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Glycosylation of the Pyrrolopyrimdine and Its Cyclisation into Tricyclic Nucleoside for Incorporation into Synthetic DNA

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Abstract: *Alkylation of DNA is commonly used during cancer chemotherapy. The most common chemotherapeutic agents are those that alkylate the O^6-position of guanine. Attempts were made in the modification of the O6-position of guanine for recognition by methylguaninemethyltranferase (MGMT) enzyme for DNA cross linking studies in an attempt to search for new cancer chemotherapeutic agents. Glycosylation of the pyrrlopyrimidine (modified guanine) was carried out successfully with 1-Chloro-2'-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranose to give the desired nucleoside. Several attempts were made inorder to cyclise the different modified nucleosides into their respective tricyclic nucleosides all of which were found to be unsuccessiful. However, this was found to be possible only with bischlorinated nucleoside using NaOH which give a fluorescence tricyclic nucleoside and the chemistry was presented here.*
Keywords: *Alkylation, cancer, chemotherapy, nucleoside, glycosylation*

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I. Introduction

Alkylation is one of the most common ways of modifying DNA arising by the addition of an alkyl group. Alkylation of DNA is commonly used during chemotherapy in cancer treatment. The most common chemotherapeutic agents are those that alkylate the O^6-position of guanine. The recognition and transfer of the alkyl group from the O^6-position of the alkylated guanine in DNA is mainly determined by the overall structure of the DNA.(1) It has been reported that alkylation at the O^6-position of guanine slightly distorts the phosphate diester backbone in double stranded DNA.(2-4). Methylguanine methyltransferase (MGMT) is a human repair protein (enzyme) which performs an important task in the repair of the O^6-alkylguanine lesions in DNA. Although it repairs alkylguanine lesion but it also promotes tumour resistance to certain alkylating agents commonly used in cancer treatment (5). MGMT is of great interest in the field of cancer chemotherapy since cancer therapy often uses alkylation which obviously works by damaging DNA to induced cancer cells apoptosis (6). Since MGMT repairs alkylation damage its expression therefore counteracts these forms of treatment. Also its high level of expression in certain types of tumour cells negates the therapeutic benefit of the cancer chemotherapy from the alkylated products.(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 search for non-toxic, pseudo-substrates. The first non-toxic MGMT inhibitor to show adequate potency was the O^6-benzylguanine (O^6-BnG) which inactivates MGMT by covalent transfer of the benzyl group to the active site cysteine.(10,11) *In vitro* and *in vivo* studies with tumour cells and in xenograpt have shown that the use of BnG to achieve MGMT inactivation followed by treatment with 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU) or temozolomide can be highly successful interms of killing tumour cell.(6,12-13) Despite the positive preliminary trial responses to O^6-BnG in combination with BCNU or temozolomide by certain tumour cells, the value of the therapy have been restricted due to lack of its specificity towards the tumour.(5, 14-17) In addition, the rate of MGMT inactivation by O^6-BnG is > 10^4 slower than the rate of normal repair reaction which clearly indicates the need for more improved potent inhibitors.(18) Other O^6-BnG analogs have been synthesised as potential MGMT inactivators but unfortunately, the potencies of most of them are only modestly better and none of them exhibit greater targeting of tumour cells than O^6-BnG.(19)

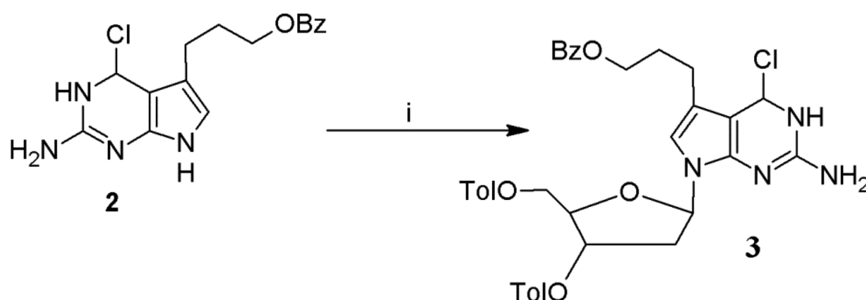
An alternative to O^6-BnG which is still in clinical trials in combination with temozolomide is the O^6-(4-bromothenyl)guanine (O^6-BTG) which result in covalent transfer of the bromothenyl group to the active site cysteine of MGMT.(6,20-23) O^6-BTG is a more oral available MGMT inactivator with a higher reactivity, but like O^6-BnG it also have lacks tumour specificity. Numerous other MGMT inactivators have also been described.(24)

The study attempts to show the chemistry involve in the glycosilation of the pyrrolopyrimidine compound (modified guanine) which later cyclised into a tricyclic nucleoside. The long term objective is to incorporate the modified nucleoside into the synthetic DNA for DNA-MGMT cross linking studies which will serve as an indicator for cancer chemotherapeutic agent.

II. Results and Discussion

Initially, the solution of pyrrolopyrimidine **2** in acetonitrile was treated with sodium hydride (NaH) to generate its sodium salt anion. The anion was glycosylated by reaction with 1-chloro-2'-deoxy-3,5-di-O-*p*-toluoyl-α-D-*erythro*-pentofuranose (α-chlorosugar, **1**) in anhydrous acetonitrile under argon (scheme 1).(25) The sugar was added in portions over 10 minutes and the reaction was left stirring overnight at room temperature. In order to ensure high stereoselectivity during the glycosylation reaction (to obtain the *β*-nucleoside), it is important that the α-chlorosugar must not be allowed to "age". Another important factor in this is the solubility of the base and its anion. Thus, the reaction of the anion of the nucleobase **2** generated using NaH in anhydrous acetonitrile was assumed to follow an S_N2 mechanism according to literature.

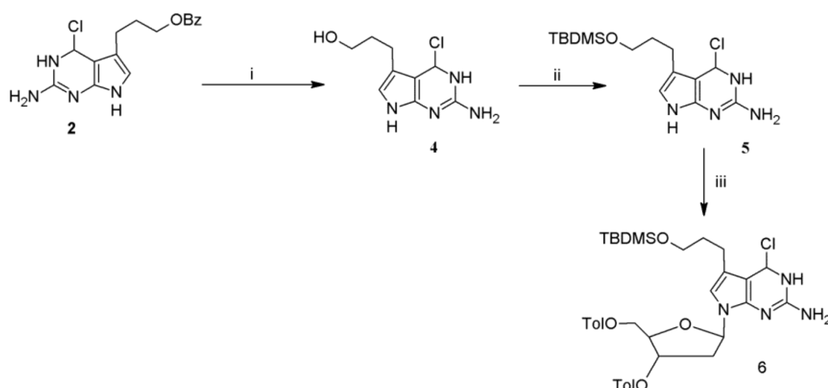
After working up the reaction, TLC analysis of the crude mixture indicated the partial formation of the nucleoside which was identified following staining of the TLC plate with anisaldehyde. Also evident from the TLC was the presence of largely unreacted pyrrolopyrimidine **2** which is presumably associated with its poor solubility in the anhydrous acetonitrile.



Scheme 1: Glycosylation of the chlorinated pyrrolopyrimidine **2**.
Reagents and reaction conditions: (i) NaH, dry MeCN, then 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-*erythro*-pentofuranose, (**1**) 7%.

In an attempt to solve the problem of nucleobase solubility during the glycosylation reaction, it was decided to add a small amount of DMSO or DMF which led to complications (that is the formation of an α-anomer). It was therefore decided that to improve the solubility of the heterocyclic base in acetonitrile the only alternative was to change the protecting group with one which would improve solubility.

Later *tert*-butyldimethylsilyloxy (TBDMS) was used instead of benzoyl protecting group inorder to improve the solubility of the heterocyclic base **2**. The TBDMS group was found to be more stable under basic conditions for glycosylation as well as deacylation reaction (removal of the *p*-toluoyl groups from the sugar),(26) and was therefore chosen to replace the benzoyl group. The problem of solubility was solved by using *tert*-butyldimethylsilyl (TBDMS). The TBDMS protected analogue **5** was prepared following de-benzoylation of **4** followed by the reaction with *tert*-butyldimethylsilyl chloride TBDMSCl (scheme 2). Glycosylation was achieved successfully using the TBDMS protected hetrocycle **5**.

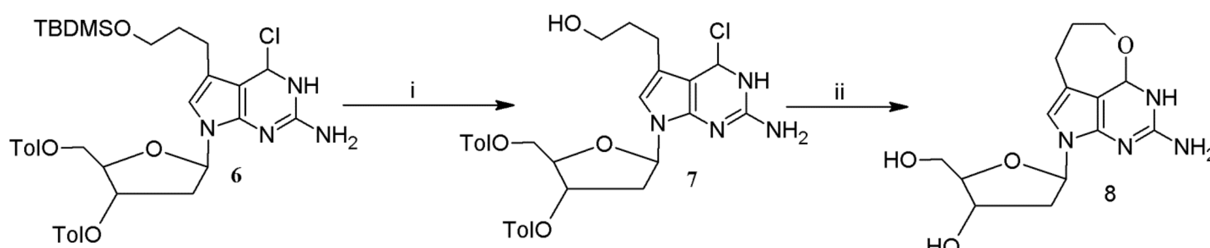


Scheme 2: Synthesis of 2-amino-5-(3-*tert*-butyldimethylsilyloxypropyl)-7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine **6** from **2**.

Reagents and reaction conditions: (i) aq. NaOH, overnight, rt, 65% (ii) TBDMS-Cl, pyridine, overnight, rt, 54%; (v) NaH, MeCN, then "α-chlorosugar", rt, 3h, 64%;

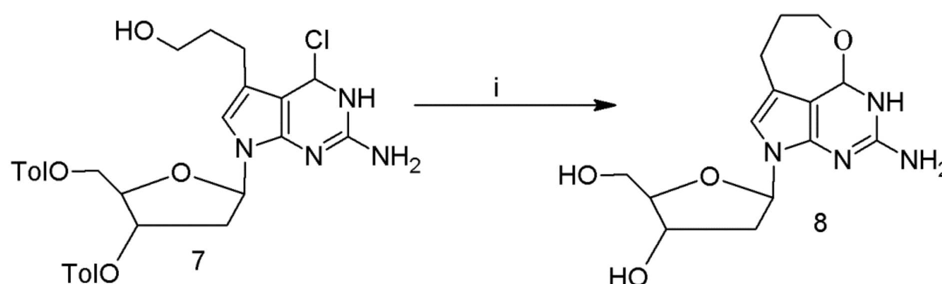
The alcohol **4** was obtained following basic hydrolysis of the benzoyl ester **2** using 1M NaOH solution, stirred overnight at room temp. The compound was extracted with ethylacetate and evaporated to obtain the pure compound in 65% yield. Compound **4** was dried by co-evaporation with anhydrous pyridine before it was treated with *tert*-butyldimethylsilyl chloride in pyridine and stirred overnight at room temperature under argon. The TBDMS protected compound **5** was obtained as a pale brown foam after silica column chromatography in 54% yield. The sodium salt of compound **5** was soluble in acetonitrile and was successfully glycosylated using the conditions previously described. The TBDMS protected nucleoside **6** was isolated after silica chromatography as a yellow foam in 64% yield. To check if the desired *β*-anomer was the product isolated, the signal of the anomeric proton was examined in the ^{1}H-NMR spectrum between 6.5 and 6.7 ppm. According to the literature, the H1' for the α-anomer is seen as pseudotriplet, while the H1' of the *β*-anomer is recognised as double doublet.(27) Therefore, the ^{1}H-NMR clearly showed a double doublet at 6.55 ppm, as well as the TBDMS signals and the expected mass of 693 for $[M + H]^+$ was seen in the ESI+ spectrum (see figure 1 and 2 respectively).

An attempt to remove the TBDMS as well as the cyclisation into the tricyclic nucleoside was made through the use of fluoride anion (triethylamine trihydrofluoride (NEt_3.3HF) in THF) which works under milder conditions.(28) The TBDMS group was removed by adding 20 equivalents of NEt_3.3HF to the solution of the nucleoside **6** in anhydrous THF. After stirring overnight, the reaction was complete (checked by TLC). Purification by silica gel column chromatography with 2% methanol in dichloromethane gave a white solid **7** in 70% yields. The ^{1}H-NMR spectroscopy showed the presence of some triethylammonium salt which necessitated repurification of the compound. It was envisaged that compound **7** would cyclise upon treatment with 1M NaOH to give nucleoside **8** (scheme 3).



Scheme 3: Removal of TBDMS and cyclisation to obtain nucleoside **8**.
Reagents and reaction conditions: (i) Et_3N.3HF, THF, rt, overnight, 70%; (ii) NaOH, rt, overnight.

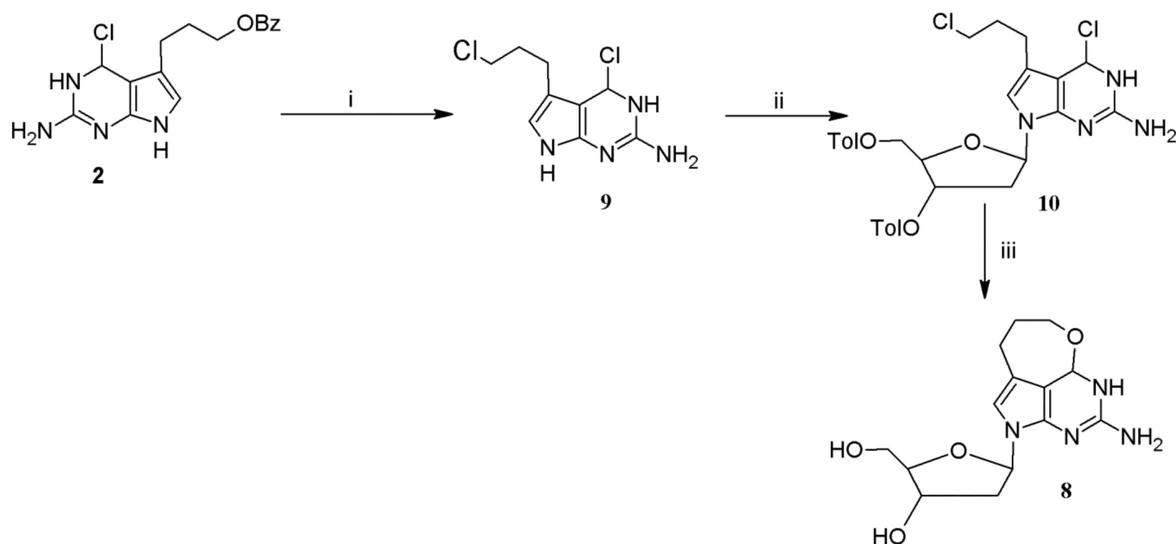
Unfortunately, the cyclisation tricyclic nucleoside **8** failed. An alternative route toward the cyclisation was considered to be by direct displacement of the chloride at the C4-position in the nucleoside scheme 4.



Scheme 4: Proposed route for direct cyclisation
Reagents and reaction conditions: (i) NaH, DMF, 70°C, 36 h.

Chlorinated nucleoside **7** was dissolved in DMF under argon, followed the addition of 4 equivalents of NaH. The mixture was stirred for two days at 50°C, from the TLC analysis three spots were observed but none was found to be fluorescent under UV. Analysis from the mass spectrometry shows a major peak at 338 ($[M + H]^+$) which did not match with either the mass of the product ($[M + H]^+ = 307$) nor the mass of the starting material ($[M + H]^+ = 343$). Because of these problems it was decided that the best way to acheive the cyclisation reaction was through the *bis*-chlorinated heterocyclic base **9**.(29) Glycosylation of the *bis*-chlorinated nucleobase **9** was successfully carried out under the same conditions as previously described. Removal of the

sugar-protecting toluoyl groups was achieved by treatment with 1 M aqueous NaOH in 1,4-dioxane in 1:1 ratio. TLC analysis showed that compound **10** (see scheme 5) was obtained after overnight stirring. It was therefore decided to proceed with the cyclisation without purifying **10** as the conditions for the cyclisation are the same as those for removing the toluoyl groups, albeit requiring a longer reaction time (3 days) to achieve the cyclisation. TLC of the reaction mixture showed the presence of two nucleosides, the tricyclic nucleoside **8** (R_f 0.45 in 10% MeOH/DCM) which is fluorescent under UV and probably the ring open nucleoside (R_f 0.35 in 10% MeOH/CH_2Cl_2) yet to be identified using mass spectra. Purification by silica gel column chromatography gave compound **8** in 19% yield. Scheme 5 gives the outline for the glycosylation as well as the cyclisation.



Scheme 5: Glycosylation of *bis*-chlorinated nucleobase and cyclisation to obtain nucleoside **14**.
Reagents and reaction conditions: (i) NaH, MeCN, then "α-chlorosugar", rt, 3h, 76%;
(ii) NaOH, 1,4-dioxane, 90˚C, 3 days, 19%.

The tricyclic nucleoside was synthesised in 19% yield; the ^{1}H-NMR (in DMSO) and mass spectrum are given in Fig. 1 and 2 respectively.

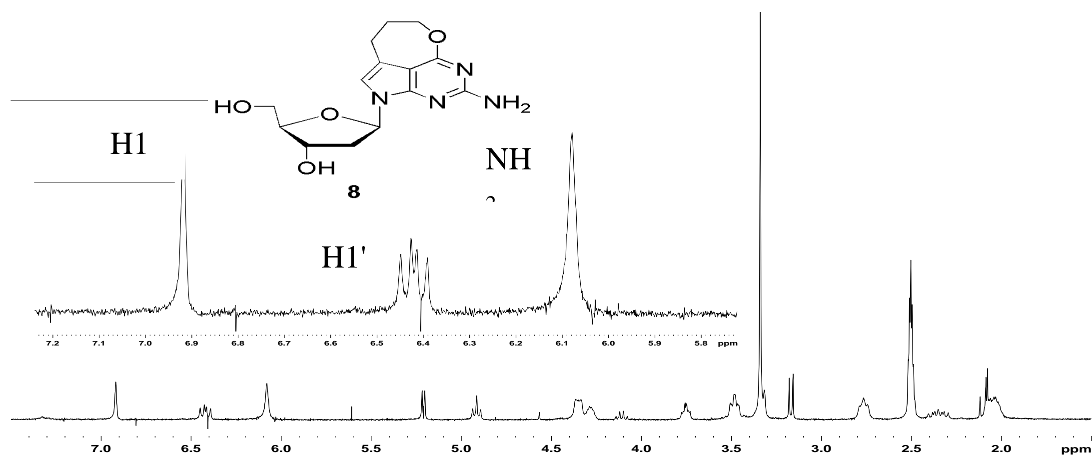


Fig. 1 ^{1}H-NMR spectrum of the tricyclic nucleoside **8**.

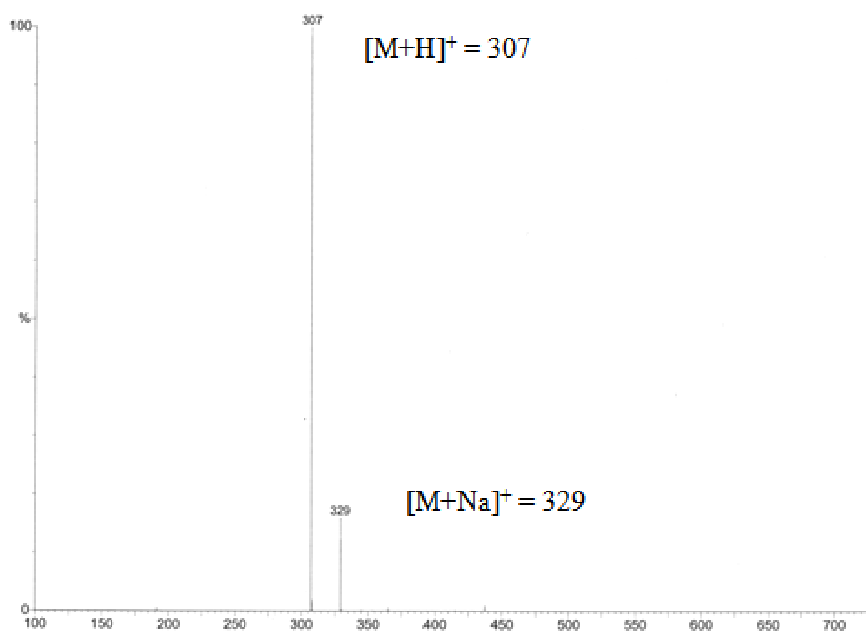


Fig.2 ESI+ mass spectrum of the tricyclic nucleoside **8**.

III. Conclusion

From this study it was clear that the most feasible pathway toward achieving the tricyclic nucleoside **8** would be via the *bis*-chlorinated nucleoside **10**. Also it was clear that the mechanism for the cyclisation proceeds by the displacement of the chloride attached to the alkyl side chain not through the displacement of the chloride attached to the heterocyclic (Cl attached to C-4 position). This is evident from previous attempted routes for the cyclisation. Direct displacement of the chloride attached to the heterocyclic base in nucleoside **7** was found not to be successful, instead a ring open nucleoside was formed. The possible explanation for this is that the S_N2 reaction at the primary alkyl halide occurs at a similar rate to displacement of chloride by hydroxide at the C-4 position.

IV. Materials and Methods

1-Chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (1) [29]

A solution of acetyl chloride (0.5 mL) in dry methanol (27 mL) was added portion-wise to a solution of 2'-deoxy-D-ribose (13.6 g, 0.1 mol) in dry methanol (243 mL) under Ar and left to stir for 25min. Anhydrous pyridine (20 mL) was then added. The reaction mixture was evaporated to a syrup. Dry pyridine (20 mL) was added and then removed *in vacuo* (repeated 3 times). The residue was dissolved in anhydrous pyridine (80 mL) and cooled to 0°C in an ice bath. Toluoyl chloride (0.2 mol, 29 mL) was then added quickly in one portion, forming a pink precipitate immediately. The reaction was stirred for 1h at 0°C and then at room temp overnight. Crushed ice (300 mL) was then added and the mixture extracted with diethyl ether (500 mL). The organic layer was washed with water (500 mL), dilute (10%) aqueous sulfuric acid (500mL) and saturated sodium bicarbonate solution (500 mL), and then concentrated to give a yellow syrup. The syrup was then dissolved in Analar glacial acetic acid (40 mL) and added to Analar acetic acid (80 mL) pre-saturated with dry hydrogen chloride gas at 10°C. Hydrogen chloride gas was then bubbled through the solution until a precipitate had formed (approx. 20min). This was then filtered and washed with anhydrous diethyl ether till the washings were pH 7. The product **1** was obtained as white flakes then dried in a desiccator (26 g, 66%), and stored in a dry container at -20°C.

δ_H ($CDCl_3$) 2.40 (6H, s, 2CH_3), 2.81-2.92 (2H, m, *H2* and *H2'*), 4.65 (2H, dd, *J* = 3.2, 4.4 Hz, *H5* and *H5'*), 4.85 (1H, dd, *J* = 3.2, 4.4 Hz, *H4*), 5.55 (1H, dd, *J* = 3.2, 4.4 Hz, *H3*), 6.57 (1H, d, *J* = 4.4 Hz, *H1*), 7.21-7.27 (4H, m, *meta CH*-Tol), 7.90 (2H, d, *J* = 7.9 Hz, *ortho CH*-Tol), 8.00 (2H, d, *J* = 7.9 Hz, *ortho CH*-Tol) ppm.

δ_C ($CDCl_3$) 21.6 (2xTol-CH_3), 44.5 (*C2'*), 63.5 (*C5'*), 73.5 (*C3'*), 84.7 (*C4'*), 95.3 (*C1'*), 126.6 and 126.8 (2xTol-*C*-C=O), 129.2 (4 *meta CH*-Tol), 129.6 (2 *ortho CH*-Tol), 129.9 (2 *ortho CH*-Tol), 144.0 and 144.2 (2xCH_3-*C*-Tol), 166.0 and 166.4 (2x*C*=O) ppm.

Mass Spec: *m/z* (ESI+) 388 $[M+H]^+$

2-Amino-5-(3-benzoyloxypropyl)-4-chloro-7*H*-pyrrolo[2,3-*d*] pyrimidine (2)[26]

Method 1

The modified guanine, 2-Amino-5-(3-benzoyloxypropyl)-3,7-dihydropyrrolo[2,3-*d*] pyrimidin-4-one[(24)] (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, 57.7mmol) 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 ($MgSO_4$). Attempts to purify the residue by silica gel column chromatography were unsuccessful.

Method 2

The modified guanine, 2-Amino-5-(3-benzoyloxypropyl)-3,7-dihydropyrrolo[2,3-*d*] pyrimidin-4-one[(3)] (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 **2** 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 (d_6-DMSO) 2.02 (2H, m, *CH_2*CH_2OBz), 3.35 (2H, t, *J* = 6.5 Hz, *CH_2*CH_2CH_2OBz), 4.36 (2H, t, *J* = 6.5 Hz, *CH_2*OBz), 6.45 (2H, s, *NH_2*), 6.81 (1H, s, *H*-6), 7.55-7.97 (5H, m, *CH*-Ph), 11.2 (1H, s, *NH*) ppm.

δ_C (d_6-DMSO) 22.1 (*CH_2*CH_2OBz), 29.3 (*CH_2*CH_2CH_2OBz), 64.1 (*CH_2*OBz), 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 $C_{16}H_{16}ClN_4O_2$ requires 331.0962 (deviation 3.8 ppm).

2-Amino-5-(3-benzoyloxypropyl)-4-chloro-7-[2'-deoxy-3,5-di-*O*-(*p*-toluoyl)-*β*-D-*erythro*-pentofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3)[(30)]

Compound **2** (330 mg, 1.0 mmol) was suspended in anhydrous acetonitrile (10 mL) under argon at room temperature, NaH (48 mg, 1.2 mmol, 60% dispersed in mineral oil) was added cautiously and the reaction left to stir for 1h. Compound **1** (α-chlorosugar, 464 mg, 1.2 mmol) was then added in three portions over 5 min. The reaction was left to stir overnight at 40°C, the solvent was removed *in vacuo*. The residue was redissolved in dichloromethane and washed with water (30 mL), dried ($MgSO_4$) and evaporated to purify the residue by silica gel column chromatography eluting with 4% ethyl acetate in dichloromethane. The product was isolated as pale yellow solid (50 mg, 7%).

TLC (2% MeOH in DCM); $R_f = 0.25$

δ_H ($CDCl_3$) 1.90 (2H, m, *CH_2*CH_2OBz), 2.40 (6H, s, 2xTol-*CH_3*), 2.58 (2H, m, *H2'* and *H2''*), 2.85 (2H, t, *J* = 5.8 Hz, *CH_2*CH_2CH_2OBz), 4.32 (2H, t, *J* = 5.8 Hz, *CH_2*OBz), 4.52-4.61 (2H, m, *H5'* and *H5''*), 4.70 (1H, q, *J* = 5.8 Hz, *H4'*) 5.06 (2H, s, *NH_2*), 5.75 (1H, d, *H3'*), 6.58 (1H, dd, *J* = 2.9, 5.8 Hz, *H1'*), 6.80 (1H, s, *H*-6), 7.30-7.35 (4H, m, *meta CH*-Tol), 7.40-7.55 (5H, m, Ph), 7.91-8.10 (4H, m, *ortho CH*-Tol) ppm.

Mass Spec: *m/z* (ESI+) 705 $[M+Na]^+$, 707 $[M+Na]^+$

Acc Mass: 705.2106; calculated for $C_{37}H_{35}N_4O_7ClNa$ requires 705.2092 (deviation 2.0 ppm).

2-Amino-5-(3-hydroxypropyl)-4-chloro-7*H*-pyrrolo[2,3-*d*] pyrimidine (4)[(31)]

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 ($MgSO_4$) and evaporated to give the pure product **4** as a yellow solid (1.3 g, 65%).

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

δ_H (d_6-DMSO) 1.70 (2H, m, *CH_2*CH_2OH), 2.65 (2H, t, *J* = 6.2 Hz, *CH_2*CH_2CH_2OH), 3.35 (2H, t, *J* = 6.2 Hz, *CH_2*OH), 4.47 (1H, s, *OH*) 5.97 (2H, s, *NH_2*), 6.40 (1H, s, *H*-6), 11.20 (1H, s, *NH*-7) ppm.

δ_C (d_6-DMSO) 22.7 (*CH_2*CH_2OH), 34.0 (*CH_2*CH_2CH_2OH), 60.7 (*CH_2*OH), 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 $[M+H]^+$, 231 $[M+H]^+$

Acc Mass: 229.1038; calculated for $C_9H_{13}ClN_4O$ requires 229.1039 (deviation -0.3 ppm).

2-Amino-5-(3-*tert*-butyldimethylsilyloxypropyl)-4-chloro-7*H*-pyrrolo[2,3-*d*] pyrimidine (5)[32]

Compound **4** (1.3 g, 5.8 mmol) was dried by co-evaporation with anhydrous pyridine (3 x 30 mL) and then redissolved in anhydrous pyridine (50 mL) under argon. *tert*-Butyldimethylsilyl chloride (1.5 g, 10.1 mmol) was then added and the reaction stirred overnight at room temperature. The product was extracted into dichloromethane (100 mL) and the organic layer was washed with 10% Na_2CO_3 (80 mL), dried ($MgSO_4$) and evaporated to dryness. Purification by silica gel column chromatography, eluting with 2% methanol in dichloromethane afforded the product **5** as a reddish brown foam (1.5 g, 54%).

TLC (2% MeOH / DCM); $R_f = 0.40$
δ_H ($CDCl_3$) 0.05 (6H, s, 2xSi-*CH₃*) 1.00 (9H, s, 3xSi-C-*CH₃*), 1.85 (2H, m, CH_2CH_2OSi), 2.80 (2H, t, J = 6.0 Hz, $CH_2CH_2CH_2OSi$), 3.68 (2H, t, J = 6.0 Hz, CH_2OSi), 6.80 (1H, s, *H*-6), 9.10 (1H, s, *NH*) ppm.
δ_C ($CDCl_3$) -5.2 (2xS-CH_3), 19.2 (*C*-Si), 22.3 (CH_2CH_2OSi), 25.9 (3C, C(CH_3)₃), 33.4 ($CH_2CH_2CH_2OSi$), 63.3 (CH_2OSi), 109.7 (*C*-5), 116.2 (*C*-4a), 119.2 (*C*-7a), 151.8 (*C*-6), 158.1 (*C*-2), 163.1 (*C*-4) ppm.
Mass Spec: *m/z* (ESI+) 341 $[M+H]^+$, 343 $[M+H]^+$
Acc Mass: 341.1553; calculated for $C_{15}H_{25}ClN_4OSi$ requires 341.1564 (deviation -3.3 ppm)

2-Amino-5-(3-*tert*-butyldimethylsilyloxypropyl)-7-[2'-deoxy-3,5-di-*O*-(*p*-toluoyl)-*β*-D-*erythro*-pentofuranosyl]-4-chloro-7*H*-pyrrolo[2,3-*d*] pyrimidine (6)[30]

Compound **5** (1.0 g, 2.9 mmol) was dissolved in anhydrous acetonitrile (60 mL) under argon at room temperature. NaH (140 mg, 3.5 mmol, 60% in mineral oil) was then added cautiously and the reaction left to stir for 1h, when α-chlorosugar **1** (1.4 g, 3.5 mmol) was added in portions over 10min. After that, the reaction was left to stir for 3h, the solvent removed *in vacuo*, redissolved in dichloromethane (100 mL) and washed with water (100 mL), 5% aq. HCl (100 mL) and brine (100 mL). The organic layer was then dried ($MgSO_4$) and evaporated to purify the residue by silica gel column chromatography, eluting with 2% methanol in dichloromethane. The product **6** was isolated as pale yellow foam (1.5 g, 64%).

TLC (2% MeOH / DCM) $R_f = 0.80$
δ_H ($CDCl_3$) 0.99 (9H, s, 3xSi-C-CH_3), 1.68 (2H, m, CH_2CH_2OSi), 2.25 (3H, s, Tol-CH_3), 2.35 (3H, s, Tol-CH_3), 2.44-2.55 (1H, m, H2'), 2.49 (3H, s, S-CH_3), 2.65-2.77 (1H, m, H2''), 2.71 (2H, t, J = 6.5 Hz, $CH_2CH_2CH_2OSi$), 3.60 (2H, t, J = 6.5 Hz, CH_2OSi), 4.43-4.69 (3H, m, H4', H5' and H5''), 4.95 (2H, s, NH_2), 5.62-5.65 (1H, m, H3'), 6.50 (1H, s, H-6), 6.55 (1H, dd, J = 3.4, 6.5 Hz, H1'), 7.08-7.17 (4H, m, *meta CH*-Tol), 7.85 (2H, d, J = 8.2 Hz, *ortho CH*-Tol), 7.89 (2H, d, J = 8.2 Hz, *ortho CH*-Tol) ppm.
δ_C ($CDCl_3$) -5.2 (2xS-CH_3), 21.7 (*C*-Si), 21.8 (2xTol-CH_3), 22.5 (CH_2CH_2OSi), 25.9 (3C, C(CH_3)₃), 33.0 ($CH_2CH_2CH_2OSi$), 37.2 (CH_2OSi), 62.6 (*C*2'), 64.2 (*C*5'), 75.3 (*C*3'), 81.8 (*C*1'), 83.5 (*C*4'), 117.3 (*C*-4a), 119.0 (*C*-7a), 119.5 (*C*-6) 126.5 and 126.8 (2xCH_3-*C*-Tol), 129.0, 129.2, 129.3, 129.6, 129.8, 129.9, 130.0 (8 *ortho* and *meta* CH-Tol), 144.1 and 144.4 (2xTol-*C*-C=O), 152.5 (*C*-5), 154.3 (*C*-4), 158.4 (*C*-2), 166.0 and 166.2 (2x*C*=O) ppm.

Mass Spec: *m/z* (ESI+) 693 $[M+H]^+$, 695 $[M+H]^+$
Acc Mass: 693.2872; calculated for $C_{36}H_{45}ClN_4O_6Si$ requires 693.2875 (deviation -0.4 ppm)

2-Amino-5-(3-hydroxypropyl)-7-[2'-deoxy-3,5-di-*O*-(*p*-toluoyl)-*β*-D-*erythro*-pentofuranosyl]-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (7)[29]

Compound **6** (1.3 g, 1.7 mmol) was dissolved in anhydrous THF (50 mL) under Ar at room temperature. Triethylamine trihydrofluoride (6 mL, 33.4 mol) was then added and the mixture was left stirring overnight. When the reaction was complete (checked by TLC) the mixture was evaporated and the residue was purified by silica gel column chromatography with 2% methanol in dichloromethane to give a white solid, **7** (700 mg, 70%).

TLC (2% MeOH / DCM); $R_f = 0.40$
δ_H ($CDCl_3$) 1.70 (2H, m, CH_2CH_2OH), 2.35 (6H, s, 2 x Tol-CH_3), 2.50-2.60 (1H, m, H2'), 2.66-2.70 (1H, m, H2''), 2.80 (2H, t, J = 6.8 Hz, $CH_2CH_2CH_2OH$), 3.50 (2H, t, J = 6.8 Hz, CH_2OH), 4.44-4.54 (2H, m, H4' and H5'), 4.68-4.76 (1H, m, H5''), 5.10 (2H, s, NH_2), 5.70 (1H, m, H3'), 6.63 (1H, dd, J = 3.4, 6.8 Hz, H1'), 6.75 (1H, s, H-6), 7.25 (4H, m, *meta CH*-Tol), 7.91 (4H, m, *ortho CH*-Tol) ppm.
δ_C ($CDCl_3$) 21.7 (2xTol-CH_3), 22.1 (CH_2CH_2OH), 31.9 ($CH_2CH_2CH_2OH$), 37.2 (C2'), 44.4 (CH_2OH), 64.3 (C5'), 75.3 (C3'), 81.9 (C1'), 82.9 (C4'), 115.5 (C-4a), 119.6 (C-7a), 119.7 (C-6), 126.5 and 126.8 (2xCH_3-*C*-

Tol), 129.1, 129.2, 129.6 and 129.8 (8 *ortho* and *meta* *C*H-Tol), 144.2 and 144.3 (2xTol-*C*-C=O), 152.3 (C-5), 154.3 (C-4), 157.1 (C-2), 166.0 and 166.2 (2x*C*=O) ppm.
Mass Spec: *m/z* (ESI+) 579 $[M+H]^+$, 581 $[M+H]^+$
Acc Mass: 579.1999; calculated for $C_{30}H_{31}ClN_4O_6$ requires 579.2010 (deviation -2.0 ppm)

4-Amino-2-(2'-deoxy-β-D-*erythro*-pentofuranosyl)-6-oxa-7,8,9-trihydro-2,3,5-triazabenzo[*cd*]azulene (8)[29]

To a solution of **10** (280 mg, 470 μmol) in 1,4-dioxane (15 mL), was added 1M aq. sodium hydroxide solution (15 mL, 15 μmol) and the mixture was refluxed at 90°C overnight. Once the solution cooled down it was neutralised with 2 mL of 0.1M aq. acetic acid solution, then evaporated and the residue purified by silica gel column chromatography eluting with 10% MeOH in dichloromethane. The product **8** was obtained as a pale yellow solid (27 mg, 19 %).

TLC (10% MeOH / DCM); R_f = 0.50 (fluorescent at 356 nm)e2

δ_H (d_6-DMSO) 2.08-2.12 (3H, m, *CH_2*CH_2O and *H2'*), 2.30-2.40 (1H, m, *H2''*), 2.77 (2H, t, *J* = 6.8 Hz, *CH_2*CH_2CH_2O), 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 Hz, *CH_2*O), 4.91 (1H, s, C5'-*OH*), 5.21 (1H, s, C3'-*OH*), 6.08 (2H, s, *NH_2*), 6.43 (1H, dd, *J* = 3.4, 6.8 Hz, *H1'*), 6.92 (1H, s, *H-6*) ppm.
δ_C (d_6-DMSO) 25.9 (*CH_2*CH_2O), 28.9 (*CH_2*CH_2CH_2O), 38.8(*C2'*), 62.1 (*C5'*), 71.1 (*C3'*), 71.8 (*CH_2*O), 81.8 (*C1'*), 86.8 (*C4'*), 97.5 (*C-6a*), 114.1 (*C-10*), 115.1 (*C-1*), 155.3 (*C-2a*), 159.7 (*C-6*), 164.9 (*C-4*) ppm.

Mass Spec: *m/z* (ESI+) 329 $[M+Na]^+$.

Acc Mass: 329.1220; calculated for $C_{14}H_{18}N_4O_4Na$ requires 329.1226 (deviation -1.7 ppm)

2-Amino-5-(3-chloropropyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (9)[29]

Method 1

Compound **2** (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 ($MgSO_4$). Attempts to purify the residue by silica gel column chromatography were unsuccessful.

Method 2

Compound **2** (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 **9** in pure form as a pale yellow solid (235 mg, 20%).

TLC (10% MeOH / DCM); R_f = 0.46
δ_H (d_6-DMSO) 2.05 (2H, m, *CH_2*CH_2Cl), 2.79 (2H, t, *J* = 6.8 Hz, *CH_2*CH_2CH_2Cl), 3.65 (2H, t, *J* = 6.8 Hz, *CH_2*Cl), 6.45 (2H, s, *NH_2*), 6.86 (1H, s, *H*-6), 11.21 (1H, s, *NH*) ppm.
δ_C (d_6-DMSO) 22.8 (*CH_2*), 32.9 (*CH_2*), 44.9 (*CH_2*Cl), 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 $[M+H]^+$, 247 $[M+H]^+$
Acc Mass: 245.0355; calculated for $C_9H_{11}Cl_2N_4$ requires 245.0361 (deviation -2.3 ppm)

2-Amino-5-(3-chloropropyl)-7-[2'-deoxy-3,5-di-*O*-(*p*-toluoyl)-*β*-D-*erythro*-pentofuranosyl]-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (10)[30]

Compound **9** (180 mg, 740 μmol) was dissolved in anhydrous MeCN (20 mL) under argon at room temperature, NaH (35 mg, 890 μmol, 60% in mineral oil) was then added cautiously and the reaction left to stir for 1h, when α-chlorosugar **1** (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 dichloromethane (20 mL) and washed with water (50 mL), 5% aq. HCl (50 mL) and brine (50 mL). The organic layer was then dried ($MgSO_4$) and evaporated to

purify the residue by silica gel column chromatography, eluting with 2% methanol in dichloromethane. The product **10** was isolated as pale yellow foam (400 mg, 76%).

TLC (2% MeOH / DCM); $R_f = 0.35$

δ_H ($CDCl_3$) 1.90 (2H, m, *CH_2*CH_2Cl), 2.35 (6H, s, 2 x Tol-*CH_3*), 2.50-2.60 (1H, m, H2'), 2.66-2.70 (1H, m, H2"), 2.80 (2H, t, J = 6.8 Hz, *CH_2*CH_2CH_2Cl), 3.50 (2H, t, J = 6.8 Hz, *CH_2*Cl), 4.44-4.54 (2H, m, H4' and H5'), 4.68-4.76 (1H, m, H5"), 5.10 (2H, s, *NH_2*), 5.70 (1H, m, H3'), 6.63 (1H, dd, J = 3.4, 6.8 Hz, H1'), 6.75 (1H, s, H-6), 7.21 (4H, m, *meta CH*-Tol), 7.91 (4H, m, *ortho CH*-Tol) ppm.

δ_C ($CDCl_3$) 21.7 (2xTol-*C*H_3), 23.1 (*C*H_2CH_2Cl), 32.7 (*C*$H_2CH_2CH_2$Cl), 37.3 (C2'), 44.4 (*C*H_2Cl), 64.2 (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 (2xCH_3-*C*-Tol), 129.1, 129.2, 129.6 and 129.8 (8 *ortho* and *meta C*H-Tol), 144.2 and 144.4 (2xTol-*C*-C=O), 152.4 (C-5), 154.3 (C-4), 158.4 (C-2), 166.0 and 166.2 (2x*C*=O) ppm.

Mass Spec: m/z (ESI+) 597 $[M+H]^+$, 599 $[M+H]^+$

Acc Mass: 597.1655; calculated for $C_{30}H_{31}Cl_2N_4O_5$ requires 597.1672 (deviation -2.8 ppm)

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