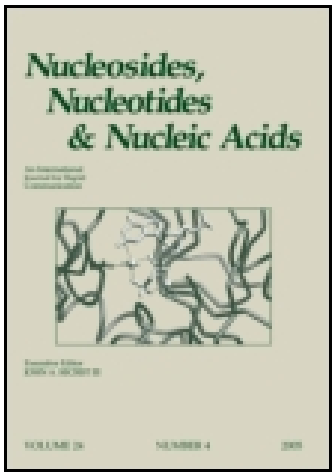

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Design and Synthesis of a Series of Chlorinated 3-Deazaadenine Analogues

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Design and Synthesis of a Series of Chlorinated 3-Deazaadenine Analogues[#]

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ABSTRACT

A series of chlorinated adenine analogues were designed with sights set on the development of potential antitumor agents. During the synthetic efforts, two unexpected compounds were identified. Their synthesis, along with synthesis of the chlorinated targets is presented herein.

Key Words: Deazaadenine; Chlorination; Purines; Nucleobases.

Colorectal cancer remains the most common fatal cancer among non-smokers. This year it is estimated that 56,500 people will die as a result of colorectal cancer, with 132,000 new cases being reported. Two pathways leading to the formation of colorectal cancer exist; mutations in the tumor suppressor p53 account for one pathway whereas the other pathway involves mutations in the DNA mismatch repair (MMR) machinery.[1,2] Although significant strides have been made regarding the

[#]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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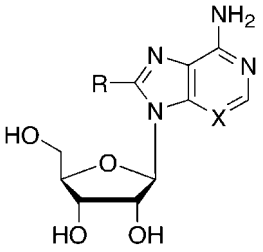

1, R=Cl, X=N
2, R=H, X=CH

Figure 1.

molecular, cellular and biochemical events associated with the development of adenocarcinomas of the colon, the recurrence of disease following surgical resection and the inability to treat advanced stage disease remain major problems often resulting in death. For the past 45 years, the mainstay therapy for advanced colorectal cancer has relied on regimens using the nucleoside analogue 5-fluorouracil (5-FU),[3] although response rates remain poor (~20%).[4] Thus, it is necessary to develop alternative and adjuvant therapeutic approaches for the treatment of this disease.

It has been reported that the nucleoside analogue 8-Cl-adenosine (**1**, Fig. 1) inhibits growth in a variety of cancer cell lines, including breast, ovary, pancreas, and colon.[5,6] In addition, it appears that 8-Cl-adenosine is working through a different mechanism than 5-FU, both as it relates to cell cycle perturbations and cell specificity. This suggests that chlorination of adenosine is significant for inhibition of transformed cell growth, however it is not clear if additional chlorinated substituents will further enhance the anti-proliferative properties of this nucleoside.

Another structural modification that has proven fruitful for nucleosides and nucleobases is the removal of the N-3 nitrogen of the purine ring as typified by **2**, 3-deazaadenosine, shown in Fig. 1. 3-Deaza analogues have exhibited potent biological activity in a variety of genre, including cancer and viruses.[7–9] This effect arises from their ability to indirectly inhibit DNA methyltransferases (DNA MeTases) by inhibition of *S*-adenosylhomocysteine hydrolase (SAHase), a key enzyme involved in methyl transfers dependent upon *S*-adenosylmethione (SAM) as a methyl donor.[10,11] With this in mind, we set our sights on the design and synthesis of a series of chlorinated 3-deazaadenine analogues with the goal of producing a synergistic biological effect.

CHEMISTRY

As shown in Fig. 2, the targets chosen for synthesis included the chlorinated nucleobases as well as their corresponding nucleosides. Since standard nucleoside synthetic pathways usually rely on coupling a preformed heterobase to various sugars, and heterobases have themselves exhibited potent medicinal properties, both the bases and the nucleosides were originally pursued.

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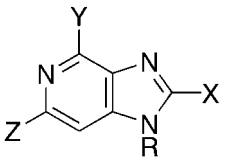

R=H, or β-D-ribofuranose

3, X=Cl; Y=NH_2; Z=H
4, X=H; Y=Z=Cl
5, X=Y=Cl; Z=H
6, X=Z=Cl; Y=NH_2
7, X=H; Y=NH_2; Z=Cl
8, X=Z=H; Y=Cl
9, X=Y=Z=Cl

Figure 2.

The target compounds were envisioned as being available from a variety of literature procedures, although over the course of this project, it was necessary to modify several of the standard protocols in order to maximize the yields and minimize unwanted byproducts.

Starting with commercially available 2,6-dichloropyridine, the N-oxide **11** was synthesized using trifluoroacetic acid (TFA) and 35% hydrogen peroxide (Sch. 1). Initially we followed the procedure as described by Rousseau and Robbins[12] but upon further investigation discovered that if we increased the reaction time from 4 to 6.5 h, the yield increased by at least 20%. Surprisingly however, an increase in reaction time greater than 6.5 h did not increase the yield. Since TFA is expensive and the amounts required for our scale were significant, we also explored the possibility of decreasing the quantity. Our efforts concluded that a 50% decrease in TFA gave a 20–25% decrease in yield, while a 25% decrease in TFA resulted in only a 10–15% decrease in yield, which was an acceptable compromise to us and we proceeded accordingly.

The *N*-oxide **11** was then subjected to nitration to form **12** according to the procedure by Cosstick[13] which was then subsequently reduced to amine **13** with glacial acetic acid and iron powder (Sch. 2). Amine **13** was converted to the nitrosoamine **14** using standard conditions, which, following treatment of **14** with acid, resulted in migration of the nitro group to give the nitro amine **15**.[12] Reduction of the migrated nitro group by the same conditions[13] as were used for the reduction of **12** was successful, however a byproduct was formed, albeit in moderate quantities, which proved to be the acetylated diamine **17**. Attempts at removal of the acetate group via concentrated ammonium hydroxide were unsuccessful however, NaOH (30% w/v) under reflux proved fruitful and we were able to convert **17** back to the desired diamine **16** quantitatively.

Unfortunately, this was not the only problem encountered with the reduction of **15**; the work-up for this procedure proved tedious due to the heavy emulsions consistently formed during the extraction process, so we searched for an alternative

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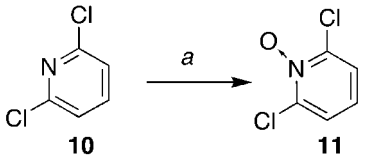


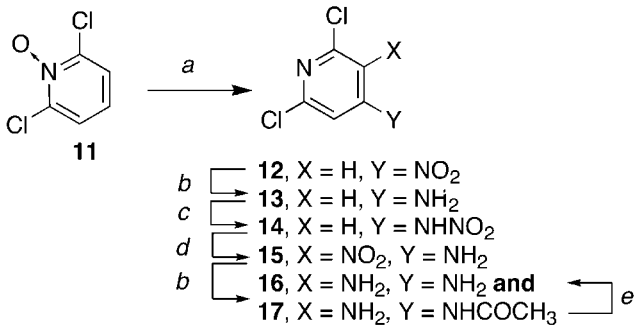
Reaction conditions; *a,* TFA, H_2O_2, reflux.

Scheme 1.

method to overcome both issues. It was discovered that using iron and aqueous HCl[14] gave equivalent yields as had been previously realized, but with a much more facile workup that resulted in neither the formation of emulsions or the unwanted acetylated byproduct. As a result, we have subsequently changed our approach to the synthesis of both **13** and **16** to employ this alternative procedure (Sch. 3).

Ring closure of diamine **16** was accomplished with refluxing formic acid, to give 2,6-dichloro-3-deazaadenine, **4** (Sch. 4 on the next page). However, poor to moderate yields plus the formation of diamide **18** as a major byproduct (as shown in Sch. 4) made this method unattractive. All attempts to convert **18** back to **4** were unsuccessful, but upon treatment with potassium hydroxide, a new heterocyclic base **19** was formed, which, to the best of our knowledge has not been previously reported. Ring closure of **16** was finally achieved using triethylorthoformate and acetic anhydride in a 1:1 ratio to give **4** in excellent yield with no sign of the byproduct **18**.[12]

Next, 2,6-dichloro-3-deazaadenine (**4**) was selectively converted to 2-chloro-3-deazaadenine **7** with methanolic ammonia at 160°C (Sch. 5, on the next page), which was then subjected to standard hydrogenation conditions to produce the 3-deaza base **20**.[15]



Reaction conditions; *a,* HNO_3, H_2SO_4, 160°C; *b,* acetic acid, Fe, reflux; *c*, HNO_3, H_2SO_4, rt; *d*, H_2SO_4,100° C; *e*, 30% NaOH, reflux.

Scheme 2.

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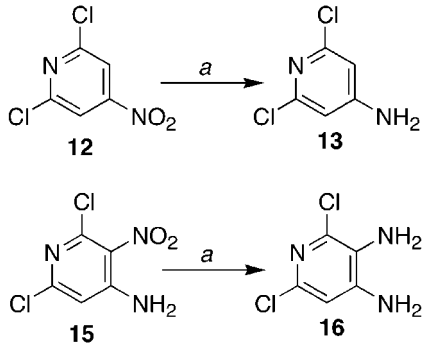
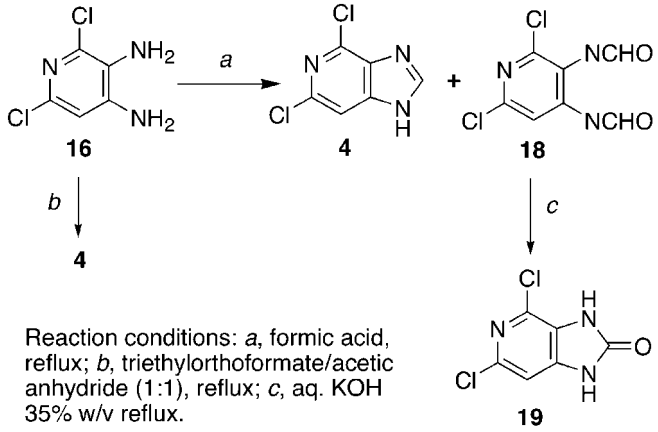


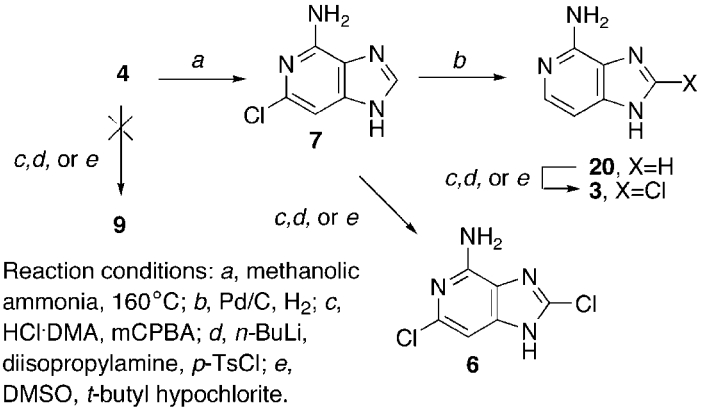
Reaction conditions; *a,* Fe, aq. HCl, EtOH.

Scheme 3.



Reaction conditions: *a*, formic acid, reflux; *b*, triethylorthoformate/acetic anhydride (1:1), reflux; *c*, aq. KOH 35% w/v reflux.

Scheme 4.



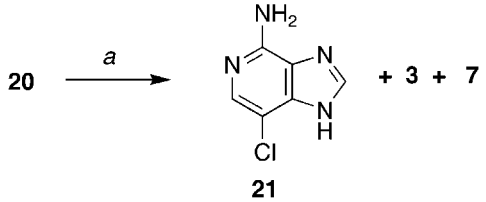
Reaction conditions: *a*, methanolic ammonia, 160°C; *b*, Pd/C, H_2; *c*, HCl·DMA, mCPBA; *d*, *n*-BuLi, diisopropylamine, *p*-TsCl; *e*, DMSO, *t*-butyl hypochlorite.

Scheme 5.

Several different standard chlorination methods were then tried to convert **20** to the 8-chloro-3-deazaadenine (**3**), as well as to convert **7** to the desired 2,8-dichloro base **6** and **4** to the trichloro base **9** (Sch. 5).[16–18] None of the conditions employed provided the trichloro base **9**. Conversion of **20** to 8-chloro-3-deazaadenine (**3**) and **7** to 2,8-dichloro-3-deazaadenine (**6**) was poor at best. Many attempts at manipulation of the reaction conditions failed to produce an improvement in the yields. Furthermore, in several cases, an intractable mixture of products formed for where the isolation and purification of the desired product could not be achieved. The conditions that afforded the highest yield and most facile purification proved to be *t*-butyl hypochlorite in DMSO.[18]

When 3-deazaadenine (**20**) was treated with *m*-CPBA, a mixture of products formed, including 8-chloro- and 2-chloro-3-deazaadenine (**3** and **7**, respectively), neither of which were recovered in significant quantities.[16] In addition, we isolated another chlorinated base (Sch. 6), which proved upon 2-D NMR structural elucidation, to be 3-chloro-3-deazaadenine (**21**). To our knowledge, this is the first report of this particular chlorinated base, and is likely to be the result of the formation of an epoxide by the *m*-CPBA at the C-2, C-3 double bond of 3-deazaadenine. The epoxide, which is susceptible nucleophilic attack by chlorine from either side, can then undergo a facile elimination of water to reestablish the aromaticity of the ring system.

Comparison of the ^{1}H NMR spectra of **21** with 8-chloro-3-deazaadenine (**3**) and 2-chloro-3-deazaadenine (**7**) showed a significant difference; 8-chloro-3-deazaadenine (**3**) exhibits two doublets centered at 7.82 ppm and 6.98 ppm for the C-2 and C-3 protons and a singlet at 6.63 ppm for the amine protons. The doublets are coupled to each other with *J*-values of 5.7 Hz, and integrated to 1:1:2 respectively. For 2-chloro-3-deazaadenine (**7**), literature values showed singlets at 6.58 ppm for the amine protons, 6.99 or 7.03 ppm for the C-3 proton and 8.37 or 8.30 ppm for the C-8 proton, and our spectra agreed with these values. In contrast, the ^{1}H NMR spectrum for 3-chloro-3-deazaadenine (**21**) exhibited signals at 8.16 ppm for the C-2 proton, 7.63 ppm for the C-8 proton and 6.32 ppm for the amine protons with an integration of 1:1:2. The C-2 proton for **21** is shifted downfield in comparison to the C-2 proton for 8-chloro-3-deazaadenine due to the presence of the C-3 chlorine and the proximity of the pyridine nitrogen. The C-3 proton in the 2-chloro-3-deazaadenine is not shifted as far as the 3-chloro since the proton is further from the pyridine nitrogen.



21

Reaction conditions: *a*, DMA, DMA·HCl, mCPBA.

Scheme 6.

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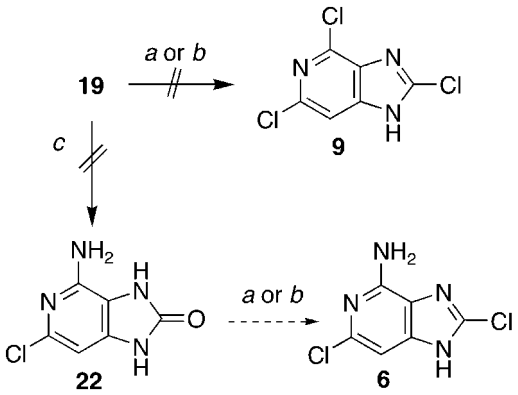

Reaction conditions: *a*, tetraethylammonium chloride, $POCl_3$; *b*, $SOCl_2$, DMF; *c*, methanolic ammonia, 140°C.

Scheme 7.

Next, in an effort to utilize the byproduct **19** produced earlier in Sch. 4, an alternate route to the trichloro base **9** was pursued, and attempts to chlorinate **19** using either $POCl_3$[19] or $SOCl_2$[20] proved unsuccessful (Sch. 7). We then tried selective conversion of the 6-chloro substituent of **19** to form the amine **22**, with sights set on subsequent transformation to **6** using methanolic ammonia, followed by chlorination. Unfortunately, ammonolysis using standard procedures was unsuccessful even after 150 h at 140°C; only starting material was recovered, therefore this route was abandoned.

During our efforts to overcome the many difficulties encountered along the way, we uncovered evidence in the literature,[21] which postulated that electron donating groups must be present on the base in order to activate the C-8 position towards chlorination. This would explain many of our difficulties, as we suspect that the loss of the N-3 nitrogen for our ring system renders the base much less reactive to substitution than is normally seen with the parent adenine. This is substantiated by two observations; one, that in all cases, the identical reaction conditions described herein have proven successful for the parent adenine ring system, and two, while the chlorination of the C-8 from amino-substituted 3-deaza precursors was successful, albeit with low yields, there was a complete lack of reaction for the more deactivated dichloro precursors.

In summary, we have presented some useful modifications to several often used literature procedures, as well as to introduce several new and potentially important 3-deaza heterocyclic systems. Further details of the synthesis of the corresponding nucleosides and any subsequent biological activity will be reported elsewhere.

EXPERIMENTAL

General. Melting points are uncorrected. Combustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. ^1H and ^{13}C spectra were recorded on a

Bruker 300 spectrometer (operated at 300 and 75 MHz, respectively) all referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet) and d (doublet). Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F_{254} precoated plates. Column chromatography was performed on Whatman silica, 200–400 mesh, 60 Å and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

2,6-Dichloropyridine-*N*-oxide (11). A mixture of trifluoroacetic acid (485.0 mL, 6.3 mol), 2,6-dichloropyridine (48.0 g, 324.0 mmol) and 35% hydrogen peroxide (85.0 mL, 971.0 mmol) were heated on a stream bath for 6.5 h.[12] The solution was cooled to room temperature and 2.4 L of water added. The flask was stored at 0°C overnight. The resulting precipitate (unreacted 2,6-dichloropyridine) was removed by filtration and the yellow-orange filtrate evaporated to a reduced volume. Chloroform (500 mL) was added and the solution treated with anhydrous potassium carbonate until carbon dioxide evolution ceased. After filtration, the filtrate was evaporated to dryness to give **11** as light yellow crystals (46.6 g, 88%), which was used directly in the next step without further purification.

3,4-Diamino-2,6-dichloropyridine (16). Iron powder (19.91 g, 356.58 mmol), H_2O (65.8 mL, 3.6 mol), and concentrated HCl (14.1 mL, 464.1 mmol) were added consecutively to a solution of **15** (14.82 g, 71.27 mmol) in ethanol (350 mL).[14] After stirring at 95°C for 16 h, the reaction mixture was cooled to room temperature, neutralized, filtered, and the filtrate evaporated to dryness. The crude product was then treated with water (300 mL) and extracted with EtOAc (3×300 mL). The organic layers were combined, dried ($MgSO_4$) and evaporated to afford **16** (11.9 g, 94%). Spectroscopic data and mp agreed with literature values.

3-Amino-4-acetoamide-2,6-dichloropyridine (17). To 4-amino-2,6-dichloro-3-nitropyridine (**15**) (5.00 g, 24.04 mmol) in glacial acetic acid (70.0 mL, 1.2 mol) was added iron powder (7.09 g, 127.02 mmol).[13] The mixture was refluxed for 2 h. After cooling to room temperature, the mixture was neutralized with 35% (w/v) potassium hydroxide solution, filtered through a celite pad. The pad was rinsed with glacial acetic acid (50 mL) and water (100 mL) and the resulting filtrate was combined with the previous filtrate and neutralized again. The product was extracted (ethyl acetate 4×100 mL) and the combined extracts dried ($MgSO_4$), filtered and evaporated. The crude solid was purified via column chromatography eluting with EtOAc:hexane (2:1) to give **17** as a brown solid (2.2 g, 51%); mp 234.1–237.3°C. ^{1}H NMR (d_6-DMSO), 9.30 (s, 1H, H5), 6.65 (s, 2H, NH_2), 6.60 (s, 1H, NH), 2.00 (s, 3H, $COCH_3$); ^{13}C NMR (d_6-DMSO) 168.95, 155.50, 148.18, 146.30, 115.33, 107.09, 22.77. Anal. Calcd. for $C_7H_7N_3Cl_2O$: C 38.21, H 3.21, Cl 32.22, N 19.10. Found: C 38.55, H 3.25, Cl 31.89, N 18.91.

2,6-Dichloro-3-deazaadenine (4). Formic acid (30.2 mL, 800 mmol) was added to **16** (109.6 mg, 615.7 μmol) and the whole was refluxed for 19 h. The mixture evaporated to produce a brown solid. The compound was purified via column chromatography eluting with EtOAc:MeOH (95:5) to produce **4** as a light brown solid

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(63 mg, 54%) whose mp was in agreement with literature value. ^{1}H NMR (d_6-DMSO), 8.52 (s, 1H, H8), 7.73 (s, 1H, H3); ^{13}C NMR (d_6-DMSO) 167.85, 149.85, 146.37, 139.73, 110.84, 108.29.

2,6-Dichloro-3-deazaadenine (4). To a mixture of triethylorthoformate-acetic anhydride 1:1 volume (50.0 mL) was added **16** (4.64 g, 26.09 mmol).[12] The solution was refluxed for 4.5 h. The excess reagents were removed in vacuo and the residue dissolved in 10% NaOH (66 mL) and warmed on a steam bath for 40 min. The resulting solution was cooled to room temperature, neutralized to pH 6 with glacial acetic acid, and cooled for 2 hours at 0°C. The resulting precipitate was removed by filtration and dried to produce **4** as a light brown solid (4.69 g, 96%); mp was in agreement with literature value. ^{1}H NMR (d_6-DMSO), 8.52 (s, 1H, H8), 7.73 (s, 1H, H3); ^{13}C NMR (d_6-DMSO) 167.85, 149.85, 146.37, 139.73, 110.84, 108.29.

3,4-Diamido-2,6-dichloropyridine (18). Using a similar method as was used for 4 but increasing the reaction time, formic acid (75.6 mL, 2.0 mol) was added to **16** (2.06 g, 11.54 mmol) and the mixture refluxed for 3 days. The mixture was evaporated and purified via column chromatography eluting with EtOAc:MeOH (95:5) to produce **18** (1.02 g, 38%) as a light brown solid. (^{1}H NMR (d_6-DMSO), 8.84 (s, 2H, CHO), 6.93 (s, 1H, H5), 6.83 (s, 2H, NH_2); ^{13}C NMR (d_6-DMSO) 168.74, 155.12, 150.40, 141.51, 141.16, 132.19, 123.05, 106.43, 96.88.

2,6-Dichloro-8-oxo-3-deazaadenine (19). Aqueous KOH 35% w/v (15 mL) was added to crude **18** (1.02 g, 4.36 mmol) and refluxed overnight and allowed to cool. The mixture was neutralized with glacial acetic acid, extracted with EtOAc (3×200 mL) and evaporated. The crude solid was purified via column chromatography eluting with CH_2Cl_2:MeOH (95:5) to produce **19** as a light brown solid (500 mg, 49%); mp 176.5–181.6°C; ^{1}H NMR (d_6-DMSO), 6.84 (s, 1H, H3), 6.74 (s, 1H, H7), 6.48 (s, 1H, H9); ^{13}C NMR (d_6-DMSO) 168.02, 158.61, 149.85, 149.20, 110.86, 106.54. HRMS Calc. for $C_6H_3N_3Cl_2O$: 202.96534, Found 202.96516.

2-Chloro-3-deazaadenine (7). To saturated methanoic ammonia stirring at −78°C (100 mL) was added 2,6-dichloro-3-deazaadenine (**4**) (3.20 g, 17.04 mmol) and heated in a bomb at 160°C for 90 h.[15] The solution was evaporated to dryness and the crude solid purified on a silica gel column eluting with EtOAc:hexane (2:1) to give **7** as a light brown solid (1.40 g, 49%); mp dec. 266°C. ^{1}H NMR (CD_3OD), 8.05 (s, 1H, H8), 6.79 (s, 1H, H3), 4.92 (s, 2H, NH_2); ^{13}C NMR (CO_3OD) 151.96, 142.67, 142.12, 115.56, 114.98, 98.48.

3-Deazaadenine (20). To a solution of crude **7** (1.38 g, 8.18 mmol) in water (239.0 mL) was added 10% NaOH (8.18 mL, 20.45 mmol) and 10% palladium on carbon catalyst (497.7 mg).[15] The mixture was hydrogenated at 34 psi for 20 h. The solution was filtered through a celite pad and the pad washed with boiling H_2O (200 mL). The combined filtrates were evaporated in vacuo to produce a crude solid, which was dissolved in EtOH and filtered to yield **20** as a yellowish brown solid

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(790.3 mg, 72%) mp was in agreement with literature value. ^{1}H NMR (d_6-DMSO), 8.09 (s, 1H, H8), 7.61 (d, 1H, H2, $J = 5.7$ Hz), 6.77 (d, 1H, H3, $J = 5.7$ Hz), 6.23 (s, 2H, NH_2); ^{13}C NMR (d_6-DMSO) 151.25, 140.38, 139.59, 124.91, 99.43.

3-Chloro-3-deazaadenine (21). DMA (10.0 mL) containing 3-deazaadenine (**20**) (268.5 mg, 2.0 mmol) was evaporated to dryness.[16] Another 10 mL of DMA was added and reduced under vacuum to a light brown oil and cooled to room temperature. The oil was then dissolved in 0.5 M HCl in DMA (4.0 mL, 2.0 mmol) and stirred for 7 min, at which point *m*-CPBA (345.5 mg, 2.0 mmol) in DMA (2.0 mL) was added. The solution was stirred for an additional 6 min, and an additional 0.5 M HCl in DMA (4.0 mL, 2.0 mmol) was added. The solution was then allowed to stir at room temperature for 25 min. The brown reaction mixture was evaporated and remaining traces of DMA removed by coevaporation with EtOH:xylene (1:2, 2×8 mL). The gummy residue was dissolved in MeOH (50.0 mL) and H_2O added. A tan precipitate formed, which was filtered and washed with H_2O. The combined filtrate and washings were extracted with diethyl ether (2×50 mL), the aqueous layer was neutralized with a 10% NaOH solution, and reduced under vacuum. The crude mixture was purified using column chromatography eluting with $CHCl_3$:MeOH (97:3 to 95:5) to yield **21** as a light tan solid (20.8 mg, 6.2%); mp dec. 240°C. ^{1}H NMR (d_6-DMSO), 8.16 (s, 1H, H8), 7.63 (s, 1H, H2), 6.32 (s, 2H, NH_2). ^{13}C NMR (d_6-DMSO) 151.0, 140.7, 137.5, 135.8, 127.05, 103.0; HRMS Calc. for $C_6H_5N_4Cl$, 168.02027, found 168.01998.

2,8-Dichloro-3-deazaadenine (6). In a manner analogous to the method[16] used with 20, 2-chloro-3-deazaadenine (7) (337.8 mg, 2.0 mmol) afforded 6 as a light tan solid (152.6 mg, 38%); mp 325.2°C (dec). ^{1}H NMR (d_6-DMSO), 8.18 (s, 1H, H3), 6.80 (s, 2H, NH_2); ^{13}C NMR (d_6-DMSO) 133.37, 132.94, 132.74, 130.68, 128.86, 127.95; MS (EI) 201.9 (M^+, 100), 204 (M^{+2}, 73), 206 (M^{+4}, 17); HRMS Calcd. for $C_6H_4N_4Cl_2$: 201.98130. Found: 201.98131.

8-Chloro-3-deazaadenine (3). To a stirred solution of **20** (50.0 mg, 86.4 mmol) in DMSO (3 mL) was added *t*-butyl hypochlorite (15.0 μL, 1.33 nmol).[18] The resulting solution was stirred at rt for 3 days, at which point three sequential portions of *t*-butyl hypochlorite (15.0 μL, 1.33 nmol) were added over a period of 3 days. The residue was dissolved in $CHCl_3$ and purified by column chromatography eluting with CH_2Cl_2:EtOH (97:3) to give **3** (16.6 mg, 31.0%) as a yellow brown solid. ^{1}H NMR (d_6-DMSO), 7.82 (1H, d, H3, $J = 5.7$ Hz), 6.98 (1H, d, H2, $J = 5.7$ Hz), 6.63 (2H, s, NH_2); ^{13}C NMR (d_6-DMSO) 139.44, 135.81, 133.43, 130.68, 127.36, 125.75; MS (EI) 168 (M^+, 100), 170 (M^{+2}, 34); HRMS Calcd. for $C_6H_5N_4Cl$: 168.02027. Found 168.02057.

REFERENCES

1. Hecht, J.R. Genetics, epidemiology, prevention, and early detection of colorectal cancer. Curr. Opin. Gastro. **1997**, *13*, 5–10.

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2. Boland, C.R.; Sinicrope, F.A.; Brenner, D.E.; Carethers, J.M. Colorectal cancer prevention and treatment. Gastro. **2000**, *118*, S115–S128.
3. Piedbois, P.; Buyse, M.; Rustum, Y.; Machover, D.; Erlichman, C. et al. Advanced colorectal cancer meta-analysis project: modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. J. Clin. Oncol. **1992**, *10*, 896–903.
4. Benson, A.B. Therapy for advanced colorectal cancer. Sem. Oncol. **1998**, *25*, 2–11.
5. Carlson, C.C.; Chinery, R.; Burnham, L.L.; Dransfield, D.T. 8-Cl-adenosine-induced inhibition of colorectal cancer growth in vitro and in vivo. Neoplasia **2000**, *2*, 441–448.
6. Taylor, C.W.; Yeoman, L.C. Inhibition of colon tumor cell growth by 8-chloro-CAMP is dependent upon its conversion to 8-chloro-adenosine. Anti-Cancer Drugs **1992**, *3*, 485–491.
7. Bader, J.P.; Brown, N.R.; Chiang, P.K.; Cantoni, G.L. 3-Deazaadenosine, an inhibitor of adenosylhomocysteine hydrolase, inhibits reproduction of rous sarcoma virus and transformation of chick embryo cells. Virology **1978**, *89*, 494–505.
8. Chiang, P.K. Conversion of 3T3-L1 fibroblasts to fat cells by an inhibitor of methylation: Effect of 3-deazaadenosine. Science **1981**, *211*, 1164–1166.
9. Chiang, P.K.; Burbelo, P.D.; Brugh, S.A.; Gordon, R.K.; Fukuda, K.; Yamada, Y. Activation of collagen IV gene expression in F9 teratocarcinoma cells by 3-deazaadenosine analogs. J. Biol. Chem. **1992**, *267*, 4988–4991.
10. Chiang, P.K.; Miura, G.A. *S*-adenosylhomocysteine hydrolase. In *Biological Methylation and Drug Design*; Humana Press: Clifton, NJ, 1986; 239–251.
11. Chiang, P.K. Biological effects of inhibitors of *S*-adenosylhomocysteine hydrolase. Pharmacol. Ther. **1998**, *77*, 115–134.
12. Rousseau, R.J.; Robins, R.K. The synthesis of various chloroimidazo[4,5-*c*]pyridines and related derivatives. J. Heterocyclic Chem. **1965**, *2*, 196–201.
13. Cosstick, R.; Li, X.; Tuli, D.K.; Williams, D.M.; Connolly, B.A. et al. Molecular recognition in the minor groove of the DNA helix. Studies on the synthesis of oligonucleotides and polynucleotides containing 3-deaza-2′-deoxyadenosine. Nucleic Acids Res. **1990**, *18*, 4771–4778.
14. Merlic, C.A.; Motamed, S.; Quinn, B. Structure determination and synthesis of fluoro nissl green: An RNA-binding fluorochrome. J. Org. Chem. **1995**, *60*, 3365–3369.
15. Montgomery, J.A.; Shortnacy, A.T.; Clayton, S.D. A comparison of two methods for the preparation of 3-deazapurine ribonucleosides. J. Heterocyclic Chem. **1977**, *14*, 195–197.
16. Ryu, E.K.; MacCoss, M. New procedure for the chlorination of pyrimidine and purine nucleosides. J. Org. Chem. **1981**, *46*, 2819–2823.
17. Hayakawa, H.; Tanaka, H.; Haraguchi, K.; Mayumi, M.; Nakajima, M. et al. Preparation of 8-chloropurine nucleosides through the reaction between their C-8 lithiated species and *p*-toluene sulfonyl chloride. Nucleosides Nucleotides **1988**, *7*, 121–128.
18. Ikehara, M.; Ogiso, Y.; Maruyama, T. Studies of nucleosides and nucleotides. LXXIII. Chlorination of adenosine and its N-6-methyl derivatives with *t*-butyl hypochlorite. Chem. Pharm. Bull. **1977**, *25*, 575–578.

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19. von Tilburg, E.W.; von Frijtag Drabbe Kunzel, J.; de Grotte, M.; Ijzerman, A.P. 2,5′-Disubstituted adenosine derivatives: Evaluation of selectivity and efficacy for the adenosine A_1, A_{2A} and A_3 receptors. J. Med. Chem. **2002**, *45*, 420–429.
20. Seyama, F.; Akahori, K.; Sakata, Y.; Misumi, S.; Aida, M.; Nagata, C. Synthesis and properties of purinophanes: Relationship between the magnitude of hypochromism and stacking geometry of purine rings. J. Am. Chem. Soc. **1988**, *110*, 2192–2201.
21. Lister, J.H. *The Purines. Supplement 1*; Wiley-Interscience: New York, 1996; 91–116.

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