Synthesis of Tricyclic Nucleoside Analogue of $O^6$-Methyl-2'-Deoxyguanosine

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Abstract—Alkylation at the O6-position of guanine leads to one of the most significant mutagenic lesions in DNA, $O^6$-alkylguanine. The human protein $O^6$-methylguanine-DNA methyltransferase (MGMT) repairs these lesions by transferring the alkyl group to an active site cysteine in an irreversible manner. The levels of MGMT are often higher in tumour cells which reduce the effectiveness of many chemotherapeutic agents that alkylate DNA. This has led to the development of inactivators of this protein for use in chemotherapy. To learn more about the repair mechanism carried out by MGMT, a high resolution MGMT-DNA structure is required which may enable the design of new inhibitors to improve current cancer treatments.

Here we described the synthesis of 6-methyl-2’-deoxyguanosine ([6], a tricyclic nucleoside analogue of $O^6$-methyl-2’-deoxyguanosine

Index Terms—Alkylation, cancer, guanine, inactivation.

I. INTRODUCTION

Alkylation at the $O^6$-position of guanine leads to one of the most significant mutagenic lesions in DNA; $O^6$-alkylguanine which has many biological effects. The mutagenicity of this analogue (causes GC to AT transition mutations) as well as cytotoxicity is well documented and its DNA repair is undertaken by $O^6$-methylguanine-DNA methyltransferase (MGMT) protein [1]-[3]. The repair mechanism involves an $S_N2$ process in which the alkyl group is transferred to SH group of the cysteine residue which renders the protein inactive with the regeneration of guanine [4]. Although MGMT performs an important task in the repair of the $O^6$-alkylguanine lesions in DNA, it also promotes tumour resistance to certain alkylating agents commonly used in cancer treatment. MGMT is thus of great interest in the field of cancer chemotherapy and treatment because since the most common method used in cancer therapy is DNA alkylation which works by damaging DNA and induction of cancer cells apoptosis. Since MGMT repairs alkylation damage, its expression therefore counteracts these forms of treatment [5]. Also its high level of expression in certain types of tumour cells negates the therapeutic benefit of the cancer chemotherapy from the alkylated products [6]. Recently ATL proteins (alkyltransferase-like proteins) were discovered which are homologues of alkyltransferases that bind to, but do not de-alkylate DNA containing $O^6$-alkylguanine lesions.

In most ATL proteins the active site cysteine is substituted by tryptophan [7].

Different approaches have been taken to target the MGMT in the development of anti-cancer therapy. The success of MGMT depletion depends upon the $O^6$-alkylation being the determinant of the cancer cell death. Several attempts to deplete the level of MGMT through the provision of larger doses of alkylating agents or combining multiple agents so that the number of $O^6$-alkylguanine adducts in DNA exceeds the number of MGMT molecules were unsuccessful due to the toxicity [8], [9]. This problem has led to the increasing interest in designing more effective inhibitors of MGMT and understanding the repair reaction process. We have been interested for some time in incorporating tricyclic analogues of $O^6$-methylguanine into DNA that may allow covalent cross-linking of such DNA to MGMT or At1 (via a suitable mutant). Such complexes will allow structural information to be derived via X-ray crystallography or would provide suitable protein-DNA complexes for identification of protein partners from crude cell lysates.

Prior to Daniels’ crystal structure in 2004, all crystal structures of MGMT or related alkyltransferases from other organisms were obtained in the absence of substrate DNA [10]. In 2001, Noll and Clarke described the formation of an MGMT-DNA complex as a result of interstrand cross-links via $N^0$, $O^6$-ethanoguanine (1) Fig. 1 [7].
Here we describe chemistry directed to the synthesis of the nucleoside 2.

II. RESULTS AND DISCUSSION

The synthesis of the key intermediate pyrrolo [2, 3-d] pyrimidinone 9 was achieved in seven steps from butane-1, 4-diol (Fig. 3).

Thus, butane-1, 4-diol 3 was monobenzoylated using benzoyl chloride in pyridine and triethylamine to give 4-benzoyloxy-1-butanol 4, in approximately 44% yield. The monobenzylation product is formed together with a bis-benzoylated diol (40%) and some of the starting material was found unreacted from the TLC of the reaction mixture. The crude product was distilled under reduced pressure and Compound 4 was isolated as colourless oil which solidifies as a white solid at room temperature.

The benzoylated alcohol 4 was oxidised using pyridinium chlorochromate (PCC) in dichloromethane to give a white solid 11 in 20% yield. Compound 9 was obtained in 50% yield using silica column chromatography.

Debenzylation was carried out by refluxing compound 9 in 1M NaOH at 50°C for one hour. The mixture was neutralised with acetic acid and after column chromatography afforded the pure pyrrolopyrimidine alcohol 10 as white solid in 63% yield.

The bis-chlorination was carried out on compound 10 by refluxing with phosphoryl chloride at 80°C for two hours. The product was purified by silica column chromatography to give a white solid 11 in 20% yield. Compound 9 was found to be insoluble in acetonitrile (usual solvent for glycosylation reaction). Furthermore, the presence of the lactam NH in addition to the pyrrolic NH could lead to both N1 and N4 glycospilation reaction). Furthermore, the presence of the lactam NH in addition to the pyrrolic NH could lead to both N1 and N4 glycosylation residue. Thus, the benzoyl protected pyrrolopyrimidine 9 was obtained after stirring at 80°C, 2h.

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Glycosylation of the bis-chlorinated nucleobase 11 was achieved by reaction of its sodium salt with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-a-D-erythro-pentofuranose (chlorosugar) in anhydrous acetonitrile. Removal of the toluoyl protecting groups was achieved by treatment with a 1:1 mixture of 1M aq. NaOH/1,4-dioxane. TLC analysis showed that compound 14 was obtained after overnight
stirring but after 3 days, two nucleosides, the tricyclic nucleoside 2 (Rf 0.45 in 10% MeOH/DCM) which is fluorescent under UV and the ring open nucleoside 15 were observed. Purification by silica column chromatography gave compound 2 in 19% yield and 15 in 42% yield (Fig. 5).

Reagents and reaction conditions
molecular sieves under Ar. Magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from hydride and then distilled and stored over 3Å molecular sieves anhydrous in the reaction procedure. Pyridine, acetonitrile and triethylamine were dried under reflux from calcium chloride and then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from hydride and then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar.

In conclusion, synthesis of compound 2 was successfully achieved via the bis-chlorinated intermediate 11. We are currently engaged in incorporating the tricyclic nucleoside into DNA which will be subsequently reported elsewhere.

III. EXPERIMENTAL

Most of the solvents were obtained from Fisher and used without further purification if they were not requested to be anhydrous in the reaction procedure. Pyridine, acetonitrile and triethylamine were dried under reflux from calcium hydride and then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar. MeOH was dried under reflux from magnesium and iodine, then distilled and stored over 3Å molecular sieves under Ar.

A. 4-(Benzyloxy)Butanol (4)

A solution of 1,4-butanediol (20.00 g, 222.2 mmol.) was dissolved in pyridine (19.00 g, 242.3 mmol.) followed by the addition of dichloromethane (33 mL) and cooled to 0°C in an ice-bath. Benzyloxyl chloride (16.00 g, 12.8 mL, 110.0 mmol) in dichloromethane (20 mL) was added dropwise and the reaction was left to stir overnight. The solution was diluted with ether (100 mL) and the organic phase was washed with water (3 x 20 mL), sodium bicarbonate solution (3 x 20 mL), dried (MgSO4) and evaporated. The resulting oil was purified by silica column chromatography (eluted with ethyl acetate: hexane 1:1) to obtain a colourless oil (22.10 g, 51%); Rf (A), 0.3; δH (CDCl3) 1.75 (2H, m, CH2CH2OH), 1.85 (2H, m, CH2CH2CH2OH), 3.71 (2H, t, J 6.5, CH2OBz), 4.35 (2H, t, J 6.5, CH2OC=O), 7.42-7.63 (3H, m, CH2-Ph), 8.14 (2H, d, J 7.3, CH, Ph); m/z (ES+) 217 ([M⁺ (Na)⁺] 100%); Acc Mass: 217.0835; calculated for C11H14O3N 217.0841 (deviation -2.8 ppm).

B. 4-(Benzyloxy)Butan-5 (5)

A solution of 4-benzyloxybutanol (4) (14.75 g, 76.0 mmol.) in dichloromethane (100 mL) was added in one portion to a solution of a stirred suspension of pyridinium chlorochromate 98% (35.88g, 166.5 mmol.), hyflosuper cel (10 g) and silica (10 g), the pyridinium chlorochromate, hyflosuper cel and silica where well stirred in dichloromethane (200 mL) in an ice-bath before adding the alcohol. After addition the bright orange suspension becomes black. The reaction was left stirring for 3 h. until complete (checked by silica TLC). The solution was separated from the black precipitate by filtration using a column containing three layers; sand, hyflosuper cel and silica. The column was eluted with dichloromethane and the filtrate was evaporated to give a colourless oil (13.71 g, 93%); Rf (A), 0.54; δH (CDCl3) 2.12 (2H, m, CH2CH2CHO), 2.60 (2H, t, J 6.5, CH2CHO), 4.61 (2H, J 6.5, CH2OBz), 7.45-7.60 and 8.05 (5H, m, CH=Ph), 9.84 (s, 1H, CHO); δC (CDCl3) 20.4 (CH2CH2CHO), 39.5 (CH2CHO), 62.9 (CH2OBz), 126.0 (1 para CH-Ph), 127.0 (2 meta CH-Ph), 128.0 (2 ortho CH-Ph), 132.0 (C-Ph), 165.4 (O=C-Ph), 200.1 (CHO); m/z (ES+) 193 ([M+H⁺] 100%).

C. 5-(Benzyloxy)-1-Nitropentan-2-ol (6)

4-Benzoyloxybutan-5 (12.90 g, 67.2 mmol.) and nitromethane (4.10 g, 67.2 mmol.) were dissolved in equal volume of ethanol (30 mL each) and cooled in an ice-bath to 0°C and sodium hydroxide (6 M, 2.8 g in 12 mL water) was added dropwise over 3 h. so that the temperature did not exceed 0°C. Ice water (50 mL) was then added to the reaction mixture followed by the addition of glacial acetic acid until the precipitated salt was dissolved and the pH was 6. The solution was extracted with ethyl acetate (175 mL) and the organic phase was washed with water (50 mL), then saturated sodium chloride solution (50 mL), then dried (MgSO4) and evaporated to give orange-yellow oil (11.70 g). Purification by silica column chromatography (eluted with dichloromethane) gave a pale-yellow oil which solidifies at room temp. (6.60 g, 26.2 mmol, 39%); Rf (B), 0.44; δH (CDCl3) 1.60 (2H, m, CH2CH2OBz), 1.94 (2H, q, J 6.2, CH2CH2CH2OBz), 4.45 (3H, m, CH2(OH)CH2NO2, CH2-OBz), 7.45-8.0 (5H, m, CH=Ph); δC (CDCl3) 24.8
(CH₃CH₂CHOBz), 30.7 (CH₃CH₂CH₂OBz), 64.9 (CH₂-OBz), 68.2 (CH-OH), 82.0 (CH₂NO₃), 129.2 and 129.5 (5 CH-Ph), 133.7 (C-Ph), 166.2 (O=C-Ph); m/z (ES⁺) 276 ([M⁺ (Na⁺)]; Acc. Mass: 276.0853; calculated for C₁₂H₁₅NO₅Na requires 276.0848 (deviation 1.7 ppm).

D. (E)-5-Nitropent-4-Enylbenzoate (7)

Methanesulfonyl chloride (2.00 g, 17.7 mmol) was added to stirred solution of 6 (4.50 g, 17.7 mmol) in dry dichloromethane (30 mL) at 0°C under an argon atmosphere. Dry triethylamine (4.9 mL, 35.4 mmol) was then added via a syringe pump at the rate 5 mL h⁻¹. When the reaction was completed, the mixture was transferred to a separating funnel with dichloromethane (30 mL) and the organic layer was sequentially washed with water (20 mL), 5% HCl (20 mL), 10% Na₂CO₃ (20 mL) and brine (20 mL). The organic layer was dried (MgSO₄) and the solvent removed in vacuo, giving the crude product 7 (2.60 g, 11.1 mmol, 63%) as an orange oil, which was used further without further purification. Rf (B) 0.44; δₙ (CDCl₃) 2.03 (2H, m, CH₂CHOBz), 2.44 (2H, q, J 6.8, CH₃CH₂CH₂OBz), 4.40 (t, J 6.8, CH₂-OBz), 6.90 (1H, t, J 6.8, CH₂NO₃), 7.31-8.09 (6H, m, 1H CHNO₂ and 5 CH-Ph); δc (CDCl₃) 25.3 (CH₂-OBz), 63.6 (CH₂-OBz), 128.4 and 129.5 (5 CH-Ph), 133.2 (C-Ph), 140.0 (CH₂NO₃), 141.3 (CH₂NO₃), 166.0 (O=C-Ph); m/z (ES⁺) 236 [M⁺ H⁺].

E. 2,6-Diamino-5-[3-Benzoylmoxy-1-Nitropentan-2-yl]Pyrimidin-4(3H)-One (8)

A solution of 2,6-diamino-4(3H)-One (8) (d6-DMSO) 1.65 (2H, CH₃CH₂OH), 2.55 (2H, t, J 6.2 Hz, CH₃CH₂CH₂OH), 3.35 (2H, t, J 6.2, CH₃CHOH), 4.47 (1H, s, OH) 5.97 (2H, s, NH₂), 6.35 (1H, s, H-6), 7.47-7.98 (5H, m, CH-Ph), 10.20 (1H, s, NH-3), 10.75 (1H, s, NH-7); δc (d6-DMSO) 22.7 (CH₂-OBz), 34.0 (CH₃CH₂CH₂OH), 60.7 (CH₃CHOH), 99.3 (C-5), 113.6 (C-4a), 118.6 (C-6), 151.7 (C-7a), 152.1 (C-4), 154.7 (C=O); m/z (ESI⁺) 209 [M⁺ H⁺]; Acc Mass: 382.1290; calculated for C₁₆H₂₂N₄O₃ requires 382.1293 (deviation -0.3 ppm).

H. 2-Amino-5-(3-Chloropropyl)-4-Chloro-7H-Pyrrolo[2, 3-d]Pyrimidine (11)

Compound 10 (1.00 g, 4.8 mmol) was suspended in phosphorl chloride (7 mL) and the solution was heated at reflux overnight at 50°C. The solution was allowed to cool and poured on to the crushed ice (30 mL), stir for 10 min. and then neutralized to pH 7 with conc. aqueous ammonia. It was left for 10 min. in an ice-bath and then filtered. Attempt to purify the compound on the silica column chromatography (10% MeOH/DCM) afforded the compound in pure form as pale yellow solid (200 mg, 20%).
mg, 890 μmol, 60% in mineral oil) was added cautiously and the reaction left to stir for 1h, when α-chlorosugar (344 mg, 890 μmol) was added in portions over 5min. After that the reaction was left to stir for 3h, the solvent removed in vacuo, redissolved in DCM (20 mL) and washed with water (50 mL), 5% aqueous HCl (50 mL) and brine (50 mL). The organic layer was then dried (MgSO₄) and evaporated to purify the residue by silica column chromatography, eluting with 2% methanol in DCM. The product 13 was isolated as pale yellow foam (400 mg, 60%); Rf (B, 0.35; δ₆ (CDCl₃) 1.90 (2H, m, CH₂CH₂Cl), 2.35 (6H, s, 2 x Tol-CH₃), 2.50-2.60 (1H, m, H₂), 2.66-2.70 (1H, m, H₂'), 2.80 (2H, t, J 6.8, CH₂CH₂Cl), 3.50 (2H, t, J 6.8, CH₂Cl), 4.44-4.54 (2H, m, H₄' and H₅'), 4.68-4.76 (1H, m, H₃'), 5.10 (2H, s, NH₂), 5.70 (1H, m, H₃'), 6.63 (1H, dd, J 3.4, 6.8, H1'), 6.75 (1H, s, H-6), 7.21 (4H, m, meta CH-Tol), 7.91 (4H, d, J 6.8, ortho CH-Tol); δ₂C (CDCl₃) 21.7 (2x Tol-CH₃), 23.1 (CH₃CH₂CH₂Cl), 32.7 (CH₂CH₂CH₂Cl), 37.3 (C2'), 44.4 (CH₂Cl), 62.2 (C5'), 75.1 (C3'), 82.0 (C1'), 83.5 (C4'), 115.5 (C-4a), 119.6 (C-7a), 119.7 (C-6), 126.5 and 126.8 (2xCH₂Tol), 129.1, 129.2, 129.6 and 129.8 (8 orto and meta CH-Tol), 144.2 and 144.4 (2x Tol-C=C=O), 152.4 (C-5), 154.3 (C-4), 158.4 (C-2), 166.0 and 166.2 (2x C=O); m/z (ESI⁺) 597 [M+H⁺]; Acc Mass: 597.1655; calculated for C₃₀H₃₁N₄O₅Cl₂ requires 597.1672 (deviation -2.8 ppm)

J. 4-Amino-2-(2’-Deoxy-β-D-Erythro-Pentofuranosyl)-6-Oxa-7,8,9-Trihydro-2,3,5-Triazabenzo[cd]Azulene (2)

To a solution of 13 (280 mg, 470 μmol) in 1,4-dioxane (15 mL), 1M aqueous NaOH solution was added (15 mL, 15 μmol) and the mixture was refluxed at 90°C for 3 days. Once the solution cooled down it was neutralised with 0.1M acetic acid aqueous solution (1 mL), then evaporated and the residue purified by silica column chromatography eluting with 10% MeOH in DCM. The product 2 was obtained as a pale yellow solid (27 mg, 235 μmol, 19%); Rf (D), 0.50 (fluorescent at 356 nm); δ₆ (d₆-DMSO) 2.08-2.12 (3H, m, CH₃CH₂OH and H₂'), 2.30-2.40 (1H, m, H₂), 2.77 (2H, t, J 7.6 Hz, CH₂CH₂CH₂O), 3.45-3.54 (2H, m, H₅' and H₅’), 3.73-3.79 (1H, m, H₄), 4.25-4.29 (1H, m, H₃), 4.34 (2H, t, J 6.8, CH₂O), 4.91 (1H, t, J 6.8, C₅’-OH), 5.21 (1H, d, J 6.8, C₃’-OH), 6.08 (2H, s, NH₂), 6.43 (1H, d, J 3.4, 6.8, H1’), 6.92 (1H, s, H-6); δ₋₁₃C (d₆-DMSO) 25.9 (CH₃CH₂O), 28.9 (CH₂CH₂CH₂O), 38.8(C2’), 62.1 (C5’), 71.1 (C3’), 71.8 (C₃’), 81.8 (C1’), 86.8 (C4’), 97.5 (C-9a), 114.1 (C-10), 115.1 (C-1), 155.3 (C-2a), 159.7 (C-5a), 164.9 (C-4); m/z (ESI⁺) 329 [M+Na⁺] ; Acc Mass: 329.1220; calculated for C₆H₁₁N₂O₂Cl₂ requires 329.1226 (deviation -1.7 ppm)

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**REFERENCES**


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