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The syntheses of tricyclic analogues of O^6-methylguanine

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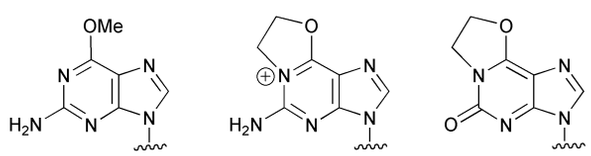
The syntheses of the novel pyrrolo[2,3-*d*]pyrimidine-based heterocycles as tricyclic analogues of O^6-methylguanine are described. Compound **5** is a weak inhibitor of human O^6-alkylguanine DNA alkyltransferase.

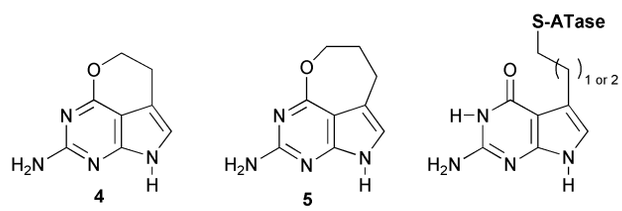
Introduction

The occurrence of the modified base O^6-methylguanine (**1**) in DNA has been implicated in the toxic, mutagenic and carcinogenic effects of exposure of DNA to alkylating agents[1] due to its potential for mispairing with thymine resulting in G to A mutations during replication.[2–5] Many organisms possess an O^6-alkylguanine-DNA alkyltransferase (ATase) which repairs this damage to the DNA by an S_N2 reaction in which the alkyl group is transferred to a cysteine residue rendering the protein inactive.[6–8] Since certain tumour cells possess elevated levels of ATase activity there has been much interest in designing effective inhibitors of the human protein and understanding the repair reaction at the molecular level.[9–19] Two crystal structures of the human protein in the absence of DNA substrate have been reported,[8,20] one of which was also obtained following alkylation of the active site cysteine, using the previously known inhibitor O^6-benzylguanine.[21] Whilst these studies have revealed a great deal about how the repair reaction takes place, there is currently no structural information available which reveals the details of the protein bound to its DNA substrate. In the absence of suitable mutant proteins,[22,23] an attractive possibility to obtain detailed structural information involves using a modified DNA substrate which could be covalently cross-linked to the protein for subsequent analysis using X-ray crystallography.[24–27]



1 **2** **3**

It has been known for some time that DNA containing the highly reactive analogue (**2**) can be cross-linked to the protein.[28] However the high reactivity of such analogues precludes their incorporation into synthetic DNA using standard automated synthesis. More recently it has been shown that the related compound (**3**) allows covalent cross-linking to the protein, but has a suitably reduced reactivity which permits its incorporation into synthetic DNA.[29] However, the exact mode of cross-linking for this analogue has not been established and furthermore it lacks the 2-amino group of the natural base which is thought to be involved in an interaction with a lysine side chain of the protein. We have been interested for some time in the design and synthesis of analogues of O^6-alkylguanine which retain the main features of the parent compound but following incorporation into synthetic DNA would allow the mechanism-based, covalent cross-linking to the human ATase. With this goal in mind, we have synthesised the novel tricyclic base analogues (**4**) and (**5**) as suitable candidates for incorporation into DNA in order to achieve cross-linking to the ATase protein (Fig. 1)



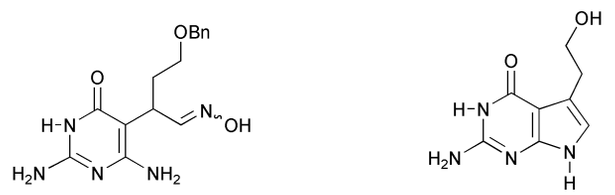
4 **5**

Fig. 1 Structures of compounds **4** and **5** and their expected mode of cross-linking with the active site cysteine of Atase.

We describe here chemistry directed to the synthesis of the heterocycles **4** and **5** and report preliminary results on their biological activity as inhibitors of human ATase.

Results and discussion

We have recently described the synthesis of the pyrimidine (**6**) as a precursor to the 5-substituted pyrrolo[2,3-*d*]pyrimidine **7** (following debenzylation).[30] We envisaged that the synthesis of the target heterocycle **4** might be achieved from a condensation reaction on **7**. Alternatively, the nitroalkane precursor (**8**) of compound **6** might be converted, following deprotection, to a bicyclic pyranopyrimidine derivative and which upon subsequent formation of the pyrrole ring would afford analogue **4**.



6 **7**

All attempts at using the Mitsunobu reaction employing triphenylphosphine and DEAD to achieve the cyclisation of the alcohol **7** were unsuccessful. In each case a mixture of products was obtained in addition to unreacted starting material. One product which we were able to characterise from this mixture was compound **9**. The formation of compound **4** from **7** during the Mitsunobu reaction would be expected to proceed *via* nucleophilic attack of the lactam oxygen on the triphenylphosphonium species derived from the alcohol. It is likely that considerable strain might arise in such a transition state leading to **4** and consequently the products which are formed are those

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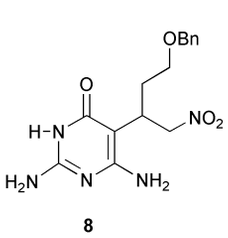
derived from alternative reactions on the phosphonium species. Thus compound **9** would be obtained following reaction with the anion derived from DEAD with the phosphonium species. Mitsunobu reactions employing other reagents *e.g.* tributylphosphine and DIAD (diisopropyl azodicarboxylate) were also unsuccessful.



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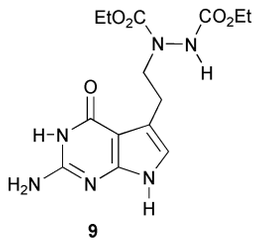
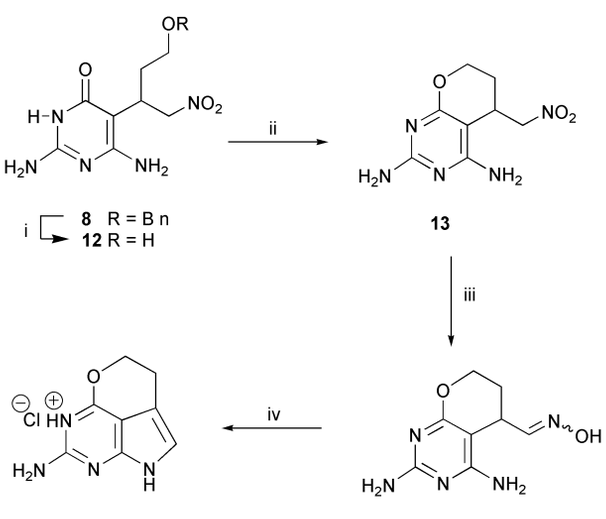


9

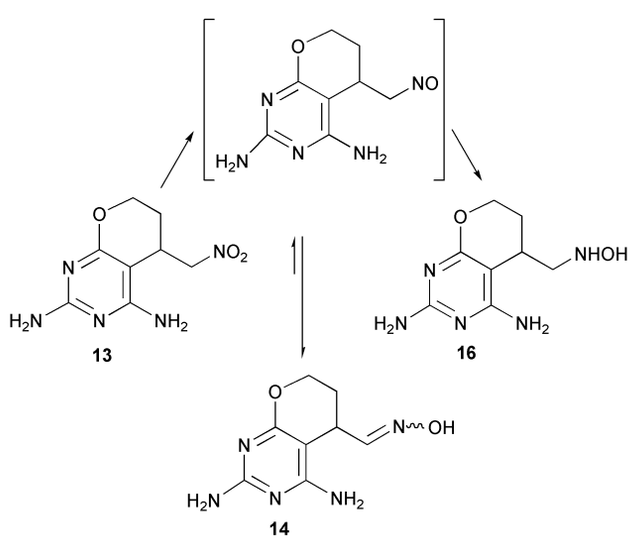
Since cyclisation of the dihydropyrano ring of the pyrrolopyrimidine **7** had been unsuccessful, as an alternative route to **4**, we considered the cyclisation of the dihydropyrano-, prior to the pyrrolo-ring using the known compound **8**. Thus compound **8** was first deprotected with boron trichloride to give **12** (Scheme 1). Initially, we investigated using the Mitsunobu reaction for this cyclisation since problems of ring strain which might prevent cyclisation (as was found previously for **7**) were not anticipated. Thus, treatment of compound **12** with an excess of triphenylphosphine and diethyl azodicarboxylate afforded the desired bicyclic compound **13**, although only in poor yield. However, when the betaine formed by prior reaction of the triphenylphosphine and diethyl azodicarboxylate was added dropwise to a solution of the pyrimidine **12**, the compound **13** was obtained in 57% yield following chromatography and recrystallisation.[31] On smaller scales (about 500 mg) however, the reaction typically afforded yields between 30 and 40% of the desired product.



Scheme 1 *Reagents and conditions*: (i) BCl_3, CH_2Cl_2, −78 °C, 6 h, 92%; (ii) PPh_3, DEAD, DMF, room temp., 16 h, 56%; (iii) $SnCl_2$, PhSH, NEt_3, CH_3OH–CH_3CN, room temp., 6 h, 56%; (iv) DMF, VCl_2 (aq), room temp., 18 h, 20%.

During the course of this work whilst engaged in the synthesis of a related heterocycle to **4**, we found to our surprise that the addition of *N*-hydroxyphthalimide to the Mitsunobu reaction of **12** achieved reproducible yields of 57% for compound **13** irrespective of the order of addition of triphenylphosphine and DEAD. We presume that the *N*-hydroxyphthalimide acts as an acid catalyst in this reaction, promoting it *via* protonation of the activated complex formed between triphenylphosphine and DEAD. Attempted bromination of compound **12** with CBr_4–Ph_3P in DMF also afforded the bicyclic compound **13**, albeit in lower yield (20%).

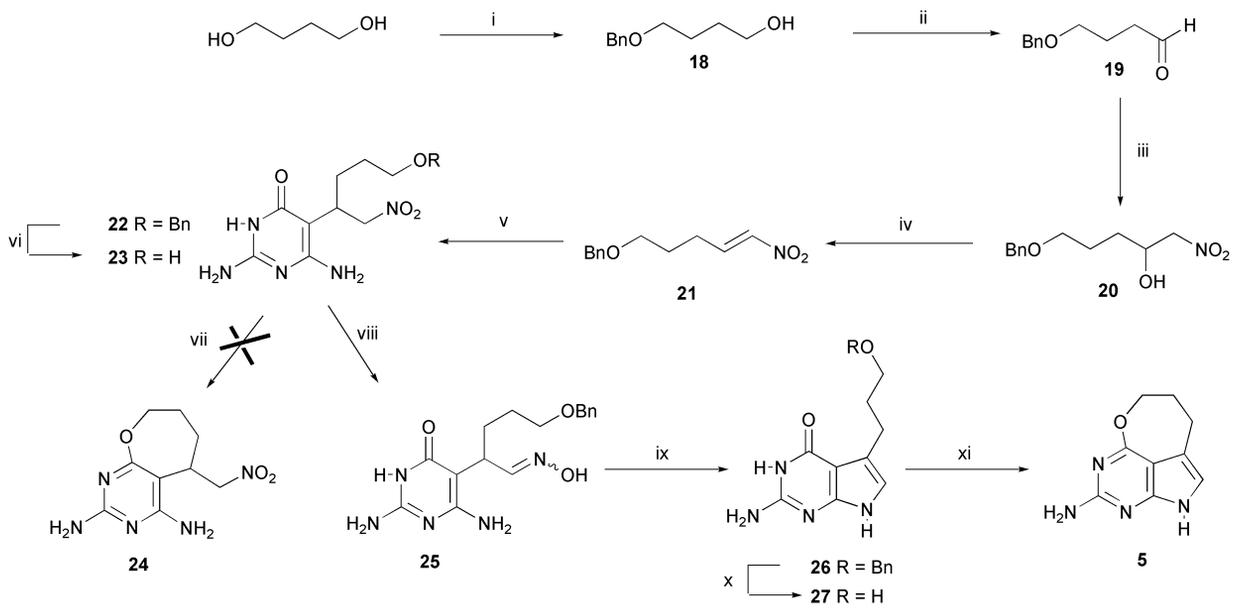
Our initial attempts at cyclisation of **13** to form the tricyclic analogue **4** using the Nef reaction[32] returned mainly starting material in addition to a product obtained in 10% yield whose NMR spectrum was consistent with that of compound **4**, that we obtained subsequently *via* a different route. However this reaction proved difficult to reproduce and the yields obtained were generally much lower than 10%. In previous work, we have shown that nitroalkanes of this type may be conveniently converted to the desired aldehyde (*via* the oxime[33]) which then spontaneously cyclises to afford a pyrrolopyrimidine.[30] Thus, treatment of compound **13** with a mixture of tin(II) chloride, thiophenol and triethylamine in methanol at room temperature afforded the oxime **14**. In our initial attempts to prepare **14**, only low yields (9%) were achieved, which were accompanied by a further reduction of **14** to the corresponding hydroxylamine derivative **16** (Scheme 2) which was obtained in 37% yield. It can be assumed that the reactions involved in this reduction are those depicted in Scheme 2, in which a fast tautomerisation of the nitroso intermediate to the oxime (**14**) may prevent the further reduction to the hydroxylamino derivative (**16**). Indeed, the hydroxylamine obtained as a by-product must arise from the nitroso derivative, since Bartra *et al.*[33] have shown that oximes do not react with the tin–thiophenol complex. They also report that polar solvents are generally recommended for the preparation of the oximes, whilst apolar solvents give rise to better yields of hydroxylamines. Consequently, we investigated other polar solvents in this reaction. Thus, in DMF, no hydroxylamine was obtained, but the yield of oxime was unchanged (9%), whilst in acetonitrile, more oxime was formed (28% yield), but the hydroxylamine was still observed.



Scheme 2 Formation of **14** and **16** using the $SnCl_2$–3PhSH–3NEt_3 mixture in MeOH–CH_3CN.

According to the observations of Bartra *et al.*[33] reduction of the nitroso intermediate to the corresponding hydroxylamine requires the presence of the tin complex. With this in mind, the tin complex was prepared in acetonitrile and added dropwise to a solution of the nitro compound **13** in order to effect conversion of the intermediate nitroso derivative to oxime **14** prior to adding excess complex. Under these conditions, the desired oxime was obtained in 56% yield using 1.2 eq of the tin complex in a mixture of methanol and acetonitrile.

When the hydrolysis of the oxime **14** to produce the corresponding aldehyde (expected to undergo spontaneous cyclisation to give **4**) was attempted by heating under reflux in water in the presence of Dowex-50 H^+-form[34] the sole product obtained was the alcohol **7**. Attempted hydrolysis of the oxime by simply heating in water returned the starting material unchanged. As an alternative, we considered generating the



Scheme 3 *Reagents and conditions*: (i) NaH, DMF, then BnCl, room temp., 18 h, 66%; (ii) PCC, CH_2Cl_2, room temp., 3 h, 87%; (iii) CH_3NO_2, EtOH, NaOH, 0 °C, 3 h, 69%; (iv) MsCl, CH_2Cl_2, 0 °C, then NEt_3, CH_2Cl_2, 0 °C, 10 h, 89%; (v) 2,6-diamino-4(*H*)-pyrimidinone, EtOAc–H_2O, 50 °C, 18 h, 81%; (vi) BCl_3, CH_2Cl_2, −78 °C, 6 h, 67%; (vii) Mitsunobu reaction; (viii) $SnCl_2$, PhSH, NEt_3, MeCN, room temp., 1 h, 65%; (ix) Dowex-50 (H^+-form), H_2O, 60 °C, 18 h, 91%; (x) BCl_3, CH_2Cl_2, −78 °C, 6 h, 84%; (xi) PPh_3, DEAD, DMF, room temp., 18 h, 33%.

aldehyde under milder conditions. To this end, a solution of oxime **14** in DMF was treated with a freshly made-up, aqueous solution of vanadium(II) chloride followed by acidification of the solution.[35] Following the removal of the vanadium salts, the desired tricyclic compound **4** crystallised from methanol in 20% yield as its white hydrochloride salt **15** (Scheme 1) which was characterised by NMR, accurate mass measurement and elemental analysis.

Interestingly, the solution of **15** in DMSO which had been prepared for NMR, soon became blue after only a few hours at room temperature and in the presence of light. We have previously observed blue coloured pigments in solutions of **7** in methanol and in addition others have also reported such findings with various 7-substituted 7-deazaguanine derivatives which were attributed to products derived following oxidation.[36] However, this darkening of the solution was not accompanied by any appreciable change in the NMR spectrum of **15**, even one week later. The stability of compound **15** was also assessed in both water and methanol. Thus, after one week the solution of **15** in water contained another component which was identified as compound **7** by TLC and mass spectral analysis. This proved the instability in water of **15** through hydrolysis of the dihydropyran ring to afford the alcohol **7**. In order to assess the stability of the neutral heterocycle **4**, the NMR solution of **15** in D_2O was neutralised with aq NaOD to monitor the proton NMR changes. This showed a shift for pyrrolo ring protons of **15** attributable to the formation of **4**, which was characterised by accurate mass spectrometry. Compound **4** was also prone to hydrolysis to compound **7** as evidenced by NMR with the reaction occurring over a similar time period as was found for its hydrochloride salt **15**.

Due to the relatively poor stability of compound **4** in water, we undertook the synthesis of its homologue, compound **5**, which was expected to have similar biological properties, but with an enhanced stability in water.

For the synthesis of compound **5** we initially used analogous chemistry to that used in the synthesis of **4**. Thus, butane-1,4-diol was monobenzylated to give **18**, which was oxidised with PCC to give aldehyde **19**. Formation of nitro alcohol **20** was achieved by the aldol-type condensation of **19** with nitromethane. The subsequent dehydration of **20** with methanesulfonyl chloride–triethylamine yielded the nitro-alkene **21** which was then reacted with 2.6-diamino-4(3*H*)-pyrimidinone *via* a Michael addition reaction to give the 5-substituted pyrimidine **22** (Scheme 3).

In common with the synthesis of **4**, there were two routes available from **22** to the tricyclic compound **5**; cyclisation of the pyrrole ring prior to the oxepine ring, and *vice versa*. It was decided to follow analogous chemistry to that used for the synthesis of **4**, so debenzylation of **22** (Scheme 3) was effected by treatment with boron trichloride to give **23**. However, attempts to cyclise the oxepino ring *via* the Mitsunobu reaction (using various combinations of different phosphine and azodicarboxylate reagents) to give **24** were unsuccessful. In each case the reaction produced a complex mixture of products, none of which were identifiable as the desired product **24**. Consequently compound **22** was converted to the corresponding oxime **25** in 65% yield, following reduction using tin(II) chloride. Hydrolysis of compound **25** using Dowex-50 H^+-form afforded the pyrrolo[2,3-*d*]pyrimidin-4-one **26** in 91% yield. Debenzylation of **26** using boron trichloride gave **27**, the precursor to the target compound **5**. On this occasion, cyclisation *via* the Mitsunobu reaction to give **5** was successful, albeit in reduced yield (typically 30%). Yields were improved slightly (36%) and purification made more straightforward when polymer-supported triphenylphosphine was employed.

In order to assess the potential of compounds **4** and **5** in DNA to act as cross-linking agents to human ATase, their activities as inactivators of the protein were screened using the standard assay[9] employing DNA containing tritiated O^6-methylguanine (experiments were kindly performed by Dr Geoff Margison, Paterson Institute for Cancer Research, Manchester). Whilst compound **4** (employed following neutralisation of its hydrochloride salt) showed no inactivation of ATase after overnight incubation, compound **5** was found to be a weak inhibitor (IC_{50} 1 mM) suggesting that it is likely to be recognised as a substrate by the protein.

We are currently engaged in the synthesis of DNA containing analogue **5** which will be reported subsequently together with its biological properties.

Experimental

The name of each product was obtained with ACD/IUPAC Name Pro software (3.5 for Microsoft Windows). Dichloromethane, pyridine and acetonitrile were dried under reflux from

calcium hydride and then distilled and stored over 3 Å molecular sieves under argon.

N,*N*-Dimethylformamide was obtained as an anhydrous solvent from Aldrich. All other reagents were purchased from commercial suppliers and used without purification.

Silica gel for flash column chromatography was used unless otherwise stated and was obtained from BDH (particle size 30–60 μm). Thin layer chromatography (TLC) was carried out on pre-coated Merck Kieselgel 60 F_{254} aluminium backed plates. The TLC eluent is reported for each case and was viewed under UV or with anisaldehyde for nucleobases. TLC systems used were A (dichloromethane), B (10% methanol in dichloromethane) and C (20% methanol in dichloromethane).

Melting points were measured on a Gallenkamp melting point apparatus. UV-visible data was obtained with a VARIAN CARY 50 probe spectrometer. Nuclear magnetic resonance (NMR) spectra were run on Bruker AC-250 and AMX-400 spectrometers. ^{1}H 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. All chemical shifts are quoted in δ relative to tetramethylsilane as an external standard. All coupling constants are quoted in Hz. All mass spectra and elemental analysis were obtained from the University of Sheffield Mass Spectrometry and Elemental Analysis Services.

2,6-Diamino-5-[3-hydroxy-1-(nitromethyl)propyl]pyrimidin-4(3*H*)-one 12

A solution of boron trichloride (1 M) in heptane (45 mL, 40.5 mmol) was added to a solution of 2,6-diamino-5-[3-(benzyloxy)-1-(nitromethyl)propyl]pyrimidin-4(3*H*)-one (**8**)[30] (1.5 g, 4.5 mmol) in CH_2Cl_2 (120 mL) at −78 °C with exclusion of moisture. The mixture was stirred for 6 h at −78 °C and a solution of CH_2Cl_2–ethanol (1 : 1, 120 mL) was added dropwise while the mixture was allowed to warm to room temp. The mixture was then evaporated to dryness, redissolved in ethanol (30 mL) and neutralised with 1 M aqueous sodium hydroxide solution. Crude product was then adsorbed on silica gel and chromatographed eluting with CH_2Cl_2–MeOH 9 : 1 and 8 : 2. The product was obtained as a pale yellow solid (1 g, 92%); mp 114–116 °C; R_f (C), 0.25; δ_H ([$^2H_6$]-DMSO) 1.51–1.64 (1H, m, CH_2), 1.80–1.94 (1H, m, CH_2), 3.27–3.36 (3H, m, CH and CH_2OH), 4.53 (1H, t, *J* 4.9, OH), 4.73 (1H, dd, *J* 5.8 and 12.2, CH_2NO$_2$), 5.06 (1H, dd, *J* 3.4 and 11.9, CH_2NO$_2$), 5.81 (2H, s, NH_2), 6.04 (2H, s, NH_2), 9.79 (1H, s, NH); δ_C ([$^2H_6$]-DMSO) 32.35, 48.56, 58.56, 76.91, 84.72, 150.74, 151.98, 159.68; MS (positive electrospray) *m*/*z* 244 ([M + H]$^+$, 100%), 266 ([M + Na]$^+$, 10%). Accurate mass measurement 244.1035, $C_8H_{14}N_5O_4$ requires 244.1046, deviation −4.4 ppm.

5-(Nitromethyl)-6,7-dihydro-5*H*-pyrano[2,3-*d*]pyrimidine-2,4-diamine 13

Method A. Diethyl azodicarboxylate (2.72 mL, 17.3 mmol) was added dropwise over 30 min to a solution of triphenylphosphine (4.64 g, 17.3 mmol) in anhydrous DMF (135 mL) under argon. The resulting pale yellow solution was stirred for 30 min, then added dropwise with a cannula to a solution of 3.5 g (14.42 mmol) of **12** in anhydrous DMF (135 mL). The mixture was then stirred overnight, the solvent evaporated and the residue adsorbed on silica gel and chromatographed eluting with CH_2Cl_2–MeOH 95 : 5 and 9 : 1. The product crystallised as white needles from acetonitrile (1.84 g, 57%); mp 238–240 °C (decomp.); R_f (C), 0.5; pH = 7.7, λ_{max}(MeOH)/nm 268.1 (log ε/dm^3 mol^{-1} cm^{-1} 4.15); λ_{min}(MeOH)/nm 250.1 (log ε/dm^3 mol^{-1} cm^{-1} 3.88); δ_H ([$^2H_6$]-DMSO) 1.75–1.98 (2H, m, CH_2), 3.32–3.37 (1H, m, CH), 4.15–4.19 (2H, m, CH_2O), 4.53 (1H, dd, *J* 3.52 and 14.67, CH_2NO$_2$), 4.61 (1H, dd, *J* 10.27 and 14.38, CH_2NO$_2$), 5.66 (2H, s, NH_2), 6.10 (2H, s, NH_2); δ_C ([$^2H_6$]-DMSO) 24.60, 27.00, 61.90, 74.90, 81.90, 162.30, 163.80, 167.10; MS (positive electrospray) *m*/*z* 226.1 ([M + H]$^+$, 100%). Accurate mass measurement 226.0948, $C_8H_{12}N_5O_3$ requires 226.0940, deviation 3.6 ppm.

Method B. Compound **12** (500 mg, 2.06 mmol) was dissolved in anhydrous DMF (40 mL) under argon and stirred at room temp. To this was added triphenylphosphine (682 mg, 2.58 mmol) and *N*-hydroxyphthalimide (433 mg, 2.58 mmol). Diethyl azodicarboxylate (DEAD) (405.5 μL, 2.58 mmol) was then added dropwise to the solution after which it was stirred for 4 h at room temp. Further triphenylphosphine (1.25 equiv), *N*-hydroxyphthalimide (1.25 equiv) and DEAD (1.25 equiv) were then added and the reaction left stirring overnight. The reaction mixture was then evaporated and the crude product purified by silica chromatography (CH_2Cl_2–MeOH 95 : 5). Further purification by recrystallisation from acetonitrile afforded white solid (282 mg, 57%).

2,4-Diamino-6,7-dihydro-5*H*-pyrano[2,3-*d*]pyrimidine-5-carbaldehyde oxime 14

Thiophenol (0.762 mL) and dry triethylamine (1.05 mL) were added to a stirred solution of anhydrous $SnCl_2$ (471 mg, 2.48 mmol) in a mixture of methanol (3.3 mL) and acetonitrile (22 mL) at room temp. The resulting solution was then added dropwise with a cannula (over 45 min) to a suspension of compound **13** (467 mg, 2.07 mmol) in acetonitrile (42 mL). After 6 h, the reaction mixture was concentrated *in vacuo* and the residue separated by column chromatography by means of CH_2Cl_2–MeOH 95 : 5 (PhSH and PhSSPh being eluted, and also Et_3NH^+) and then CH_2Cl_2–MeOH 9 : 1, to afford a white solid (241 mg, 56%) as an *E*/*Z* mixture (1 : 2.5 according to the ^{1}H NMR spectrum); mp 170–172 °C (decomp.); R_f (C), 0.34; pH = 7.3, λ_{max}(MeOH)/nm 270.1, 241.1 (log ε/dm^3 mol^{-1} cm^{-1} 3.66, 3.62); λ_{min}(MeOH)/nm 255.1, 238.1 (log ε/dm^3 mol^{-1} cm^{-1} 3.52, 3.61); spectral data of *Z*-isomer: δ_H ([$^2H_6$]-DMSO) 1.75–1.98 (2H, m, CH_2), 3.96–3.99 (1H, m, CH), 4.15–4.23 (2H, m, CH_2O), 5.77 (2H, s, NH_2), 5.98 (2H, s, NH_2), 6.73 (1H, d, *J* 8.6, CH=N), 11.50 (1H, s, OH); δ_C ([$^2H_6$]-DMSO) 23.65, 24.99, 62.49, 82.21, 151.11, 161.57, 163.65, 166.54. MS (positive electrospray) *m*/*z* 210.1 ([M + H]$^+$, 100%). Accurate mass measurement 210.0982, $C_8H_{12}N_5O_2$ requires 210.0991, deviation −4.3 ppm.

7-Amino-3,4-dihydro-1*H*-5-oxa-1,6,8-triazaacenaphthylene hydrochloride 15

To a suspension of **14** (200 mg, 0.96 mmol) in DMF (1.2 mL) under argon atmosphere was added a solution of vanadium(II) chloride (303 mg, 2.88 mmol, 3 equiv.) in 12 mL of water (addition should be rapid as VCl_2 decomposes in water). The addition of VCl_2 was accompanied by a mild exothermic reaction. The reaction mixture was stirred at room temp overnight, then 10% aqueous HCl (4 mL) was added and the reaction left stirring overnight. The solvent was then evaporated and MeOH (12 mL) was added to the black solid and the solution was filtered. The product **15** which formed after two weeks as a fine white precipitate in a green coloured filtrate was collected by centrifugation and washed with MeOH to give white crystals (40 mg, 20% yield); mp > 350 °C (decomp.); R_f (B), 0.18; Found: C, 36.38; H, 4.58; N, 21.62; Cl, 27.38. $C_8H_9N_4O{\cdot}2HCl{\cdot}H_2O$ requires C, 36.0; H, 4.8; N, 21.0; Cl, 26.5; pH = 4.5, λ_{max}(MeOH)/nm 261.01 (log ε/dm^3 mol^{-1} cm^{-1} 5.49); λ_{min}(MeOH)/nm 238.01 (log ε/dm^3 mol^{-1} cm^{-1} 5.18); λ_{sh}(MeOH)/nm 272.01 (log ε/dm^3 mol^{-1} cm^{-1} 5.42); δ_H ([$^2H_6$]-DMSO) 2.99 (2H, t, *J* 7.32, CH_2), 3.83 (2H, t, *J* 7.63, CH_2), 6.62 (1H, s, CH), 11.16 (1H, s, N$^+$H), 11.31 (1H, s, NH); δ_H ([$^2H_2$]-H_2O) 2.97 (2H, t, *J* 6.75, CH_2), 3.70 (2H, t, *J* 6.75, CH_2), 6.63 (1H, s, CH), 7.80 (1H, s, NH); δ_C ([$^2H_6$]-DMSO) 29.70, 34.45, 45.04, 99.12, 115.49, 116.51, 151.65, 158.00; δ_C (D_2O) 28.42, 44.68, 98.88, 116.04, 116.21, 117.40, 150.27, 159.36; MS (positive electrospray) *m*/*z* 215 ([M·H^{37}Cl + H]$^+$, 15%), 213 ([M·H^{35}Cl + H]$^+$,

100%), 177 ([M + H]$^+$, 20%, M = neutral compound). Accurate mass measurement 213.0534, $C_8H_{10}N_4OCl$ requires 213.0543, deviation −4.1 ppm.

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

Compound **4** was obtained by neutralising a solution of **15** 0.063 M in D_2O with 0.2 mL aq NaOD 0.05 M; δ_H (D_2O) 2.96 (2H, t, *J* 6.75, CH_2), 3.71 (2H, t, *J* 6.75, CH_2), 6.56 (1H, s, CH), 8.43 (1H, s, NH); MS of **4** in H_2O (pH = 7.10) (positive electrospray) *m*/*z* 177 ([M + H]$^+$, 100%), 213 ([M·H^{35}Cl + H]$^+$, 10%). Accurate mass measurement 177.0774, $C_8H_9N_4O$ requires 177.0776, deviation −1.5 ppm.

4-(Benzyloxy)-1-butanol 18

Butane-1,4-diol (10 g, 111.0 mmol) was dissolved in anhydrous DMF (200 mL) under argon and cooled in an ice bath. Sodium hydride (3.09 g, 122.1 mmol, 95% dispersion in mineral oil) was then cautiously added over a period of 20 min and the reaction stirred for a further 30 min. Benzyl chloride (14.05 g, 111.0 mmol) was then added dropwise and the reaction was left to stir at room temp. overnight. The precipitated solid was filtered, the filtrate evaporated and the residue co-evaporated twice with toluene. The resulting oil was then distilled under reduced pressure using an oil pump, giving **18** as a colourless oil (12.46 g, 66%); bp 110 °C (~5 mmHg); R_f (A) 0.1; δ_H ($CDCl_3$) 1.51 (4H, m, CH_2, CH_2), 3.41 (4H, *J* 1.2 and 6.4, CH_2OH, CH_2O), 4.41 (2H, s, OCH_2Ph), 7.20–7.40 (5H, m, Ph); δ_C ($CDCl_3$) 26.67, 30.01, 62.65, 70.34, 73.05, 127.67, 127.72 (2C), 128.42 (2C), 138.14; *m*/*z* (EI$^+$) 180 [M$^+$] Accurate mass measurement 180.1144, $C_{11}H_{16}O_2$ requires 180.1150 deviation 3.5 ppm.

4-(Benzyloxy)butanal 19

A solution of 4-(benzyloxy)-1-butanol (**18**) (31.67 g, 175.7 mmol) in dichloromethane (100 mL) was added in one portion to a stirred suspension of pyridinium chlorochromate (PCC) (75.75 g, 351.4 mmol) in dichloromethane (600 mL). The reaction was stirred at room temp. for 3 h, then filtered through silica to remove the black insoluble material, eluting with dichloromethane. After evaporation of the filtrate, the aldehyde (**19**) was obtained as a colourless oil (27.36 g, 87%); R_f (A) 0.42; δ_H ($CDCl_3$) 1.84 (2H, quintuplet, *J* 6.4, CH_2), 2.49 (2H, dt, *J* 1.5 and 6.1, CH_2CHO), 3.43 (2H, t, *J* 6.4, CH_2O), 4.41 (2H, s, OCH_2Ph), 7.20–7.40 (5H, m, Ph), 9.66 (1H, t, *J* 1.5, C*H*O); δ_C ($CDCl_3$) 22.56, 40.94, 69.13, 72.95, 127.62 (3C), 128.40 (2C), 138.26, 202.26; *m*/*z* (EI$^+$) 178 [M$^+$]. Accurate mass measurement 178.0990, $C_{11}H_{14}O_2$ requires 178.0994 deviation 2.3 ppm.

5-(Benzyloxy)-1-nitropentan-2-ol 20

Aldehyde **19** (12.90 g, 72.4 mmol) and nitromethane (3.92 mL, 72.4 mmol) were dissolved in an equal volume of ethanol (25 mL) and cooled in an ice bath to 0 °C. Aqueous sodium hydroxide solution (2.84 g in 10 mL) was then added dropwise to the stirred solution so that the temp. did not exceed 0 °C. The reaction was then stirred at room temp. for 3 h. Ice water was then added to the mixture followed by glacial acetic acid until the precipitated salt was dissolved and the pH was 6. The solution was then extracted with ethyl acetate (150 mL), and the organic phase washed with distilled water (50 mL), saturated sodium chloride solution (50 mL), dried ($MgSO_4$) and evaporated to give an orange–yellow oil. Purification by column chromatography, eluting with dichloromethane gave the pure nitro alcohol **20** as a pale yellow oil (11.88 g, 69%); R_f (A) 0.25; δ_H ($CDCl_3$) 1.75 (4H, m, CH_2CH_2), 3.50–3.61 (3H, m, *J* 4.0 and 5.5, C*H* and CH_2O), 4.41 (2H, m, CH_2NO$_2$), 4.51, (2H, s, OCH_2Ph), 7.20–7.40 (5H, m, Ph); δ_C ($CDCl_3$) 25.80, 31.62, 68.45, 68.87, 73.29, 80.62, 127.84, 127.98 (2C), 128.54 (2C), 136.21; *m*/*z* (CI$^+$) 240 [M + H]$^+$. Accurate mass measurement 240.1242, $C_{12}H_{18}O_4N$ requires 240.1236 deviation 2.4 ppm.

({[(4*E*)-5-Nitropent-4-enyl]oxy}methyl)benzene 21

Methanesulfonyl chloride (10.95 g, 95.6 mmol) was added in one portion to a stirred solution of compound **20** (22.88 g, 95.6 mmol) in dry dichloromethane (150 mL) at 0 °C under an argon atmosphere. Dry triethylamine (19.35 g, 191.2 mmol) in dry dichloromethane (70 mL) was then added *via* a syringe pump at a rate of 10 mL h^{-1}. When the reaction was complete, the reaction mixture was transferred to a separating funnel with dichloromethane (150 mL). The organic layer was sequentially washed with water (100 mL), 5% aqueous HCl (100 mL), 10% aqueous Na_2CO_3 (100 mL) and brine (100 mL). The organic layer was then dried ($MgSO_4$) and the solvent removed *in vacuo*, giving the crude product **21** as an orange oil, which was used without further purification. Yield (18.87 g, 89%); R_f (A) 0.32; δ_H ($CDCl_3$) 1.83 (2H, quintuplet, *J* 6.1, CH_2), 2.41 (2H, dq, *J* 1.5 and 6.1, CH_2), 3.50 (2H, t, *J* 5.8, CH_2O), 4.41 (2H, s, OCH_2Ph), 6.92 (1H, dt, *J* 1.5 and 13.4, C*H*), 7.20–7.40 (6H, m, C*H* and Ph); δ_C ($CDCl_3$) 25.46, 27.93, 68.74, 73.07, 127.72, 127.78 (2C), 128.48 (2C), 138.16, 139.76, 142.34; *m*/*z* (CI$^+$) 222 [M + H]$^+$. Accurate mass measurement 222.1136, $C_{12}H_{16}O_3N$ requires 222.1130 deviation 2.6 ppm.

2,6-Diamino-5-[4-(benzyloxy)-1-(nitromethyl)butyl]pyrimidin-4(3*H*)-one 22

A solution of 2,6-diamino-4(3*H*)-pyrimidinone (2.16 g, 17.2 mmol) and compound **21** (3.80 g, 17.2 mmol) in ethyl acetate (25 mL) and water (25 mL) was heated at 50 °C overnight. The organic layer was then washed with water (50 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was obtained as a yellow foam (4.82 g, 81%). Recrystallisation from 1,4-dioxan gave **22** as white micro-crystals; mp 144 °C; R_f (B) 0.45; λ_{max}(MeOH)/nm 274.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.40); λ_{min}(MeOH)/nm 252.00 (log ε/dm^3 mol^{-1} cm^{-1} 2.07); δ_H ([$^2H_6$]-DMSO) 1.40–1.90 (4H, m, CH_2CH_2), 3.15–3.40 (3H, m, C*H*, CH_2O), 4.41 (2H, s, OCH_2Ph), 4.72 (1H, dd, *J* 6.4 and 11.6, CH^a_2NO$_2$), 4.99 (1H, dd, *J* 2.8 and 11.6, CH^b_2NO$_2$), 5.92 (2H, s, NH_2), 6.04 (2H, s, NH_2), 7.20–7.40 (5H, m, Ph), 9.77 (1H, s, N*H*); δ_C ([$^2H_6$]-DMSO) 26.87, 27.53, 35.21, 70.35, 72.25, 78.28, 84.61, 127.76, 127.88 (2C), 128.67 (2C), 139.13, 154.00, 162.42, 163.19; *m*/*z* (EI$^+$) 347 [M$^+$] Accurate mass measurement 347.1607, $C_{16}H_{21}O_4N_5$ requires 347.1594 deviation 3.8 ppm.

2,6-Diamino-5-[4-hydroxy-1-(nitromethyl)butyl]pyrimidin-4(3*H*)-one 23

A solution of boron trichloride (1 M) in heptane (105 mL, 105.2 mmol) was added to a solution of **22** (4.07 g, 11.7 mmol) in dichloromethane (100 mL) at −78 °C under argon. The mixture was stirred at −78 °C for 6 h. A solution of ethanol in dichloromethane (1 : 1, 400 mL) was then added dropwise overnight whilst the reaction came to room temperature. The mixture was evaporated to dryness, redissolved in a small amount of ethanol and neutralised with aqueous sodium hydroxide solution (1 M). The residue from evaporation was adsorbed onto silica gel and purified by column chromatography, eluting with 10% methanol in dichloromethane. The product was obtained as a pale yellow foam (2.03 g, 67%); mp 190 °C decomp. (from 1,4-dioxan); R_f (C) 0.17; λ_{max}(MeOH)/nm 274.00 (log ε/dm^3 mol^{-1} cm^{-1} 4.14); λ_{min}(MeOH)/nm 253.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.87); δ_H ([$^2H_6$]-DMSO) 1.36 (3H, m, CH_2CH^a_2CH), 1.77 (1H, m, CH$_2$CH^bCH), 3.15–3.40 (3H, m, C*H* and CH_2OH), 4.38 (1H, t, *J* 5.12, O*H*), 4.70 (1H, dd, *J* 6.4 and 11.6, CH^a_2NO$_2$), 4.94 (1H, dd, *J* 2.8 and 11.6, CH^b_2NO$_2$), 5.91 (2H, s, NH_2), 6.02 (2H, s, NH_2), 9.75 (1H, s, N*H*); δ_C ([$^2H_6$]-DMSO) 26.87, 27.53, 35.25, 70.35, 78.28, 84.61, 154.00, 162.42, 163.19; *m*/*z* (EI+) 257 [M]$^+$. Accurate mass measurement 257.1116, $C_9H_{15}O_5N_5$ requires 257.1124 deviation 2.9 ppm.

(1*E*)-5-(Benzyloxy)-2-(2,4-diamino-6-oxo-1,6-dihydropyrimidin-5-yl)pentanal oxime 25

Benzenethiol (1.62 mL, 15.8 mmol) was added to a solution of anhydrous $SnCl_2$ (1 g, 5.27 mmol) in anhydrous acetonitrile (10 mL). Triethylamine (1.60 g, 15.6 mmol) was then added dropwise and the reaction allowed to stir for 30 min. A solution of **22** (1.22 g, 3.52 mmol) in anhydrous acetonitrile (14 mL) was then added. After 1 h, the reaction was evaporated to dryness and adsorbed onto silica gel (~5 g). Column chromatography, eluting with a gradient of methanol (5–20%) in dichloromethane, afforded the product as a beige solid (0.76 g, 65%); mp 164 °C (from MeCN); R_f (C) 0.25; λ_{max}(EtOH)/nm 276.00 (log ε/dm^3 mol^{-1} cm^{-1} 4.20); λ_{min}(MeOH)/nm 258.00 (log ε/dm^3 mol^{-1} cm^{-1} 4.10); Spectral data of *E*-isomer: δ_H ([$^2H_6$]-DMSO) 1.45 (2H, m, C*H*$_2$), 1.71 (2H, m, C*H*$_2$), 3.38 (3H, m, CH and C*H*$_2$O), 4.40 (2H, s, OC*H*$_2$Ph), 5.75 (2H, s, N*H*$_2$), 6.02 (2H, s, N*H*$_2$), 7.20–7.36 (5H, m, Ph), 7.55 (1H, d, *J* 7.0, C*H*=N), 9.81 (1H, s, N*H*), 10.14 (1H, s, O*H*); δ_C ([$^2H_6$]-DMSO) 27.42, 27.85, 35.67, 70.36, 72.22, 87.08, 127.74, 127.84 (2C), 128.68 (2C), 139.22, 152.86, 153.87, 162.24, 162.44; *m*/*z* (EI$^+$) 332 [M]$^+$ Accurate mass measurement 332.1714, $C_{16}H_{21}O_3N_5$ requires 332.1723 deviation 2.6 ppm.

2-Amino-5-[3-(benzyloxy)propyl]-3,7-dihydro-4*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one 26

Compound **25** (200 mg, 0.60 mmol) was refluxed overnight in water (30 cm^3) containing a suspension of Dowex-50 (H$^+$-form) (~1 cm^3). A hot filtration was performed to remove the Dowex before an extraction with ethyl acetate (100 cm^3) was carried out. The organic layer was dried ($MgSO_4$) and evaporation of the solvent *in vacuo* afforded the product as a beige solid (171 mg, 91%); mp 180 °C; R_f (C) 0.68; λ_{max}(MeCN)/nm 282.48infl. 260.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.53, 3.65); λ_{min}(MeCN)/nm 251.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.62); δ_H ([$^2H_6$]-DMSO) 1.86 (2H, quintuplet, *J* 6.4, C*H*$_2$), 2.60 (2H, t, *J* 7.0, C*H*$_2$CH$_2$CH$_2$O), 3.42 (2H, t, *J* 6.4, C*H*$_2$O), 4.43 (2H, s, OC*H*$_2$Ph), 6.04 (2H, s, N*H*$_2$), 6.32 (C*H*-6), 7.28–7.33 (5H, m, Ph), 10.19 (1H, s, N*H*), 10.63 (1H, s, N*H*); δ_C ([$^2H_6$]-DMSO) 23.21, 30.53, 69.84, 72.16, 99.18, 113.66, 118.43, 127.44, 127.84 (2C), 128.57 (2C), 139.20, 151.78, 152.57, 159.70; *m*/*z* (EI$^+$) 298 [M]$^+$. Accurate mass measurement 298.1419, $C_{16}H_{18}O_2N_4$ requires 298.14230 deviation 3.5 ppm.

2-Amino-5-(3-hydroxypropyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]-pyrimidin-4-one 27

A solution of boron trichloride (1 M) in heptane (54 cm^3, 54.1 mmol) was added dropwise to a solution of compound **26** (2.0 g, 6.02 mmol) in dichloromethane (100 cm^3) at −78 °C under argon. The reaction was stirred at −78 °C for 6 h. The reaction was then allowed to warm to room temp. overnight whilst a solution of ethanol in dichloromethane (1 : 1, 300 cm^3) was added dropwise. The mixture was evaporated to dryness, redissolved in a small amount of ethanol and neutralized with aqueous sodium hydroxide solution (1 M). The residue after evaporation was purified by column chromatography, eluting with 10% methanol in dichloromethane. The product was isolated as a pale yellow solid (1.0 g, 84%); mp >300 °C (decomp.) R_f (C) 0.28; λ_{max}(EtOH)/nm 282.98infl. 263.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.83, 3.95); λ_{min}(EtOH)/nm 250.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.84); δ_H ([$^2H_6$]-DMSO) 1.69 (2H, quintuplet, *J* 7.0, C*H*$_2$), 2.56 (2H, t, *J* 7.3, C*H*$_2$CH$_2$CH$_2$O), 3.39 (2H, t, *J* 6.7, C*H*$_2$O), 4.45 (1H, t, *J* 5.5, O*H*), 6.05 (2H, s, NH$_2$), 6.32 (1H, s, C*H*-6), 10.21 (1H, s, N*H*), 10.63 (1H, s, N*H*); δ_C ([$^2H_6$]-DMSO) 22.72, 34.01, 60.72, 99.30, 113.65, 118.64, 151.68, 152.74, 159.91; *m*/*z* (EI$^+$) 208 [M]$^+$. Accurate mass measurement 208.0952, $C_9H_{12}O_2N_4$ requires 208.0960 deviation 3.9 ppm.

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

Polymer-supported triphenylphosphine (Aldrich, 380 mg, 1.14 mmol) was suspended in anhydrous *N*,*N*-dimethylformamide (10 cm^3) under argon at room temp. Diethyl azodicarboxylate (200 mg, 1.2 mmol) was then added dropwise over a 20 min period, allowing the colour to disappear after each addition before continuing. The reaction was left to stir for 30 min before adding compound **27** (200 mg, 0.96 mmol) dissolved in *N*,*N*-dimethylformamide (2 cm^3) dropwise over 20 min. The mixture was then left to stir overnight. The reaction was then evaporated and the residue adsorbed onto silica gel. Purification by column chromatography, eluting with a gradient of methanol (10–20%) in dichloromethane followed by recrystallisation of the crude product from ethanol gave fine beige needles (60.8 mg, 33%); mp 220 °C (decomp); R_f (C) 0.41; Found: C, 55.79; H, 5.30; N, 28.22. $C_9H_{10}ON_4 \cdot {}^1/_4H_2O$ requires C, 55.5; H, 5.18; N, 28.8%; λ_{max}(EtOH)/nm 298.00, 263.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.77, 3.78); λ_{min}(EtOH)/nm 275.00 (log ε/dm^3 mol^{-1} cm^{-1} 3.55); δ_H ([$^2H_6$]-DMSO) 2.03 (2H, m, C*H*$_2$), 2.75 (2H, t, *J* 5.2, C*H*$_2$), 4.33 (2H, m, C*H*$_2$O), 5.86 (2H, s, N*H*$_2$), 6.63 (1H, s, C*H*-6), 10.86 (1H, s, N*H*); δ_C ([$^2H_6$]-DMSO) 26.34, 29.59, 72.16, 97.79, 113.31, 115.60, 155.41, 156.44, 169.25; *m*/*z* (EI$^+$) 190 [M]$^+$. Accurate mass measurement 190.0847 $C_9H_{10}ON_4$ requires 190.0855 deviation 4.1 ppm.

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