Chemistry of the Bleomycin-Induced Alkali-Labile DNA Lesion

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Abstract: Treatment of B-form DNA with the antitumor antibiotic bleomycin in the presence of Fe^{2+} and O_2 affords both DNA strand scission and the formation of alkali-labile lesions, the proportion of which is quite sensitive to the concentration of O_2 present. The alkali-labile lesions can undergo fragmentation cleanly in the presence of *n*-butylamine to afford DNA fragments containing 5′- and 3′-phosphate termini at the site of the alkali-labile lesion. The mechanism of decomposition of the alkali-labile lesion was studied, leading to identification of a putative intermediate that is converted readily to an (oligo)nucleotide 3′-phosphate in the presence of *n*-$BuNH_2$, as well as the identification of the byproduct of the fragmentation reaction containing the carbon atoms originally present within the alkali-labile lesion.

The bleomycin group antitumor antibiotics have now been the focus of intensive inquiry for more than three decades.[1] Aside from their clinical utility in the treatment of neoplasms,[2] this attention reflects an interest in the mechanism of their antitumor action, which involves the degradation of DNA[1,3,4] and possibly also of RNA.[5]

Bleomycin-mediated DNA degradation requires a metal ion cofactor and oxygen.[1,3,6] DNA damage has been shown to occur in a sequence-selective fashion; sites modified with the greatest efficiency involve a subset of the 5′-GC-3′ and 5′-GT-3′ sequences in the DNA substrate.[1e,7] At the level of chemical mechanism, DNA degradation is initiated by the abstraction of a H atom from the C4′ position of deoxyribose. The formed deoxyribose radical can react with dioxygen; subsequent sugar fragmentation of the resulting peroxy intermediate affords DNA strand scission as the predominant pathway for DNA degradation under ambient conditions.[1d,e,f,8]

A second DNA degradation pathway, which can predominate under conditions of low oxygen tension, involves the formation of an alkali-labile lesion. Structurally, the alkali-labile lesion is a 4′-hydroxyapurinic acid (**1**) and is known to be formed with the concomitant release of 1 equiv of nucleobase.[9] Mechanistically, these products can be envisioned as forming from the collapse of a C-4′ hydroxylated DNA intermediate (Scheme 1). A likely source of this hydroxylated DNA intermediate involves

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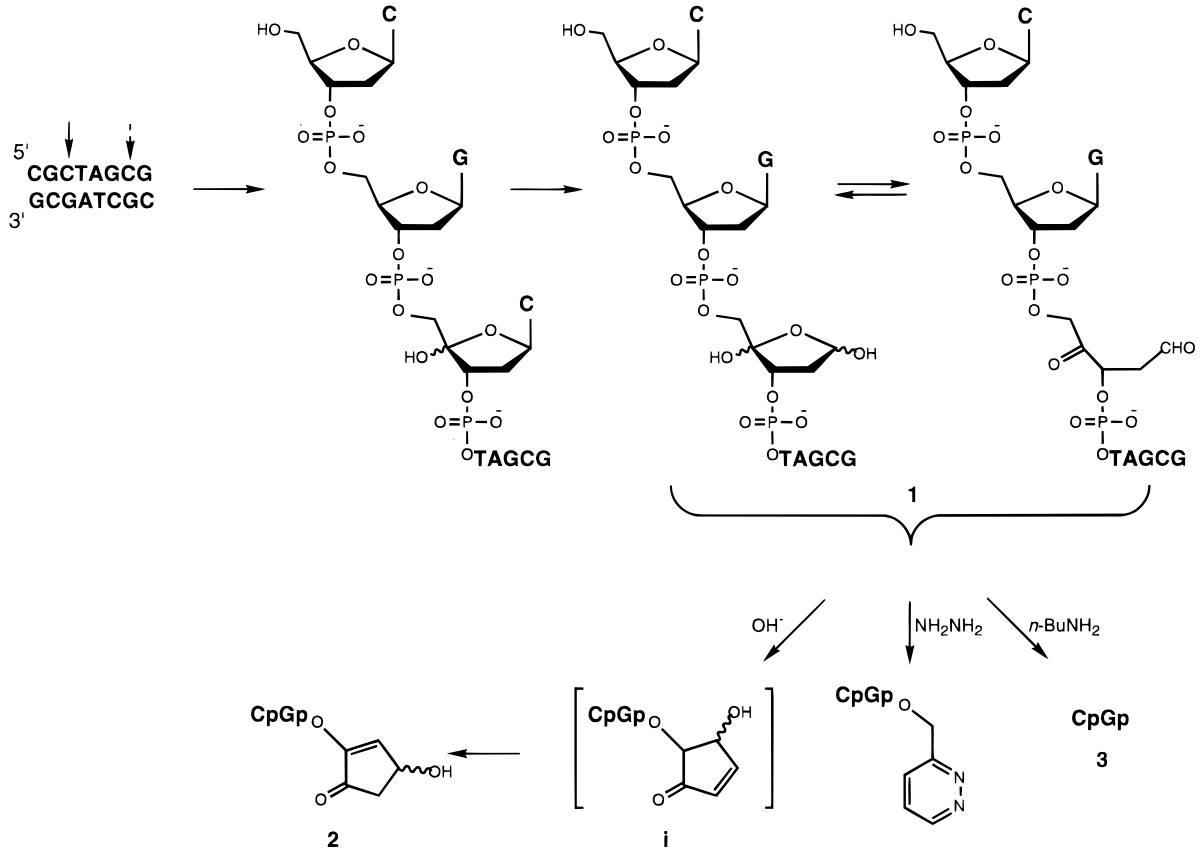
Scheme 1. Chemistry of the Bleomycin-Induced Alkali-Labile Lesion



one-electron oxidation of the deoxyribose radical intermediate formed via the agency of Fe•BLM; reaction of H_2O with the derived carbocation would afford the requisite hydroxylated intermediate.[9i]

While formation of the alkali-labile lesion does not lead directly to DNA strand scission, treatment of this lesion with any of a few different reagents does produce DNA fragments. As shown in Scheme 1, reagents that have been employed for this purpose include alkali, hydrazine, and *n*-butylamine; these afforded fragments having 4-hydroxycyclopentenones (**2**), pyridazines, and phosphates (**3**), respectively, at the 3′-termini produced at the sites of the breaks.[5e,9g]

The appearance of phosphate groups at the 3′-termini of BLM-induced DNA fragments is particularly noteworthy. While they are essentially the exclusive products formed in the presence of alkylamines,[9g] they are also observed routinely on sequencing gels following incubation of BLM-treated DNA with alkali or other bases. Because DNA fragments terminating with phosphate groups can be manipulated further, e.g. via the agency of phosphatases, kinases, and ligases, conversion of BLM-induced alkali-labile lesions to fragments terminating exclusively with phosphates potentially provides the wherewithal to exploit BLM as a reagent for the manipulation of nucleic acid structure. A limitation in realizing this goal is the dearth of information concerning the mechanism(s) by which fragments having 3′-phosphate termini are formed.

Presently, we analyze the process by which the DNA alkali-labile lesion is converted to fragments containing phosphate termini in the presence of *n*-butylamine. Identified for the first time is the byproduct of the fragmentation reaction containing the carbon atoms originally present within the alkali-labile lesion, as well as a putative intermediate in the fragmentation reaction whose treatment with *n*-$BuNH_2$ leads to (oligo)-nucleotide 3′-phosphate with great facility.

Results

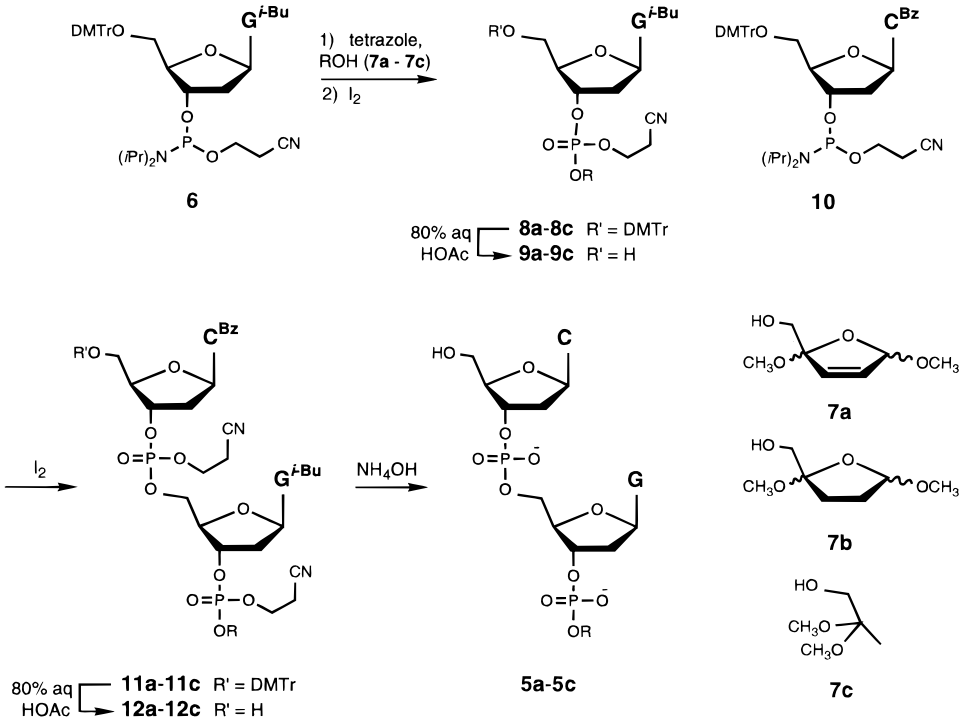
Synthesis of Dinucleotide Derivatives 5a−c. Methyl ketal derivatives (**5a**−**c**) of three dinucleotide analogues required for mechanistic analysis (**4a**−**c**) were prepared as shown in Scheme 2 by modification of the methods described for the known dinucleotide **5a**.[9g] As shown in Scheme 2, **5a** was prepared in a stepwise fashion starting with the condensation of fully protected deoxyguanosine phosphoramidite **6** with 2,5-dihydro-2,5-dimethoxyfurfuryl alcohol (**7a**); following I_2 oxidation, nucleoside phosphate triester **8a** was obtained in 92% yield. Intermediate **8a** was detritylated to afford **9a** and then condensed with protected deoxycytidine phosphosphoramidite **10**, affording fully protected dinucleotide derivative **11a** in 94% overall yield from **8a**. Stepwise deprotection afforded **12a** and then **5a** in 87% overall yield from **8a**.

Dinucleotide derivative **5b** was prepared analogously, starting from 2,5-dimethoxytetrahydrofurfuryl alcohol (**7b**). Nucleotide phosphate triester **8b** was obtained in 89% yield and converted to fully protected dinucleotide derivative **11b** in 72% overall yield. Stepwise deprotection then provided **5b** in 71% yield. Dinucleotide derivative **5c** was also accessible in the same fashion starting from 2,2-dimethoxypropanol (**7c**), the latter of which was readily obtained by treatment of methyl 2,2-dimethoxypropionate with $LiAlH_4$. All of the intermediates and final products were characterized fully, including by ^1H NMR spectroscopy and high-resolution mass spectrometry.

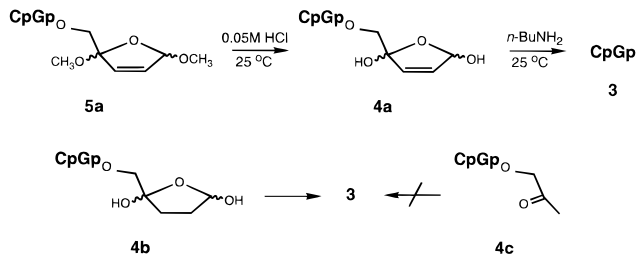
Conversion of dinucleotide derivatives **5a**−**c** to key intermediates **4a**−**c**, respectively, was carried out by treatment with aqueous HCl, as described below.

Liberation of CpGp from Dinucleotide Derivative 4a. Dinucleotide derivative **4a** was prepared by acid hydrolysis of **5a** (0.05 M HCl, 25 °C) (Scheme 3). The hydrolysis was complete within 1 h, as verified by C_{18} reverse-phase HPLC

Scheme 2. Routes Employed for the Synthesis of **5a**−**c**



Scheme 3. Conversion of Dinucleotides **4** to CpGp



analysis (Figure 1). As anticipated, the diastereomers of **5a** were separable by HPLC, while those of **4a** were not due to rapid equilibration. The aqueous solution containing **4a** was neutralized with dilute NaOH solution; HPLC analysis of the neutralized solution verified lack of decomposition. Treatment of **4a** with an excess of *n*-butylamine (**4a**, 5×10^{-5} M; *n*-$BuNH_2$, 0.2 M) effected conversion to CpGp within 10 min at 25 °C (Figure 2a; Scheme 3). The use of a smaller excess of *n*-$BuNH_2$ (**4a**, 5×10^{-4} M; *n*-$BuNH_2$, 0.02 M) also led to the clean conversion of **4a** → CpGp, although only after 3 h at 25 °C.

In comparison, treatment of 5×10^{-5} M **4a** with 0.2 M diisopropylamine at 25 °C effected only partial conversion to CpGp within 10 min (Figure 2b) along with a number of other products, although ∼60% conversion to CpGp was observed after 6 h (data not shown). Treatment of **4a** (5×10^{-5} M) with 0.2 M NaOH solution at 25 °C afforded several products, but none was identical with CpGp (Figure 2c).

Treatment of Dinucleotide Derivatives 2, 4b, and 4c with *n*-Butylamine. The surprising facility of the conversion **4a** → CpGp in the presence of *n*-$BuNH_2$ prompted the comparative study of **4b** and **4c** to help define those structural elements in **4a** that promoted the release of CpGp. In common with **4a** (Scheme 4), compound **4b** can exist as a 1,4-dicarbonyl species or in a ring-closed hydrated form, while **4c** contains only a single carbonyl functionality analogous to C-4 in compounds **4a** and **4b**.

Treatment of **4b** with 0.2 M *n*-$BuNH_2$ at 25 °C was found to effect conversion to CpGp, as noted above for **4a** (Figure 3). However, a more carefully controlled comparison of the two transformations revealed that release of CpGp from **4a** was more

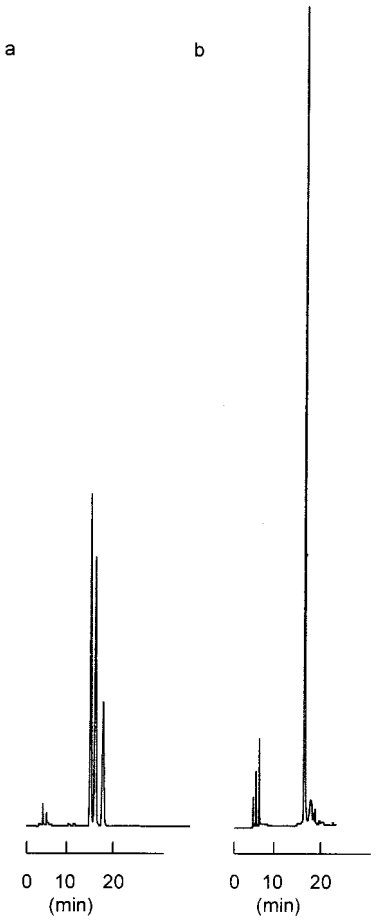


Figure 1. Preparation of **4a** by acid treatment of **5a**: (a) **5a** (retention times 15.5, 16.7, and 18.6 min) and (b) **4a** (retention time 17.5 min).

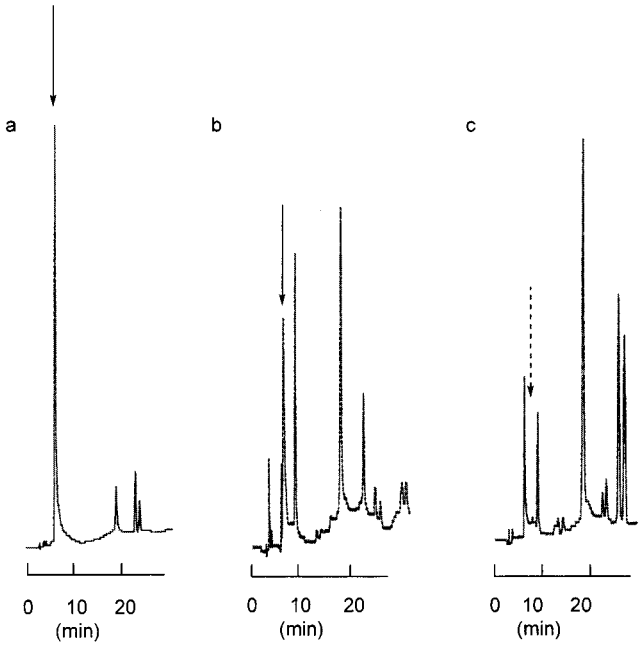


Figure 2. Analysis of the products formed from **4a** by treatment with (a) 0.2 M *n*-butylamine (room temperature, 10 min). The product that eluted at 5.8 min (arrow) comigrated with CpGp. The remaining **4a** eluted at 18.3 min; the products eluted at 22.0 and 22.8 min were not characterized. Analysis of the products formed from **4a** by treatment with (b) 0.2 M diisopropylamine (room temperature, 10 min). The product that eluted at 6 min (arrow) comigrated with CpGp. Analysis of the products formed from **4a** by treatment with (c) 0.2 M NaOH (room temperature, 10 min). The formation of CpGp was not observed.

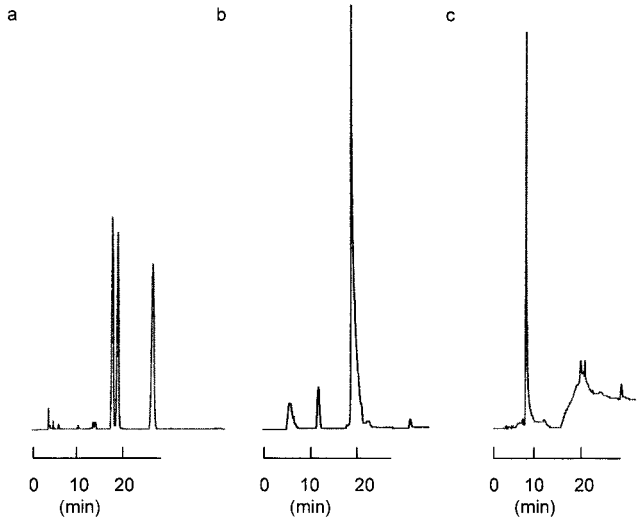
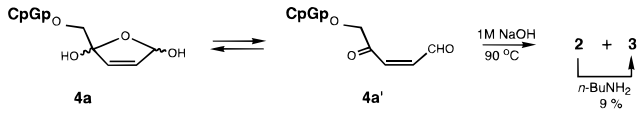


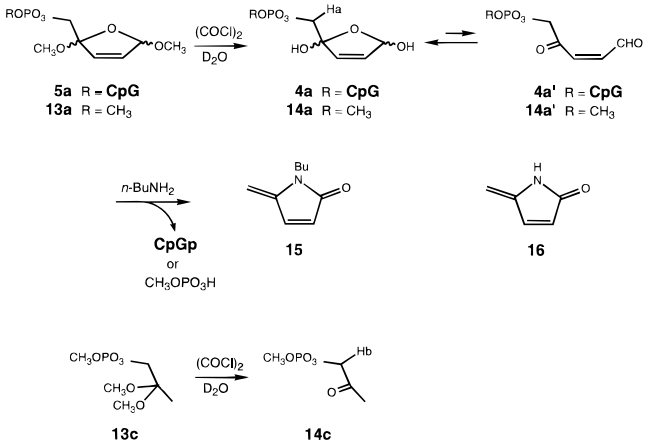
Figure 3. Preparation of **4b** and its conversion to CpGp via the agency of *n*-$BuNH_2$ (0.2 M, room temperature, 10 min): (a) **5b** (retention times 17.4, 18.5, and 25.7 min); (b) **4b** (retention time 18.6 min); and (c) CpGp (retention time 7 min).

Scheme 4. Conversion of Dinucleotide **4a** to **2** and **3**



facile than release of the dinucleotide from **4b**. Specifically, it was shown that conversion of **4a** (5×10^{-4} M) to CpGp within 3 h at 25 °C could be achieved in the presence of 30 equiv of *n*-$BuNH_2$, while 60 equiv of *n*-$BuNH_2$ were required to achieve the analogous conversion of **4b** to CpGp within 3 h. In contrast,

Scheme 5. Conversion of **4a** and **14a** to Lactam **15**



treatment of **4c** with 0.2 M *n*-$BuNH_2$ failed to effect the release of CpGp, even if the reaction mixture was heated at 90 °C. Thus the 1,4-dicarbonyl system clearly facilitated the release of CpGp, as did the additional presence of the double bond in **4a**. It may be noted that CpGp formation from **4c** would, in principle, be possible via an S_N2 displacement mechanism in which CpGp was the leaving group. The lack of formation of CpGp from **4c** under conditions that resulted in the formation of CpGp from **4a** and **4b** (Scheme 3) indicated that direct nucleophilic displacement from **4c** (and by extension from **4a** and **4b**) is not a viable route to CpGp.

As outlined in Scheme 4, it has been reported[9g] that treatment of **4a** with NaOH (1 M, 90 °C) afforded CpGp as a minor product in addition to **2**, the latter of which is thought to form via putative intermediate **i** (cf. Scheme 1). It seemed conceivable that the formation of CpGp from **4a** in the presence of *n*-$BuNH_2$ could proceed via **i** or **2**. Heating of a sample of **2** with 0.2 M *n*-$BuNH_2$ at 90 °C in a sealed tube afforded CpGp in only low (9%) yield (Scheme 4), effectively excluding this pathway as a significant source of CpGp.

Analysis of the Structure and Chemistry of 4a. To further secure the structure of **4a**, and ensure that its formation from **5a** was complete, the transformation **5a** → **4a** (Scheme 3) was studied using ^{1}H NMR spectroscopy. A solution of **5a** in D_2O was treated with $(COCl)_2$, producing DCl + $(COOD)_2$ in situ. The hydrolysis of **5a** → **4a** was confirmed by the disappearance of the signals corresponding to the methoxyl groups in **5a** (3.03−3.09 and 3.31−3.38 pm, which appeared as multiplets due to the presence of diastereomers). The formation of a peak corresponding to CH_3OD was observed.

To define the position of equilibrium between the dicarbonyl and hydrated ring closed forms of **4a** (Scheme 4), the ^{1}H NMR spectrum was simplified by preparing analogue **14a** in which the dinucleotide moiety was replaced by a methyl group. Also prepared was **14c**, the methyl analogue of dinucleotide **4c** (Scheme 5). These species were generated in situ by hydrolysis of the respective methyl ketals, the latter of which were prepared synthetically in analogy with the syntheses of **4a** and **4c** (Supporting Information, Scheme 1).

When treated with D_2O + $(COCl)_2$, the ^{1}H NMR spectrum of **13a** showed the disappearance of the signals corresponding to the methoxyl groups on the dihydrofuran ring (at 3.08, 3.14, 3.36,and 3.43 ppm) and the appearance of a signal corresponding to CH_3OD. The methylene H's on the C attached to the phosphate group (Ha in Scheme 5) appeared as a multiplet at 3.55−3.70 ppm, whereas the methylene H's of **14c** (Hb in Scheme 5) were more deshielded (4.39 ppm, d, $J = 8$ Hz). This

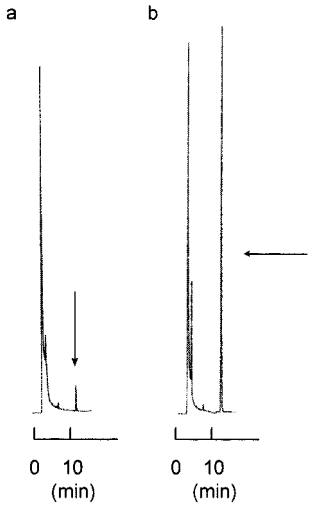
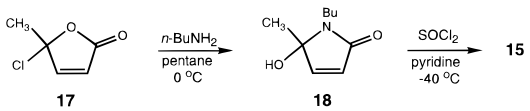


Figure 4. Analysis of the products formed from **4a** by treatment with (a) 0.2 M *n*-$BuNH_2$ and (b) 0.2 M *n*-$BuNH_2$ (adjusted to pH 5.3 with AcOH). The product that eluted at 11 min (arrows) comigrated with authentic lactam **15**.

Scheme 6. Synthesis of Lactam **15**



suggested that **14a** exists predominantly in the cyclic 2,5-dihydro-2,5-dihydroxyfuran form in aqueous solution; **4a** seems likely to exist in the same form as well.

The conversion of **4a** to products in the presence of *n*-butylamine was also studied by ^{1}H NMR spectroscopy. Accordingly, compound **5a** was dissolved in D_2O and treated with oxalyl chloride and the reaction mixture was extracted with $CDCl_3$ after 5 min of treatment with *n*-$BuNH_2$. The ^{1}H NMR spectrum of the D_2O layer reflected the formation of CpGp, the presence of which could be verified by direct comparison with the spectrum of authentic CpGp. Characterization of the $CDCl_3$ layer by ^{1}H NMR spectroscopy suggested the presence of lactam **15**; resonances were present at δ 4.82 (d, 1H, $J = 1$ Hz), 4.89 (d, 1H, $J = 1$ Hz), 6.18 (dd, 1H, $J = 6$, 1 Hz), and 6.94 (d, 1H, $J = 6$ Hz). Putative lactam **15** was also prepared in 19% yield by successive treatments of **13a** with 0.1 M HCl (25 °C, 1 h) and excess *n*-$BuNH_2$ (Scheme 5). The spectral data for **15** were entirely consistent with those of azaprotoanemonin **16** and its derivatives.[10]

The structure of **15** was further confirmed by synthesis in analogy with the reported synthesis of **16** (Scheme 6).[10] Thus treatment of chlorolactone **17** with *n*-butylamine afforded *N*-*n*-butyllactam **18**. Dehydration of **18** by treatment with $SOCl_2$ (pyridine, −40 °C) afforded **15**. This sample was shown by HPLC analysis to have the same chromatographic properties as putative **15** derived from **4a** (Figure 4a). While the conversion was carried out at a low concentration of reactants, the yield of **15** from **4a** in this experiment was nonetheless estimated to be only about 10%.

pH Dependency of the Formation of Lactam 15. A plausible mechanism for the formation of CpGp from **4a** is outlined in Scheme 7. This scheme envisions the initial formation of a cyclic amine (**ii**) that can dehydrate via an iminium species to afford α-hydroxypyrrole intermediate **iii**. The loss of CpGp from the latter intermediate would afford **15**. This

(10) Scheffold, R.; Dubs, P. *Helv. Chim. Acta* **1967**, *50*, 798.

Scheme 7. Plausible Mechanism for the Conversion of **4a** to **15** + CpGp

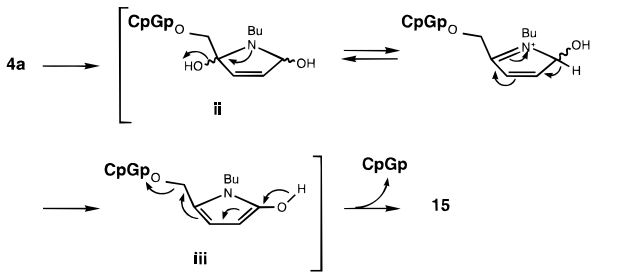
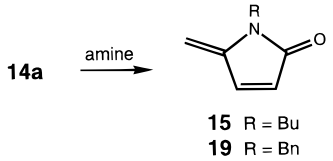


Table 1. pH-Dependent Formation of Lactams **15** and **19** from Key Intermediate **14a**



	lactam yield (%)		
amine	pH 5.3[a]	pH 7.0[a]	pH 12[b]
n-$BuNH_2$	73	82	18
$BnNH_2$	76	75	16

[a] Three equivalents of amine utilized. [b] Ten equivalents of amine utilized.

mechanism accommodates a number of experimental observations, including the more facile formation of CpGp from **4a** than **4b** and the lack of CpGp formation from **4c**. The mechanism is also consistent with the propensity of **4a** to exist in cyclic form, arguing by extension that intermediate **ii** may form with facility, and with the observation that CpGp formation was promoted more efficiently by a primary amine (*n*-$BuNH_2$) than by a secondary amine (*N*,*N*-diisopropylamine) and poorly if at all by NaOH (Figure 2).

While the foregoing mechanism appeared entirely plausible as a pathway for the production of **15** from **4a** (**14a**), the low yields of **15** seemed incompatible with the efficient formation of CpGp. A key control experiment was run to see whether CpGp could be formed under conditions that did not produce **15**. Specifically, treatment of 2,5-dihydroxy-2,5-dihydrofurfuryl alcohol (derived from **7a** by treatment with 0.1 M HCl) with amines under conditions optimal for the conversion **4a** → **15** (vide infra) did not afford **15**. This argued that CpGp did not arise from **4a** by release of 2,5-dihydroxy-2,5-dihydrofurfuryl alcohol, the latter of which then formed **15**. Because imine formation is known to be pH dependent and generally more favorable under somewhat acidic conditions,[11] the pH dependence of lactam formation was studied. As shown in Table 1, treatment of a solution of **14a** (prepared in situ from **13a** by acid hydrolysis, followed by neutralization of the solution) with 10 equiv of *n*-$BuNH_2$ afforded lactam **15** in 18% yield, i.e., quite similar to the observation made in Scheme 5. In addition to the possible pH dependency of lactam formation discussed above, it was noted that lactam **15** appeared to be unstable under the reaction conditions employed for its formation. In contrast, treatment of **14a** with 3 equiv of *n*-$BuNH_2$ at pH 5.3 afforded **15** in 73% yield; the transformation also proceeded smoothly

(11) (a) Palm, V. A.; Haldna, U. L.; Talvik, A. J. In *The Chemistry of the Carbonyl Groups: Basicity of Carbonyl Compounds*; Patai, S., Ed.; John Wiley and Sons: New York, 1966; p 421ff. (b) Dayagi, S.; Degani, Y. In *The Chemistry of the Carbon−Nitrogen Double Bond: Methods of Formation of the Carbon−Nitrogen Double Bond*; Patai, S., Ed.; John Wiley and Sons: New York, 1970; p 61ff.

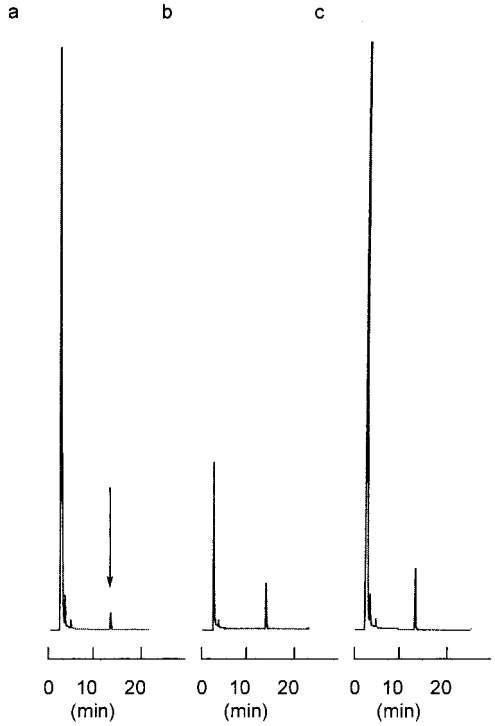


Figure 5. (a) Analysis of the products formed from d(CGCTAGCG) by successive treatments with activated Fe·BLM and *n*-butylamine (adjusted to pH 7 with AcOH). The peak at retention time 13.7 min (arrow) comigrated with lactam **15**. (b) Analysis of the products formed from dinucleotide **4a** by successive treatments with acid and *n*-butylamine (adjusted to pH 7 with AcOH). (c) Co-injection of a and b.

at pH 7.0 giving **15** in 82% yield. The use of benzylamine rather than *n*-butylamine afforded quite similar results; lactam **19** was formed to the extent of 76 and 75% at pH values of 5.3 and 7.0, respectively, but a poor yield of **19** was realized at pH 12.

The conversion of **4a** to CpGp with concomitant formation of **15** as a consequence of treatment with *n*-$BuNH_2$ at pH 5.3 and 7 was confirmed by HPLC analysis of the reaction mixtures. Although it took longer to complete the reactions (**4a**, 5×10^{-5} M; 0.2 M *n*-$BuNH_2$ at pH 5.3; 6 h), CpGp was formed in essentially quantitative yield. A high yield of **15** (~80%) was also estimated from the peak area (Figure 4b). Treatment of **4a** with AcOH solution (pH 5.3) in the absence of *n*-$BuNH_2$ did not promote any reaction of **4a** even after several hours.

Formation of Lactam 15 from the Fe·BLM-Induced Alkali-Labile DNA Lesion. The foregoing results provided convincing evidence that CpGp and **15** were formed efficiently and in comparable amounts when **4a** was treated with *n*-$BuNH_2$. Because **4a** is a putative intermediate on the pathway leading from the BLM-induced alkali-labile lesion in DNA to the formation of oligonucleotide-linked hydroxycyclopentenones[9e,g] (i.e., related to **2**), it seemed reasonable to anticipate that the alkali-labile lesion itself would afford **15** if treated with *n*-$BuNH_2$ under optimal conditions. To establish this point experimentally, the self-complementary dodecanucleotide d(CGCTAGCG) was treated with activated Fe·BLM under conditions known[9] to result in the efficient formation of the alkali-labile lesion. The DNA oligonucleotide containing the formed alkali-labile lesion was treated with 100 equiv of *n*-$BuNH_2$ at pH 7.0. As shown in Figure 5, analysis of this reaction mixture by C_{18} reverse-phase HPLC resulted in the appearance of a peak that comigrated with authentic **15**. The formation of **15** may logically be concluded to have arisen by the pathway(s) outlined in Scheme 7.

Discussion

The degradation of DNA by Fe^{II}·BLM results in the formation of two sets of products.[1,8,9] Frank strand scission is dependent on the presence of O_2 for reaction with an initially formed deoxyribose radical; this pathway leads to the formation of oligonucleotides containing a glycolate moiety appended at the 3′-end, with concomitant formation of an equal amount of a base propenal.[1,8] A second set of products, formed in slightly lesser amounts under ambient conditions but as the major product at lower oxygen tension, involves the formation of an alkali-labile lesion (Scheme 1) and a free base.[1,9] Both the base propenal and alkali-labile lesion are electrophilic, and could well react with nucleophiles (such as proteins) present under physiological conditions. In fact, the possible contribution of the base propenal to the cytoxic effects noted following administration of BLM to cells has already been suggested.[12]

It has also been noted previously that treatment of 5′-^{32}P-end-labeled DNA with Fe^{II}·BLM yields two products; the major product has been shown to contain a phosphoroglycolate moiety at its 3′-terminus, while the other has been identified as containing a phosphate group at the 3′-end. While the mechanism of formation of this phosphate group has never been elucidated, Sugiyama et al.[9g] discovered that treatment of the alkali-labile lesion with *n*-$BuNH_2$ effected its efficient conversion to a species terminating with a 3′-phosphate. Aside from its intrinsic interest from the perspective of understanding the chemical mechanism by which BLM mediates its damage of DNA, the reactivity of the alkali-labile lesion may have broader implications at the levels of the mechanisms of induction of cytotoxicity by BLM and the strategy employed for cellular repair of BLM-induced lesions. Because the experimental manipulation of DNA involves the use of oligonucleotide phosphate termini, the transformation of BLM-induced alkali-labile lesions to DNA fragments terminating with phosphate groups is of potential utility for the manipulation of BLM-treated DNA and could well find extension to the study of lesions created by other classes of DNA-damaging agents.

To elucidate the reaction mechanism of amine-induced degradation of the alkali-labile lesion, the reaction of the **4a** with amines was studied since **4a** might reasonably be expected to form in the base (amine)-promoted degradation of the alkali-labile lesion.

Conversion of **4a** to CpGp upon treatment with *n*-$BuNH_2$ proceeded under milder conditions than reported (Scheme 3).[9g] Amines produce hydroxide anion in aqueous solution and, therefore, the amine could work either directly or as a source of base in this reaction. While treatment of **4a** with *n*-butylamine effected its facile conversion to CpGp, **4a** was not converted to CpGp by treatment with NaOH solution (25 °C, 10 min). This implied that the amine itself reacted with **4a** to give CpGp. Three plausible mechanisms can be proposed to account for the production of CpGp from **4a**. These include the conversion of a nucleotide 3′-hydroxycyclopentenone intermediate (**2**) to CpGp, a possibility that can be excluded based on our finding that authentic **2** did not afford CpGp in good yield under conditions that effected the facile conversion of **4a** → CpGp (Figure 2a). A second possibility, namely that CpGp was formed by nucleophilic displacement on C-5′ of the 2,5-dihydroxy-2,5-dihydrofurfuryl moiety in **4a**, was excluded by the finding that **4c** did not afford CpGp upon treatment with *n*-butylamine. In

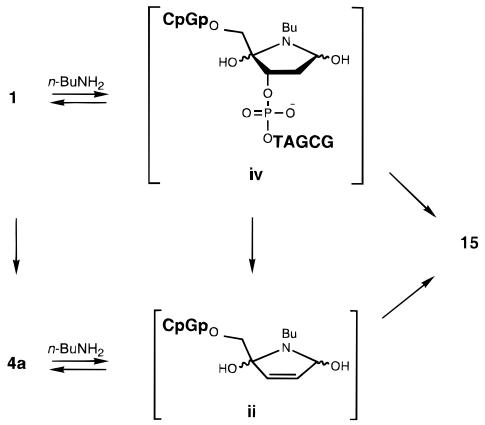
(12) (a) Johnson, F.; Pillai, K. M. R.; Grollman, A. P.; Tseng, L.; Takeshita, M. *J. Med. Chem.* **1984**, *27*, 954. (b) Grollman, A. P.; Takeshita, M.; Pillai, K. M. R.; Johnson, F. *Cancer Res.* **1985**, *45*, 1127. (c) Grollman, A. P. *Dev. Oncol.* **1988**, *53*, 79.

contrast, the available evidence is fully consistent with the production of CpGp from **4a** via intermediates **ii** and **iii** as outlined in Scheme 7.

More detailed evidence for the pathway(s) leading from **4a** → CpGp was obtained from a careful examination of structurally related dinucleotide derivatives **4a**−**c**. These species displayed different reactivities upon treatment with 0.2 M *n*-$BuNH_2$. The conversion of **4b** to CpGp proceeded to about the same extent as that of **4a** at room temperature but **4a** afforded CpGp somewhat more readily than **4b** with a smaller amount of added *n*-butylamine. On the other hand, the treatment of **4c** with 0.2 M *n*-$BuNH_2$ did not result in its conversion to CpGp at all (Scheme 3). This result indicated that the substituent at the 3′-terminus of **4c** did not function as a good leaving group via an S_N2 mechanism. Therefore, it is improbable that conversion of **4a** and **4b** to CpGp proceeded by a simple S_N2 mechanism unless the substituent at the 3′-terminus was first converted into a good leaving group. Comparison of the reactivities of **4a**, **4b**, and **4c** indicated that the reaction of *n*-$BuNH_2$ with 1,4-dicarbonyl groups (as in **4a′** and **4b′**, which exist in equilibrium) might be involved in CpGp formation. However, it was not possible to propose a specific reaction mechanism without the identification of the actual product derived from the 2,5-dihydroxy-2,5-dihydrofurfuryl moiety of **4a**. By using ^{1}H NMR spectroscopy, lactam **15** was shown to be the product formed from **4a** in parallel with CpGp (Schemes 5 and 7). The ^{1}H NMR study also gave information concerning the structures of the 2,5-dihydroxy-2,5-dihydrofurfuryl moiety of **14a** and **4a**. In acidic aqueous solution, compound **14a** exists in the cyclic rather than open form (such as **14a′**) since the chemical shift of the methylene protons (Ha in Scheme 5) was more shielded than those of **14c** (Hb), the latter of which presumably exists only in the keto form. Thus, the 2,5-dihydroxy-2,5-dihydrofurfuryl moiety of **4a** presumably also exists in the cyclic form. This finding demonstrated the ability of this 1,4-dicarbonyl system to form a five-membered ring. The structure of the product was established as that of **15** by ^{1}H NMR spectroscopy and by its preparation from **4a**. Treatment of **4a** with *n*-$BuNH_2$ afforded the lactam **15** along with CpGp. The reaction mechanism of lactam formation from **4a** can reasonably be envisioned as shown in Scheme 7. An elimination reaction similar to that posited in Scheme 7 was observed in the oxidation of furfuryl acetate (bromine and potassium acetate in acetic acid and acetic anhydride) to give protoanemonin.[13] The yield of **15** increased significantly when the reaction was carried out at lower pH (Figure 4). As suggested from the cyclic structure of **4a** in acidic solution, a cyclic amine such as putative intermediate **ii** (Scheme 7) might be expected to form more easily at lower pH.[11] Thus, it was possible to demonstrate that CpGp is liberated concomitantly with the formation of **15** and in good yield under neutral and acidic conditions as shown in Scheme 7.[14]

As anticipated, *n*-$BuNH_2$ treatment of the alkali-labile lesion (**1**) induced in d(CGCTAGCG) by activated Fe·BLM[5d,e,15] resulted in the formation of lactam **15**. The alkali-labile lesion might be expected to form cyclic amine **iv** under the reaction conditions (Scheme 8), which could collapse to afford CpGp and **15** directly, or else following initial elimination of pentanucleotide pTAGCG to give intermediate **ii**. Obviously, the initial elimination of the same pentanucleotide directly from the alkali-labile lesion could afford **4a**, whose conversion to CpGp and **15** has been documented in the foregoing experiments.

Scheme 8. Formation of **15** from the Bleomycin-Induced Alkali-Labile Lesion



The exceptional facility with which the BLM-derived alkali-labile lesion undergoes transformation to cleavage products and lactams of type **15** under physiological conditions argues that this transformation may actually obtain in biological systems. This facet of the chemistry of bleomycin seems worthy of further exploration.

The chemical transformations described herein may also find utility in manipulating DNA structure to permit, for example, study of the (bio)chemical events by which DNA lesions induced by bleomycins and other DNA damaging agents are repaired.[16,17]

Experimental Section

General Methods. Melting points were taken on a micro melting point apparatus (Yanaco, Kyoto) and are uncorrected. ^{1}H NMR spectra were obtained on a General Electric QE−300 spectrometer operating at 300 MHz or on a JEOL GX-270 (270 MHz) spectrometer. The chemical shifts are referenced to $CHCl_3$ at 7.26 ppm or HOD at 4.78 ppm. UV spectra were recorded on a Perkin-Elmer Lambda Array 3840 UV/vis spectrophotometer. IR spectra were taken on a Mattson Cygnus 100 infrared spectrophotometer or a Jasco IR A-100 infrared spectrometer. Chromatographic separations were carried out using silica gel columns (Mallinckrodt (60−200 mesh) or E. M. Merck Kieselgel 60 (70−230 mesh or 230−400 mesh)). For thin-layer chromatography, Whatman silica gel TLC plates (250 μm, fluorescent at 254 nm) or E. M. Merck TLC plates (Kieselgel 60 F_{254}, 0.2 mm) were used. HPLC analysis was carried out on a reverse-phase column (Cosmosil, Nacalai Tesque, Kyoto, 250 × 4.6 mm), and monitored by A_{260} at a flow rate of 1.0 mL/min. 5′-*O*-(4,4′-Dimethoxytrityl)-*N*-isobutyryl-2′-deoxyguanyl-3′-[2-cyanoethyl-(*N*,*N*-diisopropyl)]phosphoramidite and *N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-3′-[2-cyanoethyl-(*N*,*N*-diisopropyl)]phosphoramidite were purchased from Cruachem Inc. or Glen Research.

General Procedure for Treatment of 4a with Amines. A solution containing 1.22 mg (1.50 μmol) of **5a** in 0.25 mL of H_2O was treated with 0.25 mL of 0.1 M HCl at room temperature. The reaction mixture was analyzed on a reverse-phase HPLC column; elution was with 0.2

(13) D'Alelio, G. F.; Williams, C. J.; Wilson, C. L. *J. Org. Chem.* **1960**, *25*, 1025.

(14) The low yield of lactam observed under basic conditions was found to be due to its instability under the reaction conditions. Amine treatment of authentic **15** resulted in considerable decomposition and **15** was recovered only in 14% yield. This indicated that lactam formation under basic conditions may also proceed via the mechanism shown in Scheme 7 although the yield of **15** was low.

(15) Van Atta, R. B.; Long, E. C.; Hecht, S. M. *J. Am. Chem. Soc.* **1989**, *111*, 2722.

(16) (a) Miller, M. R.; Chinault, D. N. *J. Biol. Chem.* **1982**, *257*, 10204. (b) Seki, S.; Oda, T. *Carcinogenesis* **1986**, *7*, 77. (c) Seki, S.; Oda, T. *Carcinogensis* **1988**, *9*, 2239. (d) DiGiuseppe, J. A.; Dresler, S. L. *Biochemistry* **1989**, *28*, 9515. (e) Park, I.-S.; Koh, H. Y.; Park, J. K.; Park, S. D. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 1226. (f) Zhang, B.; Seki, S.; Ikeda, S. *Int. J. Biochem.* **1982**, *23*, 703.

(17) (a) Matsumoto, Y.; Kim, K.; Katz, D. S.; Feng, J.-A. *Biochemistry* **1998**, *37*, 6456. (b) Feng, J.-A.; Crasto, C. J.; Matsumoto, Y. *Biochemistry* **1998**, *37*, 9605. (c) Prasad, R.; Beard, W. A.; Strauss, P. R.; Wilson, S. H. *J. Biol. Chem.* **1998**, *273*, 15263.

N ammonium formate containing 6% CH_3CN. After 60 min, HPLC analysis of the reaction mixture showed the disappearance of **5a** (retention times 15.5, 16.7, and 18.6 min, Figure 1a) and the appearance of a peak corresponding to **4a** at a retention time of 17.5 min. The acidic solution (0.49 mL) was neutralized with dilute NaOH solution and diluted to 2 mL. A 0.4-mL aliquot (0.29 μmol) was treated with 5 mL of a 0.2 N aqueous solution of the appropriate amine (*n*-$BuNH_2$, $(i\text{-Pr})_2NH$, or NaOH). After 10 min, a 100-μL aliquot was treated with 100 μL of 0.2 N HCl and analyzed on a C_{18} reverse-phase HPLC column; elution was with 0.2 N ammonium formate containing 3% CH_3CN. Upon treatment with *n*-$BuNH_2$, the peak of **4a** (retention time 17.5 min) disappeared (Figure 1b) and a peak corresponding to CpGp appeared (retention time 5.8 min) (Figure 2a).

Treatment of 2 with *n*-Butylamine. A mixture of **2**[9e,g] (2 mg, 2.7 μmol) and 10 mL of 0.2 M *n*-$BuNH_2$ was heated at 90 °C in a pressure bottle. The reaction mixture was analyzed on a C_{18} reverse-phase HPLC column; elution was with 0.2 N ammonium formate containing 1% CH_3CN. After 6 h, **2** (retention times 13.2 and 15.2 min, due to the presence of diastereomers) was consumed and a peak appeared (retention time 2.7 min) corresponding to CpGp. The reaction mixture was neutralized with 1 N formic acid and lyophilized. The residue was purified by reverse-phase MPLC (LiChroprep RP-8 (40−63 μm)); elution was with 0.2 N ammonium formate to give CpGp·2 *n*-$BuNH_2$ as a colorless powder: yield 0.5 mg (9.4%); ^{1}H NMR (D_2O) δ 0.40 (t, 6H, J = 7.5 Hz), 1.36 (m, 4H), 1.61 (m, 4H), 2.32 (ddd, 1H, J = 14, 6, 2 Hz), 2.64 (ddd, 1H, J = 14, 6, 3 Hz), 2.85 (m, 1H), 2.97 (t, 4H, J = 7.5 Hz), 3.62 (m, 2H), 4.03−4.09 (m, 3H), 4.34 (m, 1H), 4.57 (m, 1H), 4.97 (m, 1H), 5.94 (d, 1H, J = 7.5 Hz), 6.08 (dd, 1H, J = 8, 6 Hz), 6.23 (t, 1H, J = 7 Hz), 7.56 (d, 1H, J = 7.5 Hz), and 8.05 (s, 1H).

Treatment of 4b with *n*-Butylamine. A solution containing 1.38 mg (1.70 μmol) of **5b** in 0.25 mL of H_2O was treated with 0.25 mL of 0.02 N HCl at room temperature. The reaction mixture was analyzed on a C_{18} reverse-phase HPLC column; elution was with 0.2 N ammonium formate containing 6% CH_3CN. After 60 min, HPLC analysis of the reaction mixture showed the disappearance of **5b** (retention times 17.4, 18.5, and 25.7 min, Figure 3a) and the appearance of a peak corresponding to **4b**. The acidic solution (0.49 mL) was neutralized with NaOH solution and diluted to 2 mL. A 0.4-mL aliquot (0.33 μmol) was treated with 5 mL of a 0.2 N aqueous solution of *n*-$BuNH_2$. After 10 min, a 100-μL aliquot was treated with 100 μL of 0.2 N HCl and analyzed on a C_{18} reverse-phase HPLC column; elution was with 0.2 N ammonium formate containing 2% CH_3CN, then with a linear gradient of 2→5% CH_3CN over a period of 5 min, followed by 0.2 N ammonium formate containing 5% CH_3CN. Upon treatment with *n*-$BuNH_2$, the peak of **4b** at retention time 18.6 min disappeared (Figure 3b) and a peak corresponding to CpGp appeared (retention time 7 min) (Figure 3c). The yield of CpGp was estimated to be ∼70% from the peak area.

Treatment of 4c with *n*-Butylamine. A solution containing 1.22 mg (1.65 μmol) of **5c** in 0.25 mL of H_2O was treated with 0.25 mL of 0.1 N HCl at room temperature. The reaction mixture was analyzed on a C_{18} reverse-phase HPLC column; elution was with 0.2 N ammonium formate containing 7% CH_3CN. After 60 min, HPLC analysis showed the disappearance of **5c** (retention time 8.2 min) and the appearance of a peak corresponding to **4c** at a retention time of 3.8 min. The acidic solution (0.49 mL) was neutralized with NaOH solution and diluted to 2 mL. A 0.4-mL aliquot (0.32 μmol) was treated with 5 mL of 0.2 N aqueous *n*-$BuNH_2$. After 10 min, a 100-μL aliquot was treated with 100 μL of 0.2 N HCl and analyzed on a C_{18} reverse-phase HPLC column; elution was with 0.2 N ammonium formate containing 4% CH_3CN. Upon treatment with *n*-$BuNH_2$, **4c** did not afford a peak corresponding to CpGp.

2,5-Dimethoxy-2,5-dihydrofurfuryl Cyanoethyl 5′-*O*-(Dimethoxytrityl)-*N*-isobutyryl-2′-deoxy-3′-guanylate (8a). 2,5-Dimethoxy-2,5-dihydrofurfuryl alcohol (**7a**)[18] (77 mg, 0.48 mmol) and 84 mg (1.2 mmol) of tetrazole were placed in a round-bottom flask equipped with a magnetic stir bar, coevaporated twice with portions of toluene, and then treated with 2 mL of dry CH_3CN and 84 mg of 4 Å molecular sieves. A solution containing 250 mg (300 μmol) of 5′-*O*-(4,4′-dimethoxytrityl)-*N*-isobutyryl-2′-deoxyguanyl-3′-[2-cyanoethyl-(*N*, *N*-diisopropyl)]phosphoramidite (**6**) in 1 mL of dry CH_3CN was added dropwise at 0 °C over a period of 5 min. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 1 h. The reaction mixture was concentrated under diminished pressure and the residue was dissolved in 2 mL of a solution containing 0.3 g of I_2 in 13:1:0.2 THF−water−pyridine. The reaction mixture was stirred at room temperature for 20 min and then concentrated under diminished pressure. The residue was dissolved in 3 mL of $CHCl_3$ and washed with 5 mL of 1% aqueous sodium bisulfite and the aqueous layer was back-extracted with $CHCl_3$. The combined organic extract was washed with brine, dried over $MgSO_4$, and then concentrated. The residue was purified on a 10-g silica gel column; elution with $CHCl_3$ and then with 20:1 $CHCl_3$−MeOH gave **8a** as a colorless solid: yield 250 mg (92%); silica gel TLC R_f 0.32 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.00−1.09 (m, 6H), 2.30 (m, 1H), 2.64 (m, 1H), 2.75 (m, 2H), 2.90 (m, 1H), 3.15 (m, 3H), 3.24−3.39 (m, 2H), 3.43 (m, 3H), 3.72 (m, 6H), 3.92−4.30 (m, 5H), 5.35 (m, 1H), 5.46 (m, 1H), 5.89 (m, 1H), 6.06−6.17 (m, 2H), 6.73−6.75 (m, 4H), 7.12−7.28 (m, 7H), 7.33−7.35 (m, 2H), 7.74−7.75 (m, 1H), and 9.10 (s, 1H); mass spectrum (FAB), *m*/*z* 915.3301 $(M + H)^+$ ($C_{45}H_{52}N_6O_{13}P$ requires 915.3330).

2,5-Dimethoxy-2,3,4,5-tetrahydrofurfuryl Cyanoethyl 5′-*O*-(Dimethoxytrityl)-*N*-isobutyryl-2′-deoxy-3′-guanylate (8b). To 200 mg of 10% palladium-on-carbon (activated by stirring under a H_2 atmosphere for 30 min in 2 mL of MeOH) was added 1.0 g (6.2 mmol) of **7a**. The reaction mixture was stirred at room temperature for 2 h under a hydrogen atmosphere. The reaction mixture was filtered and the filtrate was concentrated under diminished pressure to give 2,5-dimethoxy-2,3,4,5-tetrahydrofurfuryl alcohol (**7b**)[19] as a colorless liquid: yield 894 mg (88%); ^{1}H NMR ($CDCl_3$) δ 1.72 (br s, 1H), 1.86−2.22 (m, 4H), 3.20−3.60 (m, 8H), and 4.79 (m, 1H); mass spectrum (FAB), *m*/*z* 161.0844 $(M - H)^+$ ($C_7H_{13}O_4$ requires 161.0814). In analogy with the synthesis of **8a**, 78 mg (0.48 mmol) of **7b** was treated with 250 mg (300 μmol) of 5′-*O*-(4,4′-dimethoxytrityl)-*N*-isobutyryl-2′-deoxyguanyl-3′-[2-cyanoethyl-(*N*, *N*-diisopropyl)]phosphoramidite (**6**) in the presence of 84 mg (1.20 mmol) of tetrazole. After oxidation with I_2, **8b** was obtained as a colorless solid: yield 245 mg (89%); silica gel TLC R_f 0.37 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.00−1.10 (m, 6H), 1.88−2.10 (m, 4H), 2.24 (m, 1H), 2.67 (m, 1H), 2.77 (m, 2H), 2.98 (m, 1H), 3.18−3.37 (m, 8H), 3.74 (s, 6H), 3.88−4.27 (m, 4H), 4.32 (m, 1H), 4.99 and 5.08 (m, 1H), 5.40 (m, 1H), 6.16 (t, 1H, J = 6 Hz), 6.70−6.80 (m, 4H), 7.20−7.30 (m, 7H), 7.31−7.40 (m, 2H), 7.73−7.74 (m, 1H), and 8.91 (br, 1H); mass spectrum, *m*/*z* 917.3521 $(M + H)^+$ ($C_{45}H_{54}N_6O_{13}P$ requires 917.3486).

2,2-Dimethoxypropyl Cyanoethyl 5′-*O*-(Dimethoxytrityl)-*N*-isobutyryl-2′-deoxy-3′-guanylate (8c). Methyl 2,2-dimethoxypropionate (880 mg, 5.94 mmol) was added to a suspension of 1.13 g (29.7 mmol) of lithium aluminum hydride in 20 mL of dry ether at 0 °C; the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was treated dropwise with 1.1 mL of H_2O, 1.1 mL of 15% NaOH solution, and 3.4 mL of H_2O and the reaction mixture was stirred vigorously for 20 min. The reaction mixture was filtered through Celite and the Celite was washed thoroughly with ether. The combined ether layer was dried over Na_2SO_4 and concentrated to give **7c** as a colorless liquid: yield 550 mg (77%); ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3H), 1.72 (br, 1H), 3.25 (s, 6H), and 3.54 (d, 2H, J = 5 Hz); mass spectrum (electron impact), *m*/*z* 89 $((M - OMe)^+$, 100%); mass spectrum (electron impact), *m*/*z* 89.0625 ($C_4H_9O_2$ requires 89.0603).

In analogy with the synthesis of **8a**, 58 mg (0.48 mmol) of **7c** was treated with 250 mg (300 μmol) of 5′-*O*-(4,4′-dimethoxytrityl)-*N*-isobutyryl-2′-deoxyguanyl-3′-[2-cyanoethyl-(*N*, *N*-diisopropyl)]phosphoramidite (**6**) in the presence of 84 mg (1.20 mmol) of tetrazole. After oxidation with I_2, **8c** was obtained as a colorless solid: yield 247 mg (94%); silica gel TLC R_f 0.49 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 0.99−1.12 (m, 6H), 1.30−1.32 (m, 3H), 2.16 (m, 1H), 2.66 (m, 1H), 2.77 (m, 2H), 2.85 (m, 1H), 3.20 (m, 6H), 3.38−3.43 (m, 2H), 3.77 (s, 6H), 3.90−4.03 (m, 2H), 4.15−4.27 (m, 2H), 4.32 (m, 1H), 5.38−5.49 (m, 1H), 6.15 (m, 1H), 6.75−6.78 (m, 4H), 7.19−

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7.29 (m, 7H), 7.38−7.40 (m, 2H), 7.74−7.76 (s, 1H), and 8.44−8.48 (s, 1H); mass spectrum (FAB), *m*/*z* 875.3397 $(M + H)^+$ ($C_{43}H_{52}N_6O_{12}P$ requires 875.3381).

2,5-Dimethoxy-2,5-dihydrofurfuryl Cyanoethyl *N*-Isobutyryl-2′-deoxy-3′-guanylate (9a). A sample containing 664 mg (0.73 mmol) of **8a** was dissolved in 20 mL of 80% aqueous acetic acid and stirred at room temperature for 2 h. After concentration under diminished pressure, the residue was coevaporated twice with portions of EtOH and purified on a 20-g silica gel column; elution with $CHCl_3$ and then with 10:1 $CHCl_3$−MeOH gave **8a** as a colorless solid: yield 450 mg (99%); silica gel TLC R_f 0.11 (10:1 $CHCl_3$−MeOH);^{1}H NMR ($CDCl_3$) δ 1.25 (dd, 6H, $J = 7$, 2 Hz), 2.65−2.77 (m, 2H), 2.84 (m, 2H), 2.95 (m, 1H), 3.22 (m, 3H), 3.50 (m, 3H), 3.81 (m, 1H), 3.94 (m, 1H), 4.00−4.09 (m, 1H), 4.16−4.25 (m, 1H), 4.29−4.37 (m, 3H), 5.33 (m, 1H), 5.53 and 5.78 (s, 1H), 5.98 (d, 1H, $J = 6$ Hz), 6.18 (d, 1H, $J = 6$ Hz), 6.19 (m, 1H), 7.93 (s, 1H), 9.38 (br s, 1H), and 9.62 (br s, 1H); mass spectrum (FAB), *m*/*z* 613.2040 $(M + H)^+$ ($C_{24}H_{34}N_6O_{11}P$ requires 613.2023).

2,5-Dimethoxy-2,3,4,5-tetrahydrofurfuryl Cyanoethyl *N*-Isobutyryl-2′-deoxy-3′-guanylate (9b). A sample containing 132 mg (0.14 mmol) of compound **8b** was dissolved in 10 mL of 80% aqueous acetic acid and stirred at room temperature for 2 h. After concentration under diminished pressure, the residue was coevaporated twice with portions of MeOH and purified on a 5-g silica gel column; elution with $CHCl_3$ and then with 10:1 $CHCl_3$−MeOH gave **9b** as a colorless solid: yield 81 mg (92%); silica gel TLC R_f 0.12 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.24−1.25 (m, 6H), 1.92−2.17 (m, 4H), 2.70 (m, 2H), 2.84 (m, 2H), 2.97 (m, 1H), 3.26−3.41 (m, 6H), 3.79−4.20 (m, 4H), 4.14−4.40 (m, 3H), 5.05−5.12 (m, 1H), 5.34 (m, 1H), 6.20 (m, 1H), 7.90 (s, 1H), 9.35 (br s, 1H), and 9.59 (br s, 1H); mass spectrum (FAB), *m*/*z* 615.2147 $(M + H)^+$ ($C_{24}H_{36}N_6O_{11}P$ requires 615.2180).

2,2-Dimethoxypropionyl Cyanoethyl *N*-Isobutyryl-2′-deoxy-3′-guanylate (9c). Compound **8c** (247 mg, 0.28 mmol) was dissolved in 15 mL of 0.5% trifluoroacetic acid in $CHCl_3$ and stirred at 0 °C for 6 h. After concentration under diminished pressure, the residue was coevaporated twice with portions of $CHCl_3$ and purified on a 20-g silica gel column; elution with $CHCl_3$ and then with 10:1 $CHCl_3$−MeOH gave **9c** as a colorless solid: yield 151 mg (93%); silica gel TLC R_f 0.36 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.23 (dd, 6H, $J = 7$, 3 Hz), 1.35 (s, 3H), 2.67−2.77 (m, 2H), 2.84 (m, 2H), 2.98 (m, 1H), 3.22 (m, 6H), 3.80−3.95 (m, 2H), 4.01 (m, 2H), 4.31 (m, 3H), 4.86 (m, 1H), 5.32 (m, 1H), 6.19 (m, 1H), 7.92 (m, 1H), 9.36 (br s, 1H), and 9.61 (br s, 1H); mass spectrum (FAB) *m*/*z* 573.2054 $(M + H)^+$ ($C_{22}H_{34}N_6O_{10}P$ requires 573.2074).

2,5-Dimethoxy-2,5-dihydrofurfuryl Cyanoethyl *N*-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxy-*P*-cyanoethylcytidylyl-(3′→5′)-*N*-isobutyryl-2′-deoxy-3′-guanylate (11a). Compound **9a** (179 mg, 0.29 mmol) and 61 mg (0.87 mmol) of tetrazole were added to a round-bottom flask equipped with a magnetic stir bar, coevaporated twice with portions of toluene, and then suspended in 2 mL of dry CH_3CN and cooled to 0 °C. A solution containing 314 mg (0.38 mmol) of *N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-3′-[2-cyanoethyl-(*N*, *N*-diisopropyl)]phosphoramidite (**10**) in 4 mL of dry CH_3CN was added dropwise over a period of 5 min. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 1 h. The reaction mixture was concentrated under diminished pressure and the residue was dissolved in 2 mL of a solution containing 0.3 g of I_2 in 13:1:0.2 THF−water−pyridine. The reaction mixture was stirred at room temperature for 20 min and then concentrated under diminished pressure. The residue was dissolved in 3 mL of $CHCl_3$ and washed with 5 mL of 1% aqueous sodium bisulfite. The aqueous layer was extracted with $CHCl_3$, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified on a 10-g silica gel column; elution with $CHCl_3$ then with 10:1 $CHCl_3$−MeOH gave **11a** as a colorless solid: yield 253 mg (95%); silica gel TLC R_f 0.36 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.10−1.25 (m, 6H), 2.31 (m, 1H), 2.69−3.14 (m, 8H), 3.15−3.51 (m, 8H), 3.77, 3.78, and 3.79 (each s, 6H), 4.03−4.79 (m, 10H), 5.06 and 5.16 (each m, 1H), 5.37−5.52 (m, 1H), 5.53 and 5.77 (each m, 1H), 5.99−6.04 (m, 1H), 6.15−6.27 (m, 2H), 6.83−6.87 (m, 4H), 7.20−7.36 (m, 10H), 7.50−7.65 (m, 4H), 7.91−8.10 (m, 3H), and 9.01 (br s, 1H); mass spectrum (FAB), *m*/*z* 1361.4294 $(M + H)^+$ ($C_{64}H_{71}N_{10}O_{10}P_2$ requires 1361.4321).

2,5-Dimethoxy-2,3,4,5-tetrahydrofurfuryl Cyanoethyl *N*-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxy-*P*-cyanoethylcytidylyl-(3′→5′)-*N*-isobutyryl-2′-deoxy-3′-guanylate (11b). In analogy with the synthesis of **11a**, 60 mg (0.10 mmol) of **9b** was treated with 90 mg (0.11 mmol) of *N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-3′-[2-cyanoethyl-(*N*, *N*-diisopropyl)]phosphoramidite (**10**) in the presence of 20.5 mg (0.292 mmol) of tetrazole. After oxidation with I_2, **11b** was obtained as a colorless solid: yield 104 mg (78%); silica gel TLC R_f 0.37 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.10−1.25 (m, 6H), 2.01−2.17 (m, 5H), 2.33 (m, 1H), 2.65−2.98 (m, 7H), 3.23−3.45 (m, 8H), 3.77, 3.78, and 3.79 (each s, 6H), 3.92−4.76 (m, 10H), 5.04−5.14 (m, 2H), 5.36−5.42 (m, 1H), 6.15−6.29 (m, 2H), 6.83−6.87 (m, 4H), 7.21−7.37 (m, 10H), 7.50−7.65 (m, 4H), 7.92−8.09 (m, 3H), and 9.12 (br s, 1H); mass spectrum (FAB), *m*/*z* 1363.4481 $(M + H)^+$ ($C_{64}H_{73}N_{10}O_{20}P_2$ requires 1363.4478).

2,2-Dimethoxypropionyl Cyanoethyl *N*-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-deoxy-*P*-cyanoethylcytidylyl-(3′→5′)-*N*-isobutyryl-2′-deoxy-3′-guanylate (11c). In analogy with the synthesis of **11a**, 129 mg (0.226 mmol) of **9c** was treated with 227 mg (0.271 mmol) of *N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-3′-[2-cyanoethyl-(*N*, *N*-diisopropyl)]-phosphoramidite in the presence of 57 mg (0.813 mmol) of tetrazole. After oxidation with I_2, **11c** was obtained as a colorless solid: yield 254 mg (85%); silica gel TLC R_f 0.35 (10:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.10−1.22 (m, 6H), 1.36 and 1.38 (each s, 3H), 2.11 (m, 1H), 2.32 (m, 1H), 2.65−3.05 (m, 7H), 3.22, 3.23, and 3.24 (each s, 6H), 3.33 (m, 2H), 3.77, 3.78 and 3.79 (each s, 6H), 4.02−4.05 (m, 2H), 4.10−4.74 (m, 8H), 5.05 and 5.16 (each m, 1H), 5.36−5.42 (m, 1H), 6.13−6.28 (m, 2H), 6.83−6.88 (m, 4H), 7.20−7.35 (m, 10H), 7.49−7.66 (m, 4H), 7.92−8.07 (m, 3H), and 9.14 (br s, 1H); mass spectrum (FAB), *m*/*z* 1321.4371 $(M + H)^+$ ($C_{62}H_{71}N_{10}O_{19}P_2$ requires 1321.4372).

2,5-Dimethoxy-2,5-dihydrofurfuryl Cyanoethyl *N*-Benzoyl-2′-deoxy-*P*-cyanoethylcytidylyl-(3′→5′)-*N*-isobutyryl-2′-deoxy-3′-guanylate (12a). To 669 mg (0.491 mmol) of **11a** was added 20 mL of 80% aqueous acetic acid. The reaction mixture was stirred at room temperature for 2 h. After concentration under diminished pressure, the residue was coevaporated twice with portions of EtOH and purified on a 10-g silica gel column; elution with $CHCl_3$ and then with 10:1 $CHCl_3$−MeOH gave **12a** as a colorless solid: yield 516 mg (99%); silica gel TLC R_f 0.13 (10:1 $CHCl_3$−MeOH);^{1}H NMR (CD_3OD) δ 1.11−1.23 (m, 6H), 2.10−2.30 (m, 1H), 2.60−2.90 (m, 7H), 3.10−3.15 (m, 4H), 3.35−3.40 (m, 3H), 3.65−3.70 (m, 2H), 4.00 (m, 1H), 4.10−4.30 (m, 6H), 4.32−4.58 (m, 3H), 4.96 (m, 1H), 5.23 (m, 1H), 5.47 and 5.73 (m, 1H), 5.98 (m, 1H), 6.12−6.15 (m, 2H), 6.32 (m, 1H), 7.43−7.58 (m, 4H), 7.88−7.92 (m, 2H), 8.04 (br s, 1H), and 8.32 (m, 1H); mass spectrum (FAB), *m*/*z* 1059.3015 $(M + H)^+$ ($C_{43}H_{53}N_{10}O_{18}P_2$ requires 1059.3015).

2,5-Dimethoxy-2,3,4,5-tetrahydrofurfuryl Cyanoethyl *N*-Benzoyl-2′-deoxy-*P*-cyanoethylcytidylyl-(3′→5′)-*N*-isobutyryl-2′-deoxy-3′-guanylate (12b). To 132 mg (0.144 mmol) of **11b** was added 10 mL of 80% aqueous acetic acid. The reaction mixture was stirred at room temperature for 2 h. After concentration under diminished pressure, the residue was coevaporated twice with portions of MeOH and purified on a 5-g silica gel column; elution with $CHCl_3$ and then with 10:1 $CHCl_3$−MeOH gave **12b** as a colorless solid: yield 81 mg (91%); silica gel TLC R_f 0.11 (10:1 $CHCl_3$−MeOH); ^{1}H NMR (CD_3OD) δ 1.05−1.23 (m, 6H), 1.80−2.30 (m, 5H), 2.63−3.15 (m, 8H), 3.20, 3.21, and 3.22 (each s, 6H), 3.66 (m, 2H), 3.91−4.42 (m, 10H), 4.89−5.07 (m, 2H), 5.26 (m, 1H), 6.07 (m, 1H), 6.32 (m, 1H), 7.35−7.58 (m, 4H), 7.88−7.92 (m, 2H), 8.05 (br s, 1H), and 8.29−8.35 (m, 1H); mass spectrum (FAB), *m*/*z* 1061.3192 $(M + H)^+$ ($C_{43}H_{55}N_{10}O_{18}P_2$ requires 1061.3171).

2,2-Dimethoxypropionyl Cyanoethyl *N*-Benzoyl-2′-deoxy-*P*-cyanoethylcytidylyl-(3′→5′)-*N*-isobutyryl-2′-deoxy-3′-guanylate (12c). To 155 mg (0.12 mmol) of **11c** was added 10 mL of 0.5% trifluoroacetic acid in $CHCl_3$. The reaction mixture was stirred at 0 °C for 6 h. The solvent was concentrated under diminished pressure and the residue was coevaporated twice with portions of $CHCl_3$ and purified on a 15-g silica gel column; elution with $CHCl_3$ and then with 10:1 $CHCl_3$−

MeOH gave **12c** as a colorless solid: yield 117 mg (98%) silica gel TLC R_f 0.07 (10:1 $CHCl_3$−MeOH); ^{1}H NMR (CD_3OD) δ 1.12−1.25 (m, 6H), 1.29 and 1.31 (each s, 3H), 2.19 (m, 1H), 2.60−2.91 (m, 7H), 3.11 (m, 1H), 3.16 and 3.17 (each s, 6H), 3.66 (m, 2H), 4.00 (m, 2H), 4.10−4.50 (m, 8H), 4.97−5.10 (m, 1H), 5.28 (m, 1H), 6.08 (m, 1H), 6.33 (m, 1H), 7.35−7.56 (m, 4H), 7.88−7.91 (m, 2H), 8.06 (br s, 1H), and 8.29−8.34 (m, 1H); mass spectrum (FAB) *m*/*z* 1019.3059 $(M + H)^+$ ($C_{41}H_{53}N_{10}O_{17}P_2$ requires 1019.3065.

3′-(2,5-Dimethoxy-2,5-dihydrofurfuryl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (5a).[9g] A mixture of 201 mg (0.19 mmol) of **12a** and 5 mL of NH_4OH was heated at 55 °C in a pressure bottle for 16 h. The solvent was concentrated under diminished pressure and the residue was coevaporated twice with portions of EtOH. The residue was purified on a 5-g silica gel colum; elution with 17:1:2 and then with 7:1:2 *i*-PrOH−NH_4OH−H_2O gave **5a** as a colorless solid: yield 139 mg (88%); silica gel TLC R_f 0.09 (17:1:2 *i*-PrOH−NH_4OH−H_2O); ^{1}H NMR (D_2O) δ 1.54 (m, 1H), 2.23 (dd, 1H, J = 14, 5 Hz), 2.50−2.65 (m, 1H), 2.70−2.90 (m, 1H), 3.03, 3.09 (each s, 3H), 3.31, 3.32, and 3.38 (each s, 3H), 3.52 (br s, 2H), 3.74 (dd, 1H, J = 11, 6 Hz), 3.85−4.00 (m, 4H), 4.27 (m, 1H), 4.53 (m, 1H), 4.95 (m, 1H), 5.51 (br s, 1H), 5.87 (d, 1H, J = 8 Hz), 6.20−6.98 (m, 4H), 7.50 (d, 1H, J = 8 Hz), and 7.96 (s, 1H); mass spectrum (FAB), *m*/*z* 779.1799 $(M + H)^+$ ($C_{26}H_{37}N_8O_{16}P_2$ requires 779.1803).

3′-(2,5-Dimethoxy-2,3,4,5-tetrahydrofurfuryl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (5b). A mixture of 63 mg (59 μmol) of **12b** and 5 mL of NH_4OH was heated at 55 °C in a pressure bottle for 16 h. The solvent was concentrated under diminished pressure and coevaporated twice with portions of EtOH. The residue was purified on a 5-g silica gel column; elution with17:1:2 and then with 7:1:2 *i*-PrOH−NH_4OH−H_2O gave **5b** as a colorless solid: yield 36 mg (78%); silica gel TLC R_f 0.10 (17:1:2 *i*-PrOH−NH_4OH−H_2O); ^{1}H NMR (D_2O) δ 1.51−1.61 (m, 1H), 1.84−2.15 (m, 4H), 2.24 (dd, 1H, J = 14, 6 Hz), 2.57−2.62 (m, 1H), 2.80 (m, 1H), 3.18, 3.24, and 3.30 (each s, 6H), 3.52 (m, 2H), 3.71−3.77 (m, 1H), 3.85−3.95 (m, 1H), 3.95−4.05 (m, 3H), 4.28 (m, 1H), 4.48 (m, 1H), 4.88−5.17 (m, 2H), 5.85 (d, 1H, J = 7 Hz), 5.98 (d, 1H, J = 6 Hz), 6.14 (t, 1H, J = 7 Hz), 7.47 (d, 1H, J = 7 Hz), and 7.95 (s, 1H); mass spectrum (FAB), *m*/*z* 781.1971 $(M + H)^+$ ($C_{26}H_{39}N_8O_{16}P_2$ requires 781.1959).

3′-(2,2-Dimethoxypropionyl) 2′-Deoxycytidylyl-(3′→5′)-2′-deoxy-3′-guanylate (5c). A mixture of 75 mg (74 μmol) of **12c** and 10 mL of NH_4OH was heated at 55 °C in a pressure bottle for 16 h. The solvent was concentrated under diminished pressure and coevaporated twice with portions of EtOH. The residue was purified on a 5-g silica gel column; elution with 17:1:2 and then with 7:1:2 *i*-PrOH−NH_4OH−H_2O gave **5c** as a colorless solid: yield 47 mg (86%); silica gel TLC R_f 0.10 (17:1:2 *i*-PrOH−NH_4OH−H_2O); ^{1}H NMR (D_2O) δ 1.28 (s, 3H), 1.53 (m, 1H), 2.22 (ddd, 1H, J = 14, 6, 2 Hz), 2.56 (ddd, 1H, J = 14, 6, 3 Hz), 2.79 (m, 1H), 3.14 (s, 3H), 3.15 (s, 3H), 3.51 (m, 2H), 3.72 (m, 2H), 3.90−4.00 (m, 3H), 4.27 (m, 1H), 4.50 (m, 1H), 4.92 (m, 1H), 5.83 (d, 1H, J = 8 Hz), 5.97 (dd, 1H, J = 8, 6 Hz), 6.13 (t, 1H, J = 6 Hz), 7.45 (d, 1H, J = 8 Hz), and 7.93 (s, 1H); mass spectrum (FAB), *m*/*z* 737.1707 $(M - H)^-$ ($C_{24}H_{35}N_8O_{15}P_2$ requires 737.1697).

^{1}H NMR Study of the Formation of 4a, 14a, and 14c. Dinucleotide **5a** (1.5 mg, 1.84 μmol) was dissolved in 0.5 mL of D_2O in an NMR tube and a ^{1}H NMR spectrum was taken. To this solution was added 3 drops of $(COCl)_2$ and the reaction mixture was maintained at room temperature. After 1 h, the formation of **4a** was confirmed by the disappearance of the signals corresponding to the methoxyl groups of **5a** (3.03−3.09, 3.31−3.38, multiplets due to the presence of diastereomers). Hydrolyses of **13a** and **13c** were carried out similarly. **14a**: ^{1}H NMR (D_2O in the presence of $(COCl)_2$) δ 3.31 and 3.34 (each d, 3H, J = 11 Hz), 3.55−3.70 (m, 2H), 5.60 and 5.81 (each s, 1H), and 5.86 (m, 2H). **14c**: ^{1}H NMR (D_2O in the presence of $(COCl)_2$) δ 1.96 (s, 3H), 3.39 (d, 3H, J = 11 Hz), and 4.39 (d, 2H, J = 8 Hz).

^{1}H NMR Study of the Formation of CpGp from 4a in the Presence of *n*-BuNH$_2$. To the acidic solution of **4a** obtained above was added 32 equiv (5.8 μL, 58.8 μmol) of *n*-$BuNH_2$. The reaction mixture was shaken for 5 min and then extracted with 0.5 mL of $CDCl_3$. The $CDCl_3$ layer was separated and dried (Na_2SO_4), and ^{1}H NMR spectra of the D_2O and $CDCl_3$ layers were taken. The appearance of CpGp was observed in the D_2O layer; lactam **15** was observed in the $CDCl_3$ layer; δ 4.82 (s, 1H), 4.89 (s, 1H), 6.55 (d, 1H, J = 6 Hz), and 6.94 (d, 1H, J = 6 Hz).

1-*n*-Butyl-5-methyleneazacyclopent-3-ene (15) by Treatment of 14a with *n*-BuNH$_2$. To 485 mg (1.76 mmol) of **13a** was added 20 mL of 0.1 N HCl solution. The reaction mixture was stirred at room temperature for 1 h, then treated with 20 mL of 0.2 N *n*-$BuNH_2$ and stirred at room temperature for 30 min. The reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with brine, dried (Na_2SO_4), and concentrated under diminished pressure. The residue was purified on a 5-g silica gel column; elution with *n*-hexane and then with 1:10 AcOEt−*n*-hexane gave **15** as a colorless liquid: yield 40 mg (19%); silica gel TLC R_f 0.28 (1:8 AcOEt−*n*-hexane); IR (neat) 1700, 1630, 1400, 820, and 810 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7 Hz), 1.35 (m, 2H), 1.56 (m, 2H), 3.60 (t, 2H, J = 7 Hz), 4.82 (d, 1H, J = 1 Hz), 4.89 (d, 1H, J = 1 Hz), 6.18 (dd, 1H, J = 6, 1 Hz), and 6.94 (d, 1H, J = 6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.71, 20.05, 30.71, 38.77, 96.23, 125.04, 136.94, 145.60, and 170.38; mass spectrum (FAB), *m*/*z* 152.1061 $(M + H)^+$ ($C_9H_{14}NO$ requires 152.1075).

1-*n*-Butyl-2-oxo-5-hydroxy-5-methylazacyclopent-3-ene (18). To a solution of 2.1 g (15.8 mmol) of 2-oxo-5-chloro-5-methyloxacyclopent-3-ene (**17**)[10] in 10 mL of pentane was added 1.57 mL (1.16 g, 15.8 mmol) of *n*-$BuNH_2$ at 0 °C; the reaction mixture was maintained at 0 °C for 5 days. The reaction mixture was treated with 5 mL of H_2O and the organic layer was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with brine, dried (Na_2SO_4), and concentrated under diminished pressure. The residue was purified on a 5-g silica gel column; elution with 1:2 and then with 1:1 AcOEt−*n*-hexane gave **18** as a colorless solid: yield 481 mg (32% based on consumed **17**); silica gel TLC R_f 0.1 (1:1 AcOEt−*n*-hexane); mp 65−69 °C dec; IR ($CHCl_3$) 3400 and 1695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, J = 7 Hz), 1.35 (m, 2H), 1.54 (s, 3H), 1.42−1.72 (m, 2H), 2.71 (s, 1H, ex D_2O), 3.23 (ddd, 1H, J = 14, 10, 6 Hz), 3.39 (ddd, 1H, J = 14, 10, 6 Hz), 6.02 (d, 1H, J = 6 Hz), and 6.89 (d, 1H, J = 6 Hz); mass spectrum (FAB), *m*/*z* 170.1180 $(M + H)^+$ ($C_9H_{16}NO_2$ requires 170.1181). Lactam **18** gradually decomposed when stored at room temperature.

Lactam 15 from 18. To a solution of 108 mg (0.64 mmol) of **18** in 5 mL of dry pyridine was added 0.07 mL (114 mg, 0.96 mmol) of $SOCl_2$ at −40 °C. After being maintained at − 40 °C for 1 h, the reaction mixture was poured into 5 mL of H_2O and extracted with CH_2Cl_2. The organic layer was washed successively with H_2O and brine, then dried (Na_2SO_4) and concentrated under diminished pressure. The residue was purified on a 5-g silica gel column; elution with ether gave **15** as a colorless liquid: yield 8.2 mg (11% based on consumed **18**); UV (MeOH) λ_{max} 255 nm (log ϵ 3.94) and 294 nm (log ϵ 3.69); IR (neat) 1700, 1630, 1400, 820, and 810 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7 Hz), 1.35 (m, 2H), 1.56 (m, 2H), 3.60 (t, 2H, J = 7 Hz), 4.82 (d, 1H, J = 1 Hz), 4.89 (d, 1H, J = 1 Hz), 6.18 (dd, 1H, J = 6, 1 Hz), and 6.94 (d, 1H, J = 6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.71, 20.05, 30.71, 38.77, 96.23, 125.04, 136.93, 145.60, and 170.38; mass spectrum (FAB), *m*/*z* 152.1061 $(M + H)^+$ ($C_9H_{13}NO$ requires 152.1075).

Lactam 15 by Treatment of 14a with *n*-BuNH$_2$ (pH 5.3 and 7). (a) pH 7.0: A mixture of 72 mg (0.28 mmol) of **13a** and 1 mL of 0.1 M HCl was stirred at room temperature for 1 h; the reaction mixture was then neutralized with 0.1 M NaOH. To this was added 94 μL of *n*-$BuNH_2$ in 1 mL of H_2O, adjusted to pH 7.0 with HOAc. The reaction mixture was stirred at room temperature for 2.5 h. The reaction mixture was extracted with four portions of ether, and the ether layer was dried (Na_2SO_4) and concentrated under diminished pressure at 0 °C. The residue was purified on a 5-g silica gel column; elution with *n*-hexane and then with 5:1 pentane−ether gave **15** as a colorless liquid: yield 35 mg (82%); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, J = 7 Hz), 1.35 (m, 2H), 1.56 (m, 2H), 3.60 (t, 2H, J = 7 Hz), 4.82 (d, 1H, J = 1 Hz), 4.89 (d, 1H, J = 1 Hz), 6.18 (dd, 1H, J = 6, 1 Hz), and 6.94 (d, 1H, J = 6 Hz). **(b) pH 5.3:** A mixture of 100 mg (0.40 mmol) of **13a** and 1 mL of 0.1 M HCl was stirred at room temperature for 1 h; the reaction mixture was then neutralized with 0.1 M NaOH. To this was added 0.12 mL of *n*-$BuNH_2$ in 1 mL of H_2O, adjusted to pH 5.3 with HOAc. The reaction was carried out in analogy with that at pH 7.0 to give **15** as a colorless liquid: yield 43 mg (73%).

Lactam 19 by treatment of 14a with BnNH$_2$. (a) pH 12: A mixture of 125 mg (0.49 mmol) of **13a** and 1 mL of 0.1 M HCl was stirred at

room temperature for 1 h; the reaction mixture was then neutralized with 0.1 M NaOH. To this was added 0.54 mL of $BnNH_2$ in 1 mL of H_2O, pH 12. The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was then extracted with CH_2Cl_2, and the CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated under diminished pressure. The residue was purified on a 5-g silica gel column; elution with *n*-hexane and then with 1:4 AcOEt−*n*-hexane gave **19** as a colorless liquid: yield 15 mg (16%); IR (neat) 3060, 1705, 1685, 1630, 1400, and 820 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.80 (d, 1H, $J = 2$ Hz), 4.83 (d, 1H, $J = 2$ Hz), 4.84 (s, 2H), 6.28 (dd, 1H, $J = 6$, 2 Hz), 7.00 (d, 1H, $J = 6$ Hz), 7.18−7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 42.65, 97.58, 124.93, 126.94, 127.31, 128.62, 137.04, 137.46, 145.19, and 170.43; mass spectrum (FAB), *m*/*z* 186.0922 $(M + H)^+$ ($C_{12}H_{12}NO$ requires 186.0919). **(b) pH 7:** A mixture of 100 mg (0.40 mmol) of **13a** and 1 mL of 0.1 M HCl was stirred at room temperature for 1 h and the reaction mixture was then neutralized with 0.1 M NaOH. To this was added 0.15 mL of $BnNH_2$ in 1 mL of H_2O, adjusted to pH 7.0 with HOAc. The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was extracted with four portions of ether. The ether extract was washed with brine, dried (Na_2SO_4), and concentrated under diminished pressure. The residue was purified on a 5-g silica gel column; elution with *n*-hexane and then with 1:2 AcOEt−*n*-hexane gave **19** as a colorless liquid: yield 55 mg (75%). **(c) pH 5.3:** A mixture of 126 mg (0.50 mmol) of **13a** and 1 mL of 0.1 M HCl was stirred at room temperature for 1 h; the reaction mixture was neutralized with 0.1 M NaOH. To this was added 0.2 mL of $BnNH_2$ in 1 mL of H_2O, adjusted to pH 5.3 with HOAc. The reaction mixture was maintained at 25 °C for 1 h and then worked up as above to afford **19**: yield 70 mg (76%).

Amine Treatment of Lactam 15. To a solution of 100 mg (0.66 mmol) of **15** in 2.5 mL of H_2O was added 0.65 mL (0.48 g, 6.6 mmol) of *n*-$BuNH_2$; the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was extracted with five portions of ether and the combined organic layer was dried (Na_2SO_4) and concentrated under diminished pressure. The residue was purified on a silica gel column; elution with 3:1 pentane−ether gave **15**: yield 14 mg (14%).

HPLC Analysis of the Products Formed from 4a by Treatment with *n*-BuNH$_2$. A solution of **4a** (0.29 μmol in 0.4 mL of H_2O, obtained by the general procedure for treatment of **4a** with amines) was treated with 5 mL of 0.2 M aqueous *n*-$BuNH_2$ (pH 7.0, 10 min) or 0.2 M aqueous *n*-$BuNH_2$ (pH 5.3, 6 h). The reaction mixture was analyzed on a reverse-phase C_{18} HPLC column; elution was with 0.2 M ammonium formate containing 35% CH_3CN. HPLC gave a peak having a retention time of 11 min; this comigrated with authentic **15** (Figure 4, parts a and b).

Successive Treatments of d(CGCTAGCG) with Activated Fe·BLM and *n*-Butylamine. An incubation mixture containing 50 μL (total volume) of 10 mM K Hepes buffer, pH 7.5, 180 μM BLM, 180 μM $Fe^{III}(NH_4)(SO_4)_2$, and 500 μM d(CGCTAGCG) was treated with argon-purged H_2O_2 to give a final concentration of 0.6 mM. To this was added 20 μL of 0.1 M *n*-$BuNH_2$, adjusted to pH 7.0 with HOAc. The reaction mixture was maintained under ambient conditions for 12 h. The reaction mixture was analyzed by C_{18} reverse-phase HPLC; elution was with 0.2 M ammonium formate containing 35% CH_3CN at a flow rate of 1 mL/min.

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Supporting Information Available: Additional experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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