Refolding of Synthetic Enzymes. Each synthetic enzyme obtained from the previous step was dissolved in 25% acetic acid and passed through a Sephadex G-25 column (0.7 × 18 cm) to remove residual low molecular weight impurities remaining from the deprotection. The protein peak was collected and lyophilized. The deprotected peptide was dissolved in the reduction-denaturation buffer of Pace and Creighton[38] (0.2 M Tris-HCl, 2 mM EDTA, 0.1 M DTT, 6 M guanidine chloride, pH 8.7). The tube was purged with nitrogen, sealed, and allowed to stand for 3 h. The sample was then applied to a column of Sephadex G-25 (0.7 × 9 cm) equilibrated with 0.1 M NaCl and eluted with the same solution. The protein peak eluted first and was collected into a plastic microtube. The protein solution was kept for several hours at room temperature to allow oxidation to take place before being concentrated in a Speed-Vac apparatus to 0.1 mL and applied to a Sephadex G-25 column (0.7 × 9 cm) eluting with water. The desalted protein was collected and stored at 4 °C. Concentrations of synthetic enzymes were calculated based on the UV absorbance (ϵ_{278} = 1.91 mL/mg·cm).[47] The mutant enzyme was assumed to have the same absorbance. Assays were as described.[39-41]

Acknowledgment. The mass spectrometric measurements were made by the Rockefeller Mass Spectrometric Biotechnology Resource supported by the Divison of Research Resources, NIH (Grant RR-00862-15). We thank Drs. Meng F. Ho and Berkley Lynch for carrying out some preliminary experiments including the synthesis of several small peptide segments in an early stage of the project and Drs. Jeff W. Kelly, Hisakazu Mihara, and Peter T. Lansbury, Jr., for helpful discussion. We also thank Judi Lebovitch, Rob Cerpa, and Jennifer Banzon for performing amino acid analyses.

Registry No. S1, 132376-29-5; S2, 132408-54-9; S3, 132376-30-8; S4, 132376-31-9; S5, 132376-32-0; S6, 132408-55-0; S7, 132376-33-1; S8, 132376-34-2; S9, 132376-35-3; S10, 132376-36-4; S11, 132376-37-5; S12, 120712-40-5; S13, 132376-38-6; S14, 132376-39-7; MS3, 132376-40-0; MS4, 132376-41-1; MS5, 132408-56-1; P-I, 132408-36-7; P-II, 132408-33-4; P-III, 132408-34-5; MP-Ia, 132376-52-4; MP-Ib (trimethylsilylethyl ester), 132376-51-3; MP-Ib (free acid), 132408-57-2; RNase T_1, 132490-94-9; RNase T_1 (mutant analog), 132490-95-0; H-S4-S5-V-G-OtBu, 132376-42-2; Z-S1-S2-S3-OPip, 132376-53-5; Z-S1-S2-S3-OH, 132376-54-6; BOC-S9-OSu, 132376-43-3; BOC-S9-S10-OH, 132376-44-4; BOC-S6-S7-S8-OH, 132408-35-6; BOC-S14-CT-OBzl, 132376-46-6; BOC-S13-S14-CT-OBzl, 132376-47-7; BOC-S11-OSu, 132376-48-8; BOC-S11-S12-OH, 132376-49-9; BOC-(P-II)-(P-III)-OBzl, 132408-37-8; z-(P-I)-(P-II)-(P-III)-OBzl, 132490-97-2; BOC-(MP-Ib)-(P-II)-(P-III)-OBzl, 132408-38-9; Z-(MP-Ia)-(MP-Ib)-(P-II)-(P-III)-OBzl, 132490-96-1; Gly-OBu-*t*·HCl, 27532-96-3; BOC-Thr(Bzl)-OBzl, 54276-70-9; BOC-Cys(MeBzl)-OH, 61925-77-7; H-CT-OBzl·HCl, 132376-45-5; BOC-ETV-OH, 132376-50-2; Gly-$OCH_2CH_2SiMe_3$·HCl, 106984-76-3.

Supplementary Material Available: Procedures and characterization data for the synthesis of the mutant RNase T_1 (5 pages). Ordering information is given on any current masthead page.

(47) Takahashi, K. *J. Biochem.* **1962**, *51*, 95-108.

Synthesis and Characterization of Oligonucleotide Peptides

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Monsanto Company, St. Louis, Missouri 63167

Received October 3, 1990

The transport and reactivity of oligonucleotides may be altered by attaching pendant peptides, and it is of interest to develop general synthetic methods for such bioconjugates. Two protecting group strategies are described for the synthesis of nucleotide peptides containing a lysine residue. The preparation of a lysine-nucleopeptide phosphoramidite reagent is described, along with its use in solid-phase DNA synthesis. Di- and trinucleotides were prepared with pendant and extensively characterized by NMR. These studies showed the peptide side chains to have survived DNA synthesis conditions; we then incorporated nucleopeptide residues into longer oligonucleotides. A similar approach is described for the preparation of oligonucleotide histidines. Previously reported histidine-nucleopeptides serve as precursors to phosphoramidites and to phosphodiester DNA building blocks. Both solution- and solid-phase techniques are presented for the preparation of histidine-containing oligonucleotides. The methodology developed here allows the incorporation of nucleopeptide residues at internal positions in a DNA sequence, using standard reagents. We present a complete description of the synthesis, purification, and characterization (via mass spectral and NMR methods) of the novel compounds.

Introduction

Covalent interactions between nucleic acids and amino acids, peptides, or proteins are of increasing interest. Examples of these bioconjugates occur naturally in the genome of certain RNA and DNA viruses (including poliovirus). In these molecules, which are thought to play a role in viral replication, the protein-nucleic acid linkage occurs through a 5′-phosphodiester bond to serine, tyrosine, or threonine.[1] Another naturally occurring example of RNA-amino acid conjugates is the family of aminoacyl-tRNA molecules, in which the amino acid is esterified to the 2′ or 3′ hydroxyl group of tRNA.[2] In addition to these naturally occurring conjugates, several synthetic examples are known: DNA-enzyme conjugates have been used as sequence-specific, oligonucleotide-directed nucleases[3] and poly-L-lysine has been conjugated to synthetic oligonucleotides to improve their transport into cells.[4]

(1) Vartapetian, A. B.; Bogdanov, A. A. *Prog. Nucleic Acids Res. Mol. Biol.* **1987**, *34*, 209-251.

(2) Stryer, L. *Biochemistry*, 2nd ed.; W. H. Freeman: New York, 1981; p 620.

(3) (a) Corey, D.; Schultz, P. *Science* **1987**, *238*, 1401-1403. (b) Zuckerman, R.; Corey, D.; Schultz, P. *J. Am. Chem. Soc.* **1988**, *110*, 1614-1615. (c) Zuckerman, R.; Schultz, P. *Ibid.* **1988**, *110*, 6592-6594. (d) Zuckerman, R.; Schultz, P. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1766-1770.

(4) Lemaitre, M.; Bayard, B.; Lebleu, B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 648-652.

0022-3263/91/1956-3168$02.50/0

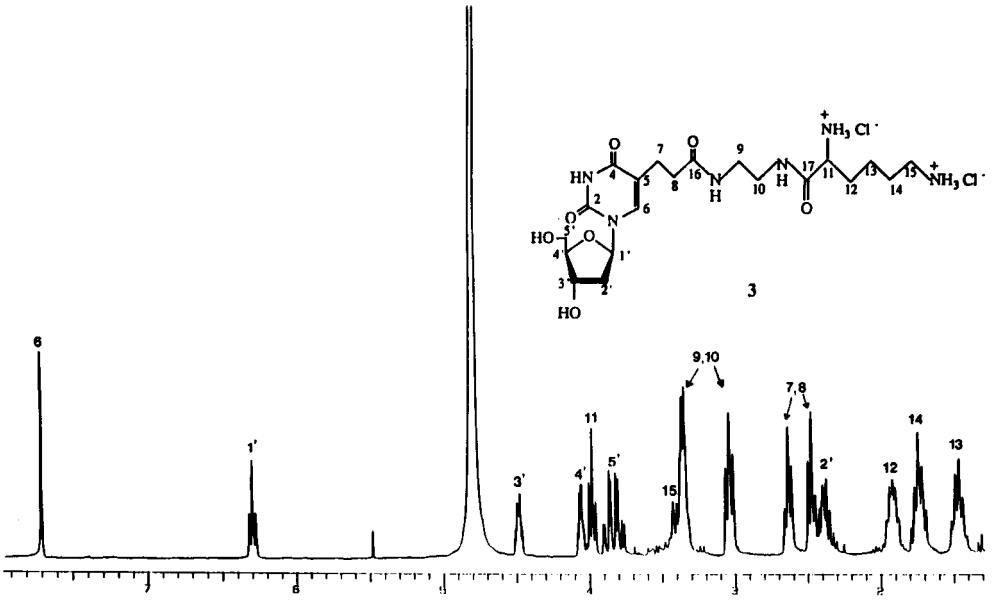


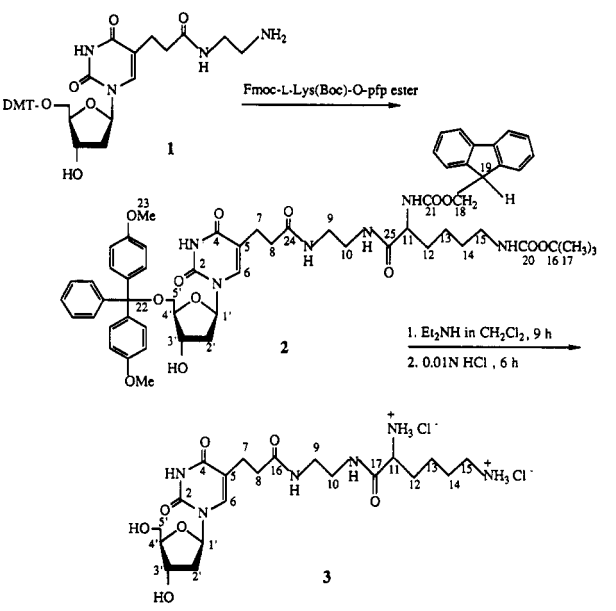
Figure 1. ^{1}H NMR spectrum of **3** (32 transients).

We have been pursuing the preparation of sequence-selective chemical ribonucleases as functional mimics of ribozymes[5] and as potential catalytic anitsense DNA probes.[6] Our approach to the synthesis of chemical nucleases was to covalently link oligonucleotides to RNA hydrolysis catalysts. For this it was necessary to identify well-defined RNA hydrolysis catalysts and to develop the synthetic methods for attaching them to DNA.

Because metal *ions* were known to catalyze RNA hydrolysis,[7] we explored the use of metal *complexes* in our program. Earlier, we reported the first examples of RNA hydrolysis by characterized metal complexes[8] and prepared hydrolytically active, covalent conjugates of thymidine and 2′-deoxyuridine with (2,2′-bipyridyl)Cu(II).[9] It was also known[10] that imidazole catalyzes the hydrolysis of RNA, so we wished to develop the synthetic methodology to allow the incorporation of pendant imidazole groups into oligonucleotides. The synthesis and characterization of a series of monoimidazole–uridine conjugates was described in an earlier report.[11] Since it is known through the work of Breslow[12] that orientational effects are of great importance in imidazole-based hydrolysis catalysts, we wished to continue the systematic development of imidazole–DNA conjugates, so that the synthetic tools would be available to optimize the number and relative orientation of catalytic groups on an oligonucleotide.

In addition to the chemistry of imidazole-based nucleopeptides, we have explored the synthesis of L-lysine–

Scheme I

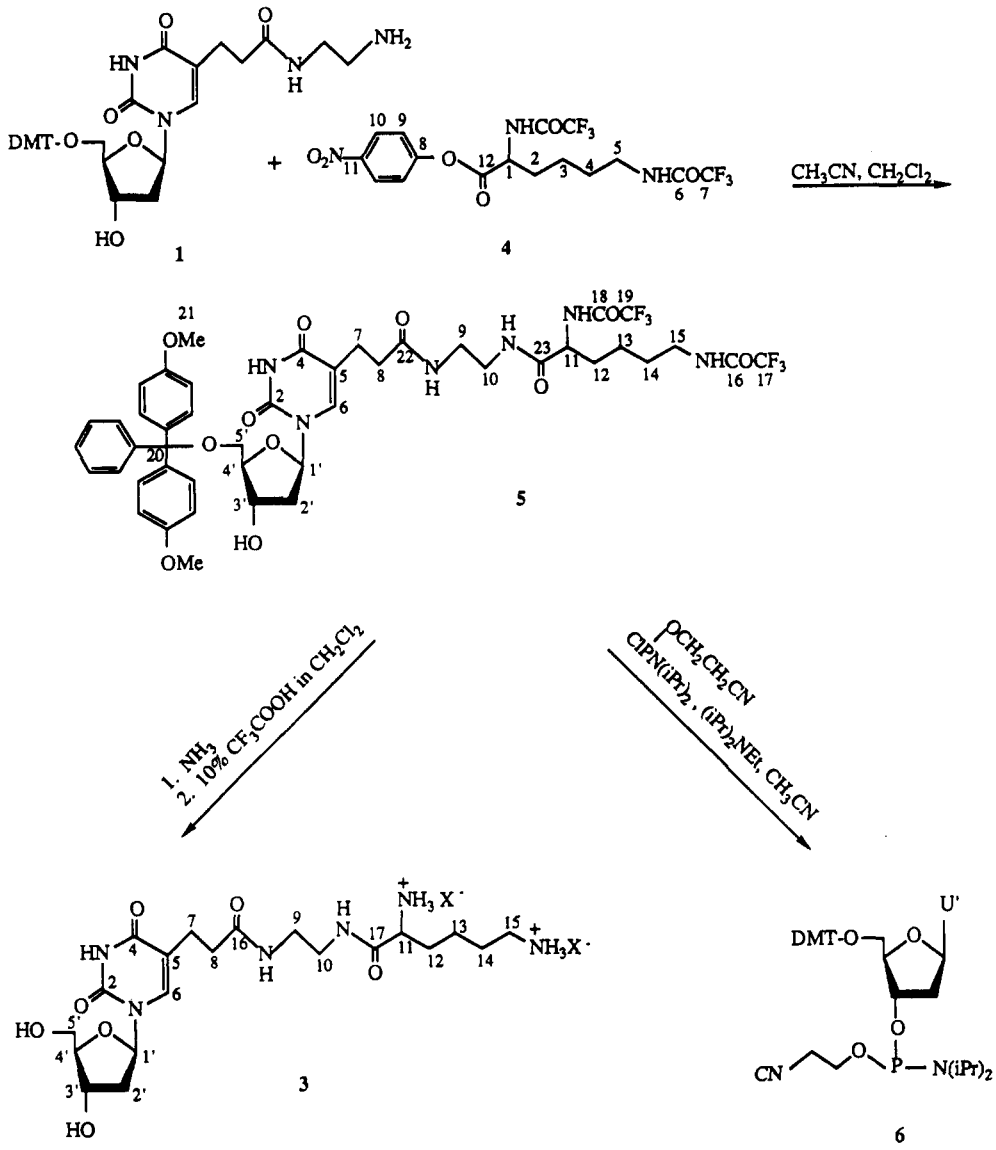


DNA conjugates because of our interest in transport of DNA into cells. Furthermore, we have previously used the formation of amide bonds, prepared from pendant amines and active esters, to link both imidazole derivatives[11] and metal complexes[9] to nucleosides, and we wished to employ the two amino groups of lysine as substrates for the attachment of multiple imidazoles or metal complexes.

The introduction of amino acid or peptide residues in oligonucleotides at internal positions in the chain was accomplished, utilizing suitably protected phosphoramidites with automated DNA synthesizers. We have used as our chemical starting point the versatile C-5 substituted 2′-deoxyuridine derivatives that are available from the pioneering work of Bergstrom and co-workers,[13] who developed the Heck reaction[14] for nucleoside and nucleotide substrates. Detailed chemical characterization of bioconjugates is rarely available, a deficiency that could hinder

(5) Cech, T. R. *Science* **1987**, *236*, 1532–1539.
(6) (a) Uhlman, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–584. (b) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165–187. (c) *OLIGODEOXYNUCLEOTIDES: Antisense Inhibitors of Gene Expression*; Cohen, J. S., Ed.; CRC Press: Boca Raton, FL, 1989.
(7) (a) Farkas, W. *Biochim. Biophys. Acta* **1968**, *155*, 401–409. (b) Butzow, J. J.; Eichhorn, G. *Biochemistry* **1971**, *10*, 2019–2027. (c) Butzow, J. J.; Eichhorn, G. *Nature* **1975**, *254*, 358–369. (d) Ikenaga, H.; Inoue, Y. *Biochemistry* **1974**, *13*, 577–582. (e) Brown, R. S.; Dewan, J. C.; Klug, A. *Biochemistry* **1985**, *24*, 4785–4801. (f) Behlen, L. S.; Sampson, J. R.; Direnzo, A. B.; Uhlenbeck, O. C. *Biochemistry* **1990**, *29*, 2515–2513.
(8) Stern, M. K.; Bashkin, J. K.; Sall, E. D. *J. Am. Chem. Soc.* **1990**, *112*, 5357–5359.
(9) Modak, A. S.; Gard, J. K.; Merriman, M. C.; Winkeler, K. A.; Bashkin, J. K.; Stern, M. K. *J. Am. Chem. Soc.* **1991**, *113*, 283–291.
(10) Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 4473–4482.
(11) Bashkin, J. K.; Gard, J. K.; Modak, A. S. *J. Org. Chem.* **1990**, *55*, 5125–5132.
(12) Anslyn, E.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 5872–5973.
(13) (a) Bergstrom, D. E.; Ogawa, M. K. *J. Am. Chem. Soc.* **1978**, *100*, 8106–8112. (b) Bergstrom, D. E.; Beal, P.; Hussain, A.; Lind, R.; Jenson, J. *J. Am. Chem. Soc.* **1989**, *111*, 374–375.
(14) Heck, R. F. *J. Am. Chem. Soc.* **1968**, *90*, 5518–5534.

Scheme II



the application of these complex molecules to biochemical or medicinal problems. We therefore present well-documented synthetic procedures for the preparation of our nucleoside- and oligonucleotide-peptides and definitive characterization of the products by mass spectrometry and multinuclear, 1- and 2-dimensional NMR. In order to determine if our protected amino acid side chains would survive automated DNA synthesis conditions, a series of di- and trinucleotides was prepared; the presence of the desired lysine or histidine groups could then be documented by NMR studies.

Results and Discussion

A. Lysine–2′-Deoxyuridine Derivatives. As shown in Schemes I and II, we synthesized L-lysine-based nucleopeptides using two different approaches to the protecting groups. Thus, the reaction of 1 with Fmoc-L-Lys(Boc)-O-pfp ester[15] yields the protected nucleopeptide 2 in 72% isolated, purified yield. On sequential deprotection with diethylamine and 0.01 N HCl, the product 3 is obtained in 92% isolated yield. High-resolution FAB mass spectra of 2 and 3 were obtained to confirm their composition. The ^1H and ^{13}C NMR spectra of 3 are given in Figures 1 and 2, and complete spectral assignments of 2 and 3 are provided in the Experimental Section, using the numbering system shown in Scheme I.

Because of the ease with which trifluoroacetyl groups are removed under standard DNA deprotection conditions, we also prepared L-lysine–DNA conjugates using an active ester of the trifluoroacetyl-protected amino acid 4. Scheme II shows the synthesis of nucleopeptide 3 from 4 via the protected nucleopeptide 5. Also depicted in Scheme II is the conversion of 5 into the reagent 6, which is a diisopropyl (β-cyanoethyl) phosphoramidite designed for use in automated DNA synthesis. Low-resolution mass spectra were obtained for compounds 5 and 6. Complete ^{13}C and ^1H NMR assignments are provided for 5; the ^{31}P NMR spectrum of 6 showed the 149 ppm chemical shift characteristic of dialkylamino phosphoramidites.

Phosphoramidite 6 was successfully employed in the solid-phase synthesis of di-, tri-, and oligonucleotides, as shown in Scheme III. Thus, coupling of 6 to solid-supported thymidine yielded dinucleotide YpT (7) after deprotection. We extended this procedure to the preparation of trinucleotide TpYpT (8) with the modified nucleoside at an internal position, and the ^1H NMR spectrum of 8

(15) Fmoc, 9-fluorenylmethyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; pfp, pentafluorophenyl; pnp, *p*-nitrophenyl.

(16) (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859–62. (b) Sinha, N. D.; Biernat, J.; Koester, H. *Tetrahedron Lett.* **1983**, *24*, 5843–6.

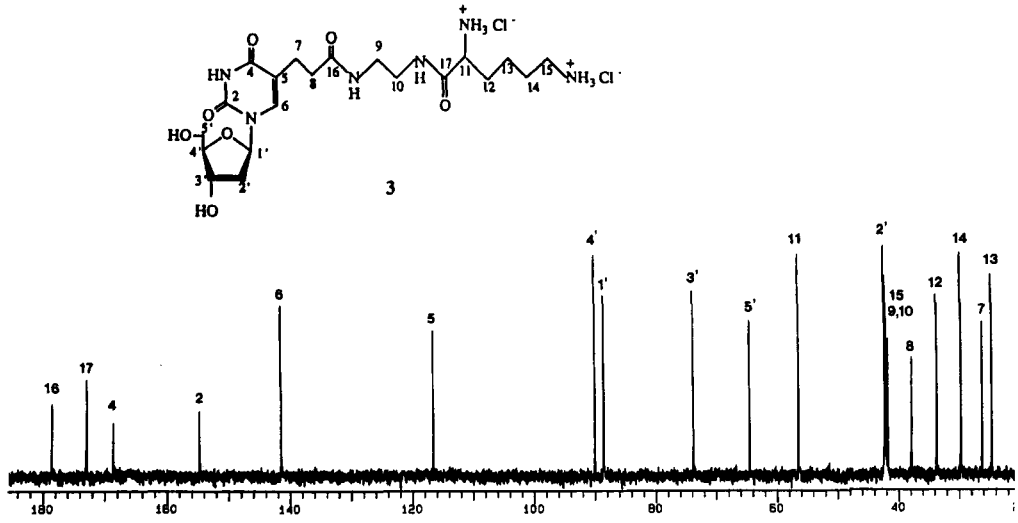
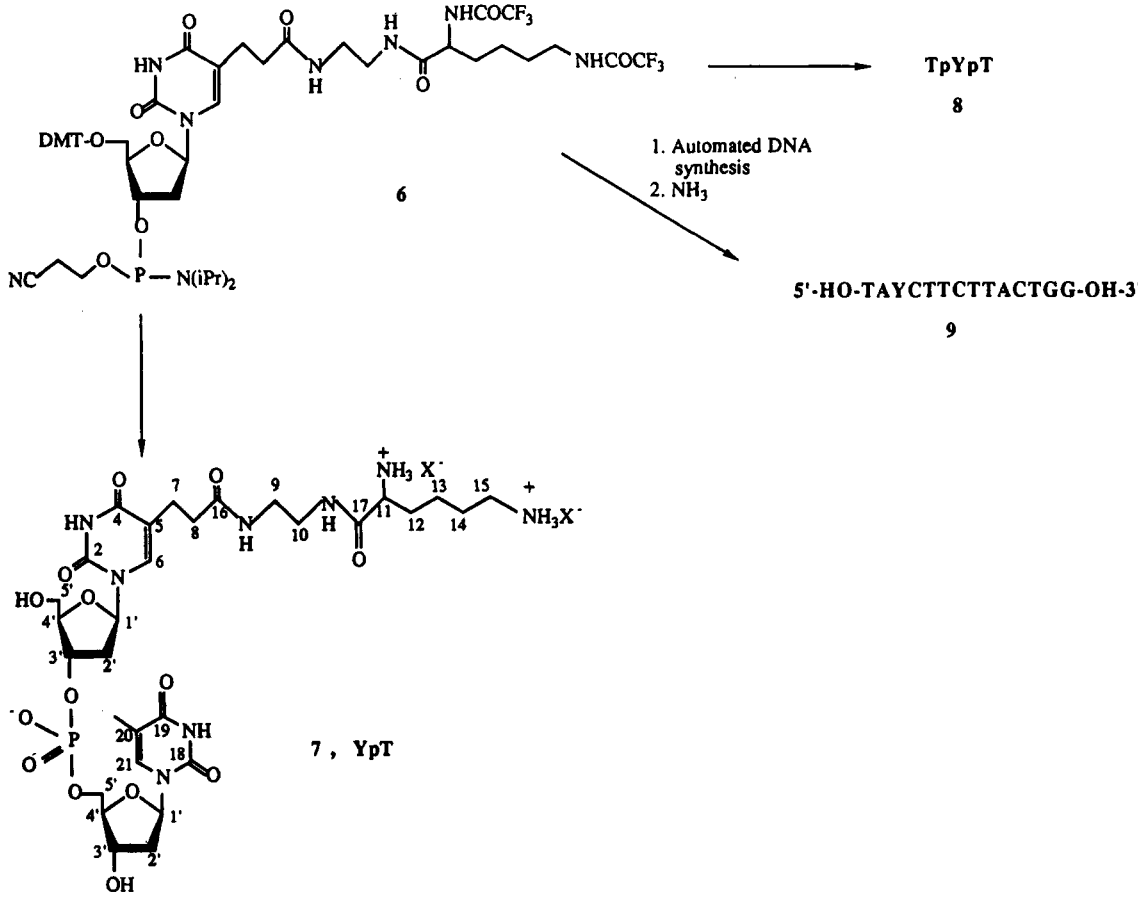


Figure 2. ^{13}C NMR spectrum of **3** resulted from accumulating 4000 transients in a 25-KHz spectral window using a 40° pulse (9 μs) and a recycle delay of 0.8 s. The accumulated free induction decay was zero filled to 32K points and Fourier transformed with 1.0-Hz line broadening.

Scheme III



is given in Figure 3. Compounds **7** and **8** were purified by reverse phase (RP) HPLC, followed by desalting on an RP column. Having demonstrated the synthesis of smaller oligonucleotides with compound **6**, we then tested its general utility by preparing 14-mer **9**. In this case, anion exchange (AX) HPLC was used to purify the product, followed by desalting on Sephadex G-25, and 90 OD units were obtained after these purification steps.

B. Histidine–2′-Deoxyuridine Derivatives. Two complementary approaches were explored for the synthesis of histidine–DNA conjugates: solution-phase phosphotriester chemistry and solid-phase phosphoramidite chemistry. Both approaches employ the previously reported[11] compound **10**. Thus, Scheme IV shows the use of **10** in the preparation of dinucleotide XpC (compound **13**), using the *o*-chlorophenyl phosphate ester technique.[17] Intermediate phosphodiester **11** was prepared in 82% yield and characterized by FABMS. The fully protected phosphotriester **12** was prepared in 49% isolated yield; FABMS showed ions corresponding to M + 3Li, M + 2Li, M + Li, and M + 3Li-Boc. After deprotection of **12** and

(17) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143–79.

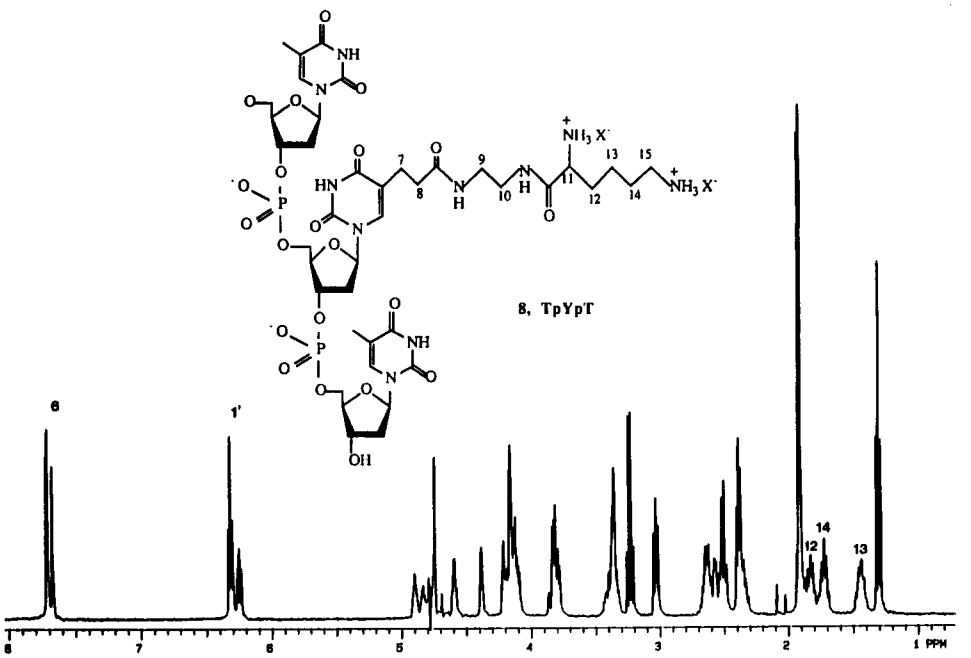
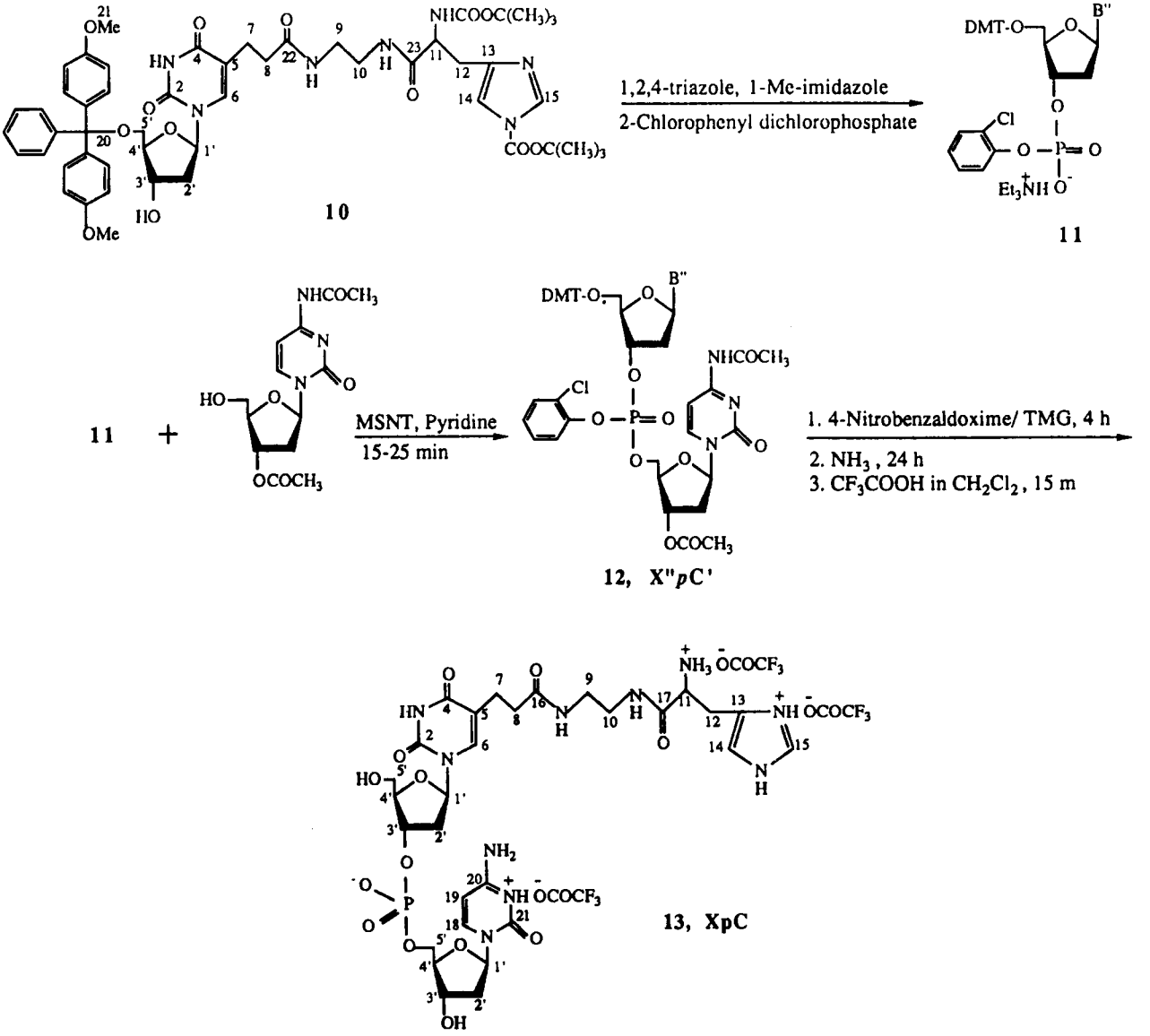


Figure 3. ^{1}H NMR spectrum of trinucleotide 8 (128 transients).

Scheme IV



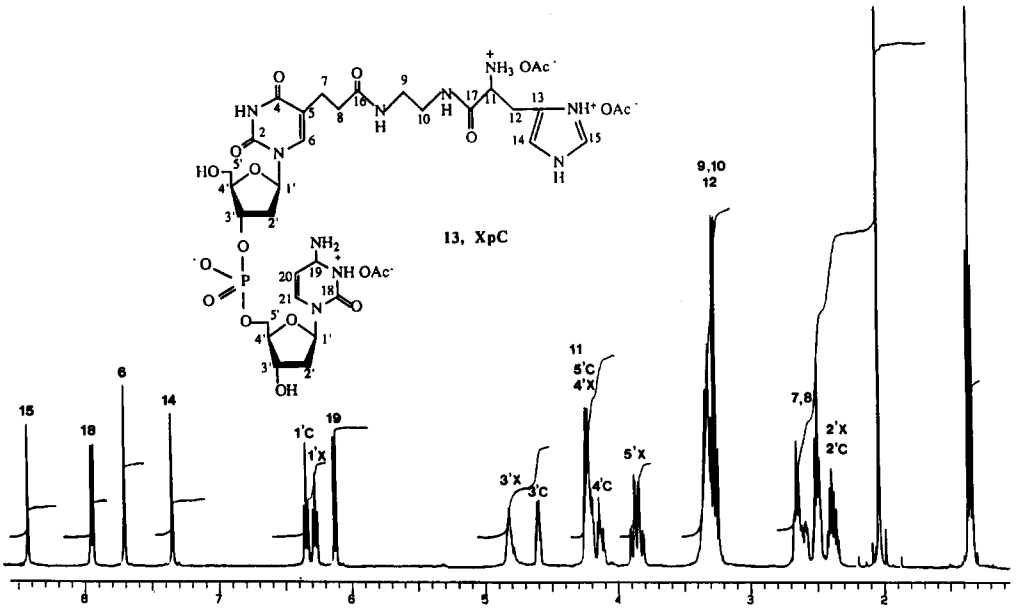


Figure 4. ^{1}H NMR spectrum of dinucleotide **13** (16 transients).

purification of the product by reverse phase HPLC, the desired dinucleotide **13** was obtained. The ^{1}H NMR spectrum of **13** is given in Figure 4, and full assignments are provided in the Experimental Section, along with ^{13}C and ^{31}P NMR data. We have found 2-D NMR spectroscopy to be invaluable in providing unequivocal assignments for the NMR spectra of nucleopeptides.[11] Due to the ambiguities with regard to the assignments of the sugar protons of **13**, we turned to a ^{1}H–^{1}H correlation experiment for this task. The double quantum filtered COSY was selected because phase-sensitive multiple quantum techniques[18] have advantages over the original absolute value experiments[19] (spectral simplification is achieved because signals are observed only for coupled spins, and the dispersive character of the diagonal is reduced). The results of this experiment performed on **13** are presented as a contour plot in Figure 5. The 5′ sugar protons of the modified nucleoside (X) at δ 3.8 are not obscured or influenced by the 3–5′ phosphate linking in the dinucleotide and were selected to serve as the starting point for making the ^{1}H–^{1}H correlations in the sugar ring. Tracing the ^{1}H connectivities through the sugar ring of the modified nucleoside 5′X to 1′X can be accomplished in spite of numerous overlapping resonances (4′X, 4′C, 5′C, and 11) and a large water resonance that obscures 3′X. On the basis of the observed correlations, the triplet at δ 6.25 is assigned as the 1′X proton. It is then possible to assign the remaining triplet at δ 6.3 to the anomeric proton on cytidine, 1′C; this assignment was confirmed by tracing the connectivities from 1′C to the 4′ cytidine proton. Additional detailed assignments are listed in the Experimental Section.

Scheme V shows the preparation of phosphoramidite **14** and its use in the solid-phase synthesis of the dinucleotide XpT, compound **15**. Phosphoramidite **14** has the characteristic ^{31}P resonances at 149.4 ppm assignable to its two diastereomers; it was further characterized by FABMS and HRMS. The ^{1}H NMR spectrum of **15** is shown in Figure 6, and the histidine group is clearly intact; peak assignments are given in the Experimental Section. Phosphoramidite **14** was then employed in the synthesis of an 11-mer oligonucleotide with the modified nucleoside at an internal position to test its general applicability. The sequence prepared, 5′-TATCTTCTXAC-3′, was purified

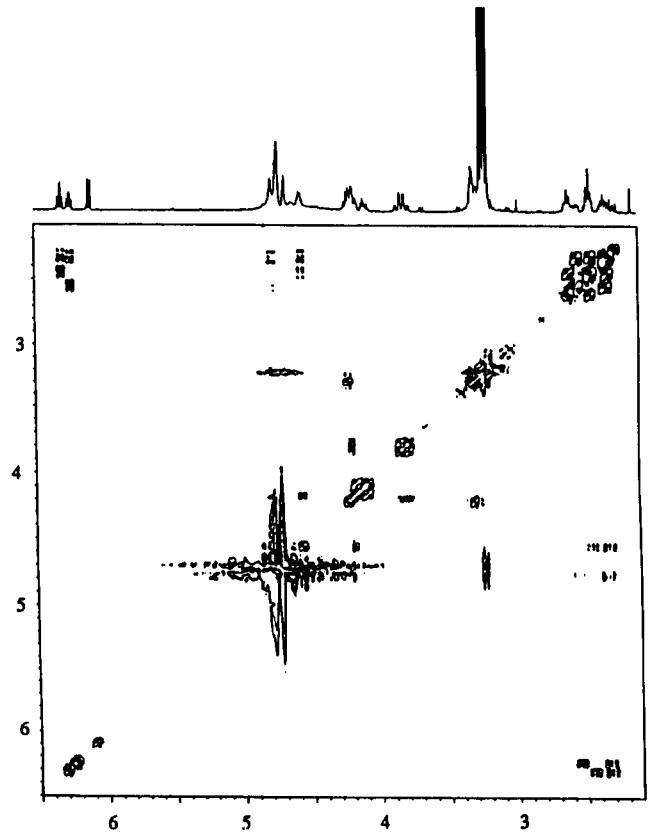


Figure 5. The double quantum filtered COSY spectrum of **13** resulted from a 2 × 512 × 2048 data matrix, with acquisition times of 128 and 256 ms in t_1 and t_2 dimensions, respectively. The delay between scans (including the 256 ms t_2 acquisition period) was 5.256 s. For each t_1 value, 4 steady-state scans were applied and 32 transients were accumulated, yielding a total measuring time of 53.8 h. Prior to Fourier transformation, the data matrix was zero filled to 2048 in the t_1 dimension and sinebell weighting functions were applied in the t_2 and t_1 domains, respectively. Data processing was performed on a Sun Microsystem Sparc1 computer. Only the region containing sugar residue protons is shown above.

(18) Rance, M.; Sorenson, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479–485.
(19) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1962**, *64*, 2229–2246.

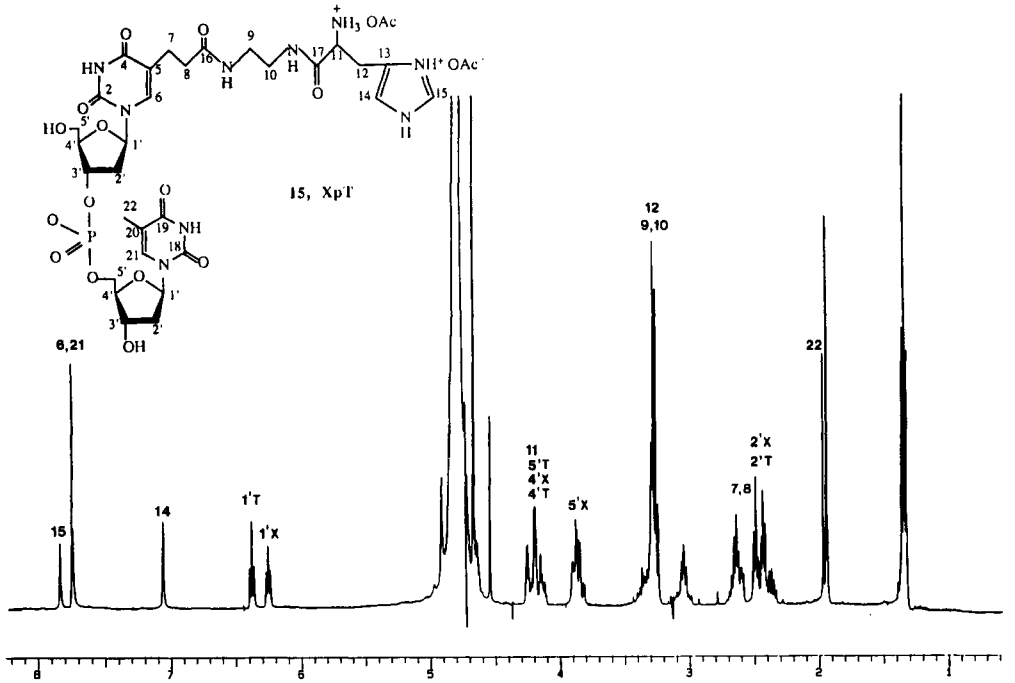
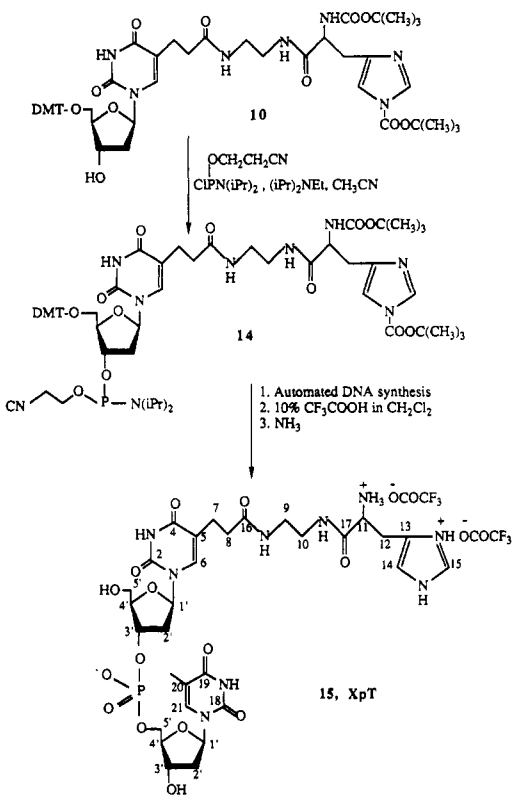


Figure 6. ^{1}H NMR spectrum of dinucleotide **15** (32 transients).

Scheme V



by AX HPLC and Sephadex desalting, yielding 90 OD units.

Conclusions

Covalently linked DNA–peptide conjugates occur widely in nature, and they are synthetic targets because of the altered transport and reactivity properties that groups such as histidine and lysine may provide to oligonucleotides. As part of our program to develop systematic and well-characterized synthetic routes to such DNA derivatives,[11] we have described the synthesis and detailed characterization of novel lysine–nucleopeptides. A phosphoramidite reagent was prepared that allowed the synthesis of di-, tri-, and oligonucleotides containing the lysine–nucleopeptide moiety. Detailed NMR spectral characterization of the small oligomers indicates that the side chains survive DNA synthesis and deprotection conditions. In addition, we have provided examples of both solution-phase and solid-phase strategies for the incorporation of histidine–nucleopeptides into oligonucleotides. Extensive NMR and mass spectral characterization of histidine dinucleotides and their precursors was also presented.

Experimental Section

General. Melting points were determined with a calibrated thermometer. All nuclear magnetic resonance spectra were recorded at 25 °C. The proton spectra resulted from Fourier transformation of the accumulated scans, consisting of 30016 data points in a 8 KHz spectral width with an aquisition time of 1.876 s. Data were acquired with a 35° pulse (10 μs) and, where necessary, the strong H_2O resonance was presaturated for 3.0 s. The free induction decays were zero filled to 32K and 0.5-Hz line broadening was applied to the data prior to Fourier transformation. Exchangeable protons are labeled (ex). The ^{1}H NMR spectra of compounds **8**, **13**, and **15** have triethylammonium acetate peaks at δ 1.3 (t), 2.0 (s) and 3.2 (q).

The FAB matrix for FABMS was a saturated solution of LiI in 3-nitrobenzyl alcohol, which is especially useful for acid-labile, protected nucleosides. Thin layer chromatography was performed on silica gel plates and spots were visualized by irradiation with UV light (254 nm). Compounds were dried over $MgSO_4$. Automated DNA synthesis was performed on a Pharmacia Gene Assembler; extended coupling times of 45 min were used for the modified phosphoramidites, and freshly prepared phosphoramidites were stored over activated 4A molecular sieves prior to use. In all cases, two 10-μmol syntheses were carried out and combined.

Preparative TLC was carried out by radial TLC using silica gel plates. Column chromatography was performed on silica gel. Reverse phase (RP) HPLC was carried out on an Alltech Econosil C18 preparative column (10 μm, 250 × 22.5 mm) for di- and trinucleotides using a linear ternary gradient flowing at 6 mL/min: solvent A (0.1 M [Et_3NH]OAc) was kept constant at 25%, while B (MeCN) and C(H_2O) were varied as follows, where *time* is in min (time, % B, % C) (*0*, 5, 70) (*33*, 35, 40) (*45*, 70, 5). Analytical RP HPLC was done on a C18 column with the same gradient,

at a flow rate of 1.5 mL/min. Longer oligonucleotides were purified on a Nucleogen-DEAE 60-7 AX HPLC column (10 × 125 mm) using a linear binary gradient flowing at 2 mL/min: solvent A (80% 0.1 M NaOAc, 20% MeCN) and solvent B (20% MeCN, 80% 1 M LiCl and 0.02 M NaOAc) were varied as follows, where *time* is in min (*time*, % A, % B) (*0*, 100, 0) (*3*, 100, 0) (*103*, 0, 100) (*108*, 0, 100). Analytical AX HPLC was run on a 4 × 125 mm Nucleogen 60-7 column at 1.5 mL/min with the following gradient: (*0*, 100, 0) (*33*, 0, 100) (*43*, 100, 0). The HPLC was monitored simultaneously at 260 and 400 nm. Desalting was carried out for di- and trinucleotides by loading them onto an analytical RP column in water, washing with water, and eluting with MeCN. Longer oligonucleotides were desalted on a 2.5 × 30 cm Sephadex G-25 medium pressure column, eluting with water and monitoring at 254 nm. When Sephadex was used for the dimers and trimers, no product was recovered off the column.

The compounds *p*-nitrophenol, L-histidine (Sigma Chemicals), Fmoc-L-Lys(Boc)-Opfp (Pharmacia), dicyclohexylcarbodiimide, diethylamine, diisopropylethylamine (Aldrich), and chloro(*N,N*-diisopropylamino)(β-cyanoethoxy)phosphine (ABN) were used without further purification.

5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-5-[3-[[2-[[6-[[(1,1-dimethylethoxy)carbonyl]amino]-2-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-1-oxohexyl]amino]ethyl]-amino]-3-oxopropyl]-2′-deoxyuridine (2). A solution of 5-[3-[(2-aminoethyl)amino]-3-oxopropyl]-5′-*O*-DMT-2′-deoxyuridine (1) (0.644 g, 1 mmol) in CH_2Cl_2 (10 mL) and CH_3CN (10 mL) was cooled to 0 °C in an ice bath and Fmoc-L-Lys(Boc)-*O*-pfp (0.951 g, 1.5 mmol) was added to the stirred reaction mixture. After 15 min the ice bath was removed and the mixture stirred at room temperature for 16 h. The reaction mixture was concentrated; it was then chromatographed on a silica gel column, eluting with a gradient of 100% CH_2Cl_2 to 12% EtOH in CH_2Cl_2. The desired product 2 (0.788 g, 0.72 mmol, 72%) eluted with 10% EtOH in CH_2Cl_2 (R_f 0.4 by TLC): mp 166 °C; ^{13}C NMR ($CDCl_3$) ppm 84.9 C1′, 40.8 C2′, 72.3 C3′, 86.2 C4′, 63.8 C5′, 164.2 CO4, 150.5 CO2, 113.7 C5, 137.3 C6, 23.1 C7, 35.6 C8, 40.1 and 39.6 C9 and C10, 54.9 C11, 29.6 C12, 22.6 C13, 32.2 C14, 39.1 C15, 79.3 C16, 28.5 C17, 67.1 C18, 47.1 C19, 156.6 C20, 156.3 C21, 86.8 C22, 55.3 C23, 172.9 C24, 172.6 C25; ^1H NMR ($CDCl_3$) δ 6.35 (t, 1 H, H1′), 2.2 (m, 2 H, H2′), 4.3 (m, 1 H, H3′), 4.2 (m, 1 H, H4′), 3.4 (m, 2 H, H5′), 7.6 (s, 1 H, H6), 2 to 2.4 (m, 4 H, H7 and H8), 3.3 (m, 4 H, H9 and H10), 4.6 (m, 1 H, H11), 3 to 3.2 (m, 2 H, H12), 1.6 (s, 9 H, H17), 4.2 (t, 1 H, H19), 3.8 (s, 6 H, H23); FABMS *m/z* 1102 (M + Li), 1074 (M + Li–CO), 798 (M + Li–DMT), 584 (M + Li – 5′-DMT-D-U); exact mass found 1102.2089, calcd for $C_{61}H_{70}N_6O_{13}Li$ 1102.20945.

5-[3-[[2-[(2,6-Diamino-1-oxohexyl)amino]ethyl]amino]-3-oxopropyl]-2′-deoxyuridine Dihydrochloride (3) from 2. A solution of 2 (0.548 g, 0.5 mmol) in CH_2Cl_2 (5 mL) was treated with diethylamine (3 mL) and left stirring for 9 h. The reaction mixture was then concentrated to remove all traces of diethylamine. The solid was triturated with diethyl ether (3 × 20 mL) and decanted to yield a white solid. The solid was then dissolved in methanol (5 mL) and treated with 0.01 N HCl, pH 2 (5 mL), for 6 h. The aqueous mixture was washed with CH_2Cl_2 (2 × 20 mL) and then lyophilized twice to yield the final product 3 (0.220 g, 0.468 mmol, 92%): ^{13}C NMR (D_2O) ppm 88.1, C1′, 41.9 C2′, 73.3 C3′, 89.5 C4′, 64.1 C5′, 168.3 CO4, 154.4, CO2, 116.1 C5, 141.2 C6, 25.8 C7, 37.4 C8, 41.8 and 41.3 C9 and C10, 56.0 C11, 33.2 C12, 24.2 C13, 29.3 C14, 41.3 C15, 178.2 C16, 172.6 C17; ^1H NMR (D_2O) δ 6.25 (t, 1 H, H1′), 2.35 (m, 2 H, H2′), 4.45 (m, 1 H, H3′), 4.0 (m, 1 H, H4′), 3.8 (m, 2 H, H5′), 7.65 (s, 1 H, H6), 2.5 (2 t's, 4 H, H7 and H8), 3.2 (2 t's, 4 H, H9 and H10), 3.95 (t, 1 H, H11), 1.9 (m, 2 H, H12), 1.4 (m, 2 H, H13), 1.7 (m, 2 H, H14), 3.3 (m, 2 H, H15); FABMS *m/z* 471 (M + H); exact mass found 477.4682, calcd for $C_{20}H_{34}N_6O_7Li$ 477.4689.

N^{α},N^{ϵ}-Bis(trifluoroacetyl)-L-Lys-*O*-pnp Ester (4). Bis-(trifluoroacetyl)-L-Lys-OH[20] (9.06 g, 27 mmol) and *p*-nitrophenol (3.92 g, 29 mmol) were dissolved in EtOAc (50 mL), and the mixture was cooled to 0 °C in an ice bath. DCC (5.72 g, 27 mmol) was added to this mixture in small portions and the mixture stirred at 0 °C for 30 min. The ice bath was removed and the mixture allowed to stir at room temperature for 12 h. The urea that precipitated was filtered off, and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 (300 mL) and washed with water (100 mL). The dried organic extract was concentrated and the product crystallized from CH_2Cl_2 and petroleum ether to yield the active ester 4 (6.36 g, 13.9 mmol, 51%): mp 145 °C; ^{13}C NMR ($CDCl_3$) δ 53.0 C1, 29.9 C2, 22.5 C3, 28.0 C4, 38.9 C5, 157.3 C6, 116.0 C7, 154.9 C8, 125.2 C9, 122.4 C10, 145.7 C11, 168.5 C12; ^1H NMR ($CDCl_3$) δ 1.35 (m, 2 H, H3), 1.5 (m, 2 H, H4), 1.9 (m, 2 H, H2), 3.2 (m, 2 H, H5), 4.5 (m, 1 H, H1), 7.2 (d, 2 H, H10, *J* = 9.1 Hz), 8.1 (d, 2 H, H9, *J* = 9.1 Hz), 7.7 (d, 1 H, NH ex), 7.3 (br s, 1 H, NH ex); FABMS *m/z* 466 (M + Li); exact mass found 466.1090, calcd for $C_{16}H_{15}F_6N_3O_6Li$ 466.1025.

5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-5-[3-oxo-3-[[2-[[1-oxo-2,6-bis[(trifluoroacetyl)amino]hexyl]amino]-ethyl]amino]propyl]-2′-deoxyuridine (5). A solution of 5-[3-[(2-aminoethyl)amino]-3-oxopropyl]-5′-O-DMT-2′-deoxyuridine (1) (1.0 g, 1.55 mmol) in CH_2Cl_2 (10 mL) and CH_3CN (10 mL) was cooled to 0 °C in an ice bath, and L-Lys(bis(trifluoroacetyl))-*O*-pnp (4) (1.07 g, 2.33 mmol) was added to the stirred reaction mixture. After 15 min the ice bath was removed and the mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo and flash chromatographed on a silica gel column, eluting with a gradient of CH_2Cl_2 to 12% EtOH in CH_2Cl_2. The desired product 5 (1.02 g, 1.06 mmol, 68%) eluted with 10% EtOH in CH_2Cl_2: mp 156 °C; ^{13}C NMR ($CDCl_3$) ppm 84.9 C1′, 40.8 C2′, 72.3 C3′, 86.2 C4′, 63.8 C5′, 164.2 CO4, 150.5 CO2, 113.7 C5, 137.3 C6, 23.1 C7, 35.6 C8, 40.1 and 39.6 C9 and C10, 54.9 C11, 29.6 C12, 22.6 C13, 32.2 C14, 39.1 C15, 157.0 C16 and C18, 116.0 C17 and C19, 86.8 C20, 55.3 C21, 172.8 C22, 172.4 C23; ^1H NMR ($CDCl_3$) δ 6.35 (t, 1 H, H1′), 2.2 (m, 2 H, H2′), 4.3 (m, 1 H, H3′), 4.2 (m, 1 H, H4′), 3.4 (m, 2 H, H5′), 7.6 (s, 1 H, H6), 2 to 2.4 (m, 4 H, H7 and H8), 3.3 (m, 4 H, H9 and H10), 4.6 (m, 1 H, H11), 3 to 3.2 (m, 2 H, H12), 1.9 (m, 2 H, H12), 1.4 (m, 2 H, H13), 1.7 (m, 2 H, H14), 3.3 (m, 2 H, H15), 3.8 (s, 6 H, H21); FABMS *m/z* 966, (M + H); exact mass found 971.86428, calcd for $C_{45}H_{50}F_6N_6O_{11}Li$ 971.86325.

5-[3-[[2-[(2,6-Diamino-1-oxohexyl)amino]ethyl]amino]-3-oxopropyl]-2′-deoxyuridine Bis(trifluoroacetate) (3) from 5. A solution of 5 (0.096 g, 0.01 mmol) in EtOH (5 mL) was treated with aqueous ammonia (10 mL) and left stirring for 12 h. The reaction mixture was then concentrated and lyophilized three times to remove all traces of ammonia. The solid was triturated with diethyl ether (3 × 20 mL) and decanted off. The solid was then suspended in CH_2Cl_2 (5 mL) and treated with 10% CF_3COOH in CH_2Cl_2 (5 mL) for 15 min. The mixture was concentrated to a glass and dissolved in water (10 mL). The aqueous layer was washed with CH_2Cl_2 (2 × 10 mL) and then lyophilized twice to yield the final product 3 (0.043 g, 0.009 mmol, 90%): ^{13}C NMR (D_2O) ppm 88.6 C1′, 42.3 C2′, 73.8 C3′, 89.9 C4′, 64.6 C5′, 168.8 CO4, 154.8 CO2, 116.6 C5, 141.6 C6, 26.2 C7, 37.8 C8, 42.2 and 41.7 C9 and C10, 56.5 C11, 33.7 C12, 24.7 C13, 29.7 C14, 42.1 C15, 178.7 C16, 173.1 C17; ^1H NMR (D_2O) δ 6.3 (t, 1 H, H1′), 2.4 (m, 2 H, H2′), 4.45 (m, 1 H, H3′), 4.05 (m, 1 H, H4′), 3.8 (m, 2 H, H5′), 7.7 (s, 1 H, H6), 2.55 (2 t's, 4 H, H7 and H8), 3.2 (2 t's, 4 H, H9 and H10), 3.95 (t, 1 H, H11), 1.9 (m, 2 H, H12), 1.45 (m, 2 H, H13), 1.7 (m, 2 H, H14), 3.4 (m, 2 H, H15); FABMS *m/z* 477 (M + Li); exact mass found 477.4682, calcd for $C_{20}H_{34}N_6O_7Li$ 477.4689.

5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-5-[3-oxo-3-[[2-[[1-oxo-2,6-bis[(trifluoroacetyl)amino]hexyl]amino]-ethyl]amino]propyl]-2′-deoxyuridine 3′-*O*-(*N,N*-Diisopropylamino)(β-cyanoethoxy)phosphine (6). Chloro(*N,N*-diisopropylamino)(β-cyanoethoxy)phosphine (0.217 g, 1.1 mmol) was weighed into an H-shaped Schlenk flask and dissolved in acetonitrile (5 mL). Diisopropylethylamine (0.280 mL, 2 mmol) was added to the reaction vessel and the mixture was stirred at room temperature for 20 min. The nucleoside 5 (0.964 g, 1 mmol) dissolved in acetonitrile (5 mL) was added to the phosphorylating mixture and left stirring for 30 min. The mixture was then filtered through the frit to the other side to remove the amine hydrochloride. The solid was washed with acetonitrile (2 × 10 mL) and concentrated to yield a glass. The glass was then chromatographed on a silica gel chromatotron plate (2000 μm). The product 6 (0.792 g, 0.68 mmol, 68%) was eluted with CH_2Cl_2/EtOAc/Et_3N 3:6:1: ^{31}P NMR (CD_3CN) ppm 2 s's at 149.1 and 149.2; FABMS *m/z* 1171 (M + Li).

(20) Kricheldorf, H. R.; Fehrle, M. *Synthesis* **1974**, 420–2.

YpT (7) and TpYpT (8). The phosphoramidite **6** was used on a DNA synthesizer to prepare the dinucleotide 5′-*O*-DMT-Y″pT-O-S-3′ and the trimer 5′-O-DMT-TpY″pT-O-S-3′. The resulting compounds **7** and **8** were cleaved off the support and deprotected with aqueous NH_3 (0.88 d); they were purified on a C18 HPLC column and desalted. Retention times for **7** (36 OD units) and **8** (26 OD units) are 14.4 and 15.5 min, respectively, on an analytical C-18 column.

Compound **7**: ^{1}H NMR (D_2O) δ 6.2 (t, 1 H, 1′Y), 6.3 (t, 1 H, 1′T), 2.4 (m, 2 H, 2′Y), 2.35 (m, 2 H, 2′T), 4.75 (m, 1 H, 3′Y), 4.65 (m, 1 H, 3′T), 4.1 (m, 1 H, 4′Y), 4.0 (m, 1 H, 4′T), 3.8 (m, 2 H, 5′Y), 4.2 (m, 2 H, 5′T), 7.7 (s, 1 H, H6), 2.55 (2 t's, 4 H, H7 and H8), 3.2 (2 t's, 4 H, H9 and H10), 3.95 (t, 1 H, H11), 1.9 (m, 2 H, H12), 1.45 (m, 2 H, H13), 1.7 (m, 2 H, H14), 3.4 (m, 2 H, H15).

5′-HO-TAYCTTCTTACTGG-OH-3′ (9). The phosphoramidite **6** was used on a DNA synthesizer to prepare the 14-mer 5′-OH-TpA′pY″pC′pTpTpC′pTpTpA′pC′pTpG″pG″-O-S-3′, which was fully deprotected with aqueous NH_3 (0.88 d) and purified by AX HPLC followed by desalting. The overall coupling efficiency was 94%, as calculated from trityl yields by Pharmacia's algorithm. The oligo **9** (90 OD units purified yield) eluted from an analytical AX column at 25.3 min.

5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-5-[3-[[2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-[1-[(1,1-dimethylethoxy)carbonyl]-1*H*-imidazol-4-yl]-1-oxopropyl]amino]ethyl]amino]-3-oxopropyl]-2′-deoxyuridine 3′-(*o*-Chlorophenyl phosphate) (11). *o*-Chlorophenyl phosphorodichloridate (0.897 g, 3.66 mmol) dissolved in acetonitrile (10 mL), 1,2,4-triazole (0.556 g, 8.052 mmol), and triethylamine (1.02 mL, 7.32 mmol) were added to the reaction vessel, and the mixture was stirred at room temperature for 20 min. The nucleoside **10** was dissolved in acetonitrile (10 mL), and 1-Me-imidazole (0.1 mL, 4.88 mmol) was added to the stirred solution. This reaction mixture was added to the phosphorylating mixture and stirred at room temperature for 20 min. The reaction was monitored on TLC, and after all of the starting material was consumed, the mixture was quenched with triethylamine (3.06 mL, 21.96 mmol) and water (10 mL) to give a homogeneous solution. The solution was stirred for 10 min and then concentrated. The residue was dissolved in dichloromethane (25 mL) and washed with saturated $NaHCO_3$ (25 mL). The aqueous layer was washed with dichloromethane (2 × 20 mL), and the combined organic extracts were dried and concentrated. The resulting glassy material was dissolved in dichloromethane (10 mL) and precipitated from petroleum ether (500 mL). The solid phosphodiester **11** (1.16 g, 0.91 mmol, 82%) was collected by centrifugation and dried: FABMS *m/z* 1171 (M − H), 1071 (M − H − Boc).

Dimer X″pC′ (12). The phosphodiester **11** (0.427 g, 3.36 mmol) was dissolved in dry pyridine (5 mL). 4-*N*,3′-*O*-Diacetyl-2′-deoxycytidine (0.095 g, 3.05 mmol) was added to it and the pyridine was removed under reduced pressure. The process of addition and removal of pyridine was carried out twice to remove traces of moisture. 1-(2-Mesitylenesulfonyl)-3-nitro-1,2,4-triazole (0.361 g, 12.2 mmol) was then added to the solution of the two nucleosides and stirred at room temperature for 20–25 min. The mixture was then quenched with 1 mL of saturated aqueous $NaHCO_3$. Dichloromethane (100 mL) was added to the reaction mixture after 5 min, and the organic phase was washed with water (50 mL). The organic extracts were dried and concentrated. The resulting glass was chromatographed on silica gel and the product **12** (0.220 g, 1.5 mmol, 49%) was eluted with CH_2Cl_2/EtOH 90:10:FABMS *m/z* 1484 (M + 3Li), 1478 (M + 2Li), 1472 (M + Li), 1384 (M + 3Li − Boc).

XpC (13). The fully protected dimer **12** (0.220 g, 0.15 mmol) was treated with a freshly prepared solution of N^1,N^1,N^3,N^3-tetramethylguanidine (0.33 M) and *o*-nitrobenzaldoxime in dry CH_3CN (1.5 mL). After 3 h at room temperature, the mixture was concentrated and the residue was washed with ether. The solid was dissolved in aqueous NH_3 and stirred at room temperature for 24 h. After concentrating the solution, the resulting solid was treated for 30 min with 50% CF_3COOH in dichloromethane (10 mL). The fully deprotected nucleoside was extracted with water (10 mL), and the aqueous layer was washed with diethyl ether (2 × 5 mL). The aqueous layer was evaporated to yield the deprotected nucleoside **13** (0.110 g, 0.143 mmol, 95%). After preparative RP HPLC, 250 OD units were obtained; analytical RP HPLC gave a retention time of 14.6 min: ^{31}P NMR (D_2O) ppm 0.19 s; ^{1}H NMR (D_2O) δ 6.25 (t, 1 H, 1′X), 6.3 (t, 1 H, 1′C), 2.45 (m, 2 H, 2′X), 2.4 (m, 2 H, 2′C), 4.8 (m, 1 H, 3′X), 4.6 (m, 1 H, 3′C), 4.1 (m, 1 H, 4′X), 4.0 (m, 1 H, 4′C), 3.8 (m, 2 H, 5′X), 4.2 (m, 2 H, 5′C), 6.1 (d, 1 H, H19), 7.9 (d, 1 H, H18), 7.7 (s, 1 H, H6), 2.4 (2 t's, 4 H, H7 and H8), 3.2 (2t's, 4 H, H9 and H10), 4.25 (m, 1 H, H11), 3.2 (m, 1 H, H12), 7.3 (s, 1 H, H14), 8.4 (s, 1 H, H15); ^{13}C NMR (D_2O) ppm 154.2 CO2, 168.3 CO4, 116.2 C5, 141.1 C6, 25.8 C7, 37.4 C8, 41.8 and 41.2 C9 and C10, 55.7 C11, 30.3 C12, 131.0 C13, 120.6 C14, 138.1 C15, 178.3 C16, 171.9 C17, 144.9 C18, 98.9 C19, 159.3 C20, 168.2 C21, 88.3 1′X, 88.9 1′C, 40.8 2′X, 42.3 2′C, 77.8 3′X (J_{CP} = 5.1 Hz), 73.3 3′C, 88.7 (J_{CP} = 6.5 Hz) 4′X, 88.1 4′C (J_{CP} = 8.9 Hz), 64.0 5′X, 67.8 5′C (J_{CP} = 5.05 Hz).

5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-5-[3-[[2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-[[1-(1,1-dimethylethoxy)carbonyl]-1*H*-imidazol-4-yl]-1-oxopropyl]amino]ethyl]amino]-3-oxopropyl]-2′-deoxyuridine 3′-*O*-(*N*,*N*-Diisopropylamino)(β-cyanoethoxy)phosphine (14). Chloro(*N*,*N*-diisopropylamino)(β-cyanoethoxy)phosphine (0.158 g, 0.81 mmol) was weighed into an H-shaped Schlenk flask equipped with a filter frit and dissolved in acetonitrile (10 mL). Diisopropylethylamine (0.196 mL, 1.52 mmol) was added to the reaction vessel and the mixture was stirred at room temperature for 20 min. The nucleoside **10** (0.75 g, 0.76 mmol) was dissolved in acetonitrile and added to the phosphorylating mixture and left stirring for 30 min. The mixture was then filtered through the frit to the other side of the H-tube, removing the amine hydrochloride. The solid was washed with acetonitrile (2 × 10 mL) and the combined MeCN solutions were concentrated to yield a glass. The glass was then purified by radial TLC. The phosphoramidite **14** (0.721 g, 0.61 mmol, 80%) eluted out with CH_2Cl_2/EtOAc/Et_3N 4.5:4.5:1: ^{31}P NMR (CD_3CN) ppm 149.4 2 s's (diastereoisomers); FABMS *m/z* 1194 (M + 2Li), 1188 (M + Li), 1094 (M + 2Li − H − Boc), 994 (M + 3Li − H − Boc); exact mass found 1188.5789, calcd for $C_{60}H_{80}N_9O_{14}PLi$ 1188.5722.

XpT (15). The phosphoramidite **14** was used on a DNA synthesizer to prepare the dinucleotide 5′-*O*-DMT-X″pT. After ammonia deprotection, the product was purified by RP HPLC and RP desalting (25 OD units purified yield). Retention time for **15** was 14.5 min by analytical RP HPLC: ^{1}H NMR (D_2O) δ 6.3 (t, 1 H, 1′X), 6.4 (t, 1 H, 1′T), 2.55 (m, 2 H, 2′X), 2.4 (m, 2 H, 2′T), 4.65 (m, 1 H, 3′T), 4.85 (m, 1 H, 3′X), 4.2 (m, 1 H, 4′X), 4.0 (m, 1 H, 4′T), 3.85 (m, 2 H, 5′X), 4.2 (m, 2 H, 5′T), 7.75 (s, 1 H, H6), 7.75 (s, 1 H, H21), 2.4 (2 t's, 4 H, H7 and H8), 3.3 (m, 4 H, H9 and H10), 4.35 (m, 1 H, H11), 3.1 (m, 1 H, H12), 7.2 (s 1 H, H14), 8.2 (s, 1 H, H15), 1.95 (s, 3 H, T-CH_3).

5′-HO-TATCTTCTXAC-OH-3′ (16). The phosphoramidite **14** was used on a DNA synthesizer to prepare the 11-mer 5′-HO-TpA′pT″pC′pTpTpC′pTpXpA′pC′-O-S-3′. Trityl release rates were slightly slower than normal, so trityl yields calculated by the Pharmacia algorithm were invalid. The oligomer on the solid support was washed for 10 min with 10% CF_3COOH in CH_2Cl_2 to effect detritylation and removal of the Boc groups. Deprotection was completed with aqueous NH_3 (0.88 d), and the product was purified by AX HPLC. Oligomer **16** (90 OD units purified yield) eluted from an analytical AX column at 23.5 min.

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Registry No. 1, 96203-41-7; 2, 132776-81-9; 3, 132776-82-0; 3·2CF_3CO_2H, 132776-97-7; 4, 132776-83-1; 5, 132776-84-2; 6 (isomer 1), 132776-85-3; 6 (isomer 2), 132776-98-8; 7, 132776-86-4; 8, 132776-87-5; 9, 132958-80-6; 10, 128900-35-6; 11, 132776-89-7; 12, 132776-90-0; 13, 132776-92-2; 14 (isomer 1), 132776-93-3; 14 (isomer 2), 132776-99-9; 15, 132776-95-5; 16, 132802-12-1; 2Fmoc-L-Lys(BOC)-*O*-pfp, 86060-98-2; $CN(CH_2)_2OPCIN(i\text{-}Pr)_2$, 124482-92-4; bis(trifluoroacetyl)-L-Lys-OH, 329-53-3; 2-chlorophenyl dichlorophosphate, 15074-54-1; 4-*N*-3′-*O*-diacetyl-2′-deoxycytidine, 70284-47-8.