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Intramolecular Carboxylate Catalysis in the Depurination of a 7-Methylguanosine Derivative[1]

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Abstract—We have compared the pH-independent rates of glycosidic hydrolysis in a carboxylate-bearing 7-methylguanosine derivative with those of a reference compound and with that of 7-methylguanosine itself. A *syn*-oriented carboxylate group affords catalysis in the hydrolysis reaction, although the instability of 7-alkylguanosines above pH 7 severely limits the useful pH range that could be studied. The effect of the carboxylate near neutral pH can be viewed in three different ways: it provides a 3-fold acceleration as compared to underivatized 7-methylguanosine, an approximately 30-fold acceleration when the decelerating effect of the ketal group is considered, and because of slow decomposition of the reference compound under the reaction conditions, we conclude that the carboxylate provides an acceleration of ≥43-fold as compared to the protio reference compound.

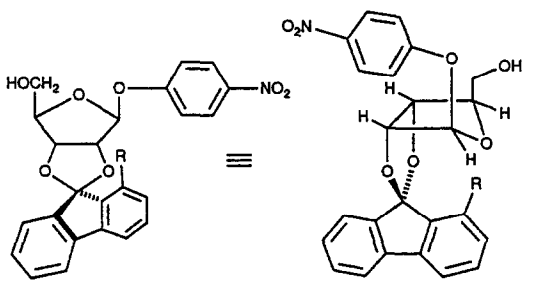
Introduction

The reaction of alkylating agents with DNA typically yields *N*-alkylated purine deoxyribonucleotides with N–glycosidic linkages that are unstable as compared to their nonalkylated counterparts; the result is often cytotoxicity and mutagenesis. DNA glycosylases, such as 7-methylguanosine glycosylase,[3] provide one of the enzymatic pathways through which damaged or misreplicated DNA is repaired via depurination of alkylated nucleotides. While little is known about the detailed chemical mechanism of glycosylase action, analogy to lysozyme suggests the importance of an active site nucleophile at the nucleosidic α-face. Hen egg white lysozyme's Asp-52 provides a carboxylate long perceived as forming an ion pair with an intermediate oxocarbenium ion. However, work by Jencks indicates that the glucosyl cation's lifetime is too short to support argument of its existence in the presence of a strong nucleophile.[4] In 1990, Withers reported the direct observation of a covalent β-glucosidase intermediate using a fluorinated substrate analog.[5] Furthermore, Hardy has demonstrated that site specific replacement of the corresponding aspartate (Asp-20) in T4 lysozyme by cysteine yields a mutant enzyme retaining almost full activity.[6] It appears increasingly likely that the role of aspartate in glycosyl transfer enzymes is that of nucleophile and not of ion pairing anion.[7]

We have previously described a glycosylase model compound based on *p*-nitrophenyl-β-D-ribofuranoside (**1b**) demonstrating carboxylate catalysis of the glycosidic hydrolysis reaction.[8] While synthetically accessible and experimentally convenient, this system was really not an ideal model for an alkylated purine nucleoside because: (1) *p*-nitrophenol might be a much better or poorer leaving group than 7-methylguanine, and (2) the mechanisms of acetal and aminal hydrolysis need not be the same. Of course, there is inherent interest in understanding the reactivity of real biomolecules over that of experimentally convenient surrogates. Consequently, we have prepared fluorenone ketal derivatives of 7-methylguanosine to evaluate carboxylate catalysis in model compounds demonstrating depurination. We now report that a catalytic carboxylate group, such as might be anticipated to occur at the active site of a DNA glycosylase enzyme, provides an acceleration to depurination of ≥43-fold as compared to a protio reference nucleoside.

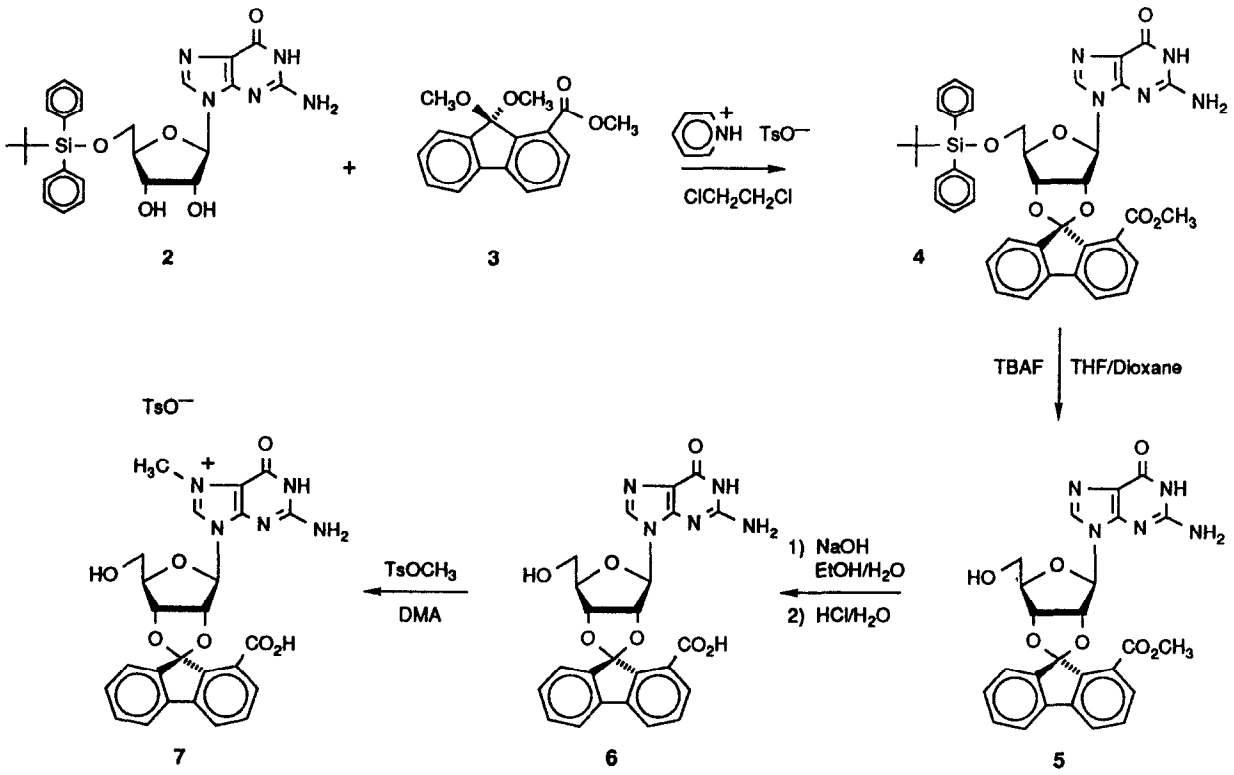
Synthesis

In order to improve on the solubility properties of guanosine and to protect against reaction at its 5'-*O* position, 5'-*O*-*t*-butyldiphenylsilylguanosine[9] (**2**) was used as the starting material for our synthesis (Scheme I). Reaction with an excess of 9,9-dimethoxy-1-methylcarboxyfluorene (**3**),[8] followed by treatment with NH_3 to decompose the imine that forms at N-2, afforded the 2',3'-*O*-guanosine adduct (**4**) in 24% yield after purification by column chromatography. The *endo* stereochemistry of this adduct was confirmed by NOE as done previously for **1a**; this *endo* product is expected for the reasons we have described previously.[8] None of the *exo* product was observed. The silyl protecting group was removed by treatment with tetra-*n*-butylammonium fluoride to give **5** in 75% crude yield, which was suitable for the next step; a sample for analysis was obtained using chromatography. The conversion of **5** to **6** was accomplished in ethanolic base, and acidification from water provided the carboxylic acid as a colorless solid. Acid **6** was methylated by treatment with methyl-*p*-toluenesulfonate in *N*,*N*-dimethyl-

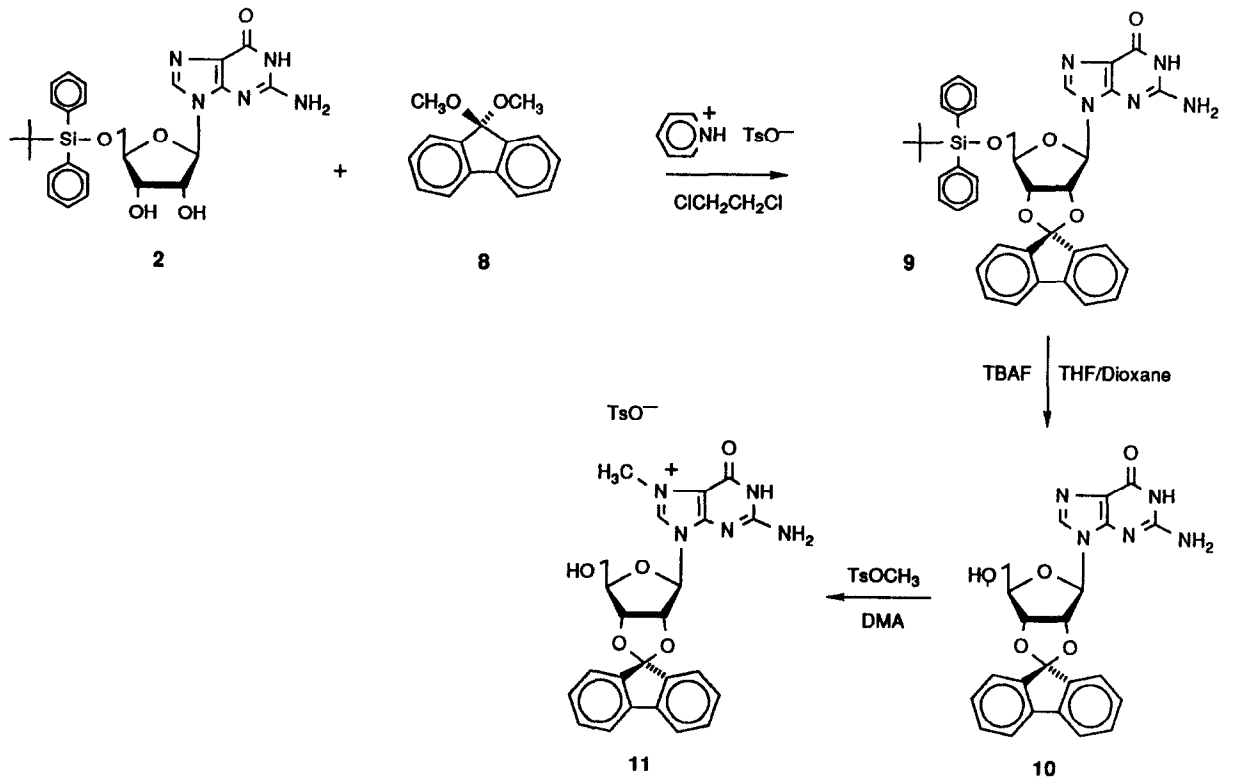


1a, R=CO_2Me
1b, R=CO_2H
1c, R=H

acetamide to afford model compound **7** in 50% yield. The expected methylation at N-7 was confirmed both by the large upfield shift of H-8 in the ^{1}H NMR spectrum and the generation of 7-methylguanine upon glycosidic hydrolysis. Reference compound **11** was synthesized via an analogous synthetic sequence as shown in Scheme II.



Scheme I. Synthesis of the guanosine based glycosylase model compound.



Scheme II. Synthesis of the guanosime based reference compound.

Hydrolysis Kinetics

The hydrolyses of 7-methylguanosine and of **7** were monitored using the UV method of Zoltewicz.[10] Because this method relies on reaction of the nucleic acid base chromophore with base, it was anticipated to be usable with **7** and **11** as with 7-methylguanosine itself. To confirm this, the rate constant at pH 6 was also determined following the formation of 7-methylguanine by HPLC, and the two methods showed acceptable (±10%) agreement. A rate constant of 2.9×10^{-2} min^{-1} was obtained for the hydrolysis of 7-methylguanosine at 100 °C in 0.1 M NaOAc pH 5 buffer, in excellent agreement with the literature value of 2.8×10^{-2} min^{-1}.[10] The glycosidic hydrolysis rates of **7** were evaluated at pH's 3–6, and that of **11** at pH 5; higher pH's were not examined because of the alkylated base's known decomposition under these conditions. The rate constants for these reactions are shown in Table 1.

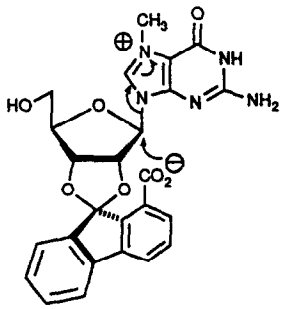
Table 1. Glycosidic hydrolysis rates (100 °C)

Compound	pH	k (min^{-1})	$t_{½}$ (min)
7-MeGuo	5	2.9×10^{-2}	24
7	3	$> 1 \times 10^{-1}$	< 6.3
7	4	1.1×10^{-1}	6.5
7	5	9.9×10^{-2}	7.0
7	6	7.9×10^{-2}	8.7
11	5	$\leq 2.3 \times 10^{-3}$	≥ 300

Because no mechanisms have been deduced for nucleoside glycosylase enzymes, any potential model compounds must be based on a hypothesis that their action will resemble that of lysozyme. While compound **7** is a better model for DNA glycosylase action than is **1b**, the fact that 7-methylguanosine undergoes base-induced imidazole ring cleavage above pH 7 makes it experimentally impossible to collect rate data over the wide range of pH's that would be desirable. However, in the pH region reported (3–6), imidazole ring opening of **7** is avoided as is rapid hydrolysis of the spiro ketal. Between pH 4 and 6, the hydrolysis of 7-methylguanosine is pH independent;[10] within the experimental error of our kinetic method, the same appears true of **7**. Although 1-carboxyfluorenone has a λ_{max} at 260 nm, no peak or significantly increased absorbance was observed at that wavelength under any of the conditions in Table 1, ruling out fluorenone ketal hydrolysis as a precursor to depurination. The kinetic stability of the fluorenone ketal results from the instability of a required cationic hydrolysis intermediate as described previously.[8]

In compound **7**, the same geometry as in **1b** is in place to study the effect of a *syn*-oriented carboxylate on depurination. Hydrolysis of **7** in this pH region is accelerated 3-fold compared to the unmodified reference (7-methylguanosine). This may be extrapolated to a 30-fold acceleration when the decelerating effect of the ketal group is considered.[11] It is worth noting that this rate acceleration is in close agreement with that observed for the hydrolysis of **1b**. These results lend credence to the validity of **1b** as a model compound for this type of study in spite of the fact that a C–O bond is cleaved in **1b** as opposed to C–N bond cleavage in the hydrolysis of **7**.

The hydrolysis of *endo*-carboxylate **7** at pH 5 and 100 °C is ≥43-times faster than that of reference compound **11**. The fact that this value is much less than the 860-fold acceleration observed using model compound **1** is likely to be due to the fact that the decomposition of **11** at pH 5 and 100 °C occurs concurrently with slow depurination. Monitoring the hydrolysis of **11** by HPLC showed decomposition peaks forming over several hours. This decomposition may precede glycosidic hydrolysis, releasing the detected 7-methylguanosine. The rate determined for this reference reaction is therefore necessarily an upper limit. It is thus entirely possible that carboxylate affords acceleration equally well towards *p*-nitrophenol and 7-methylguanine release, but the ratios we can report are different because of different rates of decomposition in the reference reactions. We propose a nucleophilic role for the *endo*-carboxylate as depicted in structure **12**. Because the approach of solvent to the α-side of the anomeric carbon in **11** is encumbered, its hydrolysis is reasonably slower.



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Conclusion

We have compared the pH-independent rates of glycosidic hydrolysis in two fluorenone ketal derivatives of 7-methylguanosine with each other and with that of 7-methylguanosine itself. A *syn*-oriented carboxylate group clearly affords catalysis in the depurination reaction, which has not been observed previously and clearly has potential relevance for the mechanism of DNA glycosylase enzymes. The effect of the carboxylate can be viewed in three different ways: it provides a 3-fold acceleration as compared to underivatized 7-methylguanosine, an approximately 30-fold acceleration when the influence of the ketal group is considered, and a ≥43-fold acceleration as compared to the protio compound. The results are qualitatively similar to those obtained previously for *p*-nitrophenylriboside, although decomposition of the 7-methylguanosine reference compound forces a lower limit for catalysis by the carboxylate group. While one cannot distinguish between nucleophilic and electrostatic stabilization rationales in the present case, nucleophilic participation would be consistent with evidence provided by model and mutant enzyme studies on lysozyme.

Experimental Section

General methods

Melting points were taken on an electrothermal melting point apparatus and are uncorrected. Microanalyses were carried out at Canadian Microanalytical Service, New Westminster, B.C. FT-NMR spectra were obtained at 11.75 T (500 MHz) or 7.0 T (300 MHz). We thank Mr Richard Weisenberger and Dr C. E. Cottrell for their assistance in obtaining high-field NMR spectra at The Ohio State University Instrumentation Center and Mr Carl Engelman for other NMR assistance. UV spectra were obtained on a Hewlett-Packard 8451A Diode Array Spectrophotometer.

endo-*2',3'*-O-*(1-Methoxycarbonyl*-o,o'-*biphenylenemethylidene)-5'*-O-t-*butyldiphenylsilylguanosine (4)*

5'-*O*-(*t*-Butyldiphenylsilyl)guanosine (2.0 g, 3.8 mmol) and pyridinium tosylate (0.40 g, 1.6 mmol) were stirred in freshly dried 1,2-dichloroethane (300 mL) and the suspension was heated to reflux. To this was added 9,9-dimethoxy-1-methylcarboxyfluorene (4.32 g, 15.2 mmol), which immediately caused the heterogeneous mixture to turn orange. The mixture was refluxed for 2 days. It was then cooled and excess 2 N NH_3 in ethanol was added to quench the catalyst and remove fluorenylidene from the N-2 amine group of the guanosine moiety; this treatment caused the solution to turn pale yellow. The solvent was removed by rotary evaporation and the residue was dissolved in chloroform, loaded onto a silica gel column and eluted first with chloroform then with chloroform/methanol (19:1). Evaporation of the appropriate fractions yielded an off-white solid which was isolated (0.58 g, 21%): ^{1}H NMR ($CDCl_3$) d 1.04 (s, 9, *t*-Bu), 3.80–4.05 (m, 5, 5'-H and ester CH_3), 4.72–4.77 (m, 1, 4'-H), 5.53–5.58 (dd, 1, 3'-H), 5.80–5.84 (dd, 1, 2'-H), 6.4–6.41 (d, 1, 1'-H), 7.17–7.87 (m, 18, Ar–H), 12.25 (br s, 1, NH); ^{13}C NMR ($(CD_3)_2SO$) d 18.7 (s), 26.5 (q), 52.5 (q), 64.6 (t), 82.6 (d), 85.4 (d), 86.7 (d), 87.9 (d), 116.9 (s), 117.3 (s), 120.7 (d), 122.9 (d), 123.1 (d), 127.6 (s), 127.7 (d), 127.8 (d), 128.7 (d), 129.2 (d), 129.8 (d), 130.3 (d), 130.9 (s), 131.7 (d), 132.6 (s), 132.9 (s), 134.9 (d), 134.93 (d), 136.1 (s), 136.5 (s), 136.8 (s), 141.1 (s), 143.8 (s), 150.6 (s), 153.6 (s), 156.7 (s), 166.5 (s); FAB mass spectrum, m/e 742 $(M + 1)^+$, 764 $(M + Na)^+$.

endo-*2',3'*-O-*(1-Methoxycarbonyl*-o,o-*biphenylenemethylidene)guanosine (5)*

2',3'-*O*-(1-Methoxycarbonyl-*o,o'*-biphenylenemethylidene)-5'-*O*-*t*-butyldiphenylsilylguanosine (**4**) (1.36 g, 1.8 mmol) was suspended in dioxane (30 mL). Tetrabutylammonium fluoride (3.3 mL of a 1.1 M solution in THF, 3.6 mmol) was added. The mixture stood without stirring for three days, resulting in an amber solution and an oil on the bottom of the flask. The solvent was evaporated under vacuum to an oily residue, which was dissolved in chloroform and eluted from a column of silica gel (1 x 8 in.) with chloroform, then with chloroform/methanol (50:1), and finally with chloroform/methanol (20:1). Product fractions were pooled and evaporated to afford a cream colored residue, which was suspended in dioxane (50 mL) and sonicated to remove TBAF. The suspension was filtered and the filtrate was evaporated to give an ivory colored gel; trituration with ethanol (20 mL) afforded a white precipitate which was collected by vacuum filtration. Rotary evaporation of the filtrate and additional triturations with ethanol gave more solid. All solid samples were pooled to give a total yield of 0.694 g (75%). Analytically pure samples were obtained by elution on preparative TLC (2 mm silica) with chloroform/methanol (9:1): m.p. 280–284 °C with dec: ^{1}H NMR ($(CD_3)_2SO$) d 3.57–3.66 (dt, 2, 5'-H), 3.99 (s, 3, ester CH_3), 4.40–4.62 (td, 1, 4'-H), 5.05–5.09 (t, 1, 5'-OH), 5.54–5.59 (dd, 1, 3'-H), 5.81–5.85 (dd, 1, 2'-H), 6.25–6.26 (d, 1, 1'-H), 6.50 (s, 2, NH_2), 7.33–8.31 (m, 8, Ar–H), 10.68 (br s, 1, NH); ^{13}C NMR ($(CD_3)_2SO$) d 52.6 (q), 61.7 (t), 82.7 (d), 85.6 (d), 86.7 (d), 87.9 (d), 116.7 (s), 117.2 (s), 120.6 (d), 123.0 (d), 128.7 (d), 129.1 (d), 130.3 (d), 130.8 (s), 131.7 (d), 135.9 (d), 136.4 (s), 136.9 (s), 141.1 (s), 143.8 (s), 150.7 (s), 153.7 (s), 156.7 (s), 166.5 (s); FAB mass spectrum, m/e 504 $(M + 1)^+$. Anal. calcd for $C_{25}H_{21}N_5O_7J1.4H_2O$: C, 56.80; H, 4.54; N, 13.25. Found: C, 57.22; H, 4.31; N, 12.83.

endo-*2',3'*-O-*(1-Hydrocarboxy*-o,o-*biphenylenemethylidene)guanosine (6)*

endo-2',3'-*O*-(1-Methoxycarbonyl-*o,o*-biphenylenemethylidene)-guanosine (**5**) (200 mg, 0.4 mmol) was suspended in ethanol (35 mL) and conc. aq. NaOH (1 mL) was added. After 24 h without stirring the solid had completely dissolved. Ethanol was removed by rotary evaporation and the remaining residue was dissolved in H_2O (40 mL). This aqueous solution was acidified to pH 3.20 with 1 M HCl. The resulting white precipitate was collected by vacuum filtration and dried to give **6** (172 mg, 88%): ^{1}H NMR ($(CD_3)_2SO$) d 3.63 (m, 2, 5'-H), 4.50 (m, 1, 4'-H), 5.09 (br s, 1, 5'-OH), 5.57 (dd, 1, 3'-H), 5.72 (dd, 1, 2'-H), 6.34 (d, 1, 1'-H), 6.63 (s, 2, NH_2), 7.3–8.0 (m, 8, Ar–H), 10.78 (br s, 1, NH), 12.5–13.5 (br s, 1, COOH); ^{13}C NMR ($(CD_3)_2SO$) d 61.8 (t), 82.7 (d), 86.2 (d), 86.3 (d), 88.0 (d), 116.7 (s), 117.8 (s), 120.5 (d), 122.9 (d), 123.0 (d), 129.1 (d), 129.3 (d), 130.2 (d), 131.6 (d), 132.0 (s), 135.9 (d), 136.4 (s), 137.6 (s), 141.3 (s), 144.3 (s), 150.7 (s), 153.8 (s), 156.8 (s), 167.1 (s); FAB mass spectrum, m/e 490 $(M + H)^+$, m/e 512 $(M + Na)^+$. High resolution FAB mass spectrum, m/e calculated for $C_{24}H_{20}N_5O_7$ $(M + H)^+$, 490.1363; measured, 490.1364.

endo-*2',3'*-O-*(1-Hydrocarboxy*-o,o-*biphenylenemethylidene)-7-methylguanosine* p-*toluenesulfonate (7)*

endo-Acid **6** (33 mg, 68 μmol) and methyl-*p*-toluenesulfonate (20 mg, 108 μmol) were stirred in dimethylacetamide (1 mL) at 70 °C in an oil bath for 5 h. The yellow solution was added to ether (40 mL) resulting in the formation of a white precipitate that was collected and dissolved in chloroform/methanol (9:1; 30 mL). The solution was evaporated to an oily yellow residue. Addition of H_2O (15 mL) with sonication resulted in a beige solid. This solid was collected by vacuum filtration and washed

with H_2O (30 mL) to give 23 mg (34 μmol, 50%) after drying *in vacuo*: ^{1}H NMR ($(CD_3)_2SO$) d 2.28 (s, 3, Ar–CH_3), 3.74 (d, 2, 5'-H), 4.02 (s, 3, N^7-CH_3), 4.66 (m, 1, 4'-H), 5.57 (dd, 1, 3'-H), 5.75 (dd, 1, 2'-H), 6.43 (d, 1, 1'-H), 7.1 (d, 2, Ts–H), 7.3–8.0 (m, 9, Ar–H), 9.35 (s, 1, 8-H); ^{13}C NMR ($(CD_3)_2SO$) d 20.7 (q), 35.6 (q), 61.6 (t), 82.6 (d), 86.0 (d), 88.4 (d), 90.9 (d), 107.4 (s), 117.6 (s), 120.5 (d), 122.6 (d), 125.4 (d), 127.8 (s). 128.0 (d), 128.9 (u), 129.6 (d), 130.2 (d), 131.5 (s), 136.5 (s), 136.6 (s), 137.1 (s), 137.6 (s), 141.1 (s), 144.1 (s), 148.9 (s), 153.9 (s), 156.2 (s). High resolution FAB mass-spectrum, m/e calculated for $C_{25}H_{22}N_5O_7$ $(M)^+$ for cation, 504.1519; measured, 504.1500.

2',3'-O-(o,o'-Biphenylenemethylidene)-5'-O-t-butyldiphenylsilyl-guanosine (9)

5'-*O*-*t*-Butyldiphenylsilylguanosine (0.15 g, 0.29 mmol) was stirred in 1,2-dichloroethane (50 mL) and heated to reflux. Pyridinium tosylate (0.03 g, 0.12 mmol) and 9,9-dimethoxyfluorene (0.65 g, 2.9 mmol) were added. The solution immediately turned orange. This mixture was refluxed overnight and the solution was cooled. An excess of 2 N NH_3 in ethanol was added and stirred for 30 min. The solution was evaporated and applied to a silica gel column and eluted with chloroform to remove fluorenone, then with $CHCl_3$/methanol (49:1). Evaporation of the appropriate fractions gave the product (0.123 g, 62%). Collection of the less homogeneous fractions and subsequent elution on a preparative TLC plate with $CHCl_3$/methanol (9:1) afforded additional product (total yield 78%): m.p. 290–291 °C dec; ^{1}H NMR ($(CD_3)_2SO$) d 0.95 (s, 9, *t*-Bu), 3.86 (d, 2, 5'-H), 4.55 (m, 1, 4'-H), 5.65 (m, 1, 3'-H), 5.82 (m, 1, 2'-H), 6.27 (s, 1, 1'-H), 6.51 (s, 2, NH_2), 7.2–7.8 (m, 18, Ar–H), 7.92 (s, 1, Ar–H), 10.65 (s, 1, NH); ^{13}C NMR ($(CD_3)_2SO$) d 18.7 (s), 26.5 (q), 64.2 (t), 82.3 (d), 85.1 (d), 87.5 (d), 88.3(d), 116.3 (s), 117.0 (s), 120.3 (d), 120.4 (d), 123.4 (d), 124.5 (d), 127.8 (d), 128.6 (d), 129.8 (d), 130.4 (d), 131.2 (d), 132.5 (s), 132.8 (s), 134.9 (d), 134,92 (d), 136.1 (s), 138.0 (s), 139.6 (s), 140.9 (s), 143.7 (s), 150.5 (s), 153.6 (s), 156.7 (s); FAB mass spectrum, m/e 684 $(M + 1)^+$, 706 $(M + Na)^+$.

2',3'-O-(o,o'-Biphenylenemethylidene)guanosine (10)

2',3'-*O*-(*o,o'*-Biphenylenemethylidene)-5'-*O*-*t*-butyldiphenylsilylguanosine (**9**) (2.22 g, 3.2 mmol) was suspended in dioxane (80 mL). To this was added tetra-*n*-butylammonium fluoride (6.5 mL of a 1.1 M solution in THF) with stirring under argon. The reaction was stirred for two days with formation of a pale yellow oil, which was concentrated by rotary evaporation. The oil was dissolved in chloroform and eluted from a column of silica gel (2 in. x 8 in.) with chloroform. Product-containing fractions were pooled and evaporated to give a cream colored semi-solid, which was sonicated in methanol (40 mL). The resulting cream colored precipitate was collected, washed with methanol and dried to give **10** (1.26 g, 87%): ^{1}H NMR ($(CD_3)_2SO$) d 3.65 (m, 2, 5'-H), 4.45 (m, 1, 4'-H), 5.1 (br s, 1, 5'-OH), 5.6 (dd, 1, 3'-H), 5.8 (dd, 1, 2'-H), 6.25 (d, 1, 1'-H), 6.5 (s, 2, NH_2), 7.2–8.0 (m, 9, Ar–H), 10.7 (br s, 1, NH). High resolution FAB mass spectrum, m/e calculated for $C_{23}H_{20}N_5O_5$ $(M + H)^+$, 446.1464; measured, 446.1462.

2',3'-O-(o,o'-Biphenylenemethylidene)-7-methylguanosine (11)

2',3'-*O*-(*o,o'*-Biphenylenemethylidene)guanosine (**10**) (111 mg, 0.25 mmol) and methyl-*p*-toluenesulfonate (60 mg, 0.3 mmol) were stirred in dimethylacetamide (2 mL) at 70 °C in an oil bath for 5 h. The yellow solution was added to ether (40 mL), resulting in a flocculent white precipitate. The precipitate was collected, yielding a brown solid that was dissolved in chloroform/methanol (9:1; 50 mL) and evaporated to a yellow oil. Addition of H_2O (25 mL) and sonication gave a white solid that was collected by vacuum filtration and washed with H_2O (30 mL) to give **11** (77 mg, 49%) after drying *in vacuo*: ^{1}H NMR ($(CD_3)_2SO$) d 2.27 (2, 3, Ts–CH_3), 3.75 (m, 2, 5'-H), 4.03 (s, 3, N^7–CH_3), 4.71 (m. 1, 4'-H), 5.57 (dd, 1, 3'-H), 5.80 (dd, 1, 2'-H), 7.0–7.9 (m, 14, Ar–H, NH_2), 9.36 (s, 1, 8-H); ^{13}C NMR ($(CD_3)_2SO$) d 20.7 (q), 35.6 (q), 61.2 (t), 83.0 (d), 85.7 (d), 89.3 (d), 92.3 (d), 107.6 (s), 115.8 (s), 120.4 (d), 120.6 (d), 123.4 (d), 124.5 (d), 125.4 (d), 128.0 (d), 128.56 (d), 128.62 (d), 130.5 (d), 131.4 (d), 136.9 (d), 137.7 (s), 138.1 (s), 139.6 (s), 140.7 (s), 143.5 (s), 145.5 (s), 148.9 (s), 153.4 (s), 155.5 (s). High resolution FAB mass spectrum, m/e calculated for $C_{24}H_{22}N_5O_5$ $(M + H)^+$, 460.1621; measured, 460.1611.

Kinetic measurements

The following buffers (0.1 M) were used for all kinetics studies: pH 3–4, sodium phosphate; pH 5, sodium acetate; pH 6, potassium phosphate. The hydrolyses of **7** were monitored as follows. A solution of the appropriate buffer (40 mL) was preheated to 100 °C and the solid guanosine derivative was added. Aliquots were withdrawn at various time intervals and quenched in an equal volume of 1 M NaOH in an ice bath. The increase in absorbance at 284 nm was used to monitor the reaction. HPLC was carried out on an IBM LC/9533 system using an IBM C-18 reversed phase column and a 201L injection loop. Elution was carried out with continuous UV detection of the eluant at 254 nm. The HPLC solvent system used (5% methanol in 0.1 M ammonium dihydrogen phosphate [no adjustment to pH made], flow rate 2.0 mL/min) gives: sodium fluorenone-1-carboxylate (4.1 min), **7** (12.68 min), 7-methylguanosine iodide (5.6 min), and 7-methylguanine (6.8 min). Kinetic runs monitored by HPLC in all cases displayed ≥95% of the 7-methylguanine predicted, based on peak integrations. The determination of first order rate constants was accomplished using the computer program ENZFITTER, available from Elsevier-Biosoft, 68 Hills Road, Cambridge, CB2 1LA, U.K.

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References and Notes

1. Taken in part from the PhD Thesis of Scott A. Van Arman, The Ohio State University, 1990.

2. Now on the faculty of the Department of Chemistry, Franklin & Marshall College.

3. For an overview, see: Pegg, A. E.; Bennett, R. A. In *Enzymes of Nucleic Acid Synthesis and Modification,* Vol. I, pp. 180–200; Jacob, S. T., Ed.; CRC Press, 1983.

4. See: Banait, N. S.; Jencks, W. P. *J. Am. Chem. Soc.* **1991**, *113*, 7951; and references therein.

5. Street, I. P.; Withers, S. G. *J. Am. Chem. Soc.* **1988**, *110*, 8551.

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