Synthesis of Novel Polycyclic Nucleoside Analogues, Incorporation into Oligodeoxynucleotides, and Interaction with Complementary Sequences

Norbert Bischofberger* and Mark D. Matteucci*,[1]

Contribution from the Department of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080. *Received July 29, 1988*

Abstract: 4-*O*-[(Triisopropylphenyl)sulfonyl]pyrimidine nucleosides were reacted with aromatic diamines leading to fused, polycyclic ring systems: *o*-phenylenediamine yielded the pyrimido[1,6-*a*]benzimidazole, 2,3-diaminonaphthalene gave the naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidine, and 1,8-diaminonaphthalene led to the pyrimido[1,6-*a*]perimidine ring system. Two of these tetracyclic nucleosides were incorporated into oligodeoxynucleotides by chemical synthesis and the interactions with the complementary sequences studied by fluorescence spectroscopy and by measuring the thermal denaturation profiles. It was found that the presence of the extra unpaired polycyclic base stabilized the duplex, stabilization being greatest when the extra base was present between the two terminal base pairs. The amount of stabilization was also dependent on the nature of the tetracyclic base and on the context. When the duplex contained two unpaired bases, a greater stabilization was observed when the two bases were present in the two different strands. These results together with fluorescent spectroscopic data provide evidence that the extra base is intercalated into the duplex.

It was observed early that certain polycyclic aromatic compounds exhibited a strong affinity to DNA and RNA and this interaction was later attributed to intercalation.[2] The nature of the intercalation phenomenon has been studied by a variety of techniques, and detailed geometrical information was obtained mainly from X-ray crystallographic studies of DNA–intercalator complexes.[3] Letsinger first described a dinucleotide covalently linked to a phenanthridinium.[4] Recently a number of intercalators were synthetically attached to oligonucleotides via a linker and the presence of the intercalator was found to increase the thermal stability of the duplex that resulted from hybridization of the oligonucleotide to its complementary strand.[5]

This enhanced thermal stability has created interest in directing oligodeoxynucleotides against specific sequences of messenger RNAs in biological systems, thereby inhibiting translation. In such an approach, it was shown that intercalators linked to oligodeoxynucleotides could specifically inhibit the cytopathic effect of influenza virus in MDCK cells[6] and were also inhibiting translation of specific mRNAs in cell-free systems.[7]

An alternative to tethering an intercalating agent to an oligodeoxynucleotide with a flexible linker would be the conversion of one of the natural bases in a sequence into a planar polycyclic ring system. Such a modified oligodeoxynucleotide could then be targeted against the complementary strand with the modified nucleoside remaining unpaired. An extended polycyclic ring system would be expected to intercalate into the duplex in a stereochemically defined manner resulting in duplex stabilization.

Duplexes containing an extrahelical unpaired base have been studied by ^{1}H NMR spectroscopy and X-ray crystallography as model systems for frame-shift mutagenesis.[8] NMR studies have shown that in solution a duplex containing an unpaired deoxyadenosine and deoxythymidine, respectively, exists with the extrahelical base being stacked into the duplex.[9] In the crystal, however, the conformation of these duplexes is close to that of B DNA, with the extra adenosines looped out from the double helix.[24] Thermal denaturation studies of these duplexes bearing the extra deoxyadenosine demonstrated that the presence of the extra base has a destabilizing effect on the duplex.[8]

We now report the synthesis of nucleoside derivatives that contain a planar polycyclic moiety as the base by a novel reaction of 4-*O*-(triisopropylphenyl)sulfonyl (TPS) - pyrimidine nucleosides with aromatic diamines. We have incorporated two of the polycyclic compounds into oligodeoxynucleotides by chemical synthesis in specific positions and studied their interactions with complementary sequences by fluorescence and UV spectroscopy. It was found that these oligodeoxynucleotides containing the extra polycyclic base hybridize specifically to their complementary sequences and the resulting duplex shows enhanced thermal stability. The amount of stabilization is dependent on the context and nature of the base. Such oligonucleotides containing an extra polycyclic base provide a convenient model system to study base stacking and intercalation. Additionally, they may be useful for the rational design of intercalating bases leading to dramatically enhanced duplex stability.

Results

Chemistry. In an attempt to synthesize the tricyclic nucleoside derivative **3**, the 4-*O*-TPS pyrimidine derivative **1**[10] was treated with *o*-phenylenediamine in refluxing THF and a slower moving spot was observed on TLC, corresponding presumably to the adduct **2** (see Figure 1). The reaction did not stop at that stage,

(1) Present address: Gilead Sciences, 344 Lakeside Drive, Foster City, CA 94404.

(2) For reviews on intercalation, see: (a) Berman, H. M.; Young, P. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87. (b) Waring, M. J. *Annu. Rev. Biochem.* **1981**, *50*, 159. (c) Peakocke, A. R. *Acridines*; Ackeson, R. M., Ed.; Interscience Publishers: New York, 1973; pp 723–758. (d) Gale, E. F.; Clundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. *The Molecular Basis of Antibiotic Action*; John Wiley & Sons: London, 1972; pp 273–306.

(3) For leading references, see: (a) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 350–367.

(4) (a) Letsinger, R. L.; Schott, M. E. *J. Am. Chem. Soc.* **1981**, *103*, 7394. (b) Letsinger, R. L.; Schott, M. E. U.S. Patent 4,547,569, 1985.

(5) (a) Asseline, U.; Toulme, F.; Thuong, N. T.; Delarue, M.; Montenay-Garestier, T.; Helene, C. *EMBO J.* **1984**, *3*, 795. (b) Asseline, U.; Thuong, N. T.; Helene, C. *J. Biol. Chem.* **1985**, *260*, 8936. (c) Asseline, U.; Delarue, M.; Lancelot, G.; Toulme, F.; Thuong, N. T.; Montenay-Garestier, T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3297. (d) Le Doan, T.; Perronault, L.; Praseuth, D.; Habhoub, N.; Decout, J.-L.; Thuong, N. T.; Lhomme, J.; Helene, C. *Nucleic Acids Res.* **1987**, *19*, 7749. (e) Le Doan, T.; Perronault, L.; Chassignol, M.; Thuong, N. T.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 8643. (f) Helene, C. *DNA–Ligand Interactions*, Guschlbauer, W., Saenger, W., Eds.; Plenum Press: New York, 1987; pp 127–140.

(6) Zerial, A.; Thuong, N. T.; Helen, C. *Nucleic Acids Res.* **1987**, *15*, 9909.

(7) (a) Toulme, J. J.; Krisch, H. M.; Loreau, N.; Thuong, N. T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1227. (b) Cazenave, C.; Loreau, N.; Thuong, N. T.; Toulme, J.-J.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 4717.

(8) (a) Patel, D. J.; Kozlowski, S. A.; Marky, L. A.; Rice, J. A.; Broka, C.; Itakura, K.; Breslauer, K. J. *Biochemistry* **1982**, *21*, 445. (b) Patel, J. D.; Kozlowski, S. A.; Marky, L. A.; Rice, J. A.; Broka, C.; Itakura, K.; Breslauer, K. J. *Biochemistry* **1982**, *21*, 451. (c) Saper, M. A.; Eldar, H.; Mizuuchi, K.; Nickol, J.; Apella, E.; Sussman, J. L. *J. Mol. Biol.* **1986**, *188*, 111.

(9) (a) Hare, D.; Shapiro, L.; Patel, D. J. *Biochemistry* **1986**, *25*, 7456. (b) Van den Hogen, Y. Th.; van Beuzekom, A. A.; van den Elst, H.; van der Marel, G. A.; van Boom, J. H.; Altona, C. *Nucleic Acids Res.* **1988**, *16*, 2971.

(10) Bischofberger, N. *Tetrahedron Lett.* **1987**, *28*, 2821.

0002-7863/89/1511-3041$01.50/0

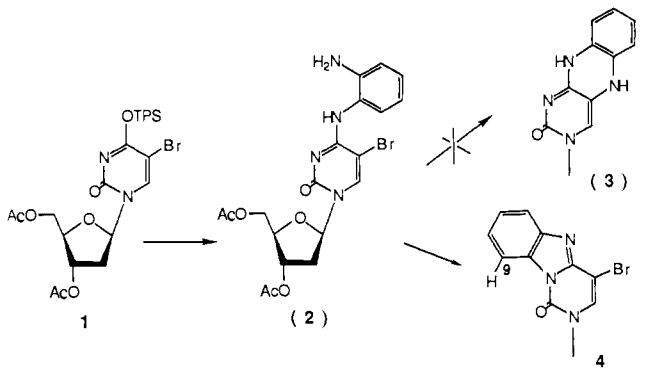


Figure 1.

however, and a new fluorescent product of higher R_f formed and was isolated in 49% yield. Quite unexpectedly, this product still contained Br but had lost NH_3 according to MS and elemental analyses. The λ_{max} in the UV spectrum was shifted to longer wavelengths relative to the starting *o*-phenylenediamine(λ_{max} = 294 nm) and exhibited a well-resolved vibrational structure. On the basis of these data, the product was subsequently assigned structure **4**. This assignment was also confirmed by the ^{1}H NMR spectrum in which the signal of the H-C9 is shifted to high field due to the anisotropy of the neighboring carbonyl group.[11]

In order to examine the generality of the rather unusual reaction, the 4-*O*-TPS – thymidine and deoxyuridine derivatives **5–7** were reacted with various aromatic diamines and the cyclized products **9** (from *o*-phenylenediamine), **10** (from 1,8-diaminonaphthalene), **11** and **12** (from 2,3-diaminonaphthalene) and the isomeric compounds **13** and **14** (from 4-nitro-*o*-phenylenediamine) were isolated in modest to good yields (see Figure 2 and Experimental Section).

When the reaction was carried out in the presence of base, none of the cyclized compounds were obtained but the reaction stopped at the intermediate stage, e.g., treatment of **6** with 2,3-diaminonaphthalene in the presence of 1 equiv of ethyldiisopropylamine yielded **15** in 60% yield. **15** was slowly converted to **11** in refluxing THF, more rapidly in the presence of acetic acid demonstrating that this second cyclization step is acid catalyzed.

Alternatively, the reaction was carried out in two steps by first treating the 4-*O*-TPS derivative with the diamine in the presence of base and then affecting the cyclization with acid. By treating *o*-phenylenediamine and **7** with potassium hexamethyldisilazane in THF at −78 °C, workup, and subsequent reflux of the crude product in THF in the presence of 0.8 equiv of acetic acid, the cyclized product **8** was obtained in 60% overall yield.

On the other hand, reaction of **7** with ethylenediamine or with 2,3-diaminopyridine led to the adducts **16** and **17**, respectively (see Figure 3), which could not be cyclized under a variety of reaction conditions. Also, attempts to extend this reaction to purine nucleosides failed. The reaction of *o*-phenylenediamine with *N*2,*O*3′,*O*5′-triisobutyryl 6-*O*-TPS-dG resulted only in the formation of **18**, which did not undergo any cyclization.

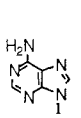
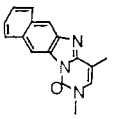
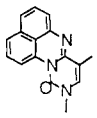
Incorporation of the Tetracyclic Nucleoside Derivatives 10 and 11 into Oligonucleotides. **10** and **11** on treatment with concentrated NH_3 rapidly gave the deacetylated derivatives, which proved to be stable to further ammonia treatment at 55 °C as observed on TLC, were directly dimethoxytritylated (DMT-Cl/pyridine) yielding **19** and **20**, and subsequently were converted to the phosphonate triethylammonium salts **21** and **22** (see Figure 2). **21** and **22** were then incorporated into oligodeoxynucleotides by the standard H-phosphonate protocol.[12]

The oligomers obtained after deblocking were purified by gel electrophoresis and desalted by reversed-phase chromatography using a C_{18} Sep-Pak column. The presence of the tetracyclic bases in the oligomers was confirmed by UV and fluorescence spectroscopies. The amount of fluorescence quenching in the single-stranded oligomers containing the fluorescent naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidine ring system was determined by snake venom phosphodiesterase digest and was found to be ~50%.

Table I. T_m Data of the Homoligomer Duplexes Containing an Inserted Naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidine Base

entry	duplex	T_m, °C
1	T_{14}/A_{14}	31
2	5′-TXT_{13}/A_{14}	40
3	5′-T_3XT_{11}/A_{14}	36
4	5′-T_5XT_9/A_{14}	34
5	5′-T_7XT_7/A_{14}	34
6	5′-T_9XT_5/A_{14}	34
7	5′-$T_{11}XT_3/A_{14}$	34
8	5′-$T_{13}XT/A_{14}$	37
9	T_{14}/5′-AXA_{13}	40

Table II. T_m Data of the Duplexes Containing Inserted Bases

		T_m (when X =), °C		
entry	duplex	 1		
1	5′-CAGTGATGTGT 3′-GTCACTACACA		42	
2	5′-CXAGTGATGTGT 3′-G-TCACTACACA	40 (−2)[a]	47 (+5)	50 (+8)
3	5′-CAGTGATGTGXT 3′-GTCACTACAC-A	40 (−2)	46 (+4)	49 (+7)
4	5′-C-AGTGATGTGT 3′-GXTCACTACACA		43 (+1)	44 (+2)
5	5′-CAGTGATGTG-T 3′-GTCACTACACXA		48 (+6) 48 (+6)	48 (+6) 48 (+6)
6	5′-CXAGTGATGTG-T 3′-G-TCACTACACXA		53 (+11)	55 (+13)
7	5′-C-AGTGATGTGXT 3′-GXTCACTACAC-A		46 (+4)	51 (+9)
8	5′-CXAGTGATGTGXT 3′-G-TCACTACAC-A		47 (+5)	
9	5′-C-AGTGATGTG-T 3′-GXTCACTACACXA		46 (+4)	

[a] Values in brackets refer to degrees stabilization relative to the unsubstituted duplex.

Fluorescence Studies. Addition of the complementary undecamer 5′-ACACATCACTG to the fluorescent 5′-CAG·TGA·TGT·GXT dodecamer caused a decrease in the fluorescence intensity whereas addition of random DNA sequences to the fluorescent dodecamer had no effect, proving that the fluorescence intensity decrease is specific to the complementary oligomer. Also, the fluorescence intensity did not further change after 1 equiv of the complementary strand had been added, indicating the formation of a duplex. The fluorescence intensity could be increased to the original level on heating as shown in Figure 4, consistent with the thermal denaturation of the duplex. The other dodecamers containing the naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidine base gave similar results, with varying levels of fluorescence quenching.

Thermal Denaturation Profiles. The melting curves of the duplexes containing one or two of the tetracyclic bases derived from monomers **21** and **22**, respectively, were measured at 2 mM oligomer concentration in buffered solution (100 mM NaCl, 10 mM Na_2HPO_4, 1 mM EDTA, pH = 7.5). For comparison and reference, the melting profiles of the corresponding duplexes containing no extra bases and of the duplexes containing an extra adenosine were also measured. From these melting curves, the

(11) Gunther, H. *NMR-Spektroskopie*; Georg Thieme Verlag: Stuttgart, FRG, 1973; pp 75–82.

(12) (a) Froehler, B. C.; Matteucci, M. D. *Tetrahedron Lett.* **1986**, *27*, 469. (b) Froehler, B. C.; Ng, P. G.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 5399. (c) Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Stromberg, R. *Tetrahedron Lett.* **1986**, *27*, 4051.

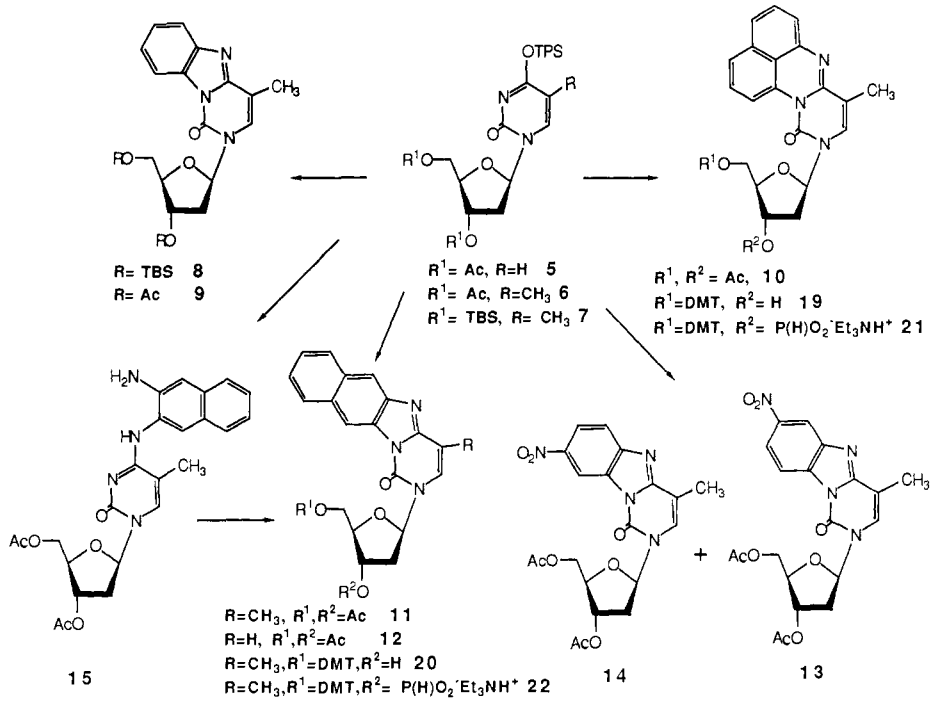


Figure 2.

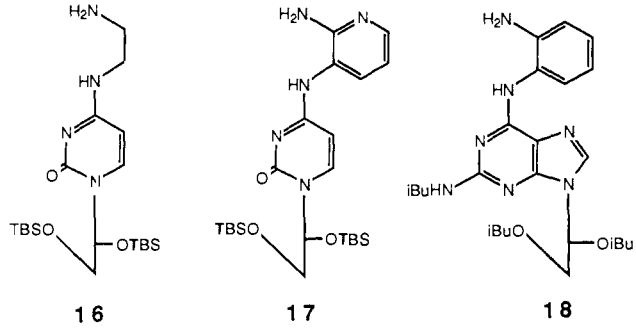


Figure 3.

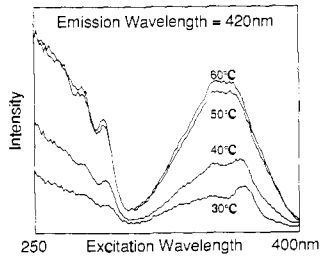


Figure 4. Fluorescence excitation spectrum of the duplex 5′-CAG·TGA·TGT·GXT/3′-GTC·ACT·ACA·CA at various temperatures.

T_m of the duplexes were determined and the results are depicted in Tables I and II.

Discussion

We have synthesized polycyclic nucleoside derivatives by a novel, one-pot procedure by reacting 4-*O*-TPS pyrimidine nucleosides with aromatic diamines. The reaction is clearly limited in scope, but it provides easy access to certain previously unknown heterocyclic ring systems.[13] The reaction is unique because two connected nucleophilic centers react with the pyrimidine nucleoside to form an extended ring system. However, reactions of pyrimidine nucleosides with electrophiles are well-known. E.g., reaction of cytidine and adenosine with bromoacetaldehyde yields etheno-cytidine and ethenoadenosine,[14] and on reaction of cytidine with 1′-methylthiaminium salts dipyrimido[1,6-*a*:4′,5′-*d*]pyrimidine derivatives are obtained.[15] Other polycyclic bases have been made from cytidine and adenosine by photochemical reactions.[16]

The products of this novel reaction are polycyclic nucleoside derivatives, and when incorporated into specific positions in DNA, they might provide an interesting model for studying intercalation-related phenomena.[17,18,25] Inspection of CPG models of a double-helical DNA in the B form containing an unpaired naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidine base reveals that the intercalated polycyclic base overlaps with the Watson–Crick hydrogen-bonded cross section.[19] The geometry of the duplex closely resembles an acridine intercalated into a DNA duplex. Moreover, compound **11** is fluorescent and consequently provides a convenient analytical handle. A crucial point in the incorporation of unnatural bases into oligodeoxynucleotides by chemical synthesis

(13) The benzimidazopyrimidine is a known ring system: (a) Nagarajan, K.; Ranga Rao, V.; Venkateswarlu, A. *Indian J. Chem.* **1970**, *8*, 126. (b) Buschauer, A.; Sattler, H.-J.; Schunack, W. *Chem. Ber.* **1984**, *117*, 2597. (c) Odijk, W. M.; Koomen, G. J. *Tetrahedron* **1985**, *41*, 1893.

(14) (a) For a review on etheno-substituted nucleotides, see: Leonard, N. J. *CRC Crit. Rev. Biochem.* **1984**, *15*, 125. (b) Kusmierek, J. T.; Jensen, D. E.; Spengler, S. J.; Stolarski, R.; Singer, B. *J. Org. Chem.* **1987**, *52*, 2374.

(15) Zoltewicz, J. A.; Wyrzykiewicz, E. *J. Org. Chem.* **1983**, *48*, 2481.

(16) (a) Inoue, H.; Imura, A.; Ohtsuka, E. *Nucleic Acids Res.* **1985**, *13*, 7119 (b) Shimada, K.; Sako, M.; Hirota, K.; Maki, Y. *Tetrahedron Lett.* **1987**, *28*, 207.

(17) Base stacking and intercalations has been studied with other model systems: (a) Rebek, J.; Askew, B.; Ballester, P., Buhr, C.; Costero, A.; Jones, S.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 6866. (b) Rebek, J.; Williams, K.; Parris, K.; Ballester, P.; Jeong, K.-S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1244.

(18) DNA intercalating abilities of perimidine derivatives have recently been investigated: Herbert, J. M.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1987**, *30*, 2081.

(19) For the concept of covalently linked Watson–Crick cross sections, see: Leonard, N. J.; Devadas, B. *J. Am. Chem. Soc.* **1987**, *109*, 623.

(20) Quigley, G. J.; Wang, A. H.-J.; Ughetto, G.; Van der Marel, G.; van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7204.

(21) Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1982**, *23*, 2257.

(22) Froehler, B. C.; Matteucci, M. D. *Nucleosides & Nucleotides* **1987**, *6*, 287.

(23) Fassman, G. D., Ed. *Handbook of Biochemistry and Molecular Biology, Nucleic Acids*, 3rd ed.; CRC Press: Boca Raton, FL, 1975; Vol. I, p 589.

(24) (a) Joshua-Tor, L.; Rabinovich, D.; Hope, H.; Frolow, F.; Apella, E.; Sussman, J. L. *Nature* **1988**, *334*, 82. (b) Miller, M.; Harrison, R. W.; Wlodawer, A.; Appella, E.; Sussman, J. L. *Nature* **1988**, *334*, 85.

(25) Polycyclic nucleoside analogues have been studied as defined probes by N. Leonard. For leading references, see: Moder, K. P.; Leonard, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 2613.

on solid support is the stability to the conditions employed, i.e., oxidation (I_2/THF/H_2O) and deblocking (concentrated NH_4OH, 55 °C). Both monomers **19** and **20** meet that requirement. On the other hand, the highly fluorescent ethenoadenosine and ethenocytidine are known to be labile to I_2[14] and would present difficulties for incorporation into oligodeoxynucleotides by chemical synthesis.

The monomers **21** and **22** were incorporated into oligodeoxynucleotide sequences into different positions and contexts. We proceeded to investigate the influence of the additional base on the thermal stability of the duplexes. The positional effect of an inserted naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidine base was studied in the homooligomer duplex A_{14}/T_{14} and the results are given in Table I. As can be seen, the highest stabilization resulted when the unnatural base was inserted between the two terminal base pairs, as in the duplexes 5′-TXT_{13}/A_{14} and 5′-$T_{13}XT/A_{14}$ (entries 2 and 8). Moving the extra base further away from the terminal base pairs reduced the amount of stabilization, and the presence of the base in the middle of the duplex as in T_7XT_7/A_{14} (entry 5) resulted in a 3 °C stabilization. This unexpected positional effect of an iserted base finds two parallel in the literature. In one study, an acridine intercalator was attached through a linker arm of flexible length to various positions on an oligodeoxynucleotide and the greatest stabilization of the duplex was found when the linker was attached to either the 5′- or 3′-end and had a length of five methylene units.[5] This best fits a model where the acridine is intercalated in the duplex between the two terminal base pairs. Another report relevant to our findings comes from an X-ray crystallographic study. Daunomycin was cocrystallized with the self-complementary oligodeoxynucleotide 5′-CGTACG and the daunomycin was found to be intercalated in the terminal CG base pairs.[20]

These results can be rationalized by taking into account the different energetic contributions of the extra base to the stability of the duplex. The presence of the additional base stabilizes the duplex due to hydrophobic and base-stacking interactions. On the other hand, it causes destabilizing geometric distortions to the double helix. Due to the cooperativity of the base-stacking interaction, the contribution of the extra base to the overall double helix stability if greater when it is positioned close to the end. Also, the unfavorable steric contribution is diminished when the extrahelical base is located close to the end, where it causes less long-range distortions than in the middle.

We next looked at the sequence specificity of the inserted unnatural base. The results are shown in Table II. It can be seen that the presence of an extra adenosine reduces the stability of the duplex by ~2 °C, consistent with other findings.[8a] Insertion of the tetracyclic bases, on the other hand, causes a stabilization of the duplex, the pyrimido[1,6-*a*]perimidine showing a higher stabilization. Clearly, context effects are present but difficult to rationalize without knowledge of the detailed geometry.

The duplexes containing two inserted bases show a peculiar phenomenon (entries 6–9, Table II). With the extra base present in opposite strands (entries 6 and 7), the duplex shows a cooperative melting curve and the amount of duplex stabilization is approximately the sum of the stabilization caused by both single insertions. However, when the two extra nucleotides are incorporated into the same single oligomer no additional increase in T_m is observed (entries 8 and 9; Figure 5). Again, these results cannot be rationalized without detailed structural information, but they are suggestive of the existence of long-range changes in the helical structure caused by the presence of the extra tetracyclic base.

In conclusion, we have prepared novel, polycyclic nucleoside derivatives and incorporated them into oligodeoxynucleotides by chemical synthesis. By studying the thermal denaturation of the duplexes containing one or two of the polycyclic nucleotide bases, we have shown that they stabilize the duplex, the amount of stabilization being greatest when the extra base is present between the two terminal base pairs. We have further shown that there is a difference in the amount of stabilization due to context and the nature of the polycyclic base. When two extra bases are present in opposite strands of the duplex, an additive effect of both single insertions is observed. While rationalization of these results must await further detailed geometric information on the duplexes, it is clear that this approach is a valuable model to study intercalation-related phenomena. The ability to synthesize stable extended polycyclic nucleoside analogues that span across the duplex by intercalation opens the possibility of using these polycycles to specifically perform modifications on a targeted complementary sequence.

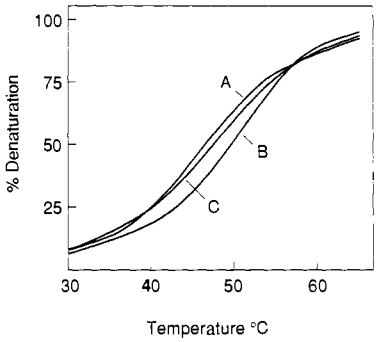


Figure 5. Melting curves of the duplexes: (A) 5′CA·GTG·ATG·TGT/3′-GXT·CAC·TAC·ACA; (B) 5′-CAG·TGA·TGT·GT/3′-GTC·ACT·ACA·CXT; (C) 5′-CA·GTG·ATG·TGT/3′-GXT·CAC·TAC·ACX·A. X is the fluorescent naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidine nucleoside.

Experimental Section

Materials and Methods. 2′-Deoxyuridine and thymidine were purchased from Sigma Chemical Co.; all other reagents were purchased from Aldrich Chemical Co. ^{1}H NMR spectra were obtained with an 80-MHz IBM NR/80 spectrometer and recorded as ppm (δ) with TMS as an internal standard. Coupling constants are given in hertz. Mass spectra were obtained by the positive-ion fast atom bombardment (FAB) technique on a Hewlett-Packard 5985C instrument. UV spectra were obtained on a Beckman UV-7 spectrophotometer with methanol as the solvent. Fluorescence spectra were obtained with an SLM 8000C spectrofluorometer. For thin-layer chromatography (TLC) EM DC-Alufolien Kieselgel-60 F_{254} plates were used; column chromatography was performed with EM Kieselgel-60 (70–230 mesh). Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. Elemental analyses were done by Chemical Analytical Services, University of California, Berkeley, CA. Oligonucleotides were synthesized on a Biosearch Model 4000 DNA synthesizer.

3′,5′-Di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]-2′-deoxy-5-bromouridine (1). **1** was obtained from 3′,5′-di-*O*-acetyl-5-bromo-2′-deoxyuridine (4.628 g, 11.8 mmol) according to the published procedure[10] as white crystals after chromatography (ether/hexane 1:1–1:0) and recrystallization from ether (5.0 g, 85%): mp 116–120 °C; ^{1}H NMR δ 8.18 (s, H-C6), 7.21 (s, 2 aromatic H), 6.09 (dd, J_1 = 6.4, J_2 = 5.6, anomeric H), 5.08–5.28 (m, H-C3′), 4.01–4.50 (m, 3 H), 3.13–2.50 (m, 5 H), 2.11, 2.08 (2 s, 2 H_3CCO), 1.18–1.39 (m, ~18 H). Anal. ($C_{28}H_{37}BrN_2O_9S$) C,H, N, Br.

3′,5′-Di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]thymidine (6). 6 was obtained from 3′,5′-di-*O*-acetylthymidine (2.82 g, 8.6 mmol) according to the published procedure[10] as white crystals after recrystallization from CH_2Cl_2/ether (3.78 g, 74%): mp 127–130 °C; ^{1}H NMR δ 7.76 (s, H-C6), 7.20 (s, 2 H), 6.12 (dd, J_1 = 8.0, J_2 = 5.8, anomeric H), 5.06–5.27 (m, 1 H), 4.01–4.54 (m, 3 H), 2.47–3.11 (m, 5 H), 2.06, 2.07 (2 s, 2 H_3CCO, H_3C-C5), 1.15–1.40 (m, ~18 H). Anal. ($C_{29}H_{40}N_2O_9S$) C, H, N.

3′,5′-Di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]-2′-deoxyuridine (5). **5** was obtained from 3′,5′-diacetyl-2′-deoxyuridine (3.03 g, 9.7 mmol) according to the published procedure[10] as a foam after chromatography (CH_2Cl_2/ethyl acetate 20:1–5:1) (3.43 g, 61%): ^{1}H NMR δ 8.02 (d, J = 7.3, H-C5), 7.21 (s, 2 aromatic H), 6.12 (d, J = 7.3, H-C5), 6.11 (t, J = 7.6, anomeric H), 5.08–5.29 (m, H-C3′), 4.04–4.46 (m, 3 H), 2.63–3.11 (m, 5 H), 2.08, 2.05 (2 s, 2 H_3CCO), 1.15–1.40 (m, ~18 H). Anal. ($C_{28}H_{38}N_2O_9S$) C, H, N.

4-Bromo-2-(3,5-di-*O*-acetyl-2-deoxy-1-β-D-ribofuranosyl)pyrimido[1,6-*a*]benzimidazol-1(2*H*)-one (4). A solution of 3′,5′-di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]-5-bromo-2′-deoxyuridine (**1**) (178 mg, 0.36 mmol) and *o*-phenylenediamine (51 mg, 0.47 mmol) in THF (3 mL) was heated to reflux for 16 h. Removal of the solvent and purification of the crude product by preparative TLC (ethyl acetate/CH_2Cl_2 1:3) yielded **4** as a foam which crystallized on addition of

methanol (79 mg, 49%): mp 70–72 °C; ^{1}H NMR δ 8.31–8.49 (m, H-C9), 7.76–8.01 (m, 2 H), 7.38–7.58 (m, 2 H), 6.61 (dd, J_1 = 8.5, J_2 = 6.1, anomeric H), 5.22–5.41 (m, 1 H), 4.29–4.51 (m, 3 H), 2.34–2.85 (m, 2 H), 2.22, 2.14 (2 s, 2 H_3CCO); UV (0.96 mg in 25 mL) λ_{max} 224 nm (ε 18 100), 240 (10 800), 284 (4800), 292 (6000), 307 (7500), 317 (9000), 330 (5800); λ_{min} 265 (3000); MS *m/z* 466, 464 (2, MH^+), 266, 264 (85), 81 (100). Anal. ($C_{19}H_{18}BrN_3O_6$) C, H, Br, N.

4-Methyl-2-(3,5-di-*O*-acetyl-2-deoxy-1-β-D-ribofuranosyl)pyrimido[1,6-*a*]benzimidazol-1(2*H*)-one (9). A solution of 3′,5′-di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]thymidine (339 mg, 0.57 mmol) and *o*-phenylenediamine (8 mg, 0.81 mmol) in THF (5 mL) was heated to reflux for 16 h. The mixture was poured into CH_2Cl (~200 mL) and extracted with 1 M citric acid solution to remove excess *o*-phenylenediamine. Concentration of the organic layer and chromatography of the crude product (CH_2Cl_2/ethyl acetate 5:2–0:1) gave **9** as a foam (193 mg, 84%) which crystallized upon addition of methanol as the methanol adduct: mp 78 °C; ^{1}H NMR δ 8.58–8.32 (m, H-C9), 7.16–7.99 (m, 4 H), 6.65 (dd, J_1 = 8.8, J_2 = 5.6, anomeric H), 5.20–5.41 (m, 1 H), 4.30–4.50 (m, 3 H), 3.49 (d, CH_3OH), 2.42 (d, J = 1.2, H_3C-C4), 2.18, 2.16 (2 s, 2 H_3CCO), 2.10–2.80 (m, 2 H); UV (1.66 mg in 50 mL) λ_{max} 235 nm (sh) (ε 15 900), 280 (11 400), 290 (13 800), 302 (sh) (11 4900), 310 (17 500), 322 (11 400); λ_{min} 260 (6000); MS *m/z* 400 (10, MH^+), 200 (100). Anal. ($C_{20}H_{21}N_3O_6$) C, H, N.

5-Methyl-3-(3,5-di-*O*-acetyl-2-deoxy-1-β-D-ribofuranosyl)pyrimido[1,6-*a*]perimidin-2(3*H*)one (10). A solution of 1,8-diaminonaphthalene (120 mg, 0.76 mmol) and 3′,5′-di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]thymidine (343 mg, 0.58 mmol) in THF (5 mL) was heated to reflux for 24 h. Purification of the crude reaction mixture by chromatography (CH_2Cl_2/ethyl acetate 20:1–1:1) and recrystallization of the product from CH_2Cl_2/methanol yielded **10** as yellow needles (205 mg, 78%): mp 158 °C; ^{1}H NMR δ 8.14 (dd, J_1 = 2.4, J_2 = 6.4, 1 H), 6.86–7.51 (m, ~6 H), 6.34 (dd, J_1 = 6.4, J_2 = 8.8, anomeric H), 5.12–5.32 (m, 1 H), 4.15–4.43 (m, 3 H), 2.0–2.75 (m, 2 H), 2.10, 2.13 (2 s, 2 H_3CCO), 2.00 (d, J = 0.8, H_3C-C5); UV (1.75 mg in 25 mL); λ_{max} 456 nm (sh) (ε 900), 438 (sh), (2400), 404 (3600), 380 (3700), 342 (sh) (8500), 332 (12 800), 324 (sh) (9600), 284 (7600); λ_{min} 294 (5400), 266 (5900). Anal. ($C_{24}H_{23}N_3O_6$) C, H, N.

2-(3,5-Di-*O*-acetyl-2-deoxy-1-β-D-ribofuranosyl)naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidin-1(2*H*)-one (12). A solution of 3′,5′-di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]-2′-deoxyuridine **5** (317 mg, 0.548 mmol) and 2,3-diaminonaphthalene (105 mg, 0.66 mmol) in THF (3 mL) was heated to reflux for 16 h. After workup (CH_2Cl_2), chromatography of the crude reaction mixture (CH_2Cl_2/ethyl acetate 1:1–0:1), and recrystallization of the fluorescent product from CH_2Cl_2/methanol, **12** was obtained as white crystals (138 mg, 58%): mp 83–88 °C; ^{1}H NMR δ 8.84, 8.22 (2 s, 2 H), 7.90–8.17 (m, 2 H), 7.62–7.37 (m, 3 H), 6.67 (d, J = 8.1, H-C3), 6.58 (t, J = 5.9, anomeric H), 5.18–5.41 (m, 1 H), 4.27–4.51 (m, 3 H), 3.48 (s, CH_3OH), 2.86–2.25 (m, 2 H), 2.15 (s, 2 H_3CO); UV (1.12 mg in 50 mL) λ_{max} 375 nm (ε 5400), 358 (10 500), 346 (12 600), 331 (9700), 287 (37 000), 276 (31 000), 265 (20 900), 243 (26 400); λ_{min} 296 (2900) 282 (16 300), 269 (17 500), 260 (18 200), 228 (17 900); MS *m/z* 436 (MH^+, 8), 236 (80). Anal. ($C_{23}H_{21}N_3O_6$; methanol complex) C, H, N.

4-Methyl-2-(3,5-di-*O*-acetyl-2-deoxy-1-β-D-ribofuranosyl)naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidin-1(2*H*)-one (11). A solution of 2,3-diaminonaphthalene (180 mg, 1.14 mmol) and 3′,5′-di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]thymidine (6; 464 mg, 0.78 mmol) in THF (5 mL) was heated to reflux for 16 h. The reaction mixture was diluted with CH_2Cl_2 (~200 mL) and extracted with 1 M citric acid solution to remove excess 2,3-diaminonaphthalene. The organic layer was concentrated and the crude product purified by chromatography (CH_2Cl_2/ethyl acetate 1:1–0:1) and recrystallized from methanol/CH_2Cl_2, yielding **11** as white crystals (231 mg, 66%): mp 128–130 °C; ^{1}H NMR δ 8.86 (2 s, 2 H), 7.90–8.17 (m, 2 H), 7.36–7.62 (m, 2 H), 7.28 (m, 1 H), 6.63 (dd, J_1 = 8.0, J_2 = 6.4, anomeric H), 5.18–5.41 (m, 1 H), 4.23–4.50 (m, 3 H), 2.40 (d, J = 1.6, H_3C-C4), 2.16, 2.15 (2 s, 2 H_3CCO), 2.0–2.70 (m, 2 H); UV (0.64 mg in 25 mL) λ_{max} 372 nm (ε 4500), 352 (9700), 348 (10 000), 340 (3700), 288 (24 200), 278 (19 500), 268 (sh) (13 900), 244 (25 800); λ_{min} 300 (2400), 283 (13 100), 272 (11 800); MS *m/z* 450 (6, MH^+), 250 (100). Anal. ($C_{24}H_{23}N_3O_6$) C, H, N.

4-Methyl-2-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-1-β-D-ribofuranosyl]pyrimido[1,6-*a*]benzimidazol-1(2*H*)-one (8). To a solution of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-4-*O*-[(2,4,6-triisopropylphenyl)-sulfonyl]thymidine (**7**; 401 mg, 0.54 mmol) and *o*-phenylenediamine (76 mg, 0.70 mmol) in THF (10 mL) was added potassium hexamethyldisilazane (1.1 mL of a 0.5 M solution in toluene, 0.55 mmol) at −78 °C. After 5 min the reaction mixture was quenched (saturated NH_4Cl solution) and worked up in ether. The crude reaction product was dissolved in THF and heated to reflux with acetic acid (40 μL, 0.40 mmol) for 8 h. The reaction mixture was concentrated and the product purified by preparative TLC (ether/hexane 2:1), yielding the fluorescent **8** as a foam (175 mg, 60%): ^{1}H NMR δ 8.36–8.57 (m, H-C9), 7.75–7.95 (m, 1 H), 7.32–7.62 (m, 3 H), 6.65 (dd, J_1 = 7.8, J_2 = 6.2, anomeric H), 4.36–4.60 (m, 1 H), 3.77–4.12 (m, 3 H), 2.38 (s, H_3C), 1.89–2.54 (m, H_2C2'), 1.75–2.05 (m, ~18 H), 0.05–0.22 (m, ~12 H); UV (1.18 mg in 25 mL) λ_{max} 220 nm (ε 27 600), 236 (sh), (15 000), 280 (10 600), 290 (12 600), 300 (13 500), 310 (16 100), 323 (10 600); λ_{min} 260 (5700), 284 (10 300), 295 (12 000), 320 (10 300); MS *m/z* 544 (MH^+, 2), 200 (100).

4-Methyl-7-nitro-2-(3,5-di-*O*-acetyl-2-deoxy-1-β-D-ribofuranosyl)pyrimido[1,6-*a*]benzimidazol-1(2*H*)-one (13) and 4-Methyl-8-nitro-2-(3,5-di-*O*-acetyl-2-deoxy-1-β-D-ribofuranosyl)pyrimido[1,6-*a*]benzimidazol-1(2*H*)-one (14). A solution of 3′,5′-di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]thymidine (1.231 g, 2.08 mmol) and 4-nitro-*o*-phenylenediamine (420 mg, 2.74 mmol) in THF (10 mL) was heated to reflux for 48 h. The mixture was diluted with CH_2Cl_2 (150 mL) and extracted with 1 M citric acid solution (3 × 50 mL). The organic layer was concentrated and the crude mixture purified by chromatography (CH_2Cl_2/ethyl acetate 5:1–0:1) yielding two fractions. **13** was obtained from the faster eluting fraction as a powder after recrystallization from CH_2Cl_2/methanol (300 mg, 32%); the slower eluting fraction yielded **14** as yellow needles after recrystallization from CH_2Cl_2/methanol (397 mg, 43%). The structure was assigned on the basis of the low-field shift of the H-C9 signal in the ^{1}H NMR spectrum due to the anisotropy of the carbonyl oxygen. **14**: mp 100–103 °C; ^{1}H NMR δ 9.32 (d, J = 1.2, H-C9), 8.41 (dd, J_1 = 9.3, J_2 = 1.2, H-C7), 7.89 (d, J = 9.3, H-C6), 7.43 (s, br, H-C3), 6.60 (t, J = 6.6, anomeric H), 5.20–5.44 (m, 1 H), 4.29–4.58 (m, 3 H), 1.99–2.88 (m, 2 H), 2.42 (s, br, H_3C-C4), 2.17 (s, 2 H_3CCO); UV (2.02 mg in 75 mL) λ_{max} 350 nm (ε 15 800), 272 (11 500); λ_{min} 296 (4900), 255 (10 500); **13**: mp 85–90 °C; ^{1}H NMR δ 8.74 (d, J = 1.5, H-C6), 8.58 (d, J = 8.8, H-C9), 8.34 (dd, J_1 = 1.5, J_2 = 8.8, H-C8), 7.39 (s, br, H-C3), 6.62 (dd, J_1 = 6.3, J_2 = 8.4, anomeric H), 5.22–5.45 (m, 1 H), 4.28–4.58 (m, 3 H), 2.00–2.87 (m, H_2C), 2.45 (s, br, H_3C-C4), 2.19 (2s, 2 H_3CCO); UV (1.53 mg in 75 mL) λ_{max} 330 nm (sh) (ε 5600), 282 (23 900), 236 (12 600); MS *m/z* 445 (MH^+, 3), 245 (25).

3′,5′-Di-*O*-acetyl-5-methyl-4-*N*-(2-amino-3-naphthyl)-2′-deoxycytidine (15). A solution of 3′,5′-di-*O*-acetyl-4-*O*-[(2,4,6-triisopropylphenyl)-sulfonyl]thymidine (203 mg, 0.34 mg), 2,3-diaminonaphthalene (75 mg, 0.47 mmol), and ethyldiisopropylamine (100 μL, 0.57 mmol) in THF (3 mL) was refluxed for 24 h. Chromatography (ethyl acetate/methanol 1:0–10:1) of the crude product mixture yielded **15** as a foam (76 mg, 47%): ^{1}H NMR δ 7.71–7.13 (m, 6 H), 6.96 (s, 1 H), 6.29 (dd, J_1 = 5.6, J_2 = 8.0, anomeric H), 5.08–5.32 (m, H-C3′), 4.12–4.42 (m, 3 H), 2.66–2.0 (m, H_2C2'), 2.12, 2.08 (2 s, 2 H_3CCO), 2.02 (s, br, H_3C-C5); UV (0.75 mg in 25 mL) λ_{max} 240 nm (ε 31 700), 282 (16 100), 340 (sh) (440), end absorption to 380; λ_{min} 262 (13 700); MS *m/z* 467 (18, MH^+), 267 (100), 250 (50).

4-Methyl-2-[5-*O*-(dimethoxytrityl)-2-deoxy-1-β-D-ribofuranosyl]-naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidin-1(2*H*)-one (20). To a solution of **11** (54 mg, 0.12 mmol) in dioxane (2 mL) was added concentrated aqueous NH_3 (3 mL). After 5 h the deacetylation was complete according to TLC, and the solution was evaporated to dryness. The residue was dissolved in pyridine (5 mL), and DMT-Cl (140 mg, 0.47 mmol) was added batchwise over a period of 24 h. The reaction was quenched by addition of methanol and evaporated and the product purified by thick-layer chromatography (CH_2Cl_2/ethyl acetate 5:1), the fluorescent band was eluted with ethyl acetate, yielding pure **20** as a yellow foam (60 mg, 75%): ^{1}H NMR δ 8.88, 8.23 (2 s, 2 H), 8.15–7.90 (m, 2 H), 7.80–6.60 (m, ~17 H), 4.55–4.80 (m, 1 H), 4.0–4.27 (m, 1 H), 3.3–3.8 (m, 2 H), 3.75 (s, 2 H_3CO), 2.30–2.67 (m, H_2C2'), 1.95 (s, br, H_3C-C4).

5-Methyl-3-[5-*O*-(dimethoxytrityl)-2-deoxy-1-β-D-ribofuranosyl]pyrimido[1,6-*a*]perimidin-2(3*H*)-one (19). To a solution of the diacetyl compound **10** (116 mg, 0.26 mmol) in THF (3 mL)/methanol (2 mL) was added 38% aqueous NH_3 (2 mL), and the resulting mixture was left at ambient temperature for 24 h. The solvents were removed, the solid residue was evaporated 2× from pyridine (5 mL) and finally dissolved in pyridine (5 mL), and DMT-Cl (156 mg, 0.46 mmol) was added. After 2 days the mixture was quenched by addition of methanol (0.5 mL) and concentrated to dryness and the residue purified by preparative TLC (CH_2Cl_2/ethyl acetate 5:1). The yellow band was collected, yielding **19** as a yellow foam (133 mg, 76%): ^{1}H NMR δ 8.17 (dd, J_1 = 6.6, J_2 = 1.9, H-C12), 6.72–7.57 (m, ~19 H), 6.41 (t, J = 7.0, anomeric H), 4.41–4.63 (m, 1 H), 3.93–4.12 (m, 1 H), 3.78 (s, 2 H_3CO), 3.34–3.54 (m, 2 H), 2.22–2.49 (m, 2 H), 1.65 (s, br, H_3C-C5).

5-*O*-(Dimethoxytrityl)-2-deoxy-1-β-[4-methyl-1-oxo-2-(2*H*)-naphth[2′,3′:4,5]imidazo[1,2-*f*]pyrimidinyl]-3-*O*-ribofuranosylphosphonate Triethylammonium Salt (22). To CH_2Cl_2 (10 mL) were sequentially added at −78 °C *N*-methylmorpholine (2 mL), PCl_3 (200 μL), and 1,2,4-triazole (860 mg). The mixture was stirred at room temperature for 1 h and

recooled to −78 °C, and a solution of **20** (135 mg, 0.20 mmol) in CH_2Cl_2 (8 mL) was added. The reaction mixture was allowed to warm to ~0 °C, quenched by addition of H_2O, diluted with CH_2Cl_2 (150 mL), and extracted 2× with 1 M biethylammonium bicarbonate solution, pH 7.5. The organic layer was dried ($MgSO_4$) and concentrated, the solid residue treated with ethyl acetate, the insoluble part [tris(triethylammonium)-phosphonate] removed by filtration, the filtrate concentrated, and the crude product purified by preparative TLC (CH_2Cl_2)/methanol/triethylamine 100:10:1). The fluorescent band was collected and eluted, yielding **22** as a white foam (141 mg, 84%): ^{1}H NMR δ 10.78 (s, NH), 8.89, 8.24 (2 s, 2 H), 6.67–8.17 (m, ~19 H), 5,10 (m, 1 H), 4.37 (m, 1 H), 3.77 (s, 2 H_3CO), 3.40 (m, 2 H), 3.09 (qua, J = 7.3, ~6 H), 2.50–2.80 (m, 2 H), 1.82 (d, J ~ 1, H_3C-C5), 1.39 (t, J = 7.3, ~9 H).

5-*O*-(Dimethoxytrityl)-2-deoxy-1-β-[5-methyl-2-oxo-(3*H*)-pyrimido-[1,6-*a*]perimidin-3-yl]-3-*O*-ribofuranosylphosphonate Triethylammonium Salt (21). To CH_2Cl_2 (10 mL) were sequentially added at −78 °C *N*-methylmorpholine (1 mL), PCl_3 (150 μL), and triazole (720 mg). The mixture was stirred at ambient temperature for 1 h, and a solution of **19** (105 mg, 0.157 mmol) in CH_2Cl_2 (3 mL) was added at −78 °C. After warming to ~0 °C, the reaction was quenched by addition of H_2O, diluted with CH_2Cl_2 (100 mL), and extracted 2× with 1 M ammonium bicarbonate solution, pH 7.5. The organic layer was dried and concentrated and the residue dispersed in ethyl acetate. The insoluble material [tris(triethylammonium)phosphonate] was removed by filtration, the filtrate concentrated, and the residue purified by preparative TLC (CH_2Cl_2/methanol/triethylamine 100:10:1), yielding **21** as a yellow foam (83 mg, 63%): ^{1}H NMR δ 8.16 (dd, J_1 = 6.8, J_2 = 1.9, H-C12), 6.68–7.56 (m, ~19 H), 6.50 (dd, J_1 = 7.9, J_2 = 5.9, anomeric H), 4.83–5.16 (m, br, 1 H), 4.17–4.38 (m, 1 H), 3.78 (s, 2 H_3CO), 3.36–3.55 (m, 2 H), 3.06 (qua, J = 7.1, 6 H), 2.41–2.64 (m, 2 H), 1.51 (s, br, H_3C-C5), 1.33 (t, J = 7.1 ~9 H).

2-(Isobutyrylamino)-6-[(2-aminophenyl)amino]-9-(3,5-di-*O*-isobutyryl-2-deoxy-1-β-D-ribofuranosyl)purine (18). A solution of *N*2,*O*3′,*O*5′-triisobutyryl-2′-deoxy-6-*O*-[(2,4,6-triisopropylphenyl)-sulfonyl]guanosine[21] (175 mg, 0.23 mmol) and *o*-phenylenediamine (100 mg, 0.92 mmol) in THF (3 mL) was heated to reflux for 24 h. The crude product was purified by chromatography (ethylacetate)/CH_2Cl_2 1:5–1:0), yielding **22** as a foam (103 mg, 76%): ^{1}H NMR δ 7.95 (s, br, 1 H), 7.77 (s, H-C8), 6.61–7.37 (m, ~4 H), 6.25 (dd, J_1 = 8.1, J_2 = 6.1, anomeric H), 5.25–5.45 (m, 1 H), 4.13–4.43 (m, ~3 H), 2.30–3.14 (m, ~5 H), 0.88–1.24 (m, ~18 H); UV (2.73 mg in 100 mL) λ_{max} 230 nm (ε 21 500), 292 (12 100), end absorption to 360; λ_{min} 262 (10 800); MS *m*/*z* 568 (MH^+, 4), 312 (100), 242 (35), 225 (52).

5-Methyl-2′-deoxy-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-4-*N*-(2-amino-3-pyridyl)cytidine (17). A solution of 3′,5′-*O*-(*tert*-butyldimethylsilyl)-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]thymidine (**7**; 115 mg, 0.15 mmol) and 2,3-diaminopyridine (100 mg, 0.92 mmol) in THF (2 mL) was heated to reflux for 18 h. The crude reaction mixture was purified by preparative TLC (ether/hexane 4:1), and the yellow band was collected and eluted with ether, yielding **17** as a foam (40 mg, 47%): ^{1}H NMR δ 7.74 (dd, J_1 = 4.8, J_2 = 0.9, H-C6″), 7.23 (m, H-C6) 6.99 (dd, J_1 = 8.0, J_2 = 0.9, H-C4″), 6.80 (dd, J_1 = 8.0, J_2 = 4.8, H-C5″), 6.40 (dd, J_1 = 6.5, J_2 = 6.0, anomeric H), 4.30–4.54 (m, 1 H), 3.74–4.00 (m, 3 H), 2.02 (s, H_3C), 1.92–2.45 (m, H_2C2'), 0.78–1.05 (m, ~18 H), 0.05–0.22 (m, ~12 H); UV (1.03 mg in 25 mL); λ_{max} 286 nm (ε 12 500), 370 (16 400), end absorption to 440; λ_{min} 252 (8500), 315 (4200); MS *m*/*z* 562 (MH^+, 2), 218 (100).

Oligonucleotide Synthesis. Polymer-bound nucleotide H-phosphonates were prepared as previously described[12] on control-pore glass by using the DBU salts of the protected nucleoside H-phosphonates.[22] For introducing the polycyclic nucleosides, a solution of ~25 mg of the triethylammonium salts **21** and **22**, respectively, in 1 mL of pyridine/acetonitrile (1:1) was used in the automated synthesis. For efficient coupling the wait time in the programmed coupling step for **21** and **22** was increased from 6 × 8 to 26 × 8 s. After oxidation and deblocking, the fragments were purified by polyacrylamide gel electrophoresis, the fluorescent (in the case of **22**) or yellow (in the case of **21**) bands were eluted and the eluate was desalted by loading onto a reversed-phase C_{18} Sep-Pak column (Waters Associates), washing with H_2O, and finally eluting the fragments with 25% aqueous acetonitrile.

Hypochromicity Measurements. Hypochromicities of the duplexes were measured at 260 nm with a Kontron Uvikon 810 spectrophotometer in a 1-cm masked cuvette. The samples were 100 mM NaCl, 10 mM Na_2HPO_4, and 1 mM EDTA at pH 7.2 and contained the oligomers at a concentration of 2 μM in a total volume of 1 mL. The extinction coefficients (ε) of the oligomers were calculated,[23] and the ε of the oligomers containing the extra tetracyclic bases were approximated by adding the values of the ε of the monomers of 260 nm (for **10**, ε(260) = 15 mL/μmol; for **11**, ε(260) = 6 mL/μmol) to the ε of the oligomers devoid of the tetracyclic bases. The samples were degassed with He, heated to ~55 °C for 4 h, allowed to cool, and maintained at 0 °C overnight. The absorbance of the samples was monitored from 15 to ~80 °C, increasing the temperature at a rate of ~0.2 °C/min. Each sample was measured at least twice, and the data were separately normalized to percent denaturation (% denaturation = $100[A_0 - A_i]/[A_f - A_i]$, where A_0 is the observed, A_i is the initial, and A_f is the final absorbance) and combined to obtain a melting curve. A linear least-squares analysis of this data gave a slope of transition and y intercept from which the T_m values were calculated.

Acknowledgment. We thank Mark Vasser, Parkash Jurani, and Peter Ng for their help with the oligodeoxynucleotide synthesis, John Chakel for the MS data, and Mike Mulkerrin for his assistance with the fluorescence spectroscopy and with the T_m data.

α-Cyclodextrin-Catalyzed Regioselective P–O(2′) Cleavages of 2′,3′-Cyclic Monophosphates of Ribonucleosides[1]

Makoto Komiyama

Contribution from the Institute of Materials Science, University of Tsukuba, Tsukuba, Ibaraki 305, Japan. *Received August 1, 1988*

Abstract: Regioselective P–O(2′) cleavages of 2′,3′-cyclic monophosphates of cytidine (1a), uridine (1b), adenosine (1c), and guanosine (1d) to the corresponding 3′-monophosphates (11a–d) are achieved at pH 11.08, 20 °C by α-cyclodextrin (α-CyD) as catalyst. The selectivities asymptotically increase with increasing concentration of α-CyD, attaining 98, 94, 76, and 67% for the cleavages of 1a–d, respectively, at the concentration of 0.05 M α-CyD. In the absence of α-CyD, however, significant concurrent cleavages of the P–O(3′) bonds take place and the selectivities for 11a–d are only 47, 50, 54, and 52%. The rate of the P–O(2′) cleavage of 1a in the α-CyD–1a complex is 14 times as large as that of free 1a, and the rate of the P–O(3′) cleavage in the complex is virtually zero. β- and γ-CyDs show no regioselective catalyses. ^{1}H NMR spectroscopy indicates that the α-CyD–1a and –1b complexes are formed by hydrogen bondings and 1's are located on the secondary hydroxyl side of the cavity of α-CyD. The selective cleavages of ribonucleotide dimers CpC, CpU, CpA, and CpG to 11a are also successfully carried out by α-CyD as catalyst.

Ribonuclease cleaves ribonucleic acids to fragments having the terminal phosphates at specific positions (mostly the 3′-positions).[2,3] This specificity is attributed to regiospecific cleavage of the P–O(2′) bond of the 2′,3′-cyclic monophosphate of the terminal

0002-7863/89/1511-3046$01.50/0