Alkylpurines as Immunepotentiating Agents. Synthesis and Antiviral Activity of Certain Alkylguanines†

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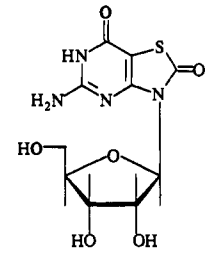
Several simple 8-substituted 9-alkyl- and 7,8-disubstituted 9-alkylguanine derivatives were synthesized as potential antiviral agents. These were tested for antiviral protection against a lethal Semliki Forest virus (SFV) infection in mice, and their antiviral properties were evaluated from a structure–activity standpoint. In this model system, 9-alkylguanines with the alkyl chain consisting of four to six carbons were found to be the most active. Substitution of the 8-position of the purine ring did not enhance activity, with the exception of the 7-alkyl-8-oxo substituent. These data were found to support the hypothesis that guanines need not contain an intact carbohydrate moiety in order to exhibit antiviral activity by virtue of immune potentiation. Hence, phosphorylation of guanosine analogs that exhibit antiviral activity by a similar mechanism does not play a significant role.

Introduction

Several acyclic nucleoside derivatives of guanine have been synthesized as potential antiviral agents.[1] Most notable among these are acyclovir,[2] the current drug of choice for treatment of herpes simplex virus (HSV) infections, and ganciclovir,[3] which has shown clinical efficacy against cytomegalovirus (CMV) infections, particularly in patients with AIDS and other serious immunodeficiencies.[4] Both compounds are reported to be selectively converted to their respective monophosphate derivatives by viral specific kinases present in the infected cells.[5] These monophosphates are subsequently converted to their triphosphates by the cellular enzymes of the host. The triphosphate form of the acyclonucleoside inhibits HSV DNA polymerase and prevents viral replication.[6] While these acyclic nucleosides are quite selective and relatively nontoxic, animal studies with ganciclovir have revealed some testicular and gastrointestinal tract toxicity.[4] Bone marrow toxicity has been observed in 14 of 31 patients undergoing bone marrow transplant and receiving ganciclovir to treat CMV infections.[7-9] Recent studies of the effects of ganciclovir on in vitro lymphocyte response show that it inhibits lymphocyte functions for which lymphocyte proliferation is necessary (e.g., response to CMV antigen, phytohemagglutinin), but does not affect lymphocyte responses that do not need DNA synthesis (e.g., production of CMV induced interleukin-2 or interferon and lymphocyte mediated toxicity).[10]

In contrast to these acyclic nucleosides of guanine are guanosine analogues that exhibit antiviral activity by virtue of their immune potentiating properties. We have reported the synthesis and activity of a variety of guanosine analogs in the thiazolo[4,5-*d*]pyrimidine,[11] pyrazolo[3,4-*d*]pyrimidine,[12] purine,[13] 7-deazapurine,[14] and 9-deazapurine[15] ring systems. Among these guanosine analogs, 7-thia-8-oxoguanosine (1)[11] has been studied in detail and shown to exhibit broad spectrum antiviral activity in vivo.[16,17] This analogue has been shown to stimulate interferon production and to activate natural killer cells,[18] macrophages,[19] and B-lymphocytes.[11] The antiviral effects of this and other related guanosine analogs may be attributed to interferon induction since administration of antibodies to this cytokine abrogates the protective properties of this nucleoside[18] against the Semliki Forest virus. The mechanism of activation of the various cells of the immune system by these guanosine analogs has not yet been fully elucidated. However, studies from our laboratory[19,20] and others[21] suggest that guanine nucleotide-binding proteins (G-proteins) may be involved in the mechanism of action of these compounds. It is conceivable that these guanosines may directly interact with the G-proteins in the cell membrane resulting in cellular activation, cytokine induction, etc.



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The immunopotentiating activity of another class of compounds, the phenylpyrimidinones, has been reported.[22] The profile of biological activity of this class of compounds is strikingly similar to that of the guanosine analogs. Indeed, Wicker and co-workers[23] demonstrated the similarities between the two classes of immunopotentiators relative to their effects on B lymphocytes and suggested that they both interact with the same cellular constituent in the target cells. Thus, it appears that carbohydrate moiety is not essential for such immunostimulation.

In view of these observations, 9-*n*-hexylguanine, a compound reported by Robins and co-workers,[24] was evaluated for antiviral protection against a lethal Semliki Forest virus (SFV) infection in mice. The striking activity

† This work is dedicated to the memory of Professor Roland K. Robins, Ph.D., deceased August 1992.

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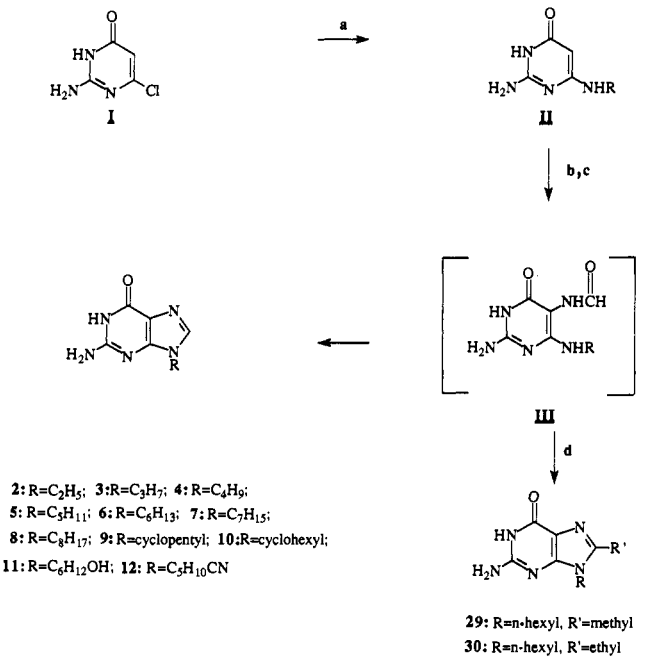
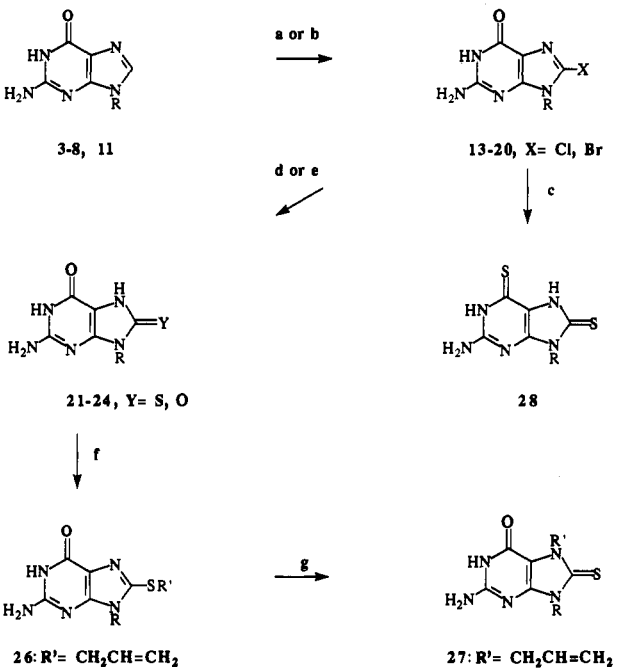
Scheme I[a]



[a]Key: (a) RNH_2, 2-ethoxyethanol, reflux, 6 h; (b) $NaNO_2$, HOAc; (c) $R'CONH_2$, $Na_2S_2O_4$, R'COOH, 70 °C; (d) CH_3COONa, >200 °C.

Scheme II[a]



[a]Key: (a) Br_2, HOAc, 50 °C; (b) HCl gas, DMF; (c) P_2S_5, pyridine; (d) thiourea, ethanol, reflux; (e) Ac_2O, AcOH, AcONa; (f) allyl bromide, DMF, K_2CO_3; (g) DMF; 130 °C, 6 d.

of this compound prompted the synthesis of a homologous series of 9-alkyl substituted guanines as potential antiviral agents. In addition to the simple 9-alkylguanines, a few selected 8-substituted and 7,8-disubstituted 9-alkylguanines were synthesized and evaluated for antiviral activity. In particular, the 8-bromo-, 8-mercapto-, and 7-methyl-8-oxoguanines were studied since earlier reports indicated that the corresponding ribonucleosides of these guanines were able to act as intracellular mitogens in murine B lymphocytes,[25] to augment the proliferation and differentiation of murine T cells in the presence of other stimulatory signals,[26,27] and to activate murine NK cells and macrophages by inducing the production of interferon.[28] The objective of the studies reported herein was to directly compare the structure–activity effects of various substituents at the 7 and/or 8 positions while maintaining an alkyl (rather than a ribosyl) moiety at the 9 position. The induction of interferon as measured indirectly in a bioassay model—the Semliki Forest virus in mice—was used to compare these substituent effects.

Chemistry

Scheme I outlines the synthetic route to 9-alkylguanines **2-12** and the 8,9-dialkyl analogs **29** and **30**. Nucleophilic displacement of the chloro group in 2-amino-4-chloro-6-hydroxypyrimidine[29] (I) with the appropriate amine resulted in the corresponding 4-(alkylamino)-2-amino-6-hydroxypyrimidine (II). This compound was subsequently converted to the 5-nitroso derivative by treatment with sodium nitrite in acetic acid. Reduction of the nitroso group with sodium hydrosulfite and ring closure to the corresponding guanines **2-12** was accomplished in a one pot procedure to yield the target guanines in yields ranging from 48 to 78%.

The synthesis of 8-haloguanines **13-20**, the 8-mercapto analogues **21-23**, and the 8-oxo analogue **24** is shown in Scheme II. Bromination of the appropriate guanines with bromine in acetic acid at 50 °C resulted in the desired 8-bromoguanines. These were isolated as solids from the reaction mixture and recrystallized to analytical purity from methanol. The yields for bromination ranged from 77 to 85% for products **13-19** while **20** was obtained in 58% yield. The 8-chloro-9-*n*-hexyl derivative **17** was prepared by treating compound **6** with HCl in DMF. Treatment of the appropriate 8-bromo derivatives **14-16** with thiourea in ethanol afforded the corresponding 8-mercaptoguanines **21-23** in yields ranging from 68 to 78%. Treatment of 8-bromo-9-*n*-hexylguanine (**16**) with phosphorous pentasulfide in refluxing pyridine resulted in thiation at both the 6 and 8 positions to provide 6,8-dithio-9-*n*-hexylguanine (**28**) in isolated yield of 77%.

Treatment of 9-*n*-hexyl-8-mercaptoguanine (**23**) with allyl bromide in DMF furnished 9-*n*-hexyl-8-(2-propenylthio)guanine (**26**) which was isolated after chromatography in 72% yield. Heating a solution of **26** in DMF under an inert atmosphere for 6 days resulted in a rearrangement to the 7-allyl-9-*n*-hexyl-8-thioxoguanine (**27**) in 80% yield after chromatographic purification. A similar rearrangement reaction has been reported for the synthesis of the ribosyl analog, 7-allyl-8-thioxo guanosine.[30]

Scheme III depicts the synthesis of 9-*n*-hexyl-7-methyl-8-oxoguanine (**25**). The synthesis of this compound was prompted by the observation that the analogous ribosyl compound, 7-methyl-8-oxoguanosine,[31] has been shown to stimulate the humoral immune response and to exhibit antiviral activity.[11] 2,6-Dichloro-7-methylpurin-8-one (**31**)[32] was alkylated with *n*-hexyl iodide in dimethylformamide solution in the presence of sodium hydride, followed by chromatographic isolation, affording 2,6-dichloro-9-*n*-hexyl-7-methylpurin-8-one (**32**) in 88% isolated yield. Selective hydrolysis of this dichloropurine with aqueous sodium hydroxide led to 2-chloro-9-*n*-hexyl-7-methylpurine-6,8-dione (**33**) in 64% yield. Amination of **33** with liquid ammonia at 150 °C provided 9-*n*-hexyl-7-methyl-8-oxoguanine (**25**) in 75% yield.

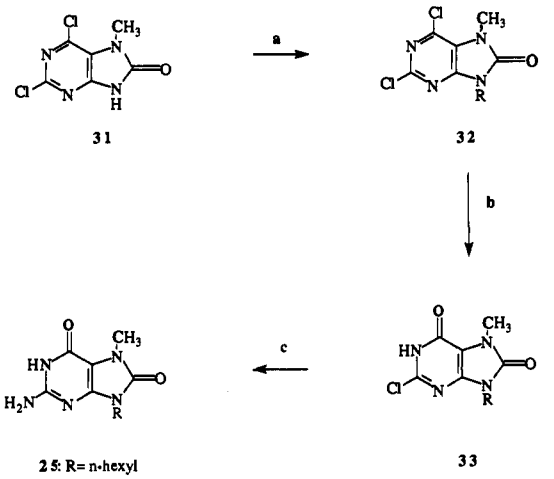
Antiviral Studies. Table I shows the results of the in vivo testing of the compounds using the Semliki Forest virus model in mice. The mice were pretreated intra-

Table I

compound	R	X	mp (°C)	antiviral activity (SFV),[a] survivors/total[b] control[c]	antiviral activity (SFV),[a] survivors/total[b] treated[d]
7-Thia-8-oxoguanosine				0/12	11/12* [e]
2	ethyl	H	>350	1/12	1/12[e]
3	*n*-propyl	H	>350	1/12	2/12
4	*n*-butyl	H	348–349	1/12	10/12[e]
5	*n*-pentyl	H	305–306	1/12	8/12[e]
6	*n*-hexyl	H	284–285	1/12	12/12[e]
7	*n*-heptyl	H	294–295	1/12	9/12[e]
8	*n*-octyl	H	>350	1/12	1/12
9	cyclopentyl	H	>350	1/12	0/12
10	cyclohexyl	H	>350	1/12	0/12
11	6-hydroxyhexyl	H	260–261	0/12	2/12*
12	5-cyanopentyl	H	>320	0/12	5/12* [e]
13	*n*-propyl	Br	269–270	1/12	3/12
14	*n*-butyl	Br	268 dec	1/12	6/12
15	*n*-pentyl	Br	277–278	1/12	8/12
16	*n*-hexyl	Br	269 dec	1/12	4/12
17	*n*-hexyl	Cl	317–318	1/12	7/12* [d]
18	*n*-heptyl	Br	277–278	1/12	1/12
19	*n*-octyl	Br	289–290	1/12	3/12
20	6-hydroxyhexyl	Br	215 dec	0/12	2/12*
21	*n*-butyl	SH	320–322	*f*	
22	*n*-pentyl	SH	314–315	1/12	8/11[e]
23	*n*-hexyl	SH	294–295	2/12	1/12
24	*n*-hexyl	oxo	304–306	3/12	1/12*
25	*n*-hexyl	oxo, 7-methyl	261–263	2/12	11/12[e]
26	*n*-hexyl	*S*-allyl	172–173	1/12	6/12*
27	*n*-hexyl	thioxo, 7-allyl	163–165	2/12	8/12* [e]
28	*n*-hexyl	6,8-dithio	243–245	0/12	0/12*
29	*n*-hexyl	methyl	336–338	3/12	0/12*
30	*n*-hexyl	ethyl	280–281	3/12	3/12*

[a] SFV Semliki Forest virus. [b] In mice that died, death occurred similar to placebo mice that died at 7.4 ± 1.5 days for these experiments. [c] A 2% aqueous sodium bicarbonate solution served as the placebo and as diluent for the compounds. [d] All compounds tested at 200 mg/kg (or 100 mg/kg if noted by *) administered 24 and 18 h before inoculation. Treatment was for 1 day only. [e] Statistically significant ($p < 0.05$), determined by the two-tailed Fisher exact test. [f] Not evaluated.

Scheme III[a]



[a] Key: (a) NaH, DMF, *n*-hexyl iodide; (b) NaOH, Δ; (c) NH_3/MeOH, Δ.

peritoneally in groups of 12 with optimum doses of the test compound and then given a lethal inoculation of virus. The level of protection conferred to the animals by the test compounds against death as the experimental endpoint was thus determined. The results indicate a few interesting trends in the structure–activity relationship. First, for the simple alkylguanines, substitution with an alkyl chain length of between four and seven carbons results in increased activity while the corresponding cyclic alkyls are devoid of activity in this system. Modification of the alkyl chain to include groups such as hydroxyl and cyano in order to enhance water solubility greatly reduces the activity. Second, substitution at the 8 position of the purine ring by a methyl, ethyl, chloro, bromo, oxo, or thioxo group does not enhance the biological activity, and in many cases even reduces it. This observation is in direct contrast to what is observed in the 8-substituted guanosines, in which guanosine itself is devoid of activity, and 8-bromo- and 8-mercaptoguanosine show weak activity in this system.

Interestingly, the activities of the 7,8-disubstituted guanines and guanosines seem to parallel each other. Thus, the 7-methyl-8-oxoguanosine and corresponding guanine derivative (bearing a hexyl group) show comparable antiviral protection in this system. This supports the hypothesis that guanines need not contain an intact carbohydrate moiety in order to exhibit this type of biological activity. It is highly unlikely, therefore, that phosphorylation of any of the guanine nucleosides which possess antiviral activity by virtue of immunepotentiation, such as those we have previously studied, plays any significant role in such demonstrated activity. Moreover, as in the case of the other guanine derivatives and analogues we have studied, these alkyl guanines were devoid of significant in vitro antiviral activity against SFV. This observation suggests that the observed in vivo antiviral activity was primarily due to immune potentiation.

Experimental Section

Chemistry. Melting points were recorded on a Haake–Buchler digital melting point apparatus and are uncorrected. Nuclear magnetic resonance proton spectra were recorded on an IBM NR300AF spectrometer at 300.1 MHz. The chemical shifts are

expressed in values (parts per million) relative to tetramethylsilane as internal standard. Ultraviolet spectra were recorded on a Beckman DU-50 spectrophotometer. Satisfactory proton and D_2O-exchange NMR spectra were obtained for all compounds. Combustion analyses were performed by Robertson Laboratories, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM reagents). E. Merck silica gel (230–400 mesh) was used for flash chromatography.

Virus Infection Models. Swiss Webster female mice (Charles River Labs, Wilmington, MA), weighing about 20 g each at the beginning of the experiment, were administered compounds or placebo. The compounds were dissolved in 2% aqueous solution of sodium bicarbonate and injected intraperitoneally 24 and 18 h before virus inoculation, a regimen optimal for treatment against various RNA viruses.[33] Viruses were pretitrated in the animals to identify doses which were 10 times the LD_{50}, and each experiment was conducted with 10 LD_{50}. Experiments ran for 21 days, at which time the animals were considered cured from the lethal phase of the infections. Statistical evaluations compared drug treated groups to respective placebo controls. Increases in survival numbers were evaluated by the two-tailed Fisher exact test.

9-Alkylguanines 2-12. General Method. A mixture of 2-amino-4-chloro-6-hydroxypyrimidine[25] (I) (0.01 mol), the appropriate amine (0.01 mol), triethylamine (0.03 mol), and 2-ethoxyethanol (50 mL) was refluxed for 6 h (except for cyclopentylamine, where 16 h was required for complete reaction). The solution was then evaporated to half its volume under reduced pressure, poured into 100–200 mL of cold water, and allowed to stand overnight. The resulting precipitate was filtered, washed with water, and dried to yield the crude 4-(alkylamino)-2-amino-6-hydroxypyrimidine which was used for the next step without further purification.

To a mixture of the 4-(alkylamino)-2-amino-6-hydroxypyrimidine (II) (0.01 mol), water (50 mL), and glacial acetic acid (50 mL) was added a solution of sodium nitrite (2 g in 20 mL water) rather rapidly. The temperature of the mixture rose very slightly. The mixture was allowed to stand 2–4 h with occasional stirring, and the red-orange nitroso derivative was filtered and washed with water, ether and dried. It was then placed in a mixture of formamide (30 mL) and 90% formic acid (20 mL) at 70 °C and was completely reduced by the addition of excess sodium hydrosulfite with stirring. The mixture was allowed to reflux for 3–4 h. The hot mixture was poured into 200–300 mL of ice–water with stirring and allowed to stand a few min. The precipitate was filtered, washed with water, dissolved in 100 mL of boiling 1 N hydrochloric acid, treated with charcoal, and filtered. The filtrate was neutralized by addition of 28% ammonium hydroxide, and the precipitate that formed was filtered, washed with acetone, and dried. For further purification, the product was dissolved in 10% boiling potassium hydroxide solution, treated with charcoal, and filtered and the filtrate acidified with glacial acetic acid. The white crystals that formed were filtered hot and washed with water and then with acetone. **General ^{1}H NMR characteristics for compounds 2-12:** (Me_2SO-d_6) 3.87–4.3 (t, 2 H, NCH_2), 6.41–6.48 (s, 2H, NH_2), 7.66–7.78 (s, 1 H, C8-H), 10.56–10.8 (s, 1 H, NH).

9-Alkyl-8-bromoguanines 13-16 and 18-20. General Method. To a stirred mixture of 9-alkylguanine (0.01 mol) in glacial acetic acid (20 mL), heated to 50 °C, was added bromine (0.01 mol), and the reaction mixture was allowed to stand 3–5 h at 50 °C with occasional stirring. The reaction mixture was then poured into 100–200 mL of ice–water with stirring and allowed to stand at room temperature for a few hours. The precipitate was filtered, washed with water and dried to afford a pure compound. For an analytical sample, the bromo compound was recrystallized from methanol. **General ^{1}H NMR characteristics for compounds 13-16 and 18-20:** virtually identical to those for compounds 2-12 with lack of the C8 proton.

9-Alkyl-8-mercaptoguanines 21-23. General Method. A mixture of 9-alkyl-8-bromoguanine (0.01 mol), thiourea (0.02 mol), and ethanol (50 mL) was stirred at reflux for 6 h. After cooling, the mixture was concentrated in vacuo, and the residue was triturated with cold water. The resulting precipitate was filtered off, washed successively with water, and dried to give 8-mercapto-9-alkylguanine. **General ^{1}H NMR characteristics for compounds 21-23:** virtually identical to those for compounds 2-12, with the 7-NH at δ 12.7 and lacking the C8 proton.

8-Chloro-9-*n*-hexylguanine (17). To a stirred solution of 9-*n*-hexylguanine (6, 2.35 g, 10 mmol) in DMF, saturated with HCl gas (24 mL), was added *m*-chloroperbenzoic acid (2.25 g, 13 mmol). After 20 min, an additional amount of peracid (0.5 g, 2.9 mmol) was added. After 2 h, the reaction mixture was evaporated to dryness in vacuo. The residue was triturated with ether (3 × 20 mL), followed by a mixture of H_2O/ether (1:1 v/v) (20 mL). The resulting solid was filtered off, washed with ether, and dried. Recrystallization from methanol afforded 1.9 g (71%) of 17 as a white crystalline solid: mp 317–318 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.79 (t, 3H, CH_3), 1.22 (m, 6H, 3 CH_2), 1.64 (t, 2H, CH_2), 3.9 (t, 2H, NCH_2), 6.64 (s, 2H, NH_2), 10.75 (s, 1H, NH). Anal. ($C_{11}H_{16}ClN_5O$) C, H, N, Cl.

9-*n*-Hexyl-8-oxoguanine (24). A suspension of 8-bromo-9-*n*-hexylguanine (1.0 g, 3.18 mmol), sodium acetate (1.35 g, 16.5 mmol), and acetic anhydride (6.7 mL) in acetic acid (35 mL) was heated at reflux for 15 h. The mixture was evaporated to dryness in vacuo, and the residue was taken up in aqueous sodium hydroxide (0.1 N, 25 mL). The resulting mixture was heated at reflux for 15 min, decolorized with activated charcoal, and filtered. The filtrate was acidified with acetic acid to pH 7 and the resulting precipitate filtered off, washed with water, and dried at elevated temperature to yield 0.5 g (63%) product as white crystals: mp 304–306; ^{1}H NMR (Me_2SO-d_6) δ 0.83 (t, 3H), 1.23 (m, 6H), 1.57 (t, 2H), 3.56 (t, 2H), 6.4 (s, 2H, NH_2), 10.53 (bs, 1H, NH), 10.60 (s, 1H, NH). Anal. ($C_{11}H_{17}N_5O_2 \cdot 0.5\ H_2O$) C, H, N.

9-*n*-Hexyl-7-methyl-8-oxoguanine (25). To a suspension of 2-chloro-9-*n*-hexyl-7-methylpurine-6,8-dione (6.6 g, 23 mmol) in methanol (10 mL), in a steel bomb, was added liquid ammonia (50 mL). The bomb was sealed and heated in an oil bath at 150 °C overnight. The bomb was cooled and opened and the ammonia allowed to evaporate. The residue was dissolved in hot methanol and decolorized with activated charcoal. The charcoal was filtered off and the filtrate cooled to yield 4.6 g (75%) of analytically pure product: ^{1}H NMR (Me_2SO-d_6) δ 0.83 (t, 3H), 1.23 (bs, 6H), 1.58 (m, 2H), 3.6 (t, 2H), (all *n*-hexyl aliphatic protons), 3.32 (s, 3H, NCH_3), 6.5 (s, 2H, NH_2), 10.71 (s, 1H, NH). Anal. ($C_{12}H_{19}N_5O_2$) C, H, N.

9-*n*-Hexyl-8-(2-propenylthio)guanine (26). Allyl bromide (0.6 g, 5 mmol) was added to a mixture of 9-*n*-hexyl-8-mercaptoguanine (1.33 g, 5 mmol) and K_2CO_3 (1.38 g, 10 mmol) in DMF (20 mL). The resulting mixture was stirred at 45 °C for 90 min and then concentrated in vacuo. The residue was extracted with CH_2Cl_2, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. It was purified by flash silica gel chromatography using CH_2Cl_2/acetone (4:1). The fractions containing the homogeneous product were evaporated to dryness to yield 1.1 g (72%) of **26** as pale yellow crystals: mp 172–173 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.8 (t, 3H, CH_3), 1.6 (m, 6H, CH_2), 1.63 (t, 2H, CH_2), 3.82 (m, 4H, 2 CH_2), 5.0 (d, 1H, CH_2), 5.16 (dd, 1H, CH_2), 5.9 (m, 1H, CH), 6.48 (s, 2H, NH_2), 10.57 (s, 1H, NH). Anal. ($C_{14}H_{21}N_5O_5$) C, H, N.

7-Allyl-9-*n*-hexyl-8-thioxoguanine (27). A solution of 9-*n*-hexyl-8-(2-propenylthio)guanine (1 g, 3.24 mmol) in DMF (20 mL) was heated at 130 °C for 6 days under argon atmosphere. The reaction mixture was monitored by TLC and then concentrated in vacuo to leave a pale yellow oil. This was purified by column chromatography on silica gel. Elution with CH_2Cl_2/acetone (4:1, v/v) gave 0.8 g (80% yield) of the product as pale yellow crystals: mp 163–165 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.83 (t, 3H, CH_3), 1.25 (m, 6H, 3 CH_2), 1.67 (t, 2H, CH_2), 4.00 (t, 2H, 9-NCH_2), 4.88 (d, 2H, 7-NCH_2), 4.97 (d, 1H, CH_2), 5.09 (d, 1H, CH_2), 5.9 (m, 1H, CH), 6.7 (2H, NH_2), 10.97 (1H, NH). Anal. ($C_{14}H_{21}N_5O_5$) C, H, N, S.

6,8-Dithioxo-9-*n*-hexylguanine (28). A mixture of 8-bromo-9-*n*-hexylguanine (3.14 g, 0.01 mol), phosphorous pentasulfide (1 g, 0.02 mol), and 25 mL of pyridine was stirred and refluxed for 12 h and then allowed to cool to room temperature. The precipitate was filtered and washed with H_2O and acetone. The crude material was dissolved in 50 mL of boiling, 10% potassium hydroxide solution with charcoal added. After filtration, the filtrate was acidified with glacial acetic acid, and the resulting precipitate was filtered and washed with water. Reprecipitation

resulted in a pale yellow product which was washed with acetone and dried at 100 °C to yield 2.2 g (77.7%) of the title compound: mp 243–245 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.83 (t, 3H, CH_3), 1.25 (m, 6H, 3 CH_2), 1.66 (t, 2H, CH_2), 3.96 (t, 2H, NCH_2), 6.96 (s, 2H, NH_2), 11.98 (s, 1H, NH), 12.82 (s, 1H, NH). Anal. ($C_{11}H_{17}N_5S_2$) C, H, N, S.

9-n-Hexyl-8-methylguanine (29). To a cooled suspension of 2-amino-4-(*n*-hexylamino)-6-hydroxypyrimidine (2.1 g, 10 mmol) in water (50 mL) was added glacial acetic acid (50 mL) with stirring. An aqueous solution of sodium nitrite (2 g in 20 mL H_2O) was added. The reaction mixture was allowed to stand for 3 h with stirring. The red-orange nitroso derivative was filtered, washed with water and ether, and dried. A solution of the product in glacial acetic acid (30 mL), heated to 70 °C, was treated with 10 g of acetamide and was completely reduced by addition of excess sodium hydrosulfite with occasional stirring. The reaction was heated at reflux for 30 min. Hot water (100 mL) and a portion of decolorizing carbon were added, and refluxing was continued for another 30 min. The hot mixture was then filtered and the filtrate allowed to cool. The resulting precipitate was filtered, washed with water, and dried to yield 5-acetamido-2-amino-4-(*n*-hexylamino)-6-hydroxypyrimidine as an almost pure compound which was used in the next step without further purification: ^{1}H NMR (Me_2SO-d_6) δ 0.84 (t, 3H, CH_3), 1.27 (m, 6H, 3 CH_2), 1.4 (t, 2H, CH_2), 1.89 (s, 3H, $COCH_3$), 3.18 (q, 2H, NCH_2), 6.24 (s, 2H, NH_2), 8.19 (s, 1H, NH), 9.99 (s, 1H, NH).

Cyclization. 5-Acetamido-2-amino-4-(*n*-hexylamino)-6-hydroxy pyrimidine (1.34 g, 5 mmol) and anhydrous sodium acetate (8 g) were mixed together and heated at 220 °C for 20 min. The resulting mixture was washed with water three times and the resulting solid filtered off and washed with water. The dry solid was subsequently dissolved in 10% boiling potassium hydroxide solution, treated with Norite and filtered and the filtrate acidified with glacial acetic acid. The resulting precipitate was filtered hot, washed with cold water and ether, and dried at 100 °C under vacuum to yield 0.8 g (64%) of 9-*n*-hexyl-8-methylguanine: mp 336–338 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.83 (t, 3H, CH_3), 1.25 (m, 6H, 3 CH_2), 1.61 (t, 2H, CH_2), 2.32 (s, 3H, $8CH_3$), 3.8 (t, 2H, NCH_2), 6.36 (s, 2H, NH_2), 10.44 (s, 1H, NH). Anal. ($C_{12}H_{19}N_5O$) C, H, N.

8-Ethyl-9-n-hexylguanine (30). 2-amino-4-(*n*-hexylamino)-6-hydroxypyrimidine (2.1 g, 10 mmol) was converted to the nitroso derivative as described for compound **29**. The nitroso derivative was filtered, washed with water and ether, and dried. The dry product was treated with a mixture of propionic acid and propionic acid anhydride (30 g, 1:1) and converted to the title compound in a manner similar to that of compound **29** to yield 0.9 g (68%) of **30** as a pale yellow powder: mp 280–281 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.82 (t, 3H, CH_3), 1.23 (m, 9H, CH_3, 3 CH_2), 2.65 (q, 2H, CH_2), 3.85 (t, 2H, NCH_2), 6.39 (s, 2H, NH_2), 10.48 (s, 1H, NH). Anal. ($C_{13}H_{21}N_5O$) C, H, N.

2,6,-Dichloro-9-n-hexyl-7-methylpurin-8-one (32). A mixture of 2,6-dichloro-7-methylpurin-8-one[32] (8.0 g, 36.5 mmol), *n*-hexyl iodide (5.93 mL, 40.2 mmol), and 60% sodium hydride in oil (1.46 g, 36.5 mmol) in 280 mL of distilled DMF was stirred at room temperature overnight. The solvent was removed in vacuo and the residue redissolved in methanol. Silica gel was added, and the slurry was dried in vacuo. Flash chromatography was performed using hexane/acetone (20:1) as eluent: yield 9.78 g (88.4%); ^{1}H NMR (Me_2SO-d_6) δ 0.84 (t, 3H), 1.27 (bs, 6H), 1.64 (m, 2H) and 3.81 (t, 2H), all *n*-hexyl aliphatic protons, 3.52 (s, 3H, NCH_3).

2-Chloro-9-n-hexyl-7-methylpurine-6,8-dione (33). To a solution of 2,6-dichloro-9-*n*-hexyl-7-methylpurin-8-one (1.53 g, 5.05 mmol) in purified dioxane (30 mL) was added freshly prepared aqueous sodium hydroxide (25.2 mL of a 2 N solution, 50.5 mmol). The mixture was allowed to reflux overnight. After cooling, the suspension was brought to pH 7 with concentrated HCl and filtered. The solids were washed with water, and the filtrate and washings were brought to pH 1–3 with concentrated HCl. The suspension was boiled for 10 min and then allowed to cool slowly. The white solid was filtered, dissolved in methanol, and absorbed onto silica gel. Chromatography was performed using hexane/acetone (3:1) as eluent: yield 0.93 g (65%); ^{1}H NMR (Me_2SO-d_6) δ 0.84 (t, 3H), 1.25 (bs, 6H), 1.62 (m, 2H) and 3.72 (t, 2H), all *n*-hexyl aliphatic protons, 3.41 (s, 3H, NCH_3).

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