Preparation of a Novel Potent Inhibitor of Methylthioadenosine Nucleosidase

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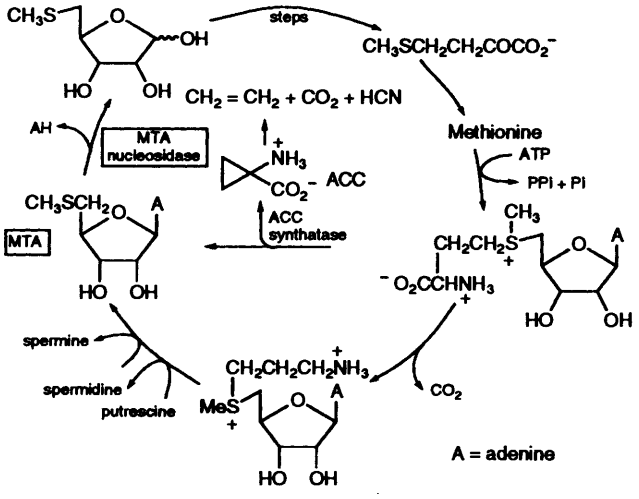
9-(4-Methylthiobutyl)adenine **7** has been prepared and shown to be a potent inhibitor of the enzyme methylthioadenosine nucleosidase.

In plants, methylthioadenosine (MTA) nucleosidase is a key enzyme involved in methionine and adenine salvage, following the synthesis of the plant growth regulators ethylene and polyamines such as spermine and spermidine (Scheme 1).[1]



Scheme 1

Attention has been focussed on this enzyme, which has been purified from lupin seeds[2] and pea seedlings,[3] in the belief that inhibiting its activity would lead to an accumulation of MTA, which is toxic. This toxicity is at least partly based on the inhibition by MTA of several enzymes including those of polyamine biosynthesis. This has been shown to lead to a decline in ethylene biosynthesis.[4] Thus, any inhibitor of MTA nucleosidase is likely to be phytotoxic and thus a possible candidate as a weed-control agent. Further, it is possible that such an inhibitor may be of low toxicity to mammals, since MTA-nucleosidase is only found in plants and some micro-organisms. It would, however, be necessary to check the effect of any inhibitor of MTA-nucleosidase on the mammalian enzyme MTA-phosphorylase.

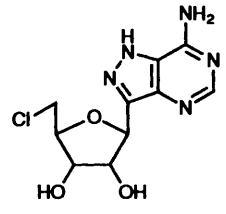
MTA-nucleosidase is weakly inhibited by 5'-isopropylthioadenosine, 5'-isobutylthioadenosine and 5'-chloroadenosine.[4] In contrast, 5'-chloroformycin **1**[5] is a potent inhibitor of MTA nucleosidase (Table 1) and was shown to effect a considerable reduction of ethylene production in plants.[4] Unfortunately, 5'-chloroformycin **1** is not readily available and is too expensive to be considered for agricultural use.

In the design of an MTA-nucleosidase inhibitor of simpler structure we directed our attention to the preparation of acyclic derivatives of adenosine, thus following a strategy that has been very successfully employed in the area of antiviral chemotherapy.[6]

Table 1 Inhibition of MTA nucleosidase by some purine derivatives[a]

Compound	Inhibition of MTA nucleosidase by substrate mimics, K_i/μmol dm^3
1	0.125
5	10.6
6	24.2
7	0.789
9	50.0
Formycin	38.0

[a] MTA nucleosidase was prepared from pea seedlings and partially purified.[3] MTA concentration in the assay was 6.25 μmol dm^{-3}. K_m of the enzyme for MTA is 1.3 μmol dm^{-3}.



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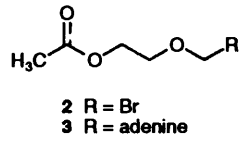
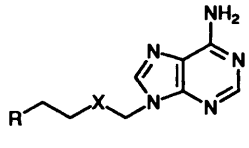


2 R = Br
3 R = adenine

Results and Discussion

The bromo ether **2** was prepared according to a literature procedure[7] and then coupled with adenine to give 9-(2-acetoxyethoxymethyl)adenine **3** which was hydrolysed to give the alcohol **4**.[8] Reaction of this alcohol with dimethyl disulfide and tributylphosphine[9] gave the thioether **5**. The corresponding chloro compound **6** was prepared from the alcohol **4** by treatment with triphenylphosphine and carbon tetrachloride.[10]

The synthesis of 9-(4-methylthiobutyl)adenine **7** was very straightforward and involved the coupling of 6-chloropurine and 4-methylthiobutan-1-ol under the influence of diethyl azodicarboxylate and triphenylphosphine followed by ammonolysis (50% overall yield).

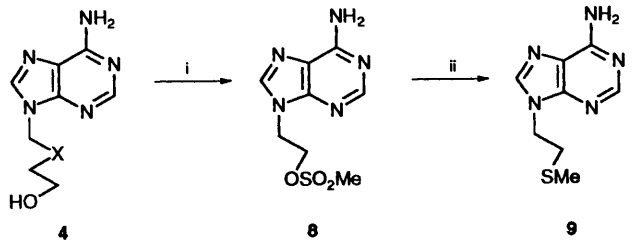


4 R = OH, X = O
5 R = SMe, X = O
6 R = Cl, X = O
7 R = SMe, X = CH_2

The three MTA analogues were tested for inhibition of MTA-nucleosidase. Interestingly, while the compounds **5** and **6** were modest inhibitors of the enzyme, the simple compound **7** proved

to be a potent inhibitor, with about one-sixth of the potency of 5'-chloroformycin (Table 1).

It was of interest to see if the length of the chain separating the methylthio group and the purine moiety was of importance. This was readily accomplished since the alcohol **4** was unexpectedly converted into the mesylate **8** on treatment with methanesulfonyl chloride (Scheme 2). The mechanism of this conversion is not clear-cut and we are investigating the details of the pathway. Treatment of the mesylate **8** with methanethiolate in methanol and dimethylformamide gave 9-(2-methylthioethyl)-adenine **9**. The same compound was prepared by treatment of methylthioethanol with 6-chloropurine, triphenylphosphine and diethyl aminoazodicarboxylate followed by reaction of the 6-chloro-9-(2-methylthioethyl)purine so formed with ammonia. The latter compound is roughly two orders of magnitude less active as an inhibitor of MTA nucleosidase than the lead compound **7** (Table 1).



4 **8** **9**

Scheme 2 *Reagents and conditions:* i, CH_3SO_2Cl; ii, $MeS^{\ominus}$, MeOH, $HCONMe_2$

Conclusions.—The simple derivative of adenosine **7** has been found to be a potent competitive inhibitor of MTA-nucleosidase: of all the literature compounds tested against the enzyme only 5'-chloroformycin has shown greater potency. In a preliminary study the compound **7** was also shown to retard the growth of pea seedlings (*Pisum sativum* var. Feltham First).[3]

Experimental

All reactions were performed under an atmosphere of nitrogen and all starting materials were obtained from commercial suppliers (Aldrich, Sigma, Lancaster and Fluka) and used without further purification unless noted otherwise.

Diethyl ether and tetrahydrofuran (THF) were distilled from sodium-benzophenone ketyl prior to use. Dry dichloromethane was obtained by distillation from calcium hydride. Light petroleum refers to the fraction boiling in the range 60–80 °C. This and ethyl acetate were distilled prior to use. Dry *N*,*N*-dimethylformamide (DMF) and dry pyridine were obtained by distillation from barium oxide and were stored over 4 Å molecular sieves.

Flash chromatography was carried out using silica gel 60 H (Merck 7385). Thin-layer chromatography (TLC) was performed on Merck 60F-254 (0.25 mm thickness, Art. 5715) glass-backed silica gel plates with visualisation by UV light (254 nm), *p*-anisaldehyde, phosphomolybdic acid, ninhydrin (all as acidic solutions in ethanol) or potassium permanganate (as a basic, aqueous solution).

Melting points were carried out on an 'Electrothermal' capillary melting point apparatus and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer 880 Grating Fourier-Transform Infrared spectrophotometer. The spectra were recorded as solutions using the solvent indicated, or as films on sodium chloride plates, or as potassium bromide discs.

^1H and ^{13}C NMR spectra were recorded on a Bruker AM-250 spectrometer at 250 and 62.9 MHz respectively. Chemical shifts are reported in ppm relative to chloroform as internal standard. *J* Values are given in Hz.

High-Resolution mass spectra were run at the SERC Mass Spectrometry Centre, Swansea, using a VG ZAB-E High-Resolution instrument.

(2-Acetoxyethoxy)methyl Bromide **2**.—1,3-Dioxolane (8.85 cm^3, 9.38 g, 0.13 mol) was added dropwise to freshly distilled acetyl bromide (9.38 cm^3, 15.6 g, 0.13 mol) cooled to 0 °C with stirring. A vigorous exothermic reaction took place during addition and the system was stirred for a further 2 h after addition was complete. Subsequent distillation gave the title compound **2** as a clear colourless oil (23.3 g, 93%), b.p. 62–64 °C/0.3 mmHg (lit.,[7] 58–60 °C/0.1 mmHg); ν_{max}(neat)/cm^{-1} 1734 (C=O, ester), 1444 (CH_2-O), 1237 and 1124 (C–O–C); δ_H($CDCl_3$) 5.66 (2 H, s, OCH_2Br), 4.26–4.21 (2 H, m, $AcOCH_2$), 3.85–3.79 (2 H, m, $AcOCH_2CH_2$) and 2.05 (3 H, s, CH_3CO); δ_C($CDCl_3$) 170.7 (C=O, ester), 75.6 (OCH_2Br), 69.2 ($AcOCH_2CH_2$), 62.2 ($AcOCH_2$) and 20.7 (CH_3CO).

9-[*(2-Acetoxyethoxy)methyl*]*adenine* **3**.—Sodium hydride (1.83 g, 76.3 mmol) was added to a suspension of adenine (5.12 g, 37.9 mmol) in dry DMF (150 cm^3) and then warmed to 70 °C for 2 h. More DMF (50 cm^3) was added and, once stirring freely, the system was cooled to 0 °C. α-Bromo ether **2** (6.23 g, 31.6 mmol) was added dropwise, and the resulting solution stirred at room temperature for 42 h. The excess of adenine was removed by filtration and the filtrate evaporated under reduced pressure. The residue was purified by silica gel chromatography, using dichloromethane–methanol (9:1) as eluent, to give the nucleoside analogue **3** as a white solid (1.12 g, 14%), R_f 0.28 CH_2Cl_2–MeOH (9:1); m.p. 138–139 °C (lit.,[8] 137–138 °C); ν_{max}(KBr)/cm^{-1} 3423 (NH_2) and 1734 (C=O); δ_H([$^2H_6$]-DMSO) 8.24 (1 H, s, ArH), 8.13 (1 H, s, ArH), 7.28 (2 H, br s, NH_2), 5.53 (2 H, s, OCH_2-Ad), 4.06–3.98 (2 H, m, $AcOCH_2$), 3.69–3.63 (2 H, m, $AcOCH_2CH_2$) and 1.88 (3 H, s, CH_3CO); δ_C(CD_3OD) 173.0 (C=O), 154.0, 143.0 (CH, purine), 74.0 (OCH_2Ad), 68.4 ($AcOCH_2CH_2$), 64.2 ($AcOCH_2$) and 20.2 (CH_3CO) [Found: $(M + H)^+$, 252.1097. $C_{10}H_{13}N_5O_3$ requires (M + H) 252.1097].

9-[*(2-Hydroxyethoxy)methyl*]*adenine* **4**.—Sodium methoxide (0.5 cm^3 of a 1 mol dm^{-3} solution in methanol) was added to a suspension of acetate **3** (0.47 g, 1.88 mmol) in methanol (20 cm^3). The starting material gradually dissolved to give a clear solution, then, after 10 min, precipitation of the product was observed. After 45 min no starting material was visible on TLC, so the product was collected by filtration and washed with dichloromethane (10 cm^3) to give the alcohol **4** (0.22 g, 56%). Concentration of the filtrate resulted in further precipitation of the alcohol (0.16 g) (total yield, 0.38 g, 98%), R_f = 0.07 CH_2Cl_2–MeOH (9:1), m.p. 198–200 °C (lit.,[8] 198–199 °C); ν_{max}(KBr)/cm^{-1} 3328 (NH_2, OH), 1689 and 1610 (purine); δ_H(D_2O) 8.20 (1 H, s, Ar-H), 8.15 (1 H, s, ArH), 5.60 (2 H, s, OCH_2Ad) and 3.62 (4 H, br s, OCH_2CH_2O); δ_C(D_2O) 154.7 (ArC), 153.0 (ArC), 149.0 (ArC), 142.2 (ArCH), 118.0 (ArC), 73.0 (OCH_2Ad), 70.0 ($HOCH_2CH_2$) and 60.0 ($HOCH_2$) [Found: $(M + H)^+$, 210.0991. $C_8H_{11}N_5O_2$ requires (M + H) 210.0991].

9-[*(2-Methylthioethoxy)methyl*]*adenine* **5**.—Tributylphosphine (1.19 cm^3, 0.97 g, 4.77 mmol) and distilled dimethyl disulfide (0.21 cm^3, 0.22 g, 2.33 mmol) were added to a solution of alcohol **4** (0.10 g, 0.48 mmol) in dry DMF (2 cm^3). After the mixture had been stirred at room temperature for 7 d, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography using dichloromethane–methanol (9:1) as eluent. This gave the title compound **5** as a white solid (0.04 g, 31%), R_f 0.16 CH_2Cl_2–MeOH (9:1); m.p. 163–166 °C; ν_{max}(KBr)/cm^{-1} 3309 (NH_2),

1667 and 1590 (purine); δ_H([$^2H_6$]-DMSO) 8.28 (1 H, s, ArH), 8.18 (1 H, s, ArH), 7.26 (2 H, br s, NH_2), 5.57 (2 H, s, OCH_2Ad), 3.67 (2 H, t, *J* 6.5, SCH_2CH_2O), 2.58 (2 H, t, *J* 6.5, SCH_2CH_2O) and 2.01 (3 H, s, CH_3S); δ_C([$^2H_6$]-DMSO) 156.0 (ArC), 152.8 (ArCH), 149.7 (ArC), 141.1 (ArCH), 118.7 (ArC), 71.6 (OCH_2Ad), 66.1 (SCH_2CH_2O), 32.3 (SCH_2CH_2O) and 14.9 (CH_3S) [Found: $(M + H)^+$, 240.0919. $C_9H_{13}N_5OS$ requires (M + H) 240.0919].

9-[2-*Chloroethoxy)methyl*]*adenine* **6**.—Carbon tetrachloride (0.13 cm^3, 0.21 g, 1.35 mmol) was added dropwise to a solution of alcohol **4** (0.28 g, 1.33 mmol) and triphenylphosphine (0.70 g, 2.66 mmol) in dry pyridine. The system was stirred overnight at room temperature after which solvent was removed under reduced pressure and the residue purified by silica gel chromatography using dichloromethane–methanol (14:1) as eluent. This gave the title compound **6** as a white solid (0.26 g, 85%), R_f 0.40 CH_2Cl_2–MeOH (9:1); m.p. 214–217 °C; ν_{max}(KBr)/cm^{-1} 3294 (NH_2), 1671 and 1600 (purine); δ_H([$^2H_6$]-DMSO) 8.29 (1 H, s, ArH), 8.20 (1 H, s ArH), 7.26 (2 H, br s, NH_2), 5.63 (2 H, s, OCH_2Ad) and 3.86–3.67 (4 H, m, $ClCH_2CH_2O$); δ_C([$^2H_6$]-DMSO) 156.0 (ArC), 152.9 (ArCH), 149.7 (ArC), 141.1 (ArCH), 71.7, 69.0 ($ClCH_2CH_2O$) and 43.2 (OCH_2Ad) [Found: $(M + H)^+$, 228.0652. $C_8H_{10}ClN_5O$ requires (M + H) 228.0652].

9-(4-*Methylthiobutyl*)*adenine* **7**.—Triphenylphosphine (1.71 g, 6.52 mmol) and DEAD (1.02 cm^3, 1.13 g, 6.48 mmol) were added to a solution of 6-chloropurine (1.01 g, 6.53 mmol) in THF (50 cm^3). After 5 min, 4-methylthiobutan-1-ol (0.61 cm^3, 0.61 g, 5.04 mmol) was added dropwise and stirred at room temperature for 26 h. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography using light petroleum–ethyl acetate (2:1) as eluent, to give 6-chloro-9(4′-methylthiobutyl)purine as an oil (0.88 g, 69%), R_f 0.61 CH_2Cl_2–MeOH (9:1); ν_{max}(film)/cm^{-1} 1595 and 1561 (purine); δ_H($CDCl_3$) 8.72 (1 H, s, ArH), 8.12 (1 H, s, ArH), 4.31 (2 H, t, *J* 7, CH_2-N), 2.52 (2 H, t, *J* 7, SCH_2), 2.11–1.98 (5 H, m, CH_2CH_2-N and CH_3S) and 1.69–1.56 (2 H, m, SCH_2CH_2) [Found: $(M + H)^+$, 257.0628. $C_{10}H_{13}ClN_4S$ requires (M + H) 257.0628].

An excess of liquid ammonia was added to this chloropurine (0.14 g, 0.55 mmol) in a Teflon tube. This was then fitted into a stainless steel vessel, sealed and left overnight at room temperature. After 18 h, the pressure was released and ammonia allowed to evaporate off. The residue was purified by silica gel chromatography using dichloromethane–methanol (29:1) as eluent to give the title compound **7** as a white solid (0.10 g, 75%), R_f 0.35 CH_2Cl_2–MeOH (9:1); m.p. 121–123 °C; ν_{max}(KBr)/cm^{-1} 3275 (NH_2), 1678 and 1603 (purine); δ_H(CD_3OD) 8.20 (1 H, s, ArH), 8.11 (1 H, s, ArH), 4.24 (2 H, t, *J* 7, CH_2Ad), 2.50 (2 H, t, *J* 7, SCH_2), 2.06–1.91 (5 H, m, CH_2CH_2Ad and CH_3S) and 1.65–1.52 (2 H, m, SCH_2CH_2); δ_C(CD_3OD) 157.3 (ArC), 153.7 (ArCH), 150.7 (ArC), 142.7 (ArCH), 120.1 (ArC), 44.5 (CH_2Ad), 34.3 (SCH_2), 30.1 (CH_2CH_2Ad), 27.0 (SCH_2CH_2) and 15.2 (CH_3S) [Found: $(M + H)^+$, 238.1126. $C_{10}H_{15}N_5S$ requires (M + H) 238.1126. Found: C, 50.5; H, 6.2; N, 29.1; S, 13.1. $C_{10}H_{15}N_5S$ requires C, 50.6; H, 6.4; N, 29.5; S, 13.5%].

9-(2-*Methylsulfonyloxyethyl*)*adenine* **8**.—Distilled methanesulfonyl chloride (0.20 cm^3, 0.30 g, 2.58 mmol) was added to a solution of alcohol **4** (0.44 g, 2.09 mmol) in pyridine (10 cm^3) cooled to 0 °C. After 10 min, the reaction was allowed to warm to room temperature and after 1 h, TLC indicated no remaining starting material. Ice was then added and the product extracted with ethyl acetate (4 × 50 cm^3). The combined extracts were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane–methanol (14:1) as eluent, to give mesylate **8** as a white crystalline solid (0.37 g, 69%), R_f 0.11 CH_2Cl_2–MeOH (14:1); m.p. 189–192 °C; ν_{max}(KBr)/cm^{-1} 3300 (NH_2), 3137 (NH_2), 1670, 1605 (purine), 1341 (SO_2) and 1164 (SO_2); δ_H([$^2H_6$]-DMSO) 8.18 (1 H, s, ArH), 8.15 (1 H, s, ArH), 7.20 (2 H, br s, NH_2), 4.64–4.56 (2 H, m, $MsOCH_2$), 4.55–4.47 (2 H, m, CH_2Ad) and 3.11 (3 H, s, CH_3SO_2-O); δ_C([$^2H_6$]-DMSO) 155.9 (ArC), 152 (ArCN), 149.5 (ArC), 140.7 (ArCH), 116.6 (ArC), 67.7 ($MsOCH_2$), 42.3 (CH_2Ad) and 36.7 (CH_3SO_2-O) [Found: $(M+H)^+$, 258.0661. $C_8H_{11}N_5O_3S$ requires (M + H) 258.0661. Found: C, 37.6; H, 4.0; N, 27.0; S, 12.4. $C_8H_{11}N_5O_3S$ requires C, 37.4; H, 4.3; N, 27.2; S, 12.5%].

9-(2′-Methylthioethyl)*adenine* **9**.—Sodium methanethiolate (0.04 g, 0.57 mmol) was added to a suspension of mesylate **8** (0.10 g, 0.39 mmol) in methanol (13 cm^3). The system was stirred at room temperature. After 6 h, dry DMF (0.5 cm^3) and a further portion of sodium methanethiolate (0.04 g, 0.57 mmol) were added. Stirring was continued at room temperature for a further 16 h when TLC indicated that no starting material remained. Water (10 cm^3) was added and, following saturation of the aqueous phase with sodium chloride, the product extracted with ethyl acetate (5 × 60 cm^3). The combined extracts were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane–methanol as eluent, to give sulfide **9** as a white solid (0.08 g, 92%), R_f 0.29 CH_2Cl_2–MeOH (9:1); m.p. 160–162 °C; ν_{max}(KBr)/cm^{-1} 3289 (NH_2), 1669 and 1601 (purine); δ_H([$^2H_6$]-DMSO) 8.15 (2 H, s, ArH), 7.17 (2 H, br s, NH_2), 4.35 (2 H, t, *J* 7, CH_2Ad), 2.94 (2 H, t, *J* 7, CH_3SCH_2) and 2.05 (3 H, s, CH_3S); δ_C([$^2H_6$]-DMSO) 155.7 (ArC), 152.3 (ArCH), 149.5 (ArC), 141.0 (ArCH), 116.6 (ArC), 41.9 (CH_2Ad), 32.7 (CH_3SCH_2) and 14.2 (CH_3S) [Found: $(M + H)^+$, 210.0813. $C_8H_{11}N_5S$ requires (M + H) 210.0813].

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