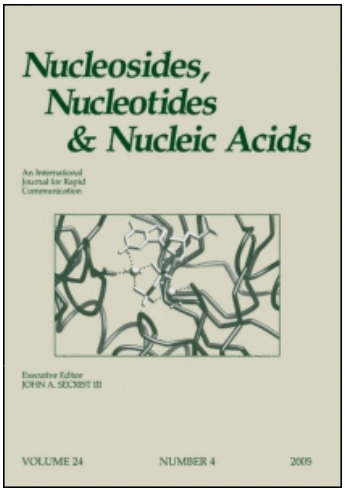

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THE SYNTHESIS AND PROPERTIES OF TRICYCLIC ANALOGUES OF S^6-METHYLTHIOGUANINE AND O^6-METHYLGUANINE

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□ *The syntheses of novel tricyclic pyrrolo[2,3-d]pyrimidine analogues of O^6-methylguanine and S^6-methylthioguanine are described. The crystal structures and pK_a values of these analogues are reported. In a standard substrate assay with the human repair protein O^6-methylguanine-DNA methyltransferase (MGMT) only the oxygen-containing analogue displayed activity.*

Keywords Pyrrolo[2,3-*d*]pyrimidines; O^6-methylguanine; MGMT; crystal structures, pK_a

INTRODUCTION

The protein O^6-methylguanine-DNA methyltransferase (MGMT) repairs toxic O^6-alkylguanine lesions in DNA by transfer of the alkyl substituent to an active site cysteine in an irreversible reaction.[1] The repair

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mechanism requires protonation of the damaged base probably by a tyrosine residue. DNA which contains S^6-methylthioguanine is also a substrate. Recently the crystal structure of an ethanoxanthosine-containing oligonucleotide[2] cross-linked to MGMT has been reported[3] and has revealed a minor groove-binding mechanism in which the target base is flipped out of the DNA duplex. Our interests in designing pseudosubstrates of O^6-methylguanine for crosslinking to MGMT have concentrated on tricyclic pyrrolo[2,3-*d*]pyrimidine analogues. Since these compounds retain the N-2 amino group of the natural substrate and would be linked to MGMT via the N7 position (replaced by C in our analogues) we expect that information derived from such complexes would complement that obtained using ethanoxanthosine-containing DNA.

SYNTHESIS

Previously, compounds **1** and **3** were synthesised in four steps from the appropriate 5-substituted pyrimidine precursors **7** and **6**, respectively (Figure 1).[4] Compounds **6**[5] and **7**[4] were prepared via Michael addition of 2,6-diamino-4(3*H*)pyrimidinone to the appropriate nitroalkenes which were in turn obtained in four steps from 1,3-propanediol or 1,4-butanediol respectively. The tricyclic pyrrolopyrimidines **4a–b**, were prepared via an alternative route in which an appropriate α-bromoaldehyde was condensed with 2,6-diamino-4(3*H*)pyrimidinone, and the resulting 5-substituted pyrrolopyrimidines subject to thiation at the 4-oxo position and cyclisation via the Mitsunobu reaction.[6]

CRYSTAL STRUCTURES AND pK_a VALUES

The x-ray crystal structures of tricyclic pyrrolo[2,3-*d*]pyrimidines **4a**, **2**, and **5b** are shown in Figure 2. These structures suggest that ring strain within the non aromatic ring in each compound is small, while the compounds

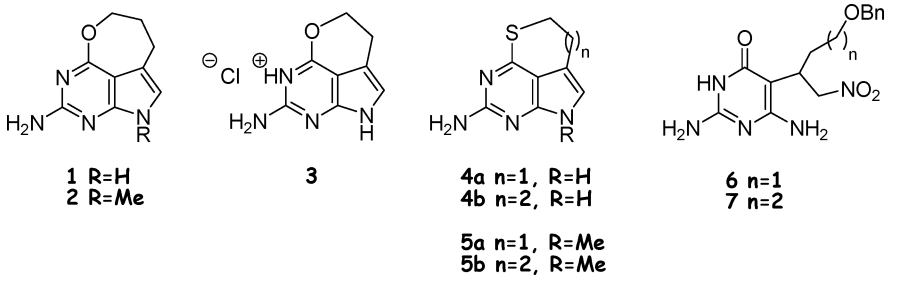

FIGURE 1 Four novel tricyclic pyrrolo[2,3-d]pyrimidine analogues of O^6-methylguanine (**1**, **3**, and **4a–b**) and 5-substituted pyrimidine precursors (**6** and **7**).

FIGURE 2 Crystal structures of 4a (left), 2 (center), and 5b (right).

(except the 6-ring oxygenated analogue **3**) display good stability in water and methanolic ammonia. The ability of MGMT to react with these analogues in DNA depends on the suitable positioning of the electrophilic methylene group within the active site and the requirement for the analogue to have a similar basicity to the natural substrate O^6-methylguanine. The electrophilic carbon, at least in structures **2** and **5b**, is displaced somewhat from the plane of the pyrrolopyrimidine moiety thereby being compatible to the expected trajectory of nucleophilic attack during DNA repair. pK_a values (determined by UV) of **2**, **5a–b** and **8** (the *N*-methyl derivative of 7-deaza-O^6-methylguanine) (Table 1) show that all analogues are more basic than O^6-methylguanine (p$K_a = 2.35$).

BIOLOGICAL ACTIVITY OF ANALOGUES AS INHIBITORS OF MGMT

In a standard assay for MGMT inhibition/inactivation, compounds **4a–b** and **3** (following neutralisation of its hydrochloride salt) showed no activity following incubation with the protein for 4 hours. (Analogue **3** is sufficiently stable over this period to display activity.) Compound **1** was a weak inactivator (IC_{50} 1mM) suggesting that it is likely to be recognised as a substrate by the protein.[4] While the analogues are designed as cross-linking agents rather than optimal inactivators of MGMT this demonstrates at least for compound **1** that recognition in DNA is likely and infact is expected to be much better following incorporation into DNA.[1]

TABLE 1 Determined pK_a values for compounds **2**, **5a–b**, and **8**

Compound	Determined pK_a value
2	4.05 ± 0.05
5a	4.31 ± 0.03
5b	4.81 ± 0.02
8	4.27 ± 0.02

CONCLUSIONS

We have synthesized a variety of analogues of O^6-methylguanine which one incorporated into DNA might function as potential cross-linking agents with MGMT. One of these analogues (compound **1**) appears to be recognised by MGMT, while the others have structures and pK_a values which suggest that they also may be valuable in this regard. We are currently engaged in the synthesis of oligodeoxyribonucleotides containing analogues **1** and **4a–b** which will be reported subsequently together with their biological properties.

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