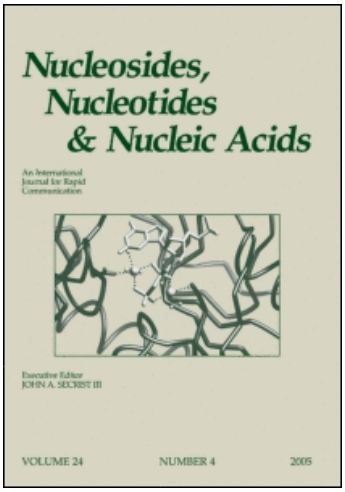

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8-Diazoguanosine, 2,8-Diaminoadenosine and Other Purine Nucleosides Derived from Guanosine

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8-DIAZOGUANOSINE, 2,8-DIAMINOADENOSINE AND OTHER PURINE NUCLEOSIDES DERIVED FROM GUANOSINE

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ABSTRACT: Diazotization of 8-aminoguanosine gave 8-diazoguanosine (2) which is stable in neutral and basic media, but decomposes to D-ribose and 8-diazoguanine in acidic conditions. 2-Amino-6,8-dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (5) was employed to synthesize 9-β-D-ribofuranosyl-2,6,8-triaminopurine (8) and a number of N6-alkyl-2-amino-8-chloro-9-β-D-ribofuranosylpurines.

Base substitution into DNA by base analogues is often a useful approach to investigate DNA biochemistry.[1] These base analogues, if incorporated into DNA, can modify the properties of DNA such as the base pairing pattern[2,3] and the double helical conformation.[4,5]

Although a number of 8-aminopurines have been diazotized to the corresponding 8-diazopurines[6], the diazo compounds have not been utilized in the study of nucleic acid metabolism. Several of these 8-diazopurines can react readily with alkylamines forming 8-triazenopurines.[7] The reactivity of the diazo group makes such nucleosides good candidates for covalent coupling to purine receptors. The result could provide an irreversible binding to specific G-proteins of the cell membrane. An example of such an interaction is 5-diazouracil, an irreversible inhibitor of dihydrothymine dehydrogenase.[8] 5-Diazouracil induces DNA repair synthesis in isolated rat hepatocytes[9] and also prevents methotrexate toxicity caused by the reduction in thymidine levels.[10]

The diazotization of 8-aminoguanine and 8-aminoadenine was first reported by Jones and Robins[11] via treatment with sodium nitrite in acidic media to give 8-diazoguanine and 8-diazoadenine, respectively. These results suggest that 8-diazoguanosine (2) might similarly be prepared.

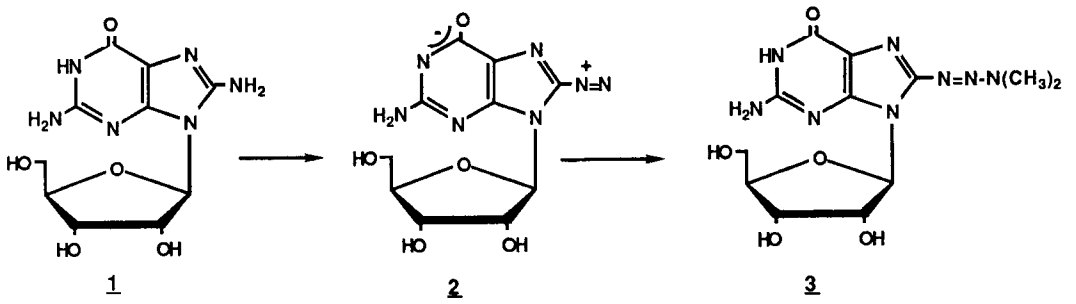
Because of the interesting biological activity of 2,8-diaminoadenine[12] and 2-aminoadenosine[13] we were also interested in the synthesis of 2,8-diaminoadenosine (8). Polyuridylic acid (poly U) binds 2,6,8-triaminopurine(TAP) in a strongly cooperative manner to form a stable 2:1 complex. This stabilization is attributed to the existence of an additional hydrogen bonding interaction. The formation of a Hoogsteen type hydrogen bonding has been suggested In the poly (U)-TAP complex between the 8-amino group of TAP and 0(2) of the uracil moiety. The stability of the complex increases as the number of amine groups on the purine ring increase[12]. Each U forms a triple hydrogen bond with TAP, thus incorporation of TAP into RNA should result in even greater changes in base-pair stacking and the geometry of the DNA or RNA molecule than the changes which have been reported for 2,6-diaminopurine.

Nucleoside base-pairing and hydrogen bonding are main factors that control duplex stability. 2,6-Diamino-9-(2-deoxy-β-D-ribofuranosyl)purine (DAPN) has recently been incorporated into DNA which is then used as a substrate for endonuclease enzymes. DAPN base-pairs with thymidine (T) forming three hydrogen bonds in a Watson-Crick arrangement which is comparable to Guanosine-cytidine (G-C) even though less stable.[13] This suggests differences in the stacking pattern and possibly in the geometry of the hydrogen bond. Chollet and Kawashima[13] have shown that these differences can result in reduction of substrate activity toward several restriction endonucleases. When only one strand contains DAPN instead of A, there is a 50% reduction in substrate activity. This substrate activity totally diminishes when both strands contain DAPN. Data also suggest that the DAPN-T pair acts as a substrate for some G-C endonucleases[14].

Results and Discussions

8-Aminoguanosine (1)[15] was carefully diazotized with sodium nitrite at -78 °C to give 8-diazoguanosine (2) (Scheme I). The IR spectrum of 2 indicates a band at 2350 cm^{-1} which is characteristic of a diazo structure.[11] The UV spectrum of 2 shows a λmax (pH 1) at 253 nm and another at 407 nm; both of these peaks disappear at pH 11. Reaction of 2 with dimethylamine gave 8-(bismethyltriazino)guanosine (3) (Scheme I).

8-Diazoguanosine (2) is quite stable in neutral and alkaline media as noted by the reversibility of the UV spectra. The 407 λmax of 2 at pH 1 disappears at pH 7 and



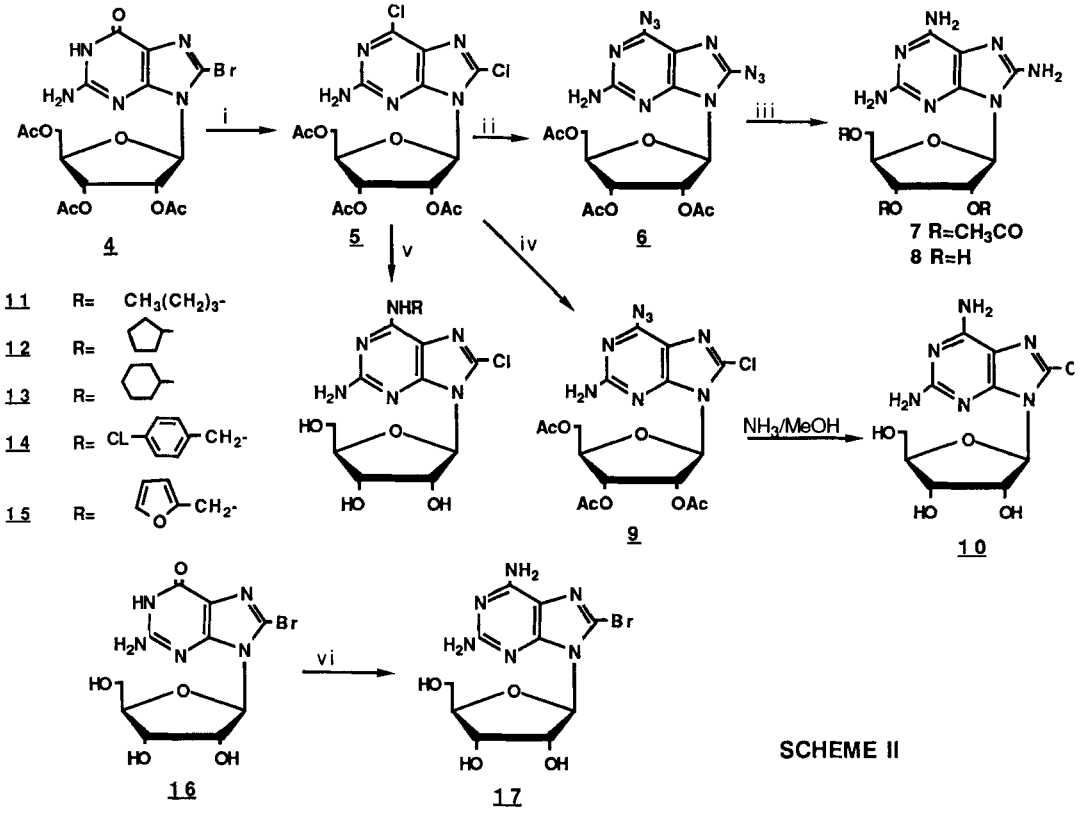
1 2 3

SCHEME I

pH 11. However, when the pH is adjusted back to 1, the absorption at 407 nm reappears indicating that the diazo group was not displaced. One explanation for this behavior is that the diazo group greatly increases the acidity of the N^1 proton. Upon dissolution in neutral to alkaline pH, this proton is removed forming an internal salt. The negative charge that is shared between the 1-nitrogen and the 6-oxygen stabilizes the 8-diazo group.

As would be expected with a strong electron withdrawing group present at the 8-position, the glycosyl bond is very acid-labile. As already stated, 2 at pH 1 has a strong absorption maximum at 407 nm but within 5 min at this pH, another pronounced band at 361 nm appears that is due to 8-diazoguanine formation[11]. And within approximately 40 min, all of 2 disappears. Based on the limited information available, we assume that the observed depurination follows an A1 type mechanism[16]. This mechanism involves the protonation of the nitrogen in the guanine moiety, followed by cleavage of the N-glycosidic bond in a slow step, ultimately producing D-ribose and 8-diazoguanine.

The synthesis of 2,8-diaminoadenosine (8) involved utilization of 2-amino-6,8-dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (5) which was synthesized from 4 in 82% yield via a modification of both the Robins procedure[17] and the original procedure reported previously from our laboratory[18] (Scheme II). Compound 8 has been synthesized recently by another procedure[19]. Reaction of 5 with sodium azide in dimethylformamide (DMF) and isobutyric acid gave 2-amino-6,8-diazido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (6) which was then catalytically hydrogenated and deprotected with aqueous sodium hydroxide to give 2,8-diaminoadenosine (8) in 69% overall yield (Scheme II). The IR spectrum of 6 showed a strong band for the azido group at 2150 cm^{-1}. This band disappeared upon hydrogenation



i) $POCl_3$, LiCl, CH_3CN, Base; ii) NaN_3, DMF, isobutyric acid; iii) $Pd/C/H_2$, NaOH,
iv) NaN_3, Me_3N, CH_3CN; v) RNH_2, $NaHCO_3/NH_3$, MeOH vi) HMDS,$ClSiMe_3$/ NH_3, HMDS, TMS-Triflate/ H_2O,MeOH.

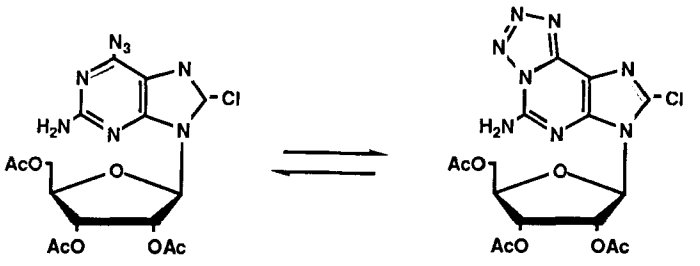
of 6. The UV spectrum of compound 6 in methanol showed a strong absorption maximum near 300 nm, whereas 8 showed a λmax (pH 1) at 252 nm (Table 1).

Treatment of compound 5 with sodium azide in the presence of base such as trimethylamine[12] resulted in nucleophilic substitution of the 6-chloro group to give 2-amino-6-azido-8-chloro-9-(2,3,5-tri-0-aceltyl-β-D-ribofuranosyl)purine (9) in 93% yield. Compound 9 equilibrates between two forms, 9a and 9b (Scheme III) due to tetrazole ring formation by the 6-azido group.

Treatment of 9 with methanolic ammonia resulted not only in deacetylation of the ribose but also nucleophilic substitution of the azido group with an amino group giving 2-amino-8-chloroadenosine (10) in over 80% yield . The strong IR absorption band of 9 at 2150 cm $^{-1}$ disappeared when treated with methanolic ammonia to give 10. Catalytic

TABLE 1: Ultra Violet Absorption Data of Guanosine Analogs

COMPOUND #	λmax (ε) at pH 1	λmax (ε) at pH 11
2	253 (---), 407	232
8	217 (21,080), 252 (12,620) 307(11,540)	211 (19,820), 259 (11,050) 290 (9,270)
10	211 (25,240), 254 (13,750) 292(12,100)	216 (29,300), 257 (11,280) 280 (12,160)
15	210 (49,770), 205 (17,810)	222 (51,320), 284 (33,050)
17	249 (14,860), 287 (9,800)	263 (10,370), 278 (10,940)
	The UV spectra were determined in methanol for the following nucleosides	
9	275 (18,450), 301 (22,170)	
11	226 (18,560), 284 (13,480)	
12	228 (19,980), 285 (15,300)	
13	227 (18,740), 285 (14.040)	
14	226 (18,960), 284 (13,480)	



9a 9b

SCHEME III

reduction of 10 gave 2,6-diamino-9-β-D-ribofuranosylpurine[20] which established the structures of 9 and 10.

Reaction of compound 5 with various N-alkylamines[18], such as cyclopentylamine, cyclohexylamine, n-butylamine, p-chlorobenzylamine, and furfurylamine, resulted in the synthesis of corresponding 2-amino-N6-alkyl-8-chloroadenosine analogues 11-15.

Treatment of 8-analogues (16) with hexamethyldisilazane(HMDS) and chlorotrimethylsilane resulted in a polysilylated product which, without purification, was treated with liquid ammonia HMDS, and TMS-triflate at 150°C followed by methanol/water to give 2-amino-8-bromoadenosine (17) in 65% yield. While there is a similarity in the UV spectra of 17 and 10, the λmax of the two compounds are different (Table 1).

Experimental

General

The 1H NMR spectra were determined using an IBM NR 300 FTNMR spectrometer (300 MHz). The chemical shift values are expressed in δ values (parts per million). The purities of the compounds were determined on a Ranin Instrument Company, high-performance liquid chromatography (HPLC) equipped with a Waters 990 photodiode-array UV-detector and a 5 microne C-18 bonded phase silica column (Partisil-ODS-2 Whatman). The elutions were performed with a linear gradient from 0-50% MeOH in H_2O. Evaporations were carried out on a rotary evaporator (Buchi Rotovapor R110). Mass spectra were determined on a Varian MAT 731 double focusing high resolution mass spectrometer with an ion Tech 11N FAB ion source operated at 7 KeV with Xe.

DEAE cellulose (DE-52) was purchased from Whatman, England. Amberlite XAD-4 and Dowex 50 X 8 (100-200 mesh) were purchased from Aldrich Chemicals, Milwaukee, WI. Avigel TG-10F (micro cellulose) was from FMC Corporation, Baltimore, MD.

Elemental analyses were performed by Robertson Laboratories, Madison, N.J.

Methods

8-Diazoguanosine. (2)

A solution of 1 [15] (1.00 g, 3.4 mmol) and $NaNO_2$ (254 mg, 3.7 mmol) in 10 ml 5% NaOH was cooled to -5 °C and 10 ml of glacial acetic acid was added. After 30 min at -5°C , the solution was precipitated in acetone. The crude material was filtered , dissolved in 60 ml of water, and decolorized with activated carbon. The pale yellow filtrate was then lyophilized. Pure 8-diazoguanosine was obtained by semi-preparative reversed-phase HPLC using water as the elution solvent. Yield :129 mg (10.7%). 1H NMR (Me_2SO-d_6), δ: 6.339 (s, $2NH_2$, 2H); 5.862 (d, H1', 1H, J = 6.93 Hz); 5.750

(brds, OH, 1H); 5.164 (brd s, OH, 1H); 4.850 (brds, OH, 1H); 4.794 (dd, H2', 1H, $J_{2',1'}$ = 6.93, $J_{2',3'}$ = 5.58 Hz); 4.073 (m, H3, 1H); 3.827 (m, H4', 1H); 3.568 (m, H5' + H5", 2H). Anal. calc. for $C_{10}H_{11}N_7O_5$,2.65 H_2O: C, 33.65; H, 4.60; N, 27.47. Found: C, 33.77; H, 4.26; N, 27.79.

8-Bismethyltriazenoguanosine. (3)

Glacial acetic acid (16 ml) was added dropwise to a -5°C solution of 1(2.00 g, 6.71 mmol) and (509 mg, 7.38 mmol) $NaNO_2$ in 20 ml of 5% aqueous NaOH. After 50 min, 6 ml of 40% aqueous dimethylamine was added in a dropwise manner. The reaction was slowly warmed to room temperature and stirred overnight. The reaction mixture was precipitated in 3 l of acetone and filtered. The solid residue was dissolved in water and applied to a 5 x 24-cm Amberlite XAD-4 column. After washing the column with 4 l of water, the product was eluted with a 0 to 25% linear gradient of ethanol in water. After the appropriate fractions were pooled and evaporated to dryness, the residue was suspended in acetone and filtered. Yield:398 mg (16.7%). ^1H NMR (Me_2SO-d_6) δ: 10.617 (s, N^1H, 1H); 6.334 (s, $2NH_2$, 2H); 5.918 (d, H1', 1H, J = 5.85 Hz) 5.284 (d, OH, 1H, J = 6.30 Hz); 4.992 (d, OH, 1H, J = 5.40 Hz); 4.942 (d, OH, 1H, J = 5.40 Hz); 4.881 (q, H2', 1H, $J_{2',1'}$ = 5.83, $J_{2',3'}$ = 4.50 Hz); 4.114 (dd, H3', 1H, $J_{3',2'}$ = $J_{3',4'}$ = 4 .50 Hz); 3.772 (m, H4', 1H), 3.607 (m, H5' + H5" + CH_3, 5H); 3.197 (s, CH_3, 3H). Anal. calc. $C_{12}H_{18}N_8O_5$ 0.5 H20: C,39.67; H, 5.27; N 30.84. Found: C39.60; H, 5.19; N, 29.80. High resolution FAB mass spectrum (MH+) obsd. 355.15 calc (MH+) 355.15.

2-Amino-6,8-dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine. (5)

To a suspension of 4[18] (20.00 g ,41.0 mmol) and anhydrous LiCl (9 g, 219 mmol) in 250 ml of anhydrous CH_3CN under a dry argon atmosphere was added dry N,N-diethylaniline (6.52 ml, 41.0 mmol) and distilled $POCl_3$ (76 ml, 820 mmol). The mixture was placed into a 120 °C oil bath and refluxed for 15 min. After cooling, the LiCl was filtered off, and the filtrate was reduced in vacuo to an oil. The oil was dissolved in CH_2Cl_2 and this solution was poured onto 100 g of ice with vigorous stirring. After the layers were separated, the aqueous phase was washed with 5 x 20 ml of CH_2Cl_2. The combined organic layers were washed with 100 ml 5% $NaHCO_3$, then dried (Na_2SO_4). The solution was concentrated under reduced pressure to approximately 40 ml, diluted with 100 ml of 2-propanol and refrigerated overnight. The crystals were filtered off

and then dried in vacuo over P_2O_5. Yield:15.44 g (81.5%). mp 138.5-140.5 °C (lit[18] 138° 140°C). ^1H NMR ($CDCl_3$) δ: 6.431 (dd, H1',1H, $J_{1',3'}$ = 4 .07, $J_{1',2'}$ = 5.51 Hz); 6.224 (dd, H2', 1H, $J_{2',1'}$ = 5.51, $J_{2',3'}$ = 4 .05 Hz); 6.182 (dd, H3', 1H, $J_{3',2'}$ = 4 .05, $J_{3',4'}$ = 5 73 Hz); 5.680 (s, $2NH_2$, 2H); 4.596 (m, H4' + H5' + H5", 3H); 2.343 (s, CH_3, 3H); 2.325 (s, CH_3, 3H), 2.195 (s, CH3, 3H). Anal. Calc. for $C_{16}H_{17}Cl_2N_5O_7$: C, 41.57; H, 3.71; N, 15.15; Cl, 15.35. Found: C, 41.82; H, 3.46; N, 14.91; Cl, 15.09.

2,8-diaminoadenosine. (8)

Dry 5 (5.00 g,10.8 mmol) and dry NaN3 (7.03 g, 108 mmol) were suspended in 150 ml of a 20:1 solution of anhydrous N',N-dimethylformamide (DMF)/isobutyric acid. After being heated at 50 °C for 72 hr, the reaction mixture was filtered and evaporated to an oil in vacuo. The oil was dissolved in CH_2Cl_2, extracted with water (3 x 50 ml), dried(Na_2SO_4), and evaporated to an oil. This crude 6 was dissolved in a minimum of ethyl acetate and then diluted with 100 ml of ethanol. This solution was hydrogenated overnight using 1 g 10% Pd/C at 45 psi H_2. After filtration of the catalyst and concentration of the filtrate under reduced pressure, the viscous solution was diluted with water and concentrated. The concentrate was cooled in ice and 3.5 ml of a 1% (w/w) NaOH solution was added. After 1 hr, the solution was neutralized with 5 ml of acetic acid and concentrated. The solution was applied onto a 5 x 40-cm Amberlite XAD-4 column and the column was washed with 4 l of water. The product was eluted from the column with 20% EtOH in water. The eluent was reduced in volume to 15 ml and lyophilized. Yield: 2.22 g (69.2%) of slightly impure material. An analytical sample was prepared by repeating the chromatography on an Amberlite XAD-4 column using a 0 to 40% EtOH in water gradient. ^1H NMR (Me_2SO-d_6) δ: 6.075 (s, NH_2, 2H); 6.049 (s, NH_2, 2H); 5.902 (s, OH, 1H); 5.732 (d, H1', 1H, J = 6.92 Hz); 5.315 (s, $8NH_2$, 2H); 5.181 (d, OH, 1H, J = 6.72 Hz); 5.053 (d, OH, 1H, J = 3.33 Hz); 4.619 (dd, H2', 1H, $J_{2',1'}$ = 6.92 Hz, $J_{2',3'}$ = 5.80 Hz); 4.080 (m, H3', 1H); 3.901 (m, H4', 1H); 3.612 (m, H5' + H5", 2H). Anal. calc. for $C_{10}H_{15}N_7O_4$ 0.25 H20: C, 39.80; H, 5.18; N, 32.49. Found: C, 39.92; H, 5.06; N, 32.21.

2-Amino-6-azido-8-chloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine. (9)

Dry 5 (3.00 g, 6.5 mmol) and NaN3 (4.22 g, 65 mmol) were suspended in 150 ml freshly distilled CH_3CN. After cooling the suspension to -20 °C under an argon

atmosphere, anhydrous Me_3N was bubbled into the mixture for 5 min. The reaction was allowed to warm to room temperature and was complete after 3 hr. The solution was evaporated to dryness and crude product was purified by flash chromatography using CH_3Cl as the elution solvent. The appropriate fractions were pooled and evaporated to dryness in vacuo. The residue was crystallized from warm 2-propanol and the resultant white precipitate was filtered and dried over P_2O_5. Yield :2.82 g (92.7%). The ^1H NMR spectrum of this compound indicates an equilibrium between the 6-azido and the cyclic tetrazole structures (~ 2:1) respectively). ^1H NMR ($CDCl_3$) δ : 6.380 (s, $2NH_2$, tetrazole), 5.096 (s, $2NH_2$, azido). Anal. calc. for $C_{16}H_{17}ClN_8O_7$: C, 40.99; H, 3.66; Cl, 7.56; N, 23.90. Found: C, 41.01; H, 3.50; Cl, 7.73; N, 23.61.

2-Amino-8-chloroadenosine. (10)

A solution of 9 (1.50 g ,3.2 mmol) in 30 ml of methanolic ammonia was sealed in a pressure bottle and stirred at -5 °C overnight. The ammonia was vented, and the solution was evaporated to dryness. Purification by flash chromatography using a stepwise gradient of MeOH in CH_3Cl yielded 772 mg of pure 10 (74.6%). ^1H NMR (Me_2SO-d_6) δ: 8.599 (s, $6NH_2$, 2H); 8.304 (s, $2NH_2$, 2H); 5.917 (d, H1', 1H, J = 5.94 Hz); 5.563 (d, OH, 1H, J = 6.03 Hz); 5.219 (d, OH, 1H, J = 5.31 Hz); 5.052 (dd, H2', 1H, $J_{1',2'}$ = 5 94 Hz, $J_{2',3'}$ = 4.02 Hz); 4.816 (t, 5'OH, 1H, $J_1 = J_2$ = 5.79 Hz); 4.223 (dd, H3', 1H, $J_{3',2'}$ = 4.02, $J_{3',4'}$ = 6.93 Hz); 3.891 (dd,H4', 1H, J_1 = 3.78, J_2 = 6.93 Hz); 3.630 (m, H5' + H5", 2H). Anal. calc. for $C_{10}H_{13}ClN_6O_4$: C, 37.92; H, 4.14; Cl, 11.19; N, 26.54. Found: C, 37.73; H, 4.38; Cl, 11.32; N, 26.23.

2-Amino-N6-(n-butyl)-8-chloroadenosine. (11)

Compound 11 was synthesized in a manner similar to 12 using n-butylamine (0.959 ml, 9.7 mmol) and 5 (1.50 g ,3.2 mmol). Yield :756 mg (63.4%). ^1H NMR (Me2SO-d6) δ: 7.581 (s, N^6H, 1H); 5.950 s ($2NH_2$, 2H); 5.720 (d, H1', 1H, J = 6.30 Hz) 5.454 (d, OH, 1H, J = 6.30 Hz); 5.155 (d, OH, 1H, J = 4.35); 5.000 (dd, H2', 1H, $J_{2',1'}$ = 6.30, $J_{2',3'}$ = 5.42 Hz); 4.120 (d, H3', 1H, J = 5.42); 3.929 (m, H4', 1H); 3.586 (m, H5' + H5", 2H); 3.364 (s, N-CH_2, 2H); 1.520 (m, CH2, 2H); 1.297 (m, CH2, 2H); 0.881 (t, CH_3, 3H, J= 7.31 Hz). Anal. calc. for $C_{14}H_{21}ClN_6O_4$: C, 45.10; H, 5.68; Cl, 9.51; N, 22.54. Found: C, 45.32; H, 5.71; Cl, 9.44; N, 22.26.

2-Amino-N^6-cyclopentyl-8-chloroadenosine. (12)

A suspension of 5 (1.50 g , .2 mmol) and $NaHCO_3$ (954 mg, 11.4 mmol) was added to a solution of cyclopentylamine (0.960 ml, 9.7 mmol) in 35 ml ethanol. The mixture was heated at reflux for 4 hr, filtered, and evaporated. The resultant oil was dissolved in methanolic ammonia and was stored at -20 °C for three days. The final product was purified by flash chromatography using a stepwise gradient of methanol in methylene chloride. The fractions containing pure 12 were pooled and evaporated to dryness, and the residue was suspended in hexane and filtered. Yield :742 mg (60.3%). ^{1}H NMR (Me_2SO-d_6) δ: 7.495 (s, N^{6}H, 1H); 5.848 (s, $2NH_2$, 2H); 5.716 (d, H1', 1H, J = 6.93 Hz); 5.668 (t, 5'OH, 1H, $J_1 = J_2$ = 4.17 Hz); 5.456 (d, OH, 1H, J = 6.36 Hz); 5.164 (d, OH, 1H, J = 4.47 Hz); 4.994 (dd, H2', 1H, $J_{2',1'}$ = 6.93 Hz, $J_{2',3'}$ = 4.88 Hz); 4.119 (dd, H3', 1H, $J_{3',2'}$ = 4.88, $J_{3',4'}$ = 6.26 Hz); 3.926 (m, H4', 1H); 3.587 (m, H5' + H5", 2H); 1.883 (brd s, CH_2, 2H); 1.667 (brd s, CH_2, 2H); 1.503 (brd s, 2(CH_2), 4H). Anal. calc. for $C_{15}H_{21}ClN_6O_4$: C, 46.82; H, 5.50; Cl, 9.21; N, 21.56. Found: C, 47.01; H, 5.50; Cl, 9.37; N, 21.56.

Amino-N^6-cyclohexyl-8-chloroadenosine. (13)

Compound 13 was prepared in a manner similar to 12 using cyclohexylamine (1.11 ml, 9.7 mmol) and 5 (1.50 g , 3.2 mmol). Yield :760 mg (67.7%). ^{1}H NMR (Me_2SO-d_6) δ: 7.351 (s, N^{6}H, 1H); 5.841 (s, $2NH_2$, 2H); 5.713 (m, H1' + 5'OH, 2H); 5.453 (d, OH, 1H, J = 6.27 Hz); 5.163 (d, OH, 1H, J = 4.35); 4.989 (dd, H2', 1H, J_1 = 5.91, J_2 = 9.06 Hz); 4.116(m, H3', 1H); 3.930 (m, H4', 1H); 3.578 (m, H5' + H5", 2H); 1.804 (d, CH_2, 2H, J = 8.97 Hz); 1.721 (d, CH_2, 2H, J = 6.96 Hz); 1.595 (m, CH, 1H); 1.294 (m, CH2, 4H); 1.157 (m, CH2, 2H). Anal. calc. for $C_{16}H_{23}ClN_6O_4$: C, 48.18; H, 5.81; Cl, 8.89; N, 21.07. Found: C, 48.77; H, 5.85; Cl, 8.73; N, 20.86.

2-Amino-N^6-(p-chlorobenzyl)-8-chloroadenosine. (14)

Compound 14 was prepared in a manner similar to 12 in that 5 (4.00 g, 8.6 mmol), $NaHCO_3$ (2.528 g), and p-chlorobenzylamine (3.158 ml, 26 mmol) were suspended in 100 ml of ethanol. Yield:1.92 g (54.7%). ^{1}H NMR (Me_2SO-d_6) δ: 8.193 (s, N^{6}H, 1H); 7.339 (s, phenyl, 4H); 5.945 (s, $2NH_2$, 2H); 5.736 (d, H1', 1H, J = 6.90 Hz); 5.648 (brd d, 5'OH, 1H, J = 4.41); 5.487 (d, OH, 1H, J = 6.30 Hz); 5.196

(d, OH, 1H, J = 4.35); 5.012 (dd, H2', 1H, $J_{2',3'}$ = 5.85, $J_{2',1'}$ = 6.90); 4.589 (brd s, CH_2, 2H); 4.135 (m, H3', 1H); 3.942 (m, H4', 1H); 3.601 (m, H5' + H5", 2H). Anal. calc. for $C_{17}H_{18}C_{12}N_6O_4$: C, 46.27; H, 4.11; Cl, 16.07; N, 19.05. Found: C, 46.48; H, 4.00; Cl, 16.13; N, 18.84.

2-Amino-N^6-furfuryl-8-chloroadenosine. (15)

Compound 15 was synthesized in a manner similar to 12 using furfurylamine (0.860 ml, 9.7 mmol) and 5 (1.5 g, 3.2 mmol). Yield: 788 mg (61.3%). ^{1}H NMR (Me_2SO-d_6) δ: 8.027 (s, N^6H, 1H); 7.528 (s, C5H, 1H); 6.357 (d, CH, 1H, J = 1.74 Hz); 6.246 (s, CH, 1H, J = 1.74 Hz); 5.974 (s, $2NH_2$, 2H); 5.724 (d, H1', 1H, J = 6.63); 5.588 (s, 5'OH, 1H); 5.474 (d, OH, 1H, J = 6.00 Hz); 5.178 (d, OH, 1H, J = 3.36 Hz); 5.006 (dd, H2', 1H, $J_{2',3'}$ = 5 79, $J_{2',1'}$ = 6.63 Hz); 4.590 (s, CH_2, 2H); 4.124 (m, H3', 1H); 3.923 (m, H4', 1H); 3.585 (m, H5' + H5", 2H). Anal. calc. for $C_{15}H_{17}ClN_6O_5$: C, 45.41; H, 4.32; Cl, 8.93; N, 21.18 . Found: C, 45.70; H, 4.21; Cl, 9.13; N, 20.96.

2-Amino-8-bromoadenosine. (17)

A mixture of dry 8-bromoguanosine (16) (10.00 g , 27.6 mmol) in a solution of 105 ml hexamethyldisilazane (HMDS) and 5.5 ml of chlorotrimethylsilane was refluxed under an argon atmosphere overnight. The yellow solution was evaporated under high vacuum to a crystalline mass that was dissolved in 50 ml of dry toluene. The solution was chilled and transferred to a bomb where it was sealed under dry argon with dry liquid ammonia (10 g) and a pre-cooled solution of 7 ml HMDS and 0.600 ml TMS-triflate. The bomb was warmed to room temperature, then placed in a 150 °C oil bath for 3 days. The bomb was cooled in dry ice, vented to the atmosphere, and allowed to warm to room temperature. The slurry was diluted with a 1:1 solution of aqueous methanol and refluxed for 4hr. After the mixture was evaporated in vacuo, the residue was suspended in 80 ml hot water and filtered hot. The precipitate was washed with hot water, then with acetone. After cooling of the filtrate the crystals were collected. Yield 6.53 g (65.2%). An analytical sample was prepared by recrystalization from water. ^{1}H NMR (Me_2SO-d_6) 8.494 (s, $6NH_2$, 2H); 7.150 (s, $2NH_2$, 2H); 5.874 (d, H1', 1H, $J_{1',2'}$ = 7.20 Hz) 4.503 (dd, H2', 1H, $J_{2',1'}$ = 7.20 Hz, $J_{2',3'}$ = 5.85 Hz), 4.122 (m, H3', 1H); 4.004 (m, H4', 1H); 3.635 (m, H5' + H5", 2H). Anal. calc. for $C_{10}H_{13}BrN_6O_4$: C, 33.26; H, 3.63; Br, 22.13; N, 23.27. Found: C, 33.27; H, 3.85; Br, 21.84; N, 23.02.

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