

Synthesis of 2-Amino-5-(3-chloropropyl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (*Bischlorinated Deazaguanine*)

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Abstract: There are many forms of DNA damage which may arise from the modifications DNA bases. Deazaguanine is a modified DNA base which is recognised by DNA repair enzyme, Methylguanine DNA-methyltransferase (MGMT). The synthesis of *bischlorinated deazaguanine* was successfully carried out via two different routes. The first route was achieved through the reaction of pyrrolopyrimidine with benzyl(triethyl)ammonium chloride, dry dimethylaniline and freshly distilled phosphoryl chloride in acetonitrile under argon atmosphere. An alternative method was employed toward achieving the *bischlorinated* through the debenzoylation of the protected pyrrolopyrimidine with 1 M NaOH at 50°C for 1h followed by double chlorination reaction using phosphoryl chloride alone at 80°C for 1h. Both routes were found to be successful with lower impurities recorded from the second route though it gives a low yield but it was shorter.

Keywords: Deazaguanine, Phosphoryl Chloride, Alkylation, Pyrrolopyrimidine, Debenzoylation

1. Introduction

There are many ways of modifying DNA in which alkylation is one of the most common arising from the addition of an alkyl group [1]. Depending on the alkylation position the resulting consequences may be different. Alkylation at the *O*⁶-position of guanine leads to one of the most significant mutagenic lesion in DNA; *O*⁶-alkylguanine which has many biological effects [2]. There are twelve different positions in DNA which react with alkylating agents to give the alkylated products. Eleven of these positions are found on the DNA-bases whilst alkylation of the phosphate diester backbone can also occur [3]. Alkylating agents are those agents that cause DNA damage by forming *O*⁶-alkylguanine adducts which if not repaired before the next cycle of cellular replication can be misread and incorrectly paired with thymine rather than cytosine [4]. DNA alkylation is also a common goal of chemotherapy in cancer treatment. There exist two main types of drugs in alkylation chemotherapy; the methylating agents and the chloroethylating agents. Methylation at the *O*⁶-position of guanine changes the Watson-Crick hydrogen bonding with the opposite cytosine [5], which leads to the loss of a proton at the N1-position of G, the N1-atom is changed from being a

donor to an acceptor and this results in distortion of the double helix which subsequently slows down the DNA polymerase during replication [6]. The main effect is that T is inserted opposite G during replication which leads to a transition mutation from *O*⁶-meG:C to *O*⁶-meG:T. Alkylation of guanine by agents containing the chloroethylating group occur in two steps; first is the alkylation which is later followed by a cyclisation reaction which gives rise to *N*¹, *O*⁶-ethanoguanine. This intermediate is reactive toward cytosine which subsequently leads to an interstrand cross-links between the bases [7]. This type of cross-linking in the DNA duplex if not properly repaired may bring a halt to DNA replication which may eventually result in cell apoptosis [6 & 8].

The study attempts to show the chemistry involve in the chlorination of the deazaguanine which later cyclised to give *O*⁶-methyldeazaguanine.

2. Results and Discussion

There are two different synthetic routes toward achieving the *bis*-chlorinated nucleobase 5 from the key intermediate 1 as shown in figure 1.

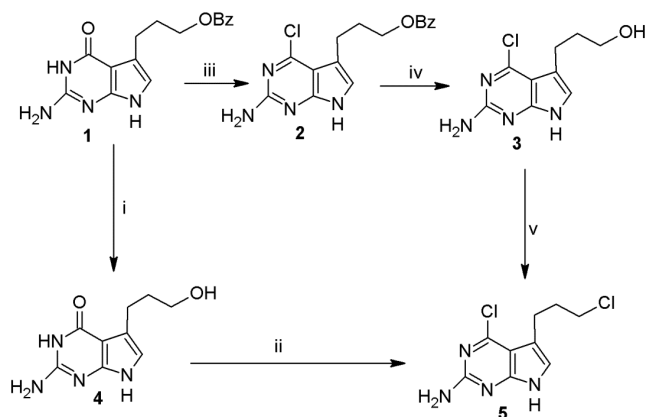


Figure 1. Synthesis of bis-chlorinated nucleobase 5.

Reagents and reaction conditions: (i) 1 M NaOH, reflux 50°C, 1h, 63%; (ii) POCl₃, reflux at 50°C, 1h, 20%; (iii) POCl₃, reflux at 80°C, 1h; (iv) 1 M NaOH, reflux 50°C, 1h; (v) POCl₃, reflux at 50°C, 1h;

Synthesis of compound 1 was reported somewhere [9]. Chlorination was undertaken by treating 1 with benzyl(triethyl)ammonium chloride (2 equivalents), dry dimethylaniline (1.4 equivalents) and freshly distilled phosphoryl chloride (6 equivalents) were dissolved in acetonitrile under argon and refluxed for 30 min [10 & 11]. After cooling to room temperature the reaction mixture was poured onto a crushed ice and neutralised using conc. aqueous ammonia solution. A black solid was obtained which was insoluble in dichloromethane, methanol as well as ethylacetate. TLC analysis in 10% Methanol/dichloromethane shows two spots at the top of the plate and one spot at the baseline. One of the top spots was expected to be dimethylaniline and that on the baseline was assumed to be the salt of benzyl(triethyl)ammonium. The ¹H-NMR spectrum of the solid material indicated signals mainly from the benzyl(triethyl)ammonium salt, with no significant presence of any pyrrolopyrimidine signals expected at 5.97, 6.35, 10.20 and 10.75 ppm. It was decided to repeat the reaction at a larger scale under the same conditions. After two hours reflux, TLC analysis showed little progress, so another batch of the same equivalent of the reagents was further added and stirring continued for another one hour still little progress had occurred and the suspension was left stirring at room temperature overnight. After overnight stirring at room temperature still not much development had occurred probably due to lack of heating. So it was decided to heat the reaction at 50°C for two hours after which the excess phosphoryl chloride was removed by distillation. The residue was cooled down, added to crushed ice and then neutralised with aqueous ammonia solution. After extraction of product with dichloromethane only dimethylaniline was observed by silica TLC. It was decided to concentrate the aqueous layer and try to dissolve the organic solids with methanol. This also proved useless or unsuccessful as only the benzyltriethylammonium chloride signals were observed from the ¹H NMR spectrum.

An alternative method was employed toward achieving the

bischlorinated compound 5. Debenzoylation was carried out by refluxing compound 1 with 1 M NaOH at 50°C for 1h. The mixture was neutralised with acetic acid and silica gel column chromatography afforded the pure pyrrolopyrimidine 4 as white solid in 63% yield. Double chlorination was carried out on compound 4 with phosphoryl chloride alone (12 equivalents at 80°C for 1h). As the temperature started to increase the reaction mixture started to change from a pink suspension to a dark brown solution which was completed within an hour. Following the reaction, excess POCl₃ was removed by distillation and the remaining contents of the flask was allowed to cool down to room temperature before it was poured onto the crushed ice and neutralised using conc. aqueous ammonia solution. The product was extracted with dichloromethane and purified by silica column chromatography to give a white solid, the chloro compound 5 identified by down field shift of pyrrolopyrimidine ¹H-NMR in 20% yield. The mechanism for this chlorination reaction is outline in Figure 2.

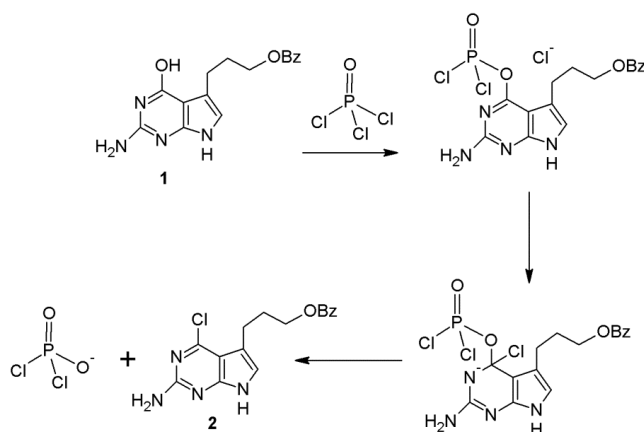


Figure 2. Mechanism for the monochlorination reaction.

Although the first route also works but it was found to proceeds with a lots of impurities of triethylammonium salt which are difficult to dissolve in many solvents and this makes the work up of the reaction a bit difficult. Therefore the later route (through the pyrrolopyrimidine alcohol 4) was found to be the shortest and most feasible route toward the bis-chlorinated nucleobase 5. In both routes the product was formed along with a side product of tricyclic nucleobase which was found to be fluorescent under UV.

3. Materials and Methods

2-Amino-5-(3-benzoyloxypropyl)-4-chloro-7H-pyrrolo[2,3-d] pyrimidine (2)

Compound 1 (3.0 g, 9.6 mmol), benzyltriethylammonium chloride (2.2 g, 9.6 mmol) and dry dimethylaniline (1.6 g, 13.4 mmol) were dissolved in dry acetonitrile (50 mL) under argon. Freshly distilled phosphoryl chloride (5 mL, 57.7 mmol) was then added and the mixture was refluxed at 90°C for 1h. The acetonitrile was removed by distillation and the residue added to crushed ice (60 mL). The pH of the mixture was adjusted to 7-8 by adding conc. aq. ammonia solution

and the product was extracted into dichloromethane (100 mL). The organic layer was then washed with distilled water (50 mL), brine (50 mL) and dried (MgSO₄). Attempts to purify the residue by silica gel column chromatography were unsuccessful.

Alternatively Compound 1 (1.0 g, 3.2 mmol) was suspended in freshly distilled phosphoryl chloride (10 mL, 39.6 mmol) and the solution was reflux 3 h at 80°C. Phosphoryl chloride was removed by distillation and the rest of the flask contents poured onto crushed ice (30 mL), stirred for 10 min. and then neutralized to pH 7 with aq. ammonia. After 10 min. the mixture was filtered and the solid obtained was purified by silica gel column chromatography. The product 3 was eluted with 10% MeOH / DCM and isolated as a pale brown solid (330 mg, 33%).

TLC (10% MeOH / DCM); R_f = 0.50

δ_H (d₆-DMSO) 2.02 (2H, m, CH₂CH₂OBz), 3.35 (2H, t, J = 6.5 Hz, CH₂CH₂CH₂OBz), 4.36 (2H, t, J = 6.5 Hz, CH₂OBz), 6.45 (2H, s, NH₂), 6.81 (1H, s, H-6), 7.55-7.97 (5H, m, CH-Ph), 11.2 (1H, s, NH) ppm.

δ_C (d₆-DMSO) 22.1 (CH₂CH₂OBz), 29.3 (CH₂CH₂CH₂OBz), 64.1 (CH₂OBz), 112.8 (C-5), 120.3 (C-6), 128.7 and 129.0 (5 CH-Ph), 133.2 (C-Ph), 159.5 (C=O) ppm.

Mass Spec: m/z (ESI+) 331 [M+H]⁺, 333 [M+H]⁺

Acc Mass: 331.0974; calculated for C₁₆H₁₆ClN₄O₂ requires 331.0962 (deviation 3.8 ppm).

2-Amino-5-(3-hydroxypropyl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (3)

Compound 2 (2.0 g, 6.1 mmol) was stirred at room temperature overnight in a mixture of methanol (40 mL) and NaOH (1M, 20 mL). The mixture was extracted with ethyl acetate (100 mL) which was dried (MgSO₄) and evaporated to give the pure product 3 as a yellow solid (1.3 g, 65%).

TLC (10% MeOH / DCM); R_f = 0.25

δ_H (d₆-DMSO) 1.70 (2H, m, CH₂CH₂OH), 2.65 (2H, t, J = 6.2 Hz, CH₂CH₂CH₂OH), 3.35 (2H, t, J = 6.2 Hz, CH₂OH), 4.47 (1H, s, OH) 5.97 (2H, s, NH₂), 6.40 (1H, s, H-6), 11.20 (1H, s, NH-7) ppm.

δ_C (d₆-DMSO) 22.7 (CH₂CH₂OH), 34.0 (CH₂CH₂CH₂OH), 60.7 (CH₂OH), 99.3 (C-5), 113.6 (C-4a), 118.6 (C-6), 151.7 (C-7a), 152.5 (C-4), 159.7 (C=O) ppm.

Mass Spec: m/z (ESI+) 229 [M+H]⁺.

Acc Mass: 229.1038; calculated for C₉H₁₃ClN₄O requires 229.1039 (deviation -0.3 ppm).

2-Amino-5-(3-hydroxypropyl)-3,7-dihydropyrrolo[2,3-d]pyrimidin-4-one (4)

This was carried out using a slight modification of the literature as reported by Dovlatyan, et al., (2006). Compound 1 (5.0 g, 15.9 mmol) added to a solution of sodium hydroxide (2.0 g, 31.9 mmol.) in water (20 mL) was heated at 50°C for 3 h. After cooling it was neutralised with aq. acetic acid. The precipitated compound was filtered and washed with water to give 4 as a white solid (3.0 g, 63%).

TLC (10% MeOH / DCM); R_f = 0.40

δ_H (d₆-DMSO) 1.65 (2H, m, CH₂CH₂OH), 2.55 (2H, t, J = 6.2 Hz, CH₂CH₂CH₂OH), 3.35 (2H, t, J = 6.2 Hz, CH₂OH),

4.47 (1H, s, OH) 5.97 (2H, s, NH₂), 6.35 (1H, s, H-6), 10.20 (1H, s, NH-3), 10.75 (1H, s, NH-7) ppm.

δ_C (d₆-DMSO) 22.7 (CH₂CH₂OH), 34.0 (CH₂CH₂CH₂OH), 60.7 (CH₂OH), 99.3 (C-5), 113.6 (C-4a), 118.6 (C-6), 151.7 (C-7a), 152.51 (C-2), 159.7 (C=O) ppm.

Mass Spec: m/z (ESI+) 209 [M+H]⁺

Acc Mass: 209.1038; calculated for C₉H₁₃N₄O₂ requires 209.1039 (deviation -0.3 ppm).

2-Amino-5-(3-chloropropyl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (5)

Compound 4 (1.0 g, 4.8 mmol), benzyltriethylammonium chloride (1.0 g, 4.8 mmol) and dry dimethylaniline (1 mL, 6.7 mmol) were dissolved in dry acetonitrile (20 mL) under argon. Freshly distilled phosphoryl chloride (3 mL, 28.9 mmol) was then added and the mixture was refluxed at 90°C for 1h. The acetonitrile was removed by distillation and the residue added to crushed ice. The pH of the mixture was adjusted to 7-8 by adding conc. aq. ammonia solution and the product was extracted into dichloromethane (20 mL). The organic layer was then washed with water (10 mL), brine (10 mL) and dried (MgSO₄). Attempts to purify the residue by silica gel column chromatography were unsuccessful.

Alternatively Compound 4 (1.0 g, 4.8 mmol) was suspended in freshly distilled phosphoryl chloride (10 mL) and the solution was heated at reflux for 1h at 90°C. The solution was allowed to cool and poured onto the crushed ice (30 mL), stir for 10 min. and then neutralized to pH 7 with conc. aq. ammonia solution. It was left for 10 min. in an ice-bath and then filtered. Attempt to purify the compound on the silica gel column chromatography (10% MeOH / DCM) afford the compound 5 in pure form as a pale yellow solid (235 mg, 20%).

TLC (10% MeOH / DCM); R_f = 0.46

δ_H (d₆-DMSO) 2.05 (2H, m, CH₂CH₂Cl), 2.79 (2H, t, J = 6.8 Hz, CH₂CH₂CH₂Cl), 3.65 (2H, t, J = 6.8 Hz, CH₂Cl), 6.45 (2H, s, NH₂), 6.86 (1H, s, H-6), 11.21 (1H, s, NH) ppm.

δ_C (d₆-DMSO) 22.8 (CH₂), 32.9 (CH₂), 44.9 (CH₂Cl), 107.2 (C-5), 112.3 (C-4a), 120.3 (C-6), 150.7 (C-7a), 155.1 (C-2), 159.1 (C-4) ppm.

Mass Spec: m/z (ESI+) 245 [M+H]⁺.

Acc Mass: 245.0355; calculated for C₉H₁₁Cl₂N₄ requires 245.0361 (deviation -2.3 ppm)

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