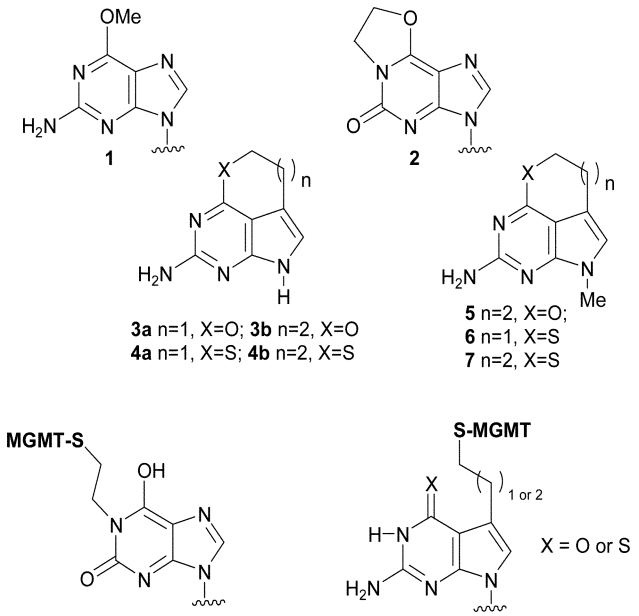

Fig. 1 Modes of cross-linking of analogues **2–7** with MGMT Cys145.

We have also been interested in designing pseudosubstrates of O^6-methylguanine for cross-linking to MGMT and have concentrated our efforts on tricyclic pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) analogues. Recently we reported the syntheses[9] of the novel analogues **3a** and **3b** for which we envisaged covalent modification *via* the C5 (C7 of 7-deazapurine) position upon

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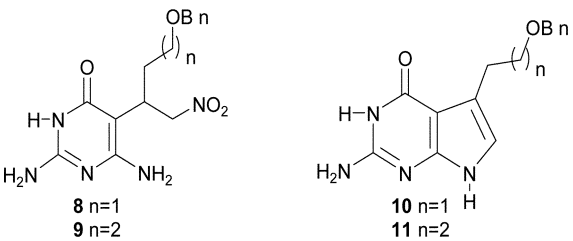
reaction with MGMT (Fig. 1). Such compounds would reveal additional information not present in the existing MGMT–DNA complex,[7,8] in particular interactions between the protein and the N1 and the amino group of the modified base (see Fig. 1) that are likely to be important during the repair of O^6-methylguanine. Unfortunately compound **3a** is too reactive for incorporation into DNA since in aq. solution it is hydrolysed to the corresponding O^6-hydroxyethyl-7-deazaguanine derivative. In contrast compound **3b** is relatively stable and displays an IC_{50} of approximately 1 mM in a standard MGMT assay.[9] Previous studies have shown that synthetic oligonucleotide substrates containing S^6-methylthio- and Se^6-methylselenoguanine analogues are also repaired by MGMT[10] which has encouraged us to prepare the corresponding thio analogues of **3a** and **3b**.

We report here the syntheses of the novel tricyclic sulfur-containing pyrrolo[2,3-*d*]pyrimidines **4a** and **4b**. We also present the crystal structures of the sulfur-containing pyrrolopyrimidines **4a** and **7** together with that of the tricyclic analogue containing the 7-membered oxygen-containing ring (compound **5**). In addition we determine the respective pK_a values of the *N*-methylated tricyclic pyrrolo[2,3-*d*]pyrimidine derivatives **5**, **6** and **7** and report on the abilities of these and their unmethylated derivatives (**3b**, **4a** and **4b**) to act as inactivators of MGMT.

To our knowledge these are the first crystal structures of tricyclic pyrrolo[2,3-*d*]pyrimidines to be described. There are few reports of similar compounds in the literature and these include related nucleoside analogues in which the third ring contains N–N[11] or N–O[12] functionality. Analogues of the former compound (triciribine) display antiviral and antineoplastic properties.

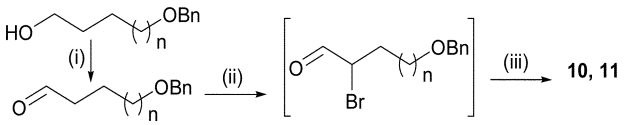
Results and discussion

Previously, compounds **3a** and **3b** were obtained in two steps from the appropriate 5-substituted pyrimidine precursors **8** and **9**, respectively.[9] Compounds **8**[13] and **9**[9] were obtained *via* Michael addition of 2,6-diamino-4(3*H*)pyrimidinone to the appropriate nitroalkenes which were in turn prepared in 4 steps from 1,3-propanediol or 1,4-butanediol respectively. Compounds **8** and **9** were subsequently converted (in 4 steps) to the desired tricyclic pyrrolopyrimidines **3a** and **3b**.[9]

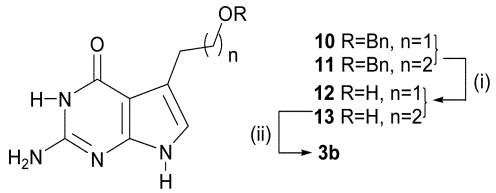


In order to develop a more efficient synthesis of **3b** that could also be applied to obtaining the novel sulfur-containing analogues **4a** and **4b**, we considered an alternative route to 5-substituted pyrrolo[2,3-*d*]pyrimidines that has been used in the synthesis of the queuine base.[14] This method, which involves the reaction of an α-bromoaldehyde with 2,6-diamino-4(3*H*)pyrimidinone, allowed the preparation of compounds **10** and **11** in three steps from the respective diols (Scheme 1).

Debenzylation of **10** and **11** afforded the alcohols **12** and **13** (Scheme 2). Compound **13** was cyclised under Mitsunobu



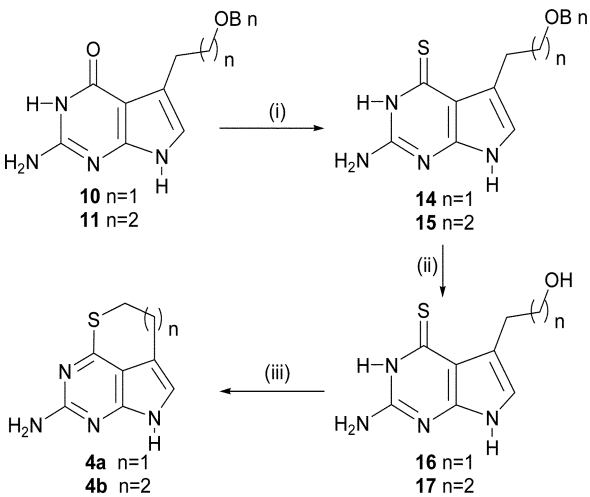
Scheme 1 *Reagents and conditions*: (i) PCC, CH_2Cl_2, $n = 1$ (ref. 13), $n = 2$ (ref. 9); (ii) TMSBr, DMSO, 0 °C to room temp., 4 h; (iii) 2,6-diamino-4(3*H*)pyrimidinone, aq. NaOAc, $n = 1$, 77%, $n = 2$, 31%.



Scheme 2 *Reagents and conditions*: (i) BCl_3, CH_2Cl_2, −78 °C, 6 h, $n = 1$ (ref. 13), $n = 2$ (ref. 9); (ii) $n = 2$, Ph_3P, DIAD, DMF, 41%.

conditions using DIAD and Ph_3P to afford the tricyclic analogue **3b** in 41% yield. This yield was slightly better than that obtained previously using the combination of DEAD and Ph_3P (30% yield).[9]

Thiation of the compounds **10** and **11** using trifluoroacetic anhydride in pyridine followed by treatment with sodium hydrosulfide[15] gave compounds **14** and **15** respectively (Scheme 3). Debenzylation using boron trichloride afforded the alcohols **16** and **17** respectively. The cyclisation of **16** and **17** using the Mitsunobu reaction gave the tricyclic sulfur-containing homologues **4a** and **4b** in 20% and 30% yield respectively. Compound **4a** was also isolated in 22% during extended treatment of compound **14** with BCl_3.



Scheme 3 *Reagents and conditions*: (i) $(CF_3CO)_2O$, pyridine, 0 °C, 1 h, then NaSH in DMF, $n = 1$, 65%, $n = 2$, 69%; (ii) BCl_3, CH_2Cl_2, −78 °C, 9 h, $n = 1$, 57%, $n = 2$, 31%; (iii) Ph_3P, DIAD, DMF, $n = 1$, 20%, $n = 2$, 30%.

In order to obtain information about the physical properties of the tricyclic analogues, compounds **3b**, **4a** and **4b** were all converted into their corresponding *N*-methylated derivatives **5**, **6** and **7** which we envisaged as simple model compounds of the respective nucleosides. Methylation was achieved using sodium hydride and MeI in DMF. Methylation of the 7-deaza analogue of O^6-methylguanine in the same way furnished the methylated pyrrolo[2,3-*d*]pyrimidine analogue **18** of O^6-methylguanine.

Previously we reported that compound **3b** is a weak inactivator of MGMT (IC_{50} approx. 1 mM).[9] To assess the likely potential of the tricyclic thio compounds in DNA to act as cross-linking agents to MGMT we used the same standard assay.[16] This assay involves pre-incubation of MGMT with the inactivator, followed by measurement of the amount of radiolabelling of the protein which occurs upon the subsequent addition of DNA containing tritiated O^6-methylguanine. However, neither of the thio-containing analogues **4a** nor **4b** displayed any activity. The repair of DNA containing O^6-methylguanine by MGMT is approximately 70 times faster than the analogous reaction with the same substrate containing S^6-methylthioguanine.[10] Thus, the apparent inactivities of **4a** and **4b** was not completely unexpected and since the rates of repair of the free base and DNA containing it vary considerably,[2] the compounds **4a** and **4b** might still be recognised once incorporated into DNA. All of the *N*-methylated compounds **5–7** were inactive. This also was not largely unexpected since these compounds are bulkier than the free bases and thereby have decreased access to the MGMT active site which normally is facilitated following binding of DNA.[2] However, **3b** does appear to be a pseudosubstrate of MGMT and clearly further studies following the incorporation of this compound and the sulfur-containing analogues into DNA are necessary.

In the context of our ultimate goal of the incorporation of these analogues into DNA for cross-linking to MGMT we were also interested in the structures, chemical stabilities and pK_a values of these compounds. In previous studies[9] we reported that the analogue **3a** is unstable in aqueous solution and undergoes hydrolysis to the ring-opened 5-hydroxyethyl analogue. Unfortunately we were unable to obtain crystals of **3a** for X-ray analysis. However, we were able to obtain crystal structures of the *N*-methylated derivatives **5** and **7** and the free base **4a** (crystals of compound **6**, the *N*-methylated derivative of **4a**, were unsuitable for X-ray analysis). The structures of compounds **4a**, **5** and **7** are displayed as ball and stick structures in Fig. 2 (see ESI† for colour TIFF and PDB files). The pyrrolopyrimidine C–O and C–S bond lengths in compounds **5** and **7** are 1.35 Å and 1.76 Å respectively. The C–O–C bond angle in compound **5** is 118.1°, whilst the C–S–C bond angle in **7** is 106.1°. Both compounds **5** and **7** display a similar C–C–C bond angle of 112° in the non aromatic ring. In comparison, the mean bond angles in compounds of general structure PhOR and PhSR found in the Cambridge Crystallographic Data Centre are 117.6° and 103.3° with corresponding Ar–X bond lengths of 1.37 Å and 1.76 Å. This suggests that the ring strain within the non aromatic ring in compounds **5** and **7** is small. Furthermore, a gauche arrangement of the methylene protons in these compounds is observed, which also minimises torsional strain. In compound **4a**, the corresponding C–S–C and S–C–C bond angles are 99.3° and 117° respectively with a C–S bond length of 1.75 Å, again suggesting that this compound is also not particularly strained. This is reflected in the stabilities of these compounds towards hydrolysis. Thus, compound **3a** is unstable in aqueous solution,[9] whereas compounds **3b**, **4a** and **4b** remain unchanged after overnight treatment with either aqueous or methanolic ammonia at room temperature. We also note in the crystal structures of these compounds that the electrophilic carbon, at least in structures **5** and **7**, is displaced somewhat from the plane of the pyrrolopyrimidine moiety. This is relevant to the preferred trajectory of nucleophilic addition of the thiolate of Cys145 of MGMT during the repair reaction. O^6-Methylguanine within G:T mispairs adopts the proximal (to the purine N7) conformation,[17] whilst as the nucleoside the distal conformation is preferred.[18] In the crystal structures of MGMT–DNA complexes the guanine lesion is not base paired and neither the proximal nor distal conformation would appear to be incompatible with repair.[8]

The mechanism by which MGMT is proposed to repair O^6-methylguanine lesions in DNA involves protonation of the modified guanine base by a Tyr114 of the protein.[2,5,8] For this reason, analogues designed to act as substrates for MGMT should ideally display pK_a values similar to or above that of O^6-methylguanine (2.35[19]). Thus the pK_a values of compounds **5–7** and **18** (the *N*-methyl derivative of 7-deaza-O^6-methylguanine) were determined by absorption spectroscopy (see experimental for details and Fig. 1 and 2 of the ESI†). The pK_a values are displayed in Table 1 and show that all of the compounds analysed are more basic than O^6-methylguanine and on this basis are not incompatible with repair by MGMT.

Table 1 Determined pK_a values for compounds **5**, **6**, **7** and **18**

Compound	Determined pK_a value
5	4.05 ± 0.05
6	4.31 ± 0.03
7	4.81 ± 0.02
18	4.27 ± 0.02

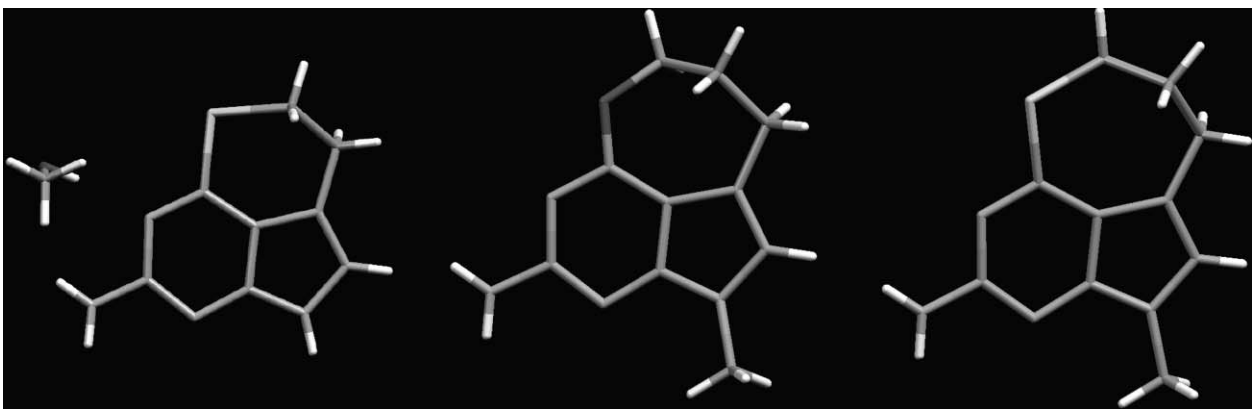


Fig. 2 Crystal structures of compounds **4a** (left), **5** (centre) and **7** (right). The crystal structure of **4a** also contains one molecule of methanol (far left).

We are currently engaged in the synthesis of DNA containing analogues **3b**, **4a** and **4b** which will be reported subsequently together with their biological properties.

Experimental

All nomenclature was generated using the IUPAC online naming service (http://www.iupac.org/nomenclature/index.html)

CH_3CN, CH_2Cl_2 and pyridine were dried by heating under reflux with CaH_2 followed by distillation. Dry DMSO and DMF were purchased from Aldrich. All dry solvents except pyridine were stored over activated 3 Å molecular sieves under Ar. All other reagents were purchased from commercial suppliers and used without purification. Silica gel for column chromatography was obtained from BDH (particle size 30–60 μm). For TLC pre-coated Merck Kieselgel 60 F_{254} aluminium backed plates were used. TLC systems used were A = 10% MeOH in CH_2Cl_2; B = CH_2Cl_2; C = 10% MeOH in EtOAc; D = 20% MeOH in CH_2Cl_2; E = 15% MeOH in CH_2Cl_2.

Melting points were measured on a Gallenkamp Melting Point Apparatus and are uncorrected. UV–Visible data were obtained with a VARIAN CARY 50 Probe Spectrometer. Nuclear magnetic resonance (NMR) spectra were run on a Bruker AC-250 and AMX-400 spectrometers. ^1H spectra were run at 250.13 MHz or 400.13 MHz respectively and ^{13}C spectra at 62.83 MHz or 100.61 MHz respectively.

5-Benzyloxy-1-pentanol

Pentane-1,5-diol (157 g, 1.45 mol) was dissolved in dry DMF (2 L) under Ar and cooled to 0 °C. NaH (40.4 g, 1.6 mol, 95% dispersion in mineral oil) was cautiously added over 60 min and the reaction was stirred for a further 30 min. Benzyl chloride (1.45 mol, 188.8 g, 215 ml) was then added dropwise and the mixture was stirred at room temp. for 24 h. The precipitated solid was filtered, the solvent evaporated and the residue was redissolved in CH_2Cl_2 (1 L), washed with water (300 ml), dried ($MgSO_4$) and evaporated. Distillation under reduced pressure gave a colourless oil (218 g, 77%); bp 110–112 °C (0.1 mm Hg) (lit[20] 123 °C (0.4 mm Hg)); R_f (A) 0.68; δ_H (d_6-DMSO) 1.26–1.51 (6H, m, 3 CH_2), 3.41 (4H, m, CH_2OH, CH_2O), 4.36 (1H, t, OH) 4.42 (2H, s, OCH_2Ph), 7.20–7.40 (5H, m, Ph) ppm; δ_C (d_6-DMSO) 22.78, 29.61, 32.82, 61.13, 70.17, 72.28, 127.76, 127.83 and 128.68, 139.21 ppm; m/z (EI^+) 194 (M^+); acc. mass: 194.1300, $C_{12}H_{18}O_2$ requires 194.1307.

5-Benzyloxypentanal

A solution of 5-benzyloxy-1-pentanol (100 g, 0.52 mol) in CH_2Cl_2 (400 ml) was added to a stirred suspension of PCC (226.5 g, 1.13 mol), Hyflo Super Cel® (230 g), silica (230 g) and CH_2Cl_2 (2 L) at 0 °C. The reaction was stirred at room temp. for 3 h, then filtered and the filtrate was purified on a 3 L column consisting of silica-Hyflo Super Cel® and silica and eluted with CH_2Cl_2 (10 L) to give a pale yellow oil (67.0 g, 68%); R_f (B) 0.42; δ_H (d_6-DMSO) 1.45–1.65 (4H, m, 2 CH_2), 2.46 (2H, m, CH_2CHO), 3.43 (2H, m, CH_2O), 4.41 (2H, s, OCH_2Ph), 7.20–7.40 (5H, m, Ph), 9.66 (1H, t, J 1.5, CHO) ppm; δ_C (d_6-DMSO) 18.94, 29.06, 43.19, 69.72, 72.27, 127.86, 128.69, and 129.63, 135.06, 203.86 ppm; m/z (EI^+) 192 (M^+); acc. mass: 192.1151, $C_{12}H_{16}O_2$ requires 192.1150.

2,7,8,9-Tetrahydro-6-oxa-2,3,5-triazabenzo[*cd*]azulen-4-amine 3b

Diisopropylazodicarboxylate (DIAD) (1.10 g, 1064 μL, 5.40 mmol) was added dropwise to Ph_3P (1.42 g, 5.40 mmol) in dry DMF (30 ml) under Ar at room temp. The mixture was then stirred at room temp. for 30 min, **13** in dry DMF (20 ml) was added dropwise over 20 min and the mixture was stirred overnight. After evaporation the residue was adsorbed onto silica and purified by column chromatography (10–20% MeOH in CH_2Cl_2) then triturated with acetonitrile (10 ml), to give a cream-coloured solid (337 mg, 41%). Data identical to those described.[9]

7-Amino-3,4-dihydro-1*H*-5-thia-1,6,8-triazaacenaphthylene 4a

Method 1. Compound **16** (65 mg, 0.31 mmol) and Ph_3P (246 mg, 0.93 mmol) were dissolved in dry DMF (30 ml) under Ar and the solution was cooled to 0 °C. DIAD (193 μL, 0.93 mmol) was then added dropwise and the mixture was then stirred overnight at room temp. The reaction mixture was then evaporated and the crude product was purified by silica gel column chromatography (5–10% MeOH in CH_2Cl_2) and then recrystallised from MeOH to give dark orange-brown needles (12 mg, 20%); found: C, 49.96; H, 4.22; N, 28.95; S, 16.76. $C_8H_8N_4S$ requires C, 49.98; H, 4.19; N, 29.14; S, 16.68%; mp > 350 °C (decomp.); R_f (E) 0.65 (fluorescent at 365 nm), **16** = 0.33 (fluorescent at 365 nm); pH = 7.87 (T = 21.4 °C); λ_{max}(MeOH)/nm 234.9, 326.2 (log ε/dm^3 mol^{-1} cm^{-1} 4.30, 3.53); λ_{min}(MeOH)/nm 219.8, 284.4 (log ε/dm^3 mol^{-1} cm^{-1} 4.05, 3.01); luminescence (MeOH; c = 2.71 × 10^{-6} M): λ_{Exc} = 240 nm, λ_{Emm} = 405 nm; λ_{Exc} = 325 nm, λ_{Emm} = 405 nm; δ_H (d_6-DMSO) 2.89 (2H, t, J 6.4, CH_2CH$_2$S), 3.27 (2H, t, J 6.4, CH$_2$S), 6.09 (2H, s, NH$_2$), 6.62 (1H, d, J 1.2, CH-2), 10.77 (1H, s, NH-1) ppm; δ_C (d_6-DMSO) 21.92, 31.02, 107.24, 109.78, 114.73, 150.09, 160.20, 160.34 ppm; m/z (ES^+) 193 ([M + H]$^+$, 100%); acc. mass: 193.0551, $C_8H_9N_4S$ requires 193.0548.

Method 2. BCl_3 (1 M) in heptane, (43.7 mL, 43.7 mmol) was added dropwise to compound **14** (1.50 g, 4.86 mmol) in dry CH_2Cl_2 (90 ml) at −78 °C under Ar. The reaction was stirred at −78 °C for 4 h, then more BCl_3 solution (19.5 mL, 19.5 mmol) was added and stirring was continued for a further 6 h. The mixture was then warmed to room temp. overnight whilst a solution of EtOH in CH_2Cl_2 (220 mL, 1 : 1) was added dropwise. The residue was purified after evaporation by silica column chromatography (10% MeOH in CH_2Cl_2) followed by recrystallisation from MeOH to give dark orange-brown needles (200 mg, 22%). Compound **16** was also obtained in this reaction (236 mg, 23%).

2,7,8,9-Tetrahydro-6-thia-2,3,5-triazabenzo[*cd*]azulen-4-amine 4b

Preparation analogous to **3b**: using **17** (360 mg, 1.61 mmol) in dry DMF (20 ml) and DIAD (649 mg, 622 μL, 3.21 mmol) and Ph_3P (842 mg, 3.21 mmol) in dry DMF (25 ml). Purification gave a light brown solid (100 mg, 30%); R_f (A) 0.5; δ_H (CD_3OD) 2.15–2.28 (2H, m, SCH$_2$CH_2), 2.92–2.97 (2H, m, SCH$_2$CH$_2$CH_2), 3.10–3.13 (2H, m, SCH_2), 6.75 (1H, t, J 1.3, CH-6) ppm; δ_C (d_4-CD_3OD) 26.43, 30.01, 31.85, 77.35, 97.25, 117.22, 124.98, 152.95, 155.25, 160.51 ppm; m/z (EI^+) 206 (M^+); acc. mass: 206.0633, $C_9H_{10}N_4S$ requires 206.0626 (deviation 3.1 ppm).

2-Methyl-7,8,9-tetrahydro-6-oxa-2,3,5-triazabenzo[*cd*]azulen-4-amine 5

To compound **3b** (320 mg, 1.68 mmol) in dry DMF (6.5 ml) under Ar at 0 °C was added NaH (84 mg, 2.1 mmol, 60% dispersion in oil) and the mixture was stirred for 30 min. MeI (265 mg, 116 μL, 1.86 mmol) was then added dropwise at 0 °C and the mixture was then stirred at room temp. overnight. MeOH (5 ml) was then added and the mixture was evaporated. The residue was purified by silica column chromatography (10% MeOH in CH_2Cl_2) and recrystallised from CH_2Cl_2–EtOAc to give cream-coloured needles (320 mg, 93%); mp 217–219 °C; R_f (A) 0.5; found: C, 58.60; H, 5.94; N, 26.28. $C_{10}H_{12}ON_4$ requires C, 58.8; H, 5.9; N, 27.4%; λ_{max} (MeOH)/nm 270.02 (log ε/dm^3 mol^{-1} cm^{-1} 3.65); λ_{min} (MeOH)/nm 280.06 (log ε/dm^3 mol^{-1} cm^{-1} 3.51); λ_{sh} (MeOH)/nm 261.06 (log ε/dm^3 mol^{-1} cm^{-1} 3.62); δ_H (d$_6$-DMSO) 2.02 (2H, m, CH_2), 2.74 (2H, t, *J* 5.6, CH_2), 3.50 (3H, s, NCH_3), 4.32 (2H, m, OCH_2), 6.01 (2H, s, NH_2), 6.67 (1H, s, C*H*-6) ppm; δ_C (CD_3OD) 26.21, 29.19, 31.79, 78.64, 98.43, 117.82, 125.70, 152.98, 155.25, 160.54 ppm; *m*/*z* (EI$^+$) 204 (M$^+$); acc. mass: 204.1016, $C_{10}H_{12}ON_4$ requires 204.1011.

7-Amino-1-methyl-3,4-dihydro-1*H*-5-thia-1,6,8-triazaacenaphthylene 6

Preparation analogous to **5**: using **4a** (200 mg, 1.04 mmol), NaH (48 mg, 1.20 mol, 60% dispersion in oil) and MeI (75.5 μL, 1.20 mmol) in dry DMF (3 ml). Chromatography gave an orange foam (188 mg, 88%). Recrystallisation from MeOH afforded orange needles; mp 48–50 °C, R_f (A) 0.48 (product; fluorescent at 365 nm), **6** = 0.35 (fluorescent at 365 nm); pH = 7.92 (*T* = 23.8 °C); λ_{min}(MeOH)/nm 222.0, 291.7 (log ε/dm^3 mol^{-1} cm^{-1} 3.92, 3.31); λ_{max}(MeOH)/nm 238.2, 326.4 (log ε/dm^3 mol^{-1} cm^{-1} 4.17, 3.48); luminescence (MeOH; $c = 2.52 \times 10^{-6}$ M): λ_{Exc} = 240 nm, λ_{Emm} = 410 nm; λ_{Exc} = 325 nm, λ_{Emm} = 410 nm; δ_H (d$_6$-DMSO) 2.89 (2H, t, *J* 6.4, CH_2CH$_2$S), 3.28 (2H, t, *J* 6.4, CH$_2$S), 3.35 (3H, s, CH$_3$N), 6.26 (2H, s, NH$_2$), 6.66 (1H, d, *J* 1.2, CH-2) ppm; δ_C (d$_6$-DMSO) 21.58, 30.44, 30.81, 107.19, 109.46, 119.13, 149.22, 160.37, 160.40 ppm; *m*/*z* (ES+) 207 ([M + H]$^+$, 100%); acc. mass: 206.062397, $C_9H_{10}N_4S$ requires 206.062618.

2-Methyl-7,8,9-tetrahydro-6-thia-2,3,5-triazabenzo[*cd*]azulen-4-amine 7

Preparation analogous to **5**: using **4b** (99 mg, 0.48 mmol), NaH (24 mg, 0.6 mmol, 60% dispersion in oil) and MeI (36 μL, 0.5 mmol) in dry DMF (2 ml). Chromatography gave a light brown solid (65 mg, 62%); R_f (A) 0.6; δ_H (CD_3OD) 2.21–2.27 (2H, m, SCH$_2$CH_2), 2.91–2.95 (2H, m, SCH$_2$CH$_2$CH_2), 3.10–3.12 (2H, m, SCH_2), 3.57 (1H, s, NCH_3), 6.71 (1H, t, *J* 1.3, C*H*-6) ppm; δ_C (CD_3OD) 29.24, 30.09, 31.30, 111.59, 117.61, 129.49, 152.68, 153.53, 158.32 ppm; *m*/*z* (ES$^+$) 221 ([M + H]$^+$); acc. mass: 221.0681, $C_{10}H_{13}N_4S$ requires 221.0861.

2-Amino-5-[2-(benzyloxy)ethyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 10

A solution of 4-(benzyloxy) butanal[13] (7.25 g, 40.7 mmol) in dry CH_3CN (100 ml) was cooled to 0 °C. Bromotrimethylsilane (6.4 mL, 47.0 mmol) and dry DMSO (3.35 mL, 47.0 mmol) were then added dropwise *via* a syringe and the solution was stirred at room temp. for 4 h. A suspension of 2,6-diamino-4(3*H*)pyrimidinone (5.88 g, 44.75 mmol) and sodium acetate (3.67 g, 44.75 mmol) in water (100 ml) was then added and the reaction stirred overnight. The mixture was extracted with EtOAc (4 × 1 L) and the organic layers were washed with brine (500 ml), dried (Na_2SO_4) and evaporated. Purification by silica chromatography (10% MeOH in EtOAc) gave a cream coloured solid (8.94 g, 77%). Data as described.[13]

2-Amino-5-[3-(benzyloxy)propyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one 11

Preparation analogous to **10**: using 5-benzyloxypentanal (6 g, 31.2 mmol), dry CH_3CN (100 ml), bromotrimethylsilane (35.9 mmol, 4.88 ml), dry DMSO (34.3 mmol, 2.44 ml) and 2,6-diamino-4(3*H*)pyrimidinone (34.3 mmol, 4.51 g) and sodium acetate (34.3 mmol, 4.51 g) in water (100 ml). Work-up and purification gave a pink-white solid (2.85 g, 31%). Data as described.[9]

2-Amino-5-(3-benzyloxyethyl)-3,7-dihydro-pyrrolo[2,3-*d*]pyrimidin-4-thione 14

Sodium hydrosulfide hydrate (NaSH·xH_2O) (14.8 g, 263.8 mmol) was dried over a naked flame, suspended in dry DMF (200 ml) and stirred overnight. Compound **10** (2.5 g, 8.8 mmol) was then dried by co-evaporation of dry pyridine (3 × 20 ml). The resulting syrup was dissolved in dry pyridine (120 ml) under Ar and cooled to 0 °C. Trifluoroacetic anhydride (9.9 mL, 70.4 mmol) was then added dropwise and the mixture stirred for 1 h at 0 °C. The suspension of NaSH in DMF was then added and stirring continued at room temp. overnight. The mixture was then poured into saturated aq. ammonium bicarbonate solution (500 ml) and stirred vigorously at room temp. overnight. The mixture was then evaporated and the residue was extracted into MeOH (600 ml). The MeOH was then evaporated and the residue was triturated with aq. triethylammonium acetate (0.1 M, 500 mL; pH 5.2), dried under vacuum and purified by silica column chromatography (5% MeOH in CH_2Cl_2) to give an orange foam (1.7 g, 65%); R_f (D) 0.68 (product) (**10** = 0.52[11]); λ_{max}(MeOH)/nm 235, 264 (log ε/dm^3 mol^{-1} cm^{-1} 3.24, 3.17); λ_{min}(MeOH)/nm 228, 251, 299 (log ε/dm^3 mol^{-1} cm^{-1} 3.23, 3.13, 2.96); λ_{sh}(MeOH)/nm 343 (log ε/dm^3 mol^{-1} cm^{-1} 2.98); δ_H (d$_6$-DMSO) 3.19 (2H, t, *J* 7.0, CH_2CH$_2$OBn), 3.69 (2H, td, *J* 4.2 and 2.7, CH_2OBn), 4.48 (2H, d, *J* 1.8, OCH_2Ph), 6.40 (2H, s, NH$_2$), 6.63 (1H, s, CH-6), 7.19–7.34 (5H, m, C*H*-Ph), 11.04 (1H, s, NH-3), 11.22 (1H, s, NH-7) ppm; δ_C (d$_6$-DMSO) 26.43, 70.84, 71.62, 115.87, 116.19, 127.21, 127.36, 128.15, 128.90, 138.88, 151.84, 152.10, 175.29 ppm; *m*/*z* (ES+) 301 ([M + H]$^+$, 100%); acc. mass: 301.1123, $C_{15}H_{17}N_4OS$, requires 301.1126.

2-Amino-5-(3-benzyloxypropyl)-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-thione 15

Preparation analogous to **14**: using **11** (16 g, 53.6 mmol), trifluoroacetic anhydride (90.1 g, 428.8 mmol, 61 ml), dry pyridine (660 ml) and dried NaSH·xH_2O (80 g, 1.42 mol) in dry DMF (2 L). Work-up and purification gave a light brown solid (11.6 g, 69%); R_f (D) 0.74 0.37 (lit.[9] **11** = 0.68); δ_H (d$_6$]-DMSO) 1.85–1.95

(2H, m, CH_2), 2.88 (2H, t, *J* 7.6, CH_2), 3.44 (2H, t, *J* 6.4, CH_2O), 4.44 (2H, s, OCH_2Ph), 6.39 (2H, s, NH_2), 6.53 (1H, s, H-6), 7.31 (5H, m, Ph), 10.97 (1H, s, N*H*), 11.41 (1H, s, N*H*); δ_C (d_6-DMSO) 23.76, 30.40, 69.40, 71.72, 111.06, 117.15, 119.41, 127.27, 127.45, 127.64, 127.82 and 128.19, 138.78, 148.54, 151.81, 175.33; *m/z* (ES^+) 315 ($[M + H]^+$); acc. mass 315.1274, $C_{16}H_{19}ON_4S$ requires 315.1280.

2-Amino-5-(2-hydroxyethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidine-4-thione 16

BCl_3 (1 M) in heptane, (33.6 mL, 33.6 mmol) was added dropwise to a solution of compound **14** (1.12 g, 3.73 mmol) in dry CH_2Cl_2 (80 ml) at −78 °C under Ar and the reaction was stirred at that temp. for 9 h. The mixture was then warmed to room temp. overnight whilst a mixture of EtOH–CH_2Cl_2 (200 mL, 1 : 1) was added dropwise. The mixture was then evaporated, redissolved in ethanol (50 ml) and neutralized with aq. sodium hydroxide solution (4 M). The residue was purified after evaporation by silica column chromatography (10% MeOH in CH_2Cl_2) to give a pale brown solid (447 mg, 57%); R_f (E) 0.30, **14** = 0.62; λ_{max}(MeOH)/nm 234.6, 270.6, 347.1 (log ε/dm^3 mol^{-1} cm^{-1} 4.11, 3.84, 3.86); λ_{min}(MeOH)/nm 225.0, 251.6, 302.2 (log ε/dm^3 mol^{-1} cm^{-1} 4.00, 3.56, 3.24); λ_{sh}(MeOH)/nm 285.2 (log ε/dm^3 mol^{-1} cm^{-1} 3.73); δ_H (d_6-DMSO) 2.98 (2H, t, *J* 7.0, CH_2CH_2OH), 3.60 (2H, t, *J* 7.0, CH_2OH), 4.43 (1H, t, *J* 5.2, OH), 6.41 (2H, bs, NH_2), 6.59 (1H, bs, CH-6), 11.01 (1H, bs, NH-3), 11.43 (1H, bs, NH-7) ppm; δ_C (d_6-DMSO) 29.80, 62.06, 111.14, 116.56, 117.97, 148.40, 151.77, 175.27 ppm; *m/z* (ES^+) 211 ($[M + H]^+$, 100%); acc. mass: 211.0651, $C_8H_{11}N_4OS$ requires 211.0654.

2-Amino-5-(3-hydroxypropyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-thione 17

Preparation analogous to **16**: using **15** (0.5 g, 1.59 mmol), CH_2Cl_2 (25 ml), BCl_3 in heptane (14.2 ml, 14.18 mmol). Work-up and purification gave a pale brown solid (110 mg, 31%); R_f (D) 0.53; δ_H (d_6-DMSO) 1.70–1.80 (2H, m, CH_2), 2.79 (2H, t, *J* 6.4, CH_2), 3.35 (2H, t, *J* 6.4, CH_2OH), 4.32 (1H, t, O*H*), 6.37 (2H, bs, NH_2), 6.55 (1H, s, H-6), 10.92 (1H, bs, N*H*), 11.36 (1H, bs, N*H*); δ_C (d_6-DMSO) 22.72, 34.01, 60.72, 99.30, 113.65, 118.64, 151.68, 152.74, 175.91; *m/z* (ES^+) 247 ($[M + Na]^+$); acc. mass: 247.0635, $C_9H_{12}ON_4SNa$ requires 247.0630.

2-Amino-4-methoxy-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 18

Preparation analogous to **5**: using 2-amino-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine.[21] Yield 91%. Data as described.[21]

pK_a determination of compounds 5, 6, 7 and 18

UV spectra of solutions of the respective compounds in the appropriate buffer (1 mL, 50 μM) were measured at low pH (0.1) and then at high pH (8.0) to determine the wavelength where the largest difference in absorbance was observed between the two spectra. The absorbance at this wavelength (which, for **5** was 294 nm, for **6** and **7** was 292 nm and for **18** was 264 nm) was then measured over a range of pH values.[22] A succinic acid buffer was used for pH values above 3.40, a chloroacetic acid buffer was used for pH values between 2.20 and 3.40 and for pH measurements below 2.20, dilutions of hydrochloric acid were used as described.[23] Absorption spectra were measured at 20 °C (after 5 min pre-incubation). The absorbance values were plotted against pH and the pK_a values were determined following non-linear regression fitting to eqn (1) using Kaleidagraph software (Synergy Software, Reading, PA, USA).

$$A = A_I + (A_M - A_I) \cdot \left(\frac{K_a}{K_a + [H^+]} \right) \quad (1)$$

Where A is the absorbance at the measured wavelength, A_I is the absorbance of the cation and A_M is the absorbance of the neutral molecule.

Crystal structure determination of compound 4a ($C_8H_8N_4S{\cdot}CH_3OH$)¶

Crystal data. $C_9H_{12}N_4OS$, M = 224.29, monoclinic, a = 8.6545(12), b = 15.734(2), c = 7.7979(11) Å, U = 1055.1(3) Å^3, T = 150(2) K, space group $P2_1/c$, Z = 4, μ(Mo–Kα) = 0.286 mm^{-1}, 11534 reflections measured, 2391 unique (*R*int = 0.0276) which were used in all calculations. The final *wR*(*F*2) was 0.0948 (all data).

Crystal structure determination of compound 5

Crystal data. $C_{10}H_{12}N_4O$, M = 204.24, orthorhombic, a = 7.9420(17), b = 13.862(3), c = 8.4623(19) Å, U = 931.6(4) Å^3, T = 150(2) K, space group $Pna2_1$, Z = 4, μ(Mo–Kα) = 0.100 mm^{-1}, 9204 reflections measured, 1141 unique (*R*int = 0.0369) which were used in all calculations. The final *wR*(*F*2) was 0.1186 (all data).

Crystal structure determination of compound 7

Crystal data. $C_{10}H_{12}N_4S$, M = 220.30, monoclinic, a = 7.8532(12), b = 12.4259(19), c = 11.0895(17) Å, U = 1024.4(3) Å^3, T = 150(2) K, space group $P2_1/c$, Z = 4, μ(Mo–Kα) = 0.286 mm^{-1}, 8569 reflections measured, 2325 unique (*R*int = 0.0305) which were used in all calculations. The final *wR*(*F*2) was 0.1328 (all data).

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