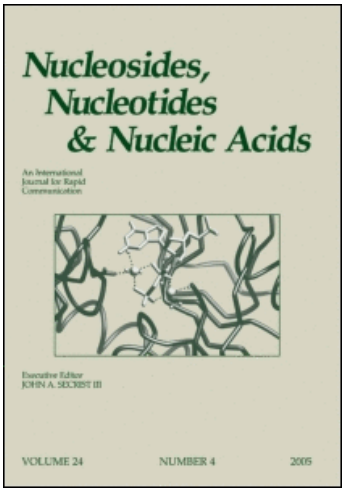


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

AN IMPROVED SYNTHESIS OF THE DINUCLEOTIDES pdCpA AND pdCpdA

Xue-Feng Zhu[a]; A. Ian Scott[a]
[a] Center for Biological NMR, Department of Chemistry, Texas A&M University, TX, U.S.A.

Online publication date: 28 February 2001

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(3), 197–211 (2001)

AN IMPROVED SYNTHESIS OF THE DINUCLEOTIDES pdCpA AND pdCpdA

Xue-Feng Zhu and A. Ian Scott*

Center for Biological NMR, Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, TX 77842-3012

ABSTRACT

An improved route was developed for the preparation of the dinucleotide hybrid 5′-*O*-phosphoryl-2′-deoxycytidylyl-(3′ → 5′)adenosine (pdCpA) **7**. This simple and concise synthesis involves the successive coupling of 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetra- isopropylphosphorodiamidite with 4-*N*-benzoyl-5′-*O*-(4, 4′-dimethoxytrityl)-2′-deoxy-cytidine **1** and 6-*N*,6-*N*,2′-*O*,3′-*O*-tetrabenzoyladenosine **2** as the key step. Some dinucleotide derivatives bearing different protecting groups were also synthesized and the selective deprotection conditions were studied in detail. The utility and efficiency of this approach has been further demonstrated by its application to the synthesis of total DNA dinucleotide pdCpdA **17** and total RNA dinucleotide **21**.

INTRODUCTION

Recently, a general biosynthetic method for site-specific incorporation of unnatural amino acids with novel steric or electronic properties into proteins has been developed independently by the groups of Schultz (1–7) and Chamberlin (8–11). This technique is a powerful tool for probing protein structure and function and has many other important applications (12–15). The crucial step of the technique involves the construction of a misacylated suppressor tRNA bearing the desired unnatural amino acid (16,17). Hecht and co-workers originally developed one successful approach to this problem, which depends on the T4 RNA ligase mediated

*Corresponding author.

coupling of 2′(3′)-*O*-aminoacylated dinucleotide 5′-*O*-phosphorylcytidylyl-(3′ → 5′)adenosine (pCpA) to 3′-truncated run-off tRNA lacking the 3′-terminal pCpA [tRNA(-CA)] (18–20). Chamberlin (8) and Schultz (2) simplified this method by replacing the cytidine unit of pCpA with a 2′-deoxycytidine unit, resulting in the synthesis of dinucleotide hybrid 5′-*O*-phosphoryl-2′-deoxycytidylyl-(3′ → 5′)adenosine (pdCpA). It was shown that 2′(3′)-*O*-aminoacylated pdCpA derivatives are still substrates of T4 RNA ligase and therefore can be coupled with a truncated tRNA as efficiently as pCpA (2). Furthermore, the resulting aminoacylated tRNA analogs conserve good compatibility with other enzymes involved in protein biosynthesis (10).

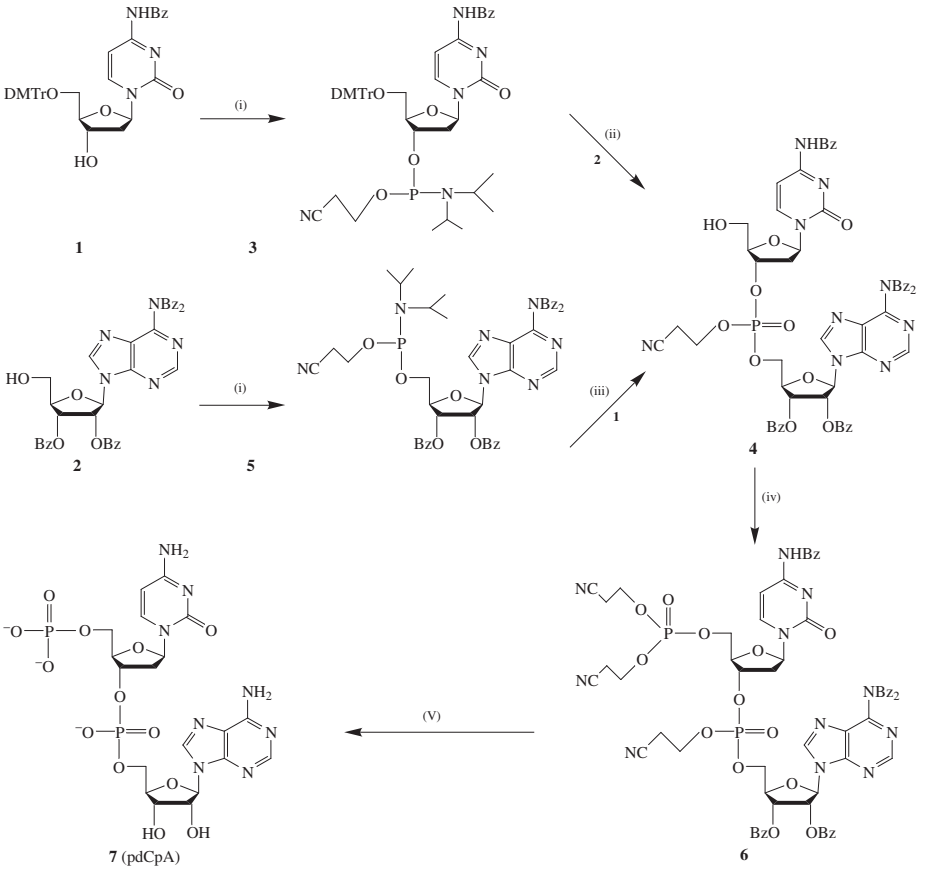
Although the substitution of pCpA by pdCpA offers several advantages, such as synthetic simplicity and product stability, the current synthetic routes to pdCpA are tedious and rather inefficient (2,10,21). In this paper, we describe an improved procedure which produces pdCpA in high yield under mild reaction conditions. Furthermore, 5′-*O*-phosphoryl-2′-deoxycytidylyl-(3′ → 5′)-2′-deoxy adenosine (pdCpdA), a potential substitute for pCpA and pdCpA, was also efficiently prepared by this strategy.

RESULTS AND DISCUSSION

The synthetic route to pdCpA is shown in Scheme 1. In order to reduce the number of synthetic steps and increase the overall yield, we developed a one-pot reaction to produce the dinucleotide 4-*N*-benzoyl-5′-*O*-(4, 4′-dimethoxytrityl)-2′-deoxycytidylyl-{3′−O^P-2-cyanoethyl)]→ 5′}-6-*N*,6-*N*, 2′-*O*, 3′-*O*-tetrabenzoyl-adenosine (structure not shown in Scheme 1), a key intermediate for pdCpA. Our strategy is based on employing 2- cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropyl-phosphorodiamidite, rather than previously reported 2,2,2-trichloroethyl phosphorodichloridite, as a bifunctional phosphitylating reagent (22,23). Although the 2,2,2-trichloroethyl group has also been widely used as a phosphate(phosphite) protecting group, the major problem associated with its use is difficulty in complete removal (normally by reductive elimination with zinc-copper couple). In addition, the phosphitylation of 2,2,2-trichloroethyl phosphorodichloridite with nucleosides should be performed at low temperature (−78°C) (24). On the other hand, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite possesses two reactive centers (the two iPr_2N groups) attached to the same phosphorus atom, allowing it to react with *two different* properly protected nucleosides in the presence of 1*H*-tetrazole thus forming a dinucleotide linked by a phosphite triester under very mild conditions. Another significant advantage of the use of 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphoro- diamidite is that in the final step of the dinucleotide preparation, all protecting groups from the heterocyclic bases (*N*-benzoyl) and from the phosphates (2-cyanoethyl) can be cleaved at the same time by treatment with aqueous ammonia.

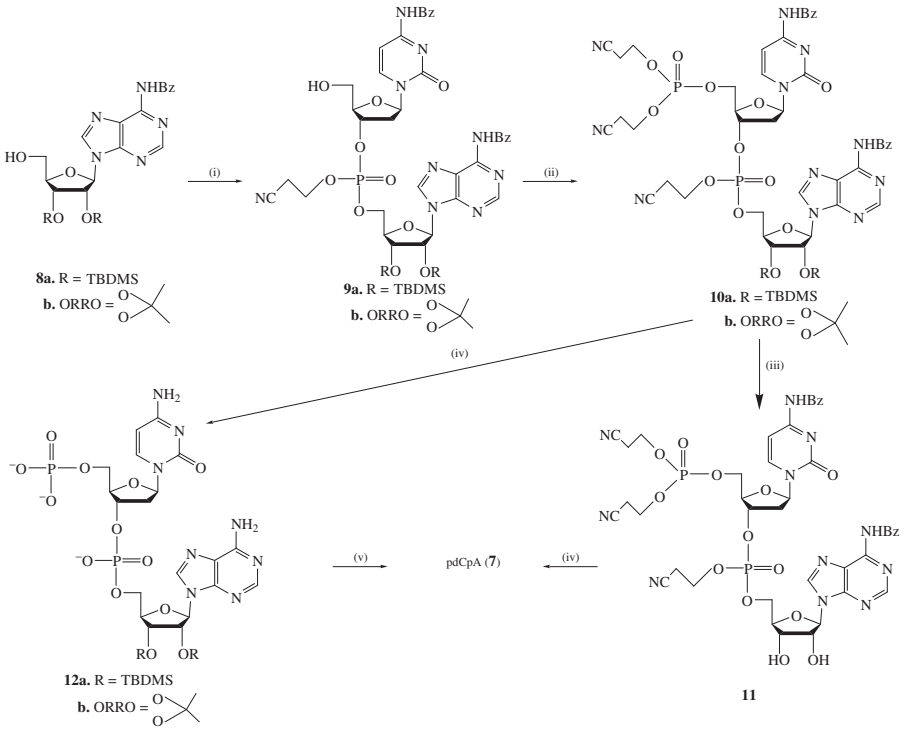
4-*N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine **1** was chosen as the dC component, and 6-*N*,6-*N*,2′-*O*,3′-*O*-tetrabenzoyladenosine **2** obtained from

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



Scheme 1. *Reagents and Conditions*: (i) 1*H*-tetrazole, $(iPr_2N)_2POCH_2CH_2CN$, CH_2Cl_2, rt, 2 h; (ii) (a) 1*H*-tetrazole, **2**, CH_2Cl_2, rt, 4 h, (b) I_2/THF/H_2O/Pyridine, rt, 10 min, (c) 1% TFA in CH_2Cl_2, rt, 5 min; (iii) (a) 1*H*-tetrazole, **1**, CH_2Cl_2, rt, 4 h, (b) I_2/THF/H_2O/Pyridine, rt, 10 min, (c) 1% TFA in CH_2Cl_2, rt, 5 min. (iv) 1*H*-tetrazole, $iPr_2NP(OCH_2CH_2CN)_2$, CH_2Cl_2, rt, 3 h, (b) I_2/THF/H_2O/Pyridine, rt, 10 min; (v) NH_4OH/CH_3OH/1,4-dioxane, rt, 24 h. DMTr=di(*p*-methoxyphenyl)phenylmethyl.

6-*N*-benzoyl- adenosine in high yield (83%) was employed as the "A" component rather than the 6-*N*,2′-*O*,3′-*O*-tribenzoyl derivative because of its better solubility and easy separation from the product by flash chromatography. Thus, 2′-deoxycytidine **1** was condensed with 2- cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite in the presence of 1*H*-tetrazole to give phosphoramidite **3**, which, without isolation, was subsequently coupled to the protected adenosine **2**. After iodine oxidation of the resulting phosphite triester, selective removal of the trityl group by 1% TFA in dichloromethane provided the phosphotriester **4** in 67% overall yield. It was also observed that inverting the addition sequence of **1** and **2** (via intermediate **5**) has little effect on the yield of **4** (60%). Conversion of **4** into the corresponding 5′-phosphorylated product **6** was readily achieved by condensing **4** with excess di(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite (2 equiv.)

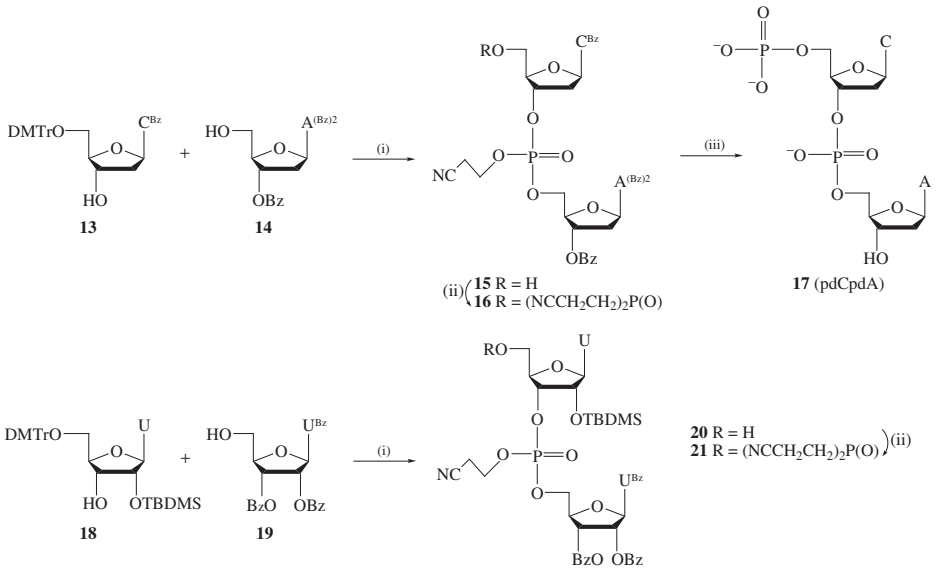


Scheme 2. *Reagents and Conditions*: (i) (a) **3** (prepared *in situ* as shown in Scheme 1), 1*H*-tetrazole, **8**, CH_2Cl_2, rt, 3 h, (b) I_2/THF/H_2O/Pyridine, rt, 10 min, (c) 1% TFA in CH_2Cl_2, rt, 5 min; (ii) (a) 1*H*-tetrazole, $iPr_2NP(OCH_2CH_2CN)_2$, CH_2Cl_2, rt, 3.5 h, (b) I_2/THF/H_2O/Pyridine, rt, 10 min; (iii) 1N HCl, rt, 3.5 h; (iv) NH_4OH/CH_3OH/1,4-dioxane, rt; (v) From **12a**, TBAF/THF, rt, 2 h, From **12b**, 1N HCl, 1.5 h, rt.

followed by iodine oxidation. After the simultaneous removal of benzoyl and 2-cyanoethyl groups with ammonium, pdCpA **7** was obtained in high overall yield (Scheme 1).

The generality of this method was demonstrated by the preparation of analogs of **4** bearing different protecting groups removable under neutral or acidic conditions, thus allowing selective deprotection of the amino groups of the nucleotide bases or of the two hydroxy groups of the ribose moiety, as depicted in Scheme 2. These deprotection protocols are required to introduce a wide range of functionality in the "unnatural" substrates to be used in future studies based on a theoretical prediction that the RNA World could sustain formation of the carbon-carbon bond and carbon-oxygen bond (25). Among them, partially protected dinucleotide **11** is an especially important one for our current investigation of developing programmable molecular machines used for organic synthesis. Dinucleotide **11** is a key intermediate in the synthesis of misacylated dinucleotides that are crucial precursors of our polyprenoid and polysaccharide synthesis.

The 2′,3′-di-*O*-TBDMSoxy analogue **10a** was initially chosen as a promising candidate. Following a procedure similar to the one described for synthesis of



Scheme 3. *Reagents and Conditions*: (i) (a) 1*H*-tetrazole, **13**, $(iPr_2N)_2POCH_2CH_2CN$, 14 or 1*H*-tetrazole, **18**, $(iPr_2N)_2POCH_2CH_2CN$, **19**, CH_2Cl_2, rt, 3 h, (b) I_2/THF/H_2O/Pyridine, rt, 10 min, (c) 1% TFA in CH_2Cl_2, rt, 5 min; (ii) (a) 1*H*-tetrazole, $iPr_2NP(OCH_2CH_2CN)_2$, CH_2Cl_2, rt, 3.5 h, (b) I_2/THF/H_2O/Pyridine, rt, 10 min; (iii) NH_4OH/CH_3OH/1,4-dioxane, rt. C^{Bz} = 4-*N*-benzoylcytosin-1-yl; $A^{(Bz)2}$ =6-*N*,6-*N*-dibenzoyladenin-9-yl; U = uracil-1-yl; U^{Bz} = 3-*N*-benzoyluracil-1-yl.

6, dinucleotide derivative **10a** was obtained in 75% overall yield from **8a**. However, the selective deprotection of 2′,3′-di-*O*-TBDMS groups from **10a** proved unexpectedly difficult. Many attempts were made to transform **10a** into diol **11** by using a variety of deprotection conditions (TBAF/THF, TBAF-HOAc/THF, HF-Pyridine/THF), but were all unsuccessful. One possible explanation for this result is that nucleophilic fluoride ions attack cyanoethyl groups of phosphate ester and cleave them as a result of β-elimination since, when studied in model reactions, **8a** can be completely converted into 6-*N*-benzoyl adenosine under exactly the same conditions as mentioned above. On the basis of these observations, we now replaced 2′,3′-di-*O*-TBDMS groups with an isopropylidene group, which is also an acid-labile protecting group, to allow the synthesis of isopropylidene acetal **10b** in 69% overall yield from **8b**. Several methods were also tried for removing the acetonide unit from **10b**. Both ferric chloride hexahydrate (26) and 95% TFA gave a complex mixture of products, while aqueous acetic acid and conc.HCl-MeOH (1:70) were ineffective. Finally, it was found that stirring **10b** in 1N HCl for 3.5 h at rt accomplished complete conversion (TLC monitoring), providing diol **11** in 90% yield after flash chromatography separation. As expected, dinucleotides **10a** and **10b** were smoothly converted into 2′,3′-*O* protected pdCpA **12a** and **12b** by ammonium hydroxide treatment. Both **11** and **12** can be further transformed into pdCpA **7** in good yield under the conditions shown in Scheme 2.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

In order to further show the utility of this useful one-pot procedure, we successfully applied it to the synthesis of the fully protected pdCpdA **16** and the total RNA dinucleotide **21** in high yields (Scheme 3). Upon treatment with aqueous ammonia, dinucleotide **16** can be transformed into pdCpdA **17**. The synthesis of pdCpdA as a substitute of pdCpA is especially interesting. As we know, both 2′-and 3′-*O*-aminoacylated pdCpA derivatives are substrates of T4 RNA ligase, therefore we can infer that 3′-*O*-aminoacylated pdCpdA derivatives should also be substrates. If this assumption is true, pCpA can be further replaced by pdCpdA rather than pdCpA, from which 2′- and 3′-*O*-aminoacylated mixtures are always obtained. In addition, pdCpdA derivatives are useful in determining the functionality of the free 3′(2′)-*O*H group of 2′(3′)-*O*-aminoacylated pdCpA in the *in vitro* protein synthesizing system. These investigations are currently under way in our laboratory.

In conclusion, we have developed a novel and general approach to the synthesis of the fully-protected phosphotriester **4** by using 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropyl- phosphorodiamidite as the coupling reagent. With good overall yields and very mild reaction conditions, it allows the synthesis of analogs of **4**, as illustrated by the preparation of DNA/RNA dinucleotides hybrids **10**, **11** and **12**, all of which are key precursors of pdCpA. In addition, the synthetic approach described herein is versatile, as shown by the synthesis of total DNA dinucleotides **15**, **16** and total RNA dinucleotides **20** and **21**.

EXPERIMENTAL

^{1}H (500 MHz), ^{13}C (125 MHz) and ^{31}P (202.5 MHz) NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or D_2O on a Bruker ARX-500 spectrometer. All compounds, except **7**, **12a**, **12b** and **17**, are diastereoisomers at 3′ → 5′ phosphorus atom and instrumentally analyzed without separation, therefore, the number of protons and carbons are doubled in NMR spectra. Coupling constants are expressed in Hz. LSI mass spectra were obtained on a VG analytical 70s high-resolution double-focussing magnetic-sector mass spectrometer; MALDI mass spectra were obtained on a Perseptive Biosystems Voyager XL-ELITE mass spectrometer. UV spectra were recorded on a Varian DMS 90 UV- Visible spectrometer. λ are quoted in nm and band intensities in parenthesis, as ε. High-performance liquid chromatography (HPLC) was performed on a Hewlett Packard HP 1100 series HPLC system equipped with a HP-3390A integrator. The column employed was a 250 mm × 22 mm reverse phase C-18 preparative (C-18 10U, Alltech) using a solvent system consisting of 10 mM aqueous AcOH : CH_3CN (linear gradient from 90:10 to 10:90 in 60 min) at a flow rate of 5 ml/min.

2-Cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (24), di(2-cyanoethyl) *N*,*N*- biisopropylphosphoramidite (27) and nucleosides (**1**, **8b**, **13**, **14**, **18**, **19**) were prepared following published procedures. Nucleoside **2** was prepared by Schultz protocol. However, we modified this procedure by using

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

N-benzoyladenosine, rather than adenosine, as the starting material, resulting in higher yield of **2**. The synthesis of **8a** was reported in our previous paper (28). Thin-layer chromatographies (TLC) were performed on pre-coated 0.25 mm silica gel 60F254 polyester plates (20 × 20 cm, Aldrich). Flash column chromatographies were carried out on Mallinckrodt Silica Gel 150 (60-200 mesh). All solvent and reagents were purified when necessary by standard literature methods. All air- and water-sensitive reactions were conducted in anhydrous solvent and under nitrogen or argon atmosphere. In the work-up stage, organic solutions were dried over anhydrous sodium sulfate. All evaporations were carried out under reduced pressure using a rotary evaporator, unless stated otherwise.

4-*N*-benzoyl-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-*N*,6-*N*,2′-*O*, 3′-*O*-tetra-benzoyl adenosine (4) To a solution of **1** (180 mg, 0.284 mmol) in dichloromethane (1 ml) at rt was added 1*H*-tetrazole (25 mg, 0.357 mmol) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (107 mg, 0.355 mmol). After stirring for 2 h at rt, 1*H*-tetrazole (25 mg, 0.357 mmol) and **2** (243 mg, 0.355 mmol) were added. The resulting mixture was stirred at rt for 4 h and then iodine (270 mg, 1.063 mmol) in THF-H_2O-pyridine (66:33:1, 2.7 ml) was added. After 10 min, the reaction mixture was poured into ethyl acetate (50 ml), washed with 0.2 M sodium bisulfite (10 ml × 2) and brine (10 ml), dried, filtered and concentrated. The residue was then stirred with 1% TFA solution in dichloromethane (25 ml) at rt for 5 min. The reaction was quenched by adding saturated aqueous sodium hydrogen carbonate (5 ml). After separation, the organic phase was washed with brine (5 ml), dried, filtered and evaporated. The crude product was purified by flash column chromatography using dichloromethane-methanol (100:0 to 100:4) as the eluent to give **4** (215 mg, 67%, 2 diastereoisomers, 1:1 ratio) as a white solid. UV (CH_2Cl_2) λ 252 nm (ε 41 900), λ_{max} 232 nm (ε 47 600). $\delta_H(CDCl_3)$ 1.96 (2H, br s), 2.34 (1H, m), 2.43 (1H, m), 2.71-2.80 (6H, m), 3.72-3.83 (4H, m), 4.17-4.31 (6H, m), 4.51-4.58 (4H, m), 4.70 (2H, m), 5.05 (1H, m), 5.14 (1H, m), 6.13 (2H, m), 6.18 (1H, m), 6.21 (1H, m), 6.25 (1H, m), 6.29 (1H, m), 6.53 (1H, d, *J* 4.9), 6.55 (1H, d, *J* 4.9), 7.33-7.38 (16H, m), 7.45-7.48 (8H, m), 7.51-7.58 (8H, m), 7.82 (8H, d, *J* 7.6), 7.89-7.91 (8H, m), 7.93 (4H, d, *J* 7.4), 8.22 (1H, d, *J* 7.5), 8.26 (1H, d, *J* 7.5), 8.49 (1H, s), 8.50 (1H, s), 8.67 (1H, s), 8.69 (1H, s) and 9.08 (2H, br s); $\delta_C(CDCl_3)$ 19.6 (2C), 39.6 (1C), 39.7 (1C), 61.3 (1C), 61.5 (1C), 62.5 (1C), 62.6 (1C), 66.7 (1C), 66.8 (1C), 70.6 (1C), 70.7 (1C), 73.9 (1C), 74.1 (1C), 78.7 (1C), 78.9 (1C), 81.2 (1C), 81.3 (1C), 86.3 (1C), 86.5 (1C), 86.8 (2C), 88.0 (1C), 88.2 (1C), 96.6 (2C), 113.1 (2C), 116.5 (1C), 116.6 (1C), 127.6 (arom.C, 2C), 127.8 (arom.C, 2C), 128.2 (arom.C, 2C), 128.4 (arom.C, 2C), 128.6 (arom.C, 8C), 128.8 (arom.C, 8C), 129.0 (arom.C, 4C), 129.4 (arom.C, 8C), 129.8 (arom.C, 4C), 129.9 (arom.C, 4C), 132.8 (arom.C, 2C), 133.2 (arom.C, 4C), 133.3 (arom.C, 2C), 133.8 (arom.C, 4C), 133.9 (arom.C, 2C), 134.0 (arom.C, 2C), 143.6 (1C), 143.8 (1C), 146.1 (2C), 151.9 (1C), 152.0 (1C), 152.5 (2C), 152.6 (2C), 152.7 (1C), 152.8 (1C), 162.0 (1C), 162.1 (1C), 165.2 (4C), 165.3 (1C), 165.4 (1C), 172.4 (2C) and 172.5 (2C); HRMS $[M+1]^+$, Found: 1130.3085. $C_{57}H_{49}N_9O_{15}P$ requires m/z, 1130.3086.

4-*N*-benzoyl-5′-*O*-[di(2-cyanoethyloxy)phosphoryl]-2′-deoxycytidylyl-3′-O^P-2-cyanoethyl)]→ 5′}-6-*N*,6-*N*,2′-*O*,3′-*O*-tetrabenzoyladenosine (6) To a solution of **4** (330 mg, 0.292 mmol) in dichloromethane (2 ml) at rt was added 1*H*-tetrazole (41 mg, 0.584 mmol) and di(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite (158 mg, 0.584 mmol). The resulting mixture was stirred at rt for 4 h, then iodine (297 mg, 1.169 mmol) in THF-H_2O-pyridine (66:33:1, 3 ml) was added. After 10 min, the reaction mixture was diluted with ethyl acetate (60 ml), washed with 0.2 M sodium bisulfite (10 ml × 2) and brine (10 ml), dried, filtered and concentrated. Flash column chromatography of the residue using dichloromethane-methanol (100:2 to 100:4) as the eluent yielded **6** (377 mg, 98%, 2 diastereoisomers, 1:1 ratio) as a white solid. UV (CH_2Cl_2) λ_{max} 257 nm (ε 40 000), λ 236 nm (ε 36 200). δ_H($CDCl_3$) 2.26 (1H, m), 2.31 (1H, m), 2.67-2.86 (14H, m), 4.18-4.43 (18H, m), 4.55-4.57 (4H, m), 4.70 (2H, m), 5.14 (2H, m), 6.13-6.16 (2H, m), 6.18 (1H, m), 6.21 (1H, m), 6.26 (1H, m), 6.29 (1H, m), 6.54 (1H, d, *J* 4.8), 6.56 (1H, d, *J* 4.9), 7.33-7.39 (16H, m), 7.45-7.59 (16H, m), 7.82 (8H, m), 7.86 (4H, m), 7.88-7.95 (8H, m), 7.98 (2H, m), 8.50 (1H, s), 8.52 (1H, s) and 8.68 (2H, s); δ_C($CDCl_3$) 19.6 (3C), 19.7 (3C), 39.1 (2C), 62.7 (6C), 66.7 (2C), 66.9 (1C), 67.1 (1C), 70.6 (1C), 70.7 (1C), 74.0 (2C), 77.5 (1C), 77.7 (1C), 81.2 (1C), 81.3 (1C), 83.5 (2C), 86.8 (1C), 86.9 (1C), 87.4 (1C), 87.5 (1C), 96.8 (2C), 116.5 (2C), 116.6 (3C), 116.7 (3C), 127.6 (arom.C, 2C), 127.7 (arom.C, 2C), 128.2 (arom.C, 2C), 128.4 (arom.C, 2C), 128.5 (arom.C, 4C), 128.6 (arom.C, 4C), 128.8 (arom.C, 8C), 129.0 (arom.C, 4C), 129.4 (arom.C, 8C), 129.8 (arom.C, 4C), 129.9 (arom.C, 4C), 133.2 (arom.C, 8C), 133.8 (arom.C, 4C), 133.9 (arom.C, 4C), 143.8 (1C), 143.9 (1C), 144.5 (2C), 152.0 (4C), 152.5 (1C), 152.6 (1C), 152.7 (1C), 152.8 (1C), 162.3 (1C), 162.4 (1C), 165.1 (4C), 165.3 (2C) and 172.3 (4C); HRMS $[M+Na]^+$, Found: 1338.3084. $C_{63}H_{55}N_{11}NaO_{18}P_2$ requires m/z, 1338.3099.

5′-*O*-Phosphoryl-2′-deoxycytidylyl-(3′→5′)adenosine (7) To a solution of **6** (300 mg, 0.228 mmol) in dioxane (0.45 ml) and methanol (3 ml) was added concentrated ammonium hydroxide (3.45 ml). The flask was capped and allowed to stir for 24 h at rt. Then the solvent was removed under vacuum and the residue was further lyophilized. The crude product was purified by HPLC. After the appropriate fractions were collected and lyophilized, pdCpA **7** (130 mg, 90%) was obtained as a white solid. (On a gram-scale preparation, the crude product should be pre-purified on a Dowex anion exchange column using 0.025 M to 1 M aqueous ammonium acetate as gradient eluent before the HPLC separation). UV (H_2O) λ_{max} 260 nm (ε 21 000). δ_P(D_2O) 2.32 and 3.46; δ_H(D_2O)1.85 (1H, m), 2.29-2.33 (1H, m), 3.85 (2H, m), 3.97 (2H, m), 4.14 (1H, m), 4.20 (1H, m), 4.38 (1H, m), 4.69 (1H, m), 4.74 (1H, m), 5.92-5.96 (2H, m), 6.01 (1H, d, *J* 7.9), 7.83 (1H, d, *J* 7.8), 8.16 (1H, s) and 8.40 (1H, s); δ_C(D_2O) 38.0 (1C), 63.9 (1C), 64.2 (1C), 69.5 (1C), 73.2 (1C), 75.2 (1C), 83.1 (1C), 84.5 (1C), 85.6 (1C), 86.6 (1C), 94.8 (1C), 117.7 (1C), 140.1 (1C), 142.7 (1C), 148.2 (1C), 149.2 (2C), 152.5 (1C) and 159.8 (1C); HRMS $[M+1]^+$, Found: 637.1190 $C_{19}H_{27}N_8O_{13}P_2$ requires m/z, 637.1173.

4-*N*-benzoyl-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-N -benzoyl-2′,3′-di-*O*-(*tert*-butyl-dimethylsilyl)adenosine (9a) Following a similar procedure described for **4**, **9a** (515 mg, 82%, 2 diastereoisomers, 1:1 ratio) was obtained from **8a** (0.60 mmol scale) as a white solid. UV (CH_2Cl_2) λ_{max} 260 nm (ε 21 200), λ 234 nm (sh ε 11 900). δ_H($CDCl_3$) -0.11 (3H, s), -0.01 (3H, s), 0.04 (3H, s), 0.08 (3H, s), 0.12 (3H, s), 0.13 (6H, s), 0.15 (3H, s), 0.86 (9H, s), 0.89 (9H, s), 0.94 (9H, s), 0.95 (9H, s), 2.31 (1H, m), 2.45 (1H, m), 2.70-2.82 (6H, m), 3.79-3.87 (4H, m), 4.22-4.36 (10H, m), 4.41-4.56 (6H, m), 4.83 (1H, m), 4.90 (1H, m), 5.10 (1H, m), 5.21 (1H, m), 6.01 (2H, m), 6.23 (2H, m), 7.46-7.52 (10H, m), 7.56-7.59 (4H, m), 7.89 (4H, d, *J* 7.1), 8.06 (4H, m), 8.29-8.34 (4H, m), 8.76 (1H, s), 8.77 (1H, s), 9.23 (2H, br s) and 9.45 (2H, br s); δ_C($CDCl_3$) -5.0 (1C), -4.9 (1C), -4.8 (1C), -4.7 (1C), -4.6 (2C), -4.4 (1C), -4.3 (1C), 17.7 (1C), 17.8 (1C), 17.9 (2C), 19.6 (1C), 19.7 (1C), 25.5 (3C), 25.6 (3C), 25.7 (6C), 39.8 (2C), 61.2 (1C), 61.4 (1C), 62.4 (2C), 66.1 (1C), 66.7 (1C), 70.7 (1C), 71.2 (1C), 74.6 (1C), 74.9 (1C), 78.5 (1C), 78.7 (1C), 81.8 (1C), 82.3 (1C), 86.5 (2C), 87.6 (2C), 89.8 (1C), 89.9 (1C), 96.8 (2C), 116.4 (1C), 116.5 (1C), 123.8 (1C), 124.1 (1C), 127.7 (arom.C, 4C), 127.9 (arom.C, 2C), 128.0 (arom.C, 2C), 128.7 (arom.C, 2C), 128.8 (arom.C, 2C), 128.9 (arom.C, 4C), 132.8 (arom.C, 2C), 132.9 (arom.C, 2C), 133.1 (arom.C, 2C), 133.3 (arom.C, 1C), 133.4 (arom.C, 1C), 142.0 (1C), 142.4 (1C), 145.3 (2C), 149.6 (1C), 149.7 (1C), 151.3 (1C), 151.5 (1C), 152.4 (2C), 154.8 (2C), 162.3 (1C), 162.4 (1C), 165.1 (1C), 165.3 (1C) and 166.7 (2C); HRMS $[M+Na]^+$, Found: 1068.3842. $C_{48}H_{64}N_9NaO_{12}PSi_2$ requires m/z, 1068.3848.

4-*N*-benzoyl-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-*N*-benzoyl-2′,3′-*O*- iso-propylideneadenosine (9b) Following a similar procedure described for **4, 9b** (720 mg, 70%, 2 diastereoisomers, 1:1 ratio) was obtained from **8a** (1.20 mmol scale) as a white solid. UV (CH_2Cl_2) λ_{max} 260 nm (ε 20 800), λ 232 nm (sh ε 10 400). δ_H($CDCl_3$) 1.42 (6H, s), 1.63 (6H, s), 2.26 (1H, m), 2.37 (1H, m), 2.63-2.81 (6H, m), 3.81 (4H, m), 4.17- 4.34 (12H, m), 4.51 (2H, m), 5.04-5.16 (4H, m), 5.49 (2H, m), 6.17 (2H, m), 6.25 (2H, s), 7.46-7.49 (10H, m), 7.55-7.60 (4H, m), 7.89 (4H, d, *J* 7.5), 8.03 (4H, d, *J* 7.5), 8.25-8.29 (4H, m), 8.76 (1H, s), 8.77 (1H, s), 9.29 (2H, br s) and 9.51 (2H, br s); δ_C($CDCl_3$) 19.5 (1C), 19.6 (1C), 25.3 (2C), 27.0 (2C), 39.7 (1C), 39.8 (1C), 61.4 (2C), 62.4 (2C), 67.6 (1C), 67.7 (1C), 78.7 (1C), 78.8 (1C), 80.9 (1C), 81.0 (1C), 84.0 (1C), 84.2 (1C), 85.4 (2C), 86.4 (1C), 86.5 (1C), 87.7 (1C), 87.8 (1C), 90.7 (1C), 90.8 (1C), 96.9 (2C), 114.8 (1C), 114.9 (1C), 116.5 (1C), 116.6 (1C), 123.8 (1C), 124.0 (1C), 127.8 (arom.C, 4C), 128.0 (arom.C, 2C), 128.1 (arom.C, 2C), 128.6 (arom.C, 2C), 128.7 (arom.C, 2C), 128.8 (arom.C, 4C), 132.8 (arom.C, 2C), 132.9 (arom.C, 2C), 133.1 (arom.C, 2C), 133.2 (arom.C, 1C), 133.3 (arom.C, 1C), 142.4 (1C), 142.6 (1C), 145.3 (2C), 149.8 (2C), 151.2 (1C), 151.4 (1C), 152.6 (2C), 154.9 (2C), 162.4 (2C), 165.2 (1C), 165.4 (1C) and 166.9 (2C); HRMS $[M+1]^+$, Found: 858.2605. $C_{39}H_{41}N_9O_{12}P$ requires m/z, 858.2612.

4-*N*-benzoyl-5′-*O*-[di(2-cyanoethyloxy)phosphoryl]-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-*N*-benzoyl-2′,3′-di-*O*-(*tert*-butyldimethylsilyl)

adenosine (10a) Following a similar procedure described for **6**, **10a** (650 mg, 92%, 2 diastereoisomers, 1:1 ratio) was obtained from **9a** (0.57 mmol scale) as a white solid. UV (CH_2Cl_2) λ_{max} 260 nm (ε 20 400), λ 232 nm (ε 14 700). δ_H($CDCl_3$) -0.15 (3H, s), -0.11 (3H, s), 0.01 (3H, s), 0.02 (3H, s), 0.10 (6H, s), 0.12 (6H, s), 0.82 (9H, s), 0.84 (9H, s), 0.92 (18, s), 2.19 (1H, m), 2.25 (1H, m), 2.70-2.84 (12H, m), 4.13-4.48 (28H, m), 4.82 (1H, m), 4.87 (1H, m), 5.05 (1H, m), 5.10 (1H, m), 5.99 (2H, m), 6.17 (1H, m), 6.21 (1H, m), 7.47-7.50 (10H, m), 7.54-7.56 (4H, m), 7.88 (4H, m), 7.95-8.03 (6H, m), 8.27 (1H, s), 8.30 (1H, s), 8.76 (1H, s), 8.77 (1H, s), 8.88 (2H, br s) and 9.26 (2H, br s); δ_C($CDCl_3$) -4.8 (2C), -4.7 (2C), -4.6 (2C), -4.4 (2C), 17.9 (2C), 18.0 (2C), 19.6 (3C), 19.7 (3C), 25.7 (6C), 25.8 (6C), 39.3 (2C), 62.5 (2C), 62.7 (6C), 66.8 (1C), 67.0 (1C), 71.1 (1C), 71.3 (1C), 74.6 (1C), 74.8 (1C), 77.3 (2C), 82.0 (1C), 82.3 (1C), 83.6 (2C), 87.5 (2C), 89.9 (2C), 96.8 (2C), 116.5 (3C), 116.6 (3C), 123.9 (1C), 124.1 (1C), 127.7 (arom.C, 4C), 128.0 (arom.C, 4C), 128.8 (arom.C, 4C), 129.0 (arom.C, 4C), 132.8 (arom.C, 4C), 133.3 (arom.C, 2C), 133.5 (arom.C, 2C), 142.3 (1C), 143.4 (1C), 144.4 (2C), 149.8 (2C), 151.4 (1C), 151.5 (1C), 152.5 (2C), 153.9 (2C), 162.3 (2C), 164.9 (2C), and 166.1 (2C); HRMS $[M+Na]^+$, Found: 1254.4039. $C_{54}H_{71}N_{11}NaO_{15}P_2Si_2$ requires m/z, 1254.4043.

4-*N*-benzoyl-5′-*O*-[di(2-cyanoethyloxy)phosphoryl]-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine (10b) Following a similar procedure described for **6**, **10b** (548 mg, 99%, 2 diastereoisomers, 1:1 ratio) was obtained from **9b** (0.53 mmol scale) as a white solid. UV (CH_2Cl_2) λ_{max} 260 nm (ε 21 700), λ 234 nm (sh ε 8 600). δ_H($CDCl_3$) 1.38 (6H,s), 1.59 (3H, s), 1.60 (3H, s), 2.16 (1H, m), 2.22 (1H, m), 2.67-2.81 (14 H, m), 4.15-4.49 (24 H, m), 4.99-5.05 (2H, m), 5.11-5.13 (2H, m), 5.45-5.48 (2H, m), 6.09-6.13 (2H, m), 6.23-6.24 (2H, m), 7.45-7.48 (10H, m), 7.53-7.58 (4H, m), 7.87 (4H, d, *J* 7.4), 7.95 (1H, d, *J* 7.5), 7.96 (1H, d, *J* 7.5), 8.00 (4H, m), 8.26 (1H, s), 8.28 (1H, s), 8.78 (1H, s), 8.79 (1H, s), 8.92 (2H, s) and 9.45 (2H, br s); δ_C($CDCl_3$) 19.6 (3C), 19.7 (3C), 25.3 (2C), 27.0 (2C), 39.2 (2C), 62.5 (2C), 62.8 (6C), 66.8 (1C), 67.8 (1C), 77.5 (2C), 81.0 (2C), 83.5 (2C), 84.1 (2C), 85.5 (2C), 87.6 (2C), 90.8 (2C), 97.0 (2C), 114.87 (1C), 114.9 (1C), 116.6 (3C), 116.7 (3C), 123.8 (1C), 123.9 (1C), 127.8 (arom.C, 4C), 128.0 (arom.C, 2C), 128.1 (arom.C, 2C), 128.8 (arom.C, 4C), 128.9 (arom.C, 4C), 132.9 (arom.C, 4C), 133.2 (arom.C, 4C), 142.6 (1C), 142.7 (1C), 144.4 (2C), 149.8 (2C), 151.3 (2C), 152.5 (2C), 154.6 (2C), 162.6 (2C), 165.1 (2C) and 166.8 (2C); HRMS $[M+Na]^+$, Found: 1066.2639. $C_{45}H_{47}N_{11}NaO_{15}P_2$ requires m/z, 1066.2626.

4-*N*-benzoyl-5′-*O*-[di(2-cyanoethyloxy)phosphoryl]-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-*N*-benzoyladenosine (11) Dinucleotide **10b** (35 mg, 0.033 mmol) was stirred with 1M HCl (2 ml) at rt. After 3.5 h, the starting material was completely disappeared (TLC monitoring), then the reaction mixture was neutralized by slowly adding saturated aqueous sodium hydrogen carbonate. The resulting solution was extracted with ethyl acetate (15 ml × 4). The combined extracts were washed with H_2O (5 ml) and brine (5 ml), dried , filtered and concentrated. The residue was subjected to flash chromatography using

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

dichloromethane-methanol (100:4) as eluent to afford **11** (30 mg, 90%, 2 diastereoisomers, 1:1 ratio) as a white solid. UV (CH_3CN) λ_{max} 258 nm (ε 20 100), λ 232 nm (sh ε 15 300). δ_H(DMSO-d_6) 2.40 (2H, m), 2.66 (2H, m), 2.87-2.94 (12H, m), 4.16-4.39 (28H, m), 4.73 (2H, m), 5.04 (2H, m), 5.74 (2H, br s), 6.08 (2H, d, *J* 5.2), 6.15-6.19 (2H, m), 7.36 (2H, br s), 7.52 (8H, m), 7.62 (4H, m), 7.99 (4H, d, *J* 7.7), 8.04 (4H, d, *J* 7.8), 8.13 (2H, d, *J* 6.8), 8.66 (2H, s), 8.76 (2H, s), 11.18 (2H, br s) and 11.23 (2H, br s); δ_C(DMSO-d_6) 19.0 (3C), 19.1 (3C), 38.0 (2C), 62.8 (6C), 66.5 (2C), 67.6 (2C), 69.9 (2C), 72.8 (1C), 72.9 (1C), 77.2 (1C), 77.3 (1C), 82.3 (1C), 82.4 (1C), 83.0 (1C), 83.1 (1C), 86.9 (2C), 87.8 (2C), 96.5 (2C), 118.4 (6C), 125.8 (2C), 128.5 (arom.C, 16C), 132.5 (arom.C, 2C), 132.8 (arom.C, 2C), 133.1 (arom.C, 2C), 133.3 (arom.C, 2C), 143.2 (2C), 145.1 (2C), 150.4 (2C), 151.7 (2C), 152.2 (2C), 154.2 (2C), 163.3 (2C), 165.7 (2C) and 167.3 (2C); HRMS $[M+1]^+$, Found: 1004.2513. $C_{42}H_{44}N_{11}O_{15}P_2$ requires m/z, 1004.2494.

5′-*O*-Phosphoryl-2′-deoxycytidylyl-(3′ → 5′)-2′,3′-*O*-(*tert*-butyldimethylsilyl)adenosine (12a) Following a similar procedure described for **7**, **12a** (68 mg, 97%) was obtained from **10a** (0.081 mmol scale) as a white solid, m/z (MALDITOF) 865.25 ($[M+1]^+$). The structure of **12a** was further confirmed by transforming it into pdCpA **7** upon treatment with 1M TBAF in THF.

5′-*O*-Phosphoryl-2′-deoxycytidylyl-(3′→5′)-2′,3′-*O*-isopropylideneadenosine (12b) Following a similar procedure described for **7**, **12b** (62 mg, 95%) was obtained from **10b** (0.096 mmol scale) as a white solid. UV (H_2O) λ_{max} 260 nm (ε 20 000). δ_P(D_2O) -0.13 and 1.21; δ_H(D_2O) 1.30 (3H, s), 1.51 (3H, s), 1.87 (1H, m), 2.23 (1H, m), 3.85 (2H, m), 3.95 (2H, m), 4.08 (1H, m), 4.50 (1H, m), 4.61 (1H, m), 5.05 (1H, dd, *J* 2.3 and 6.1), 5.31 (1H, dd, *J* 2.9 and 6.1), 5.87 (1H, m), 6.04 (1H, d, *J* 7.9), 6.08 (1H, d, *J* 2.9), 7.85 (1H, d, J 7.9), 8.12 (1H, s) and 8.28 (1H, s); δ_C(D_2O) 23.7 (1C), 25.5 (1C), 38.1 (1C), 64.0 (1C), 65.0 (1C), 75.6 (1C), 80.4 (1C), 83.2 (1C), 84.6 (1C), 84.7 (1C), 85.7 (1C), 90.0 (1C), 94.7 (1C), 114.4 (1C), 117.8 (1C), 140.7 (1C), 143.0 (1C), 147.7 (1C), 148.2 (1C), 148.8 (1C), 152.0 (1C), 159.5 (1C); HRMS $[M+1]^+$, Found: 677.1459. $C_{22}H_{31}N_8O_{13}P_2$ requires m/z, 677.1486.

4-*N*-benzoyl-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-*N*,6-N, 3′-O-tribenzoyl-2′-deoxy-adenosine (15) Following a similar procedure described for 4, 15 (187 mg, 62%, 2 diastereoisomers, 1:1 ratio) was obtained from **13** (0.30 mmol scale) as a white solid. δ_H($CDCl_3$) 2.28 (1H, m), 2.44 (1H, m), 2.64-2.86 (8H, m), 3.15 (1H, m), 3.17 (1H, m), 3.76 (4H, m), 4.17-4.53 (14H, m), 5.02 (1H, m), 5.16 (1H, m), 5.75 (2H, m), 6.18 (1H, m), 6.22 (1H, m), 6.62 (2H, m), 7.24-7.34 (8H, m), 7.45-7.48 (14H, m), 7.59-7.61 (4H, m), 7.81-7.86 (12H, m), 8.03 (4H, m), 8.18 (1H, d, *J* 7.4), 8.23 (1H, d, *J* 7.4 Hz), 8.45 (1H, s), 8.50 (1H, s), 8.66 (1H, s), 8.67 (1H, s) and 8.86 (2H, br s); δ_C($CDCl_3$) 19.5 (2C), 37.0 (1C), 37.2 (1C), 39.6 (1C), 39.7 (1C), 53.4 (2C), 61.2 (1C), 61.4 (1C), 62.5 (2C), 67.4 (2C), 74.5 (1C), 74.6 (1C), 78.5 (1C), 79.0 (1C), 83.2 (1C), 83.4 (1C), 84.5 (1C), 84.7 (1C), 86.2 (1C), 86.3 (1C), 87.6 (1C), 87.7 (1C), 96.8 (2C), 116.6 (1C), 116.7 (1C), 127.7 (arom.C, 4C), 128.6 (arom.C, 4C), 128.7 (arom.C, 8C), 128.8 (arom.C, 8C), 129.4 (arom.C, 8C), 129.7 (arom.C, 4C), 133.0 (arom.C, 2C), 133.1 (arom.C, 4C), 133.7 (arom.C, 2C),

133.8 (arom.C, 4C), 143.6 (1C), 143.7 (1C), 145.3 (2C), 151.7 (1C), 151.8 (1C), 152.3 (4C), 152.6 (1C), 152.7 (1C), 154.9 (2C), 162.4 (2C), 165.9 (2C), 172.3 (2C) and 172.4 (2C); HRMS $[M+Na]^+$, Found: 1032.2721. $C_{50}H_{44}N_9NaO_{13}P$ requires m/z, 1032.2694.

4-*N*-benzoyl-5′-*O*-[di(2-cyanoethyloxy)phosphoryl]-2′-deoxycytidylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-6-*N*,6-N,3′-O-tribenzoyl-2′-deoxyadenosine (16) Following a similar procedure described for **6**, **16** (562 mg, 95%, 2 diastereoisomers, 1:1 ratio) was obtained from **15** (0.495 mmol scale) as a white solid. UV (CH_2Cl_2) λ_{max} 257 nm (ε 35 000), λ 234 nm (sh ε 27 500). $\delta_H(CDCl_3)$ 2.22 (1H, m), 2.33 (1H, m), 2.66-2.87 (16H, m), 3.17 (1H, m), 3.21 (1H, m), 4.12-4.50 (24H, m), 5.10 (1H, m), 5.15 (1H, m), 5.77 (2H, m), 6.20 (2H, m), 6.61 (2H, m), 7.30 (2H, m), 7.34 (8H, m), 7.42-7.50 (12H, m), 7.59 (4H, m), 7.80-7.82 (8H, m), 7.88 (4H, m), 7.99 (1H, d, *J* 7.4), 8.00 (1H, d, *J* 7.2), 8.03-8.05 (4H, m), 8.47 (1H, s), 8.49 (1H, s) 8.67 (2H, s) and 9.30 (2H, br s); $\delta_C(CDCl_3)$ 19.6 (3C), 19.7 (3C), 37.0 (1C), 37.1 (1C), 39.2 (2C), 46.4 (2C), 62.7 (6C), 66.6 (1C), 66.7 (1C), 67.6 (1C), 67.7 (1C), 74.4 (1C), 74.5 (1C), 77.6 (2C), 83.5 (2C), 84.7 (2C), 87.4 (1C), 87.5 (1C), 96.9 (2C), 116.6 (3C), 116.7 (3C), 127.7 (4C), 127.75 (1C), 127.8 (1C), 128.6 (2C), 128.7 (2C), 128.8 (8C), 128.9 (4C), 129.0 (4C), 129.4 (8C), 129.8 (4C), 132.9 (2C), 133.1 (4C), 133.2 (2C), 133.8 (1C), 133.9 (1C), 133.95 (1C), 134.0 (1C), 143.6 (2C), 143.5 (2C), 151.8 (1C), 151.9 (1C), 152.4 (4C), 152.7 (2C), 154.1 (2C), 162.6 (2C), 166.0 (2C) and 172.3 (4C); HRMS $[M+Na]^+$, Found: 1218.2978. $C_{56}H_{51}N_{11}NaO_{16}P_2$ requires m/z, 1218.2888.

5′-*O*-Phosphoryl-2′-deoxycytidylyl-(3′→5′)-2′-deoxyadenosine (17) Following a similar procedure described for **7, 17** (95 mg, 92%) was obtained from **16** (0.167 mmol scale) as a white solid. UV (H_2O) λ_{max} 260 nm (ε 21 700). $\delta_P(D_2O)$ 0.17 and 1.59; $\delta_H(D_2O)$ 1.47 (1H, m), 2.03 (1H, m), 2.41 (1H, m), 2.69 (1H, m), 3.78 (2H, m), 3.88 (2H, m), 4.02 (1H, m), 4.06 (1H, m), 4.52 (1H, m), 4.59 (1H, m), 5.84 (1H, d, *J* 7.3), 5.85 (1H, m), 6.21 (1H, m), 7.53 (1H, d, *J* 7.6), 7.93 (1H, s) and 8.22 (1H, s); $\delta_C(D_2O)$ 37.6 (1C), 38.0 (1C), 63.8 (1C), 64.4 (1C), 70.2 (1C), 75.2 (1C), 82.5 (1C), 84.1 (1C), 84.9 (1C), 85.0 (1C), 95.5 (1C), 117.3 (1C), 138.9 (1C), 140.8 (1C), 147.7 (1C), 151.5 (2C), 154.1 (1C) and 163.4 (1C); HRMS $[M+1]^+$, Found: 621.1234. $C_{19}H_{26}N_8O_{12}P_2$ requires m/z, 621.1224.

2′-*O*-*tert*-butyldimethylsilyluridylyl-{3′-*O*^{P}-2-cyanoethyl)]→5′}-3-*N*,2′-*O*,3′-*O*-tribenzoyl-uridine (20) Following a similar procedure described for **4, 20** (288 mg, 70%, 2 diastereoisomers, 62:38 ratio) was obtained from **18** (0.40 mmol scale) as a white solid. UV (CH_2Cl_2) λ 254 nm (ε 30 800), λ_{max} 240 nm (ε 32 300). $\delta_H(CDCl_3)$ major isomer: 0.09 (3H, s), 0.10 (3H, s), 0.86 (9H, s), 2.79 (2H, m), 3.87 (1H, m), 3.95 (1H, m), 4.33-4.41 (3H, m), 4.47-4.56 (3H, m), 4.68-4.71 (1H, m), 4.90 (1H, m), 5.63-5.71 (3H, m), 5.81 (1H, m), 5.97 (1H, d, *J* 8.2), 6.30 (1H, d, *J* 5.8), 7.29-7.32 (2H, m), 7.35-7.39 (2H, m), 7.39-7.44 (2H, m), 7.50-7.57 (2H, m), 7.60 (1H, m), 7.67 (1H, m), 7.76 (1H, d, *J* 8.1), 7.85 (2H, d, *J* 7.4), 7.92 (4H, m) and 8.90 (1H, s); minor isomer: 0.06 (3H, s), 0.08 (3H, s), 0.84 (9H, s), 2.79 (2H, m), 3.87 (1H, m), 3.95 (1H, m), 4.33-4.41 (3H, m), 4.47- 4.56 (3H, m), 4.68-4.72 (1H, m), 4.94 (1H, m), 5.63-5.71 (3H, m), 5.81 (1H, m), 5.82 (1H,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

d, *J* 8.2), 6.25 (1H, d, *J* 5.4), 7.29-7.32 (2H, m), 7.35-7.39 (2H, m), 7.39-7.44 (2H, m), 7.50-7.57 (2H, m), 7.60 (1H, m), 7.67 (1H, m), 7.73 (1H, d, *J* 8.2), 7.84 (2H, d, *J* 7.1), 7.92 (4H, m) and 8.84 (1H, s); $\delta_C(CDCl_3)$ major isomer: -4.9 (2C), 18.0 (1C), 19.8 (1C), 25.6 (3C), 60.9 (1C), 63.0 (1C), 63.1 (1C), 67.2 (1C), 70.6 (1C), 73.3 (1C), 76.3 (1C), 81.1 (1C), 81.2 (1C), 83.2 (1C), 91.7 (1C), 102.5 (1C), 103.7 (1C), 116.5 (1C), 128.1 (arom.C, 1C), 128.3 (arom.C, 1C), 128.5 (arom.C, 2C), 128.6 (arom.C, 2C), 129.2 (arom.C, 2C), 129.8 (arom.C, 2C), 129.9 (arom.C, 2C), 130.5 (arom.C, 2C), 131.1 (arom.C, 1C), 134.0 (arom.C, 2C), 135.2 (arom.C, 1C), 139.9 (1C), 141.7 (1C), 149.4 (1C), 150.3 (1C), 161.6 (1C), 163.0 (1C), 165.4 (1C), 165.5 (1C) and 168.3 (1C); minor isomer: -5.0 (2C), 18.0 (1C), 19.7 (1C), 25.6 (3C), 61.1 (1C), 62.8 (1C), 62.9 (1C), 67.1 (1C), 70.5 (1C), 73.4 (1C), 76.3 (1C), 81.0 (1C), 81.1 (1C), 83.2 (1C), 92.1 (1C), 102.5 (1C), 103.6 (1C), 116.6 (1C), 128.1 (arom.C, 1C), 128.3 (arom.C, 1C), 128.5 (arom.C, 2C), 128.6 (arom.C, 2C), 129.2 (arom.C, 2C), 129.8 (arom.C, 2C), 129.9 (arom.C, 2C), 130.5 (arom.C, 2C), 131.1 (arom.C, 1C), 134.0 (arom.C, 2C), 135.2 (arom.C, 1C), 140.1 (1C), 142.1 (1C), 149.3 (1C), 150.2 (1C), 161.7 (1C), 162.9 (1C), 165.4 (1C), 165.6 (1C) and 168.4 (1C); HRMS $[M+Na]^+$, Found: 1052.2783. $C_{48}H_{52}N_5NaO_{17}PSi$ requires m/z, 1052.2763.

2′-*O*-*tert*-butyldimethylsilyl-5′-*O*-[di(2-cyanoethyloxy)phosphoryl]-uridylyl-{3′-*O*^{P}-2-cyanoethyl)]→ 5′}-3-*N*,2′-*O*,3′-*O*-tribenzoyluridine (21)
Following a similar procedure described for **6**, **21** (170 mg, 96%, 2 diastereoisomers, 62:38 ratio) was obtained from **20** (0.146 mmol scale) as a white solid. UV (CH_2Cl_2) λ 256 nm (ε 32 000), λ_{max} 240 nm (ε 33 800). $\delta_H(CDCl_3)$ major isomer: 0.09 (3H, s), 0.11 (3H, s), 0.86 (9H, s); minor isomer: 0.08 (3H, s), 0.10 (3H, s), 0.85 (9H, s); the remaining protons have the identical chemical shifts: 2.71-2.86 (6H, m), 4.29-4.62 (13H, m), 4.94 (1H, m), 5.64-5.81 (4H, m), 5.97 (1H, m), 6.30 (1H, d, *J* 4.6), 7.28-7.31 (2H, m), 7.35-7.38 (2H, m), 7.40-7.44 (2H, m), 7.49- 7.56 (3H, m), 7.60 (1H, m), 7.72 (1H, d, *J* 8.2), 7.84 (2H, m), 7.92 (4H, m) and 9.29 (1H, br s); $\delta_C(CDCl_3)$ major isomer: -4.98 (1C), -4.96 (1C), 18.0 (1C), 19.7 (3C), 25.5 (3C), 62.8 (3C), 63.1 (1C), 66.1 (1C), 67.2 (1C), 70.5 (1C), 73.4 (1C), 74.9 (1C), 80.1 (1C), 81.0 (1C), 88.0 (1C), 91.1 (1C), 102.7 (1C), 103.5 (1C), 116.7 (3C), 128.2 (arom.C, 1C), 128.4 (arom.C, 1C), 128.5 (arom.C, 2C), 128.6 (arom.C, 2C), 129.2 (arom.C, 2C), 129.7 (arom.C, 2C), 129.8 (arom.C, 2C), 130.5 (arom.C, 2C), 131.13 (arom.C, 1C), 133.9 (arom.C, 2C), 135.2 (arom.C, 1C), 140.1 (1C), 140.6 (1C), 149.4 (1C), 150.2 (1C), 161.7 (1C), 163.0 (1C), 165.3 (1C), 165.4 (1C) and 168.4 (1C); minor isomer: -4.94 (1C), -4.92 (1C), 18.0 (1C), 19.6 (3C), 25.5 (3C), 62.8 (3C), 63.0 (1C), 66.5 (1C), 67.4 (1C), 70.4 (1C), 73.3 (1C), 74.9 (1C), 80.1 (1C), 81.0 (1C), 88.0 (1C), 92.2 (1C), 102.8 (1C), 103.5 (1C), 116.7 (3C), 128.1 (arom.C, 1C), 128.4 (arom.C, 1C), 128.5 (arom.C, 2C), 128.6 (arom.C, 2C), 129.2 (arom.C, 2C), 129.7 (arom.C, 2C), 129.8 (arom.C, 2C), 130.53 (arom.C, 2C), 131.1 (arom.C, 1C), 133.9 (arom.C, 2C), 135.23 (arom.C, 1C), 140.1 (1C), 141.1 (1C), 149.5 (1C), 150.1 (1C), 161.7 (1C), 162.9 (1C), 165.3 (1C), 165.4 (1C) and 168.6 (1C); HRMS $[M+Na]^+$, Found: 1238.2907. $C_{54}H_{59}N_7NaO_{20}P_2Si$ requires m/z, 1238.2957.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ACKNOWLEDGMENTS

This work was supported by grants from the Texas Advanced Technology Research Program and the Robert A. Welch Foundation. We thank Dr. Clotide Pichon-Santander for helpful discussion and Dr. Howard J. Williams for his valuable suggestion of NMR data assignments.

REFERENCES

1. Noren, C.J.; Anthony-Cahill, S.J.; Griffith, M.C.; Schultz, P.G. *Science* **1989**, *244*, 182–188.
2. Robertson, S.A.; Noren, C.J.; Anthony-Cahill, S.J.; Griffith, M.C.; Schultz, P.G. *Nucleic Acids Res.* **1989**, *17*, 9649–9660.
3. Mendel, D.; Ellman, J.A.; Schultz, P.G. *J. Am. Chem. Soc.* **1991**, *113*, 2758–2760.
4. Robertson, S.A.; Ellman, J.A.; Schultz, P.G. *J. Am. Chem. Soc.* **1991**, *113*, 2722–2729.
5. Ellman, J.A.; Mendel, D.; Schultz, P.G. *Science* **1992**, *255*, 197–200.
6. Mendel, D.; Ellman, J.A.; Chang, Z.; Veenstra, D.L.; Kollman, P.A.; Schultz, P.G. *Science* **1992**, *256*, 1798–1802.
7. Cload, S.T.; Liu, D.R.; Froland, W.A.; Schultz, P.G. *Chemistry & Biology* **1996**, *3*, 1033–1038.
8. Bain, J.D.; Glabe, C.G.; Dix, T.A.; Chamberlin, A.R. *J. Am. Chem. Soc.* **1989**, *111*, 8013–8014.
9. Bain, J.D.; Wacker, D.A.; Kuo, E.E.; Chamberlin, A.R. *Tetrahedron* **1991**, *47*, 2389–2400.
10. Bain, J.D.; Diala, E.S.; Glabe, C.G.; Wacker, D.A.; Lyttle, M.H.; Dix, T.A.; Chamberlin, A. R. *Biochemistry* **1991**, *30*, 5411–5421.
11. Steward, L.E.; Collins, C.S.; Gilmore, M.A.; Carlson, J.E.; Ross, J.B.A.; Chamberlin, A.R. *J. Am. Chem. Soc.* **1997**, *119*, 6–11.
12. Hecht, S.M. *Acc. Chem. Res.* **1992**, *25*, 545–552.
13. Cornish, V.W.; Mendel, D.; Schultz, P.G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 621–633.
14. England, P.M.; Lester, H.A.; Davidson, N.; Dougherty, D.A. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11025–11030.
15. Hohsaka, T.; Kajihara, D.; Ashizuka, Y.; Murakami, H.; Sisido, M. *J. Am. Chem. Soc.* **1999**, *121*, 34–40.
16. Noren, C.J.; Anthony-Cahill, S.J.; Suich, D.J.; Noren, K.A.; Griffith, M.C.; Schultz, P.G. *Nucleic Acids Res.* **1990**, *18*, 83–88.
17. Bain, J.D.; Wacker, D.A.; Kuo, E.E.; Lyttle, M.H.; Chamberlin, A.R. *J. Org. Chem.* **1991**, *56*, 4615–4625.
18. Heckler, T.G.; Chang, L.H.; Zama, Y.; Naka, T.; Hecht, S.M. *Tetrahedron* **1984**, *40*, 87–94.
19. Heckler, T.G.; Chang, L.H.; Zama, Y.; Naka, T.; Chorghade, M.S.; Hecht, S.M. *Biochemistry* **1984**, *23*, 1468–1473.
20. Roesser, J.R.; Xu, C.; Payne, R.C.; Surratt, C.K.; Hecht, S.M. *Biochemistry* **1989**, *28*, 5185–5195.
21. Niinomi, T.; Sisido, M. *Chem. Lett.* **1993**, 1305–1308.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

22. Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 3626.
23. Gu, Q.-M.; Prestwich, G.D. *J. Org. Chem.* **1996**, *61*, 8642–8647.
24. Imai, J.; Torrence, P.F. *J. Org. Chem.* **1981**, *46*, 4015–4021.
25. Scott, A.I. *Tetrahedron Lett.* **1997**, *38*, 4961–4964.
26. Sen, S.E.; Roach, S.L.; Boggs, J.K.; Ewing, G.J.; Magrath, J. *J. Org. Chem.* **1997**, *62*, 6684–6686.
27. Uhlmann, E.; Engls, J. *Tetrahedron Lett.* **1986**, *27*, 1023–1026.
28. Zhu, X.-F.; Williams, H.J.; Scott, A.I. *J. Chem. Soc. Perkin Trans. 1* **2000**, (*15*), 2305–2306.

Received May 10, 2000
Accepted October 27, 2000

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!