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Solution-Phase Synthesis of Branched DNA Hybrids Based on Dimer Phosphoramidites and Phenolic or Nucleosidic Cores

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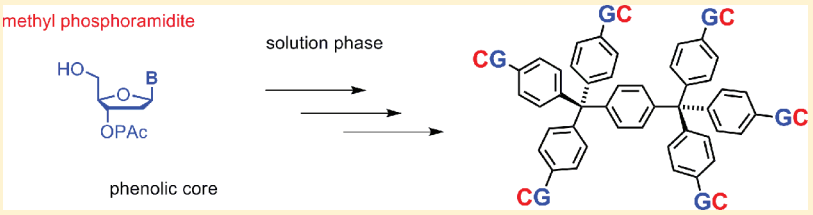
Supporting Information



ABSTRACT: Branched oligonucleotides with "CG zippers" as DNA arms assemble into materials from micromolar solutions. Their synthesis has been complicated by low yields in solid-phase syntheses. Here we present a solution-phase synthesis based on phosphoramidites of dimers and phenolic cores that produces six-arm or four-arm hybrids in up to 61% yield. On the level of hybrids, only the final product has to be purified by precipitation or chromatography. A total of five different hybrids were prepared via the solution-phase route, including new hybrid $(TCG)_4TTPA$ with a tetrakis(triazolylphenyl)adamantane core and trimer DNA arms. The new method is more readily scaled up than solid-phase syntheses, uses no more than 4 equiv of phosphoramidite per phenolic alcohol, and provides routine access to novel materials that assemble via predictable base-pairing interactions.

INTRODUCTION

Synthesis has been the driving force behind countless advances in science and technology. This is also true for the synthesis of nucleic acids. The development of automated solid-phase synthesis of oligodeoxynucleotides in the late 1970s and 1980s[1] enabled decisive advances in biotechnology, genetics, and diagnostics. Likewise, synthetic methodologies are driving new developments in sequencing[2] and synthetic biology.[3,4] Currently, chain assembly on solid support, based on phosphoramidites of protected nucleosides as monomers, is the dominating methodology for synthesizing both DNA and RNA strands.[5] This methodology routinely produces linear oligonucleotides of any given sequence up to 150 nucleotides in length in fully automated fashion.

The advent of DNA nanotechnology[6] has increased the demand for conventional synthetic oligonucleotides[7] but has also spawned research into branched oligonucleotides not found in biology.[8,9] Among the branched structures are conjugates between DNA and dendrimers,[10] transition-metal complexes,[11] gold nanoparticles,[12,13] cross-links,[14] or organic branching elements.[15,16] We were recently able to show that branched oligonucleotide hybrids featuring rigid organic branching elements as cores and a direct phosphodiester linkage to the DNA assemble at much higher temperatures than linear control strands.[17] When self-complementary DNA sequences are appended to the termini of tetrahedral or pseudo-octahedral cores, such as tetrakis(*p*-hydroxyphenyl)-methane (TPM) or hexakis(*p*-hydroxyphenyl)xylene (HPX), dinucleotides of the sequence CG suffice to induce assembly into a material from dilute aqueous solution upon addition of divalent cations.[17,18] The strong assembly forces observed in these multivalently binding hybrids have led to the term "CG zippers" for dinucleotide arms in hybrids, such as **1** and **2** (Figure 1). Diffusion of intercalators into assemblies demonstrated that the materials are porous and produced fluorescent solids with interesting optical properties.[18]

The stability of phenolic phosphodiester linkages in hybrids of oligodeoxynucleotides to the deprotection conditions used in standard DNA synthesis protocols had been established prior to our work.[19] But, no more than minute amounts of branched oligonucleotides with rigid phenolic cores had been available, hampering an exploration of the interesting materials formed by hybrids. Compounds like **1** and **2** were synthesized in low yield on controlled pore glass (cpg), using a combination of 5′- and 3′-phosphoramidite building blocks, phenolic cores, and on-support phosphitylation, immediately followed by coupling of alcohols.[17]

Optimization of the solid-phase syntheses proved challenging. The lability of the phosphoramidite-terminated chain, the simultaneous assembly of three or five oligonucleotide chains on a nonswellable support, together with issues arising from the properties of aromatic triester intermediates made it difficult to generate quantities suitable for systematic crystallization screens

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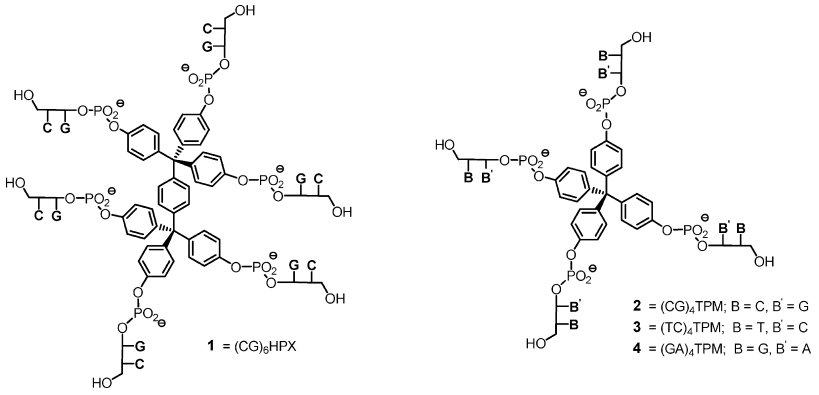


Figure 1. Branched DNA hybrids with six or four DNA chains attached to a phenolic core.

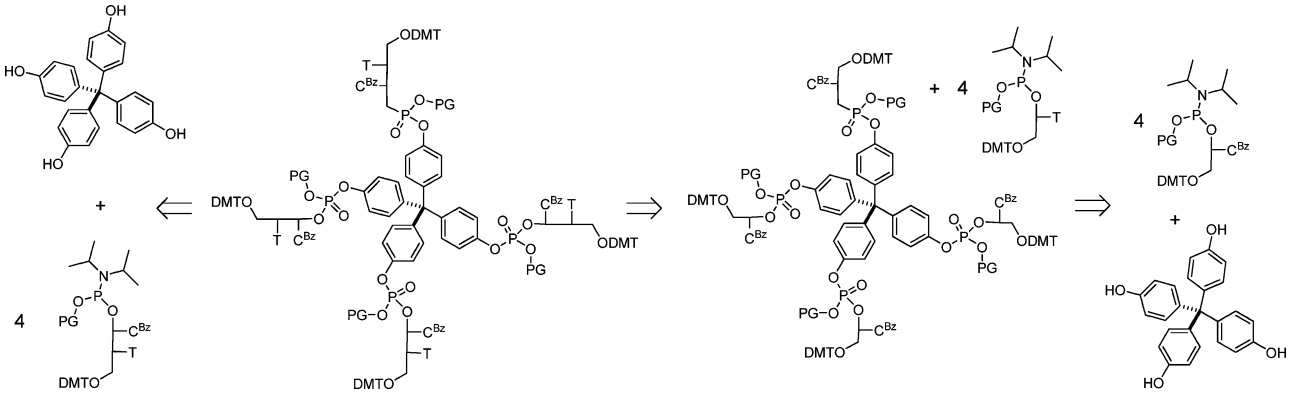
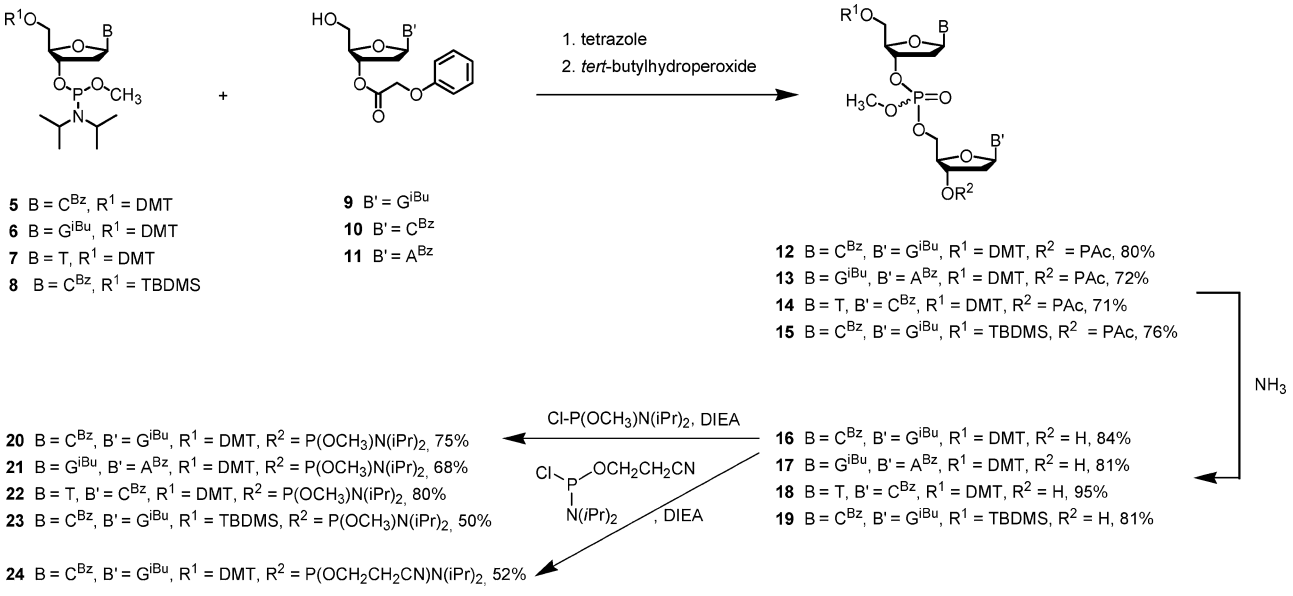


Figure 2. Retrosynthetic approaches to a protected DNA hybrid, starting from a phenolic core and either dinucleotide building blocks or phosphoramidites of mononucleosides; PG = protecting group.

Scheme 1. Synthesis of 3′-Phosphoramidites of Dimer



or studies on the absorption of gases or guest molecules. Therefore, we decided to establish a solution-phase synthesis, well knowing that this approach, classically employed for DNA chain assembly,[20] had become unpopular among most nucleic acid chemists.[21] Here we report an efficient solution-phase synthesis of hybrids **1**–**4**, based on the coupling of 3′-phosphoramidites of dimer fragments and unmodified cores, together with observations on the chemistry of these phenolic diesters. All intermediates on the hybrid stage were isolated without chromatography.

■ RESULTS AND DISCUSSION

Two strategies for the assembly of fully protected branched DNA hybrids were tested: block coupling of dimers to the core, or stepwise elaboration of the DNA chains from monomer

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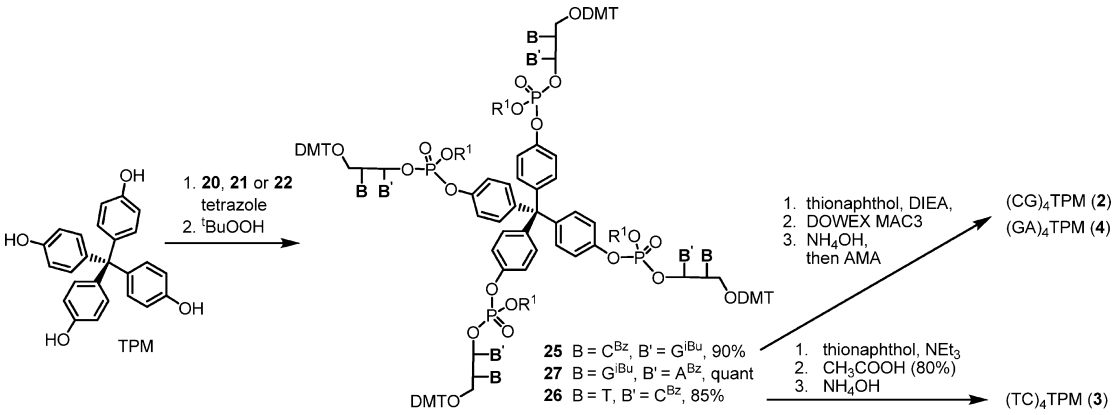
Scheme 2. Synthesis of the TPM Hybrids $(CG)_4$TPM (2), $(TC)_4$TPM (3), and $(GA)_4$TPM (4)[a]



[a]AMA = ammonium hydroxide/methylamine (1:1).

phosphoramidites, as shown in Figure 2 for a TPM hybrid with DNA arms of the sequence 5′-TC-3′. Initial experiments focused on the latter approach, as this promised low costs, being based on inexpensive commercial phosphoramidites. Attempts to employ the methodology of van der Marel and colleagues, originally developed for solution-phase synthesis of short linear DNA chains,[21b] were not satisfactory. Besides incomplete coupling to phenolic cores, severe losses during handling and purification of intermediates that were apparently caused by the lability of the cyanoethyl groups of the phosphotriesters limited overall yields to below 5% in the very best cases and usually to yields ≤1%.

We then focused on block coupling, based on dimer phosphoramidites, to reduce the number of steps on the hybrid level and to facilitate purification. Failure products lacking entire chains should be easier to separate from products than failures lacking only a single nucleotide. Several approaches to the synthesis of dimer or trimer phosphoramidites as building blocks in solid-phase DNA synthesis exist.[22] We chose the method of Virnekäs et al.,[23] originally developed for trimer phosphoramidites, because it gave satisfactory yields in exploratory experiments and because it uses methyl protecting groups for the phosphotriesters. The methyl groups should be more stable than the cyanoethyl groups tested first. They should be removable by S_N2 displacement with soft nucleophiles.

Three different DNA sequences were tested: the established self-complementary 5′-CG-3′ "zipper" dimer,[17,18] and the two nonselfcomplementary sequences 5′-TC-3′ and 5′-GA-3′ that were used as sticky ends in designed nanoporous DNA crystals.[24] Two different 5′-protecting groups were tested: the conventional dimethoxytrityl (DMT) group, and the *tert*-butyldimethylsilyl (TBDMS) group that can be removed under basic conditions, thus avoiding acidic conditions on the hybrid level. Tailored silyl-protecting groups have been developed for solid-phase RNA synthesis,[25] where multiple deprotections are required. However, TBDMS group are well established as 2′-protecting groups of oligoribonucleotide chains, where a single deprotection step is employed after assembly of the entire chain.[26] Also, both methyl and cyanoethyl protecting groups were tested for the 3′-terminal phosphite/phosphate (dimers **20** and **24**). The cyanoethyl group can be removed under milder conditions, reducing the likelihood of cleaving phenolic esters during the process. Scheme 1 shows the route to the dimer phosphoramidites.

Chain assembly started from commercial phosphoramidites **5**−**8**. In our case, they were prepared by phosphitylation of the 3′-unprotected nucleosides, as detailed in the Supporting Information (Scheme S1). Unexpectedly, the diastereomeric mixture of silyl-protected phosphoramidites (**8**) crystallized from pentane. Phosphoramidites **5**−**8** were coupled to 5′-alcohols **9**−**11**, and the resulting phosphite triesters were oxidized in situ with TBHP to give fully protected dimers **12**−**15** in 71−80% yield, after chromatography. The 3′-terminal phenoxyacetyl (PAC) groups were then removed via aminolysis to yield **16**−**19**. Careful monitoring of this reaction is recommended to avoid the loss of base protecting groups. Two equivalents of ammonia gave better yields than the 10 equiv reported in ref 23. Phosphitylation of the 3′-alcohols then gave the desired dimer building blocks **20**−**24** in 50−80% yield after column chromatography (31−54% overall yield from the monomeric starting materials). Lower yields for **23** and **24** may be due to unoptimized conditions.

We then turned to the assembly of hybrids. First, the less challenging four-arm hybrids with TPM as core were synthesized (Scheme 2). Initially, all attempts to obtain **25**−**27** in high yield were unsuccessful. Independent of how many equivalents of phosphoramidite were used and how long the coupling reaction was allowed to proceed, incomplete conversions were observed, as monitored by MALDI-TOF mass spectra of crudes, measured after detritylation (the DMT-protected species gave too little signal). Invariably, the spectra also showed peaks for products with just three or two DNA chains.

A detailed study then demonstrated that over time decomposition or hydrolysis of the initial phosphite triester set in, competing with the coupling to the remaining free hydroxy groups. The modest solubility of the core further complicated the synthesis. Five steps were taken to overcome these problems. First, the concentration of the tetrazole catalyst was reduced, thus reducing the rate of the decomposition reaction. Second, the core was freeze-dried from a shock-frozen hot solution in dioxane, producing a fine powder that helped to bring the TPM rapidly into solution upon reacting with the phosphoramidites. Third, to remove residual water traces, the core was dried in vacuo at 130 °C for 2 h, and molecular sieves (3 Å) were added to the mixture of educts for coupling. Fourth, an unusual temperature protocol was followed because a temperature study had shown that decomposition became more dominant at elevated temperature than in the cold. During the

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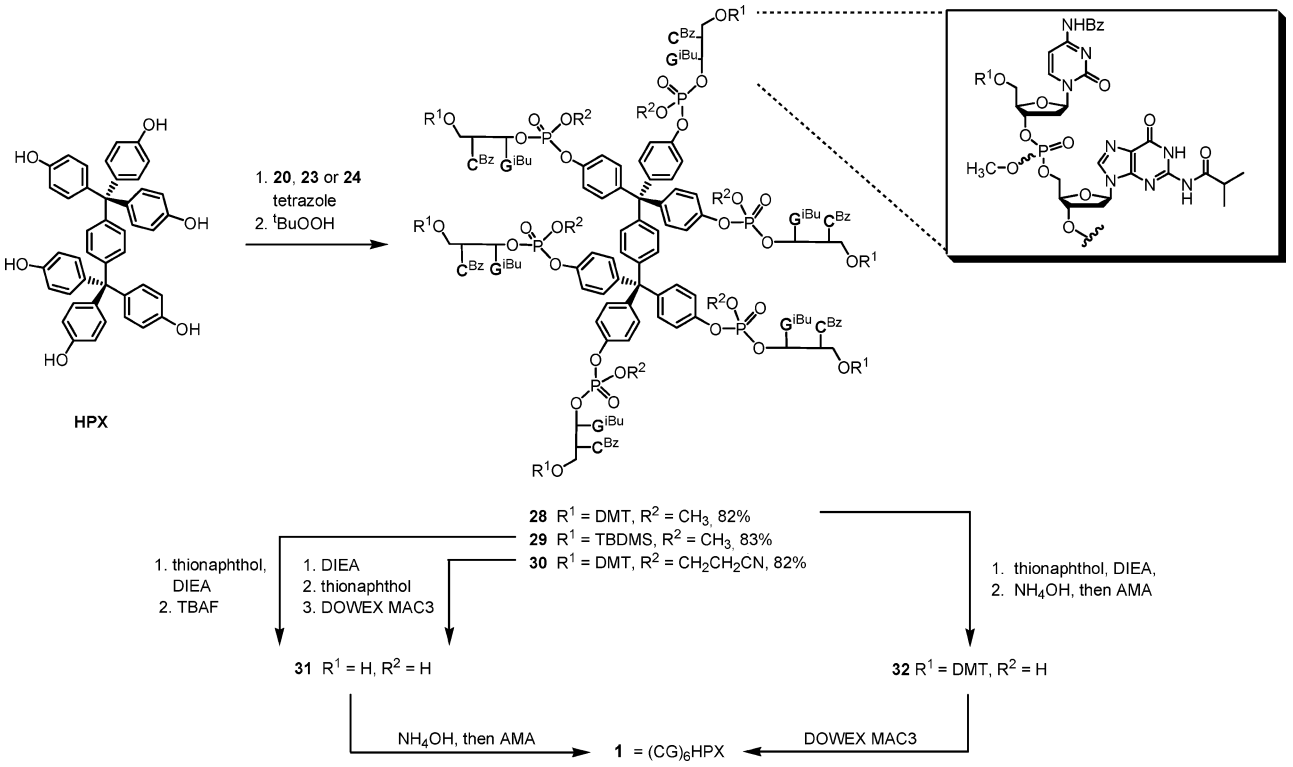
Scheme 3. Synthesis of HPX Hybrid $(CG)_6$HPX (1)



initial phase of the reaction, the solution was sonicated at room temperature until the core had dissolved, and then the reaction mixture was cooled to allow the coupling to progress to completion. Finally, two consecutive cycles of coupling with subsequent oxidation were employed to drive the assembly of the hybrids to completion, where necessary, with isolation of the first crude via precipitation. With these measures, hybrids **25**–**27** were obtained in 85–99% yield.

Deprotection of the hybrids proved challenging. Difficulties in deprotecting the phenolic triester were expected, based on the literature.[27] Partial loss of DNA chains on one of the three stages of the deprotection schemes was difficult to suppress. The acidic step for the removal of the DMT groups was studied first. For each of the three hybrids **25**–**27**, less than 5% three-arm product was observed at the end of the reaction, both with trichloroacetic acid (TCA, 3% in CH_2Cl_2) followed by methanol and with 80% AcOH. The same was true for the detritylation of the analogous six-arm hybrid with HPX core (Scheme 3). Only when the methyl groups were removed first, and the resulting diesters were treated with strongly acidic cation-exchange resin Amberlyst 15 (H^+ form) did depurination set in noticeably, as monitored by MALDI-TOF mass spectrometry. The final two basic steps (deprotection of the phosphotriesters to phosphodiesters and removal of nucleobase protecting groups) caused more side reactions, leading to partial loss of DNA chains for purine-containing sequences. The side products lacked one or several DNA chains, usually featuring phosphate groups instead. These "failures" were not the result of incomplete couplings, though, because MALDI-TOF mass spectra of protected intermediates showed little evidence of such species (Figure 3) and because incomplete coupling should result in unphosphorylated, free phenols.

For TPM hybrids **25**–**27**, two different protocols were tested. For purine-containing **25** and **27**, the methyl protecting groups were removed first. For this, thionaphthol was identified as a less toxic and more selective substitute for thiophenol. This

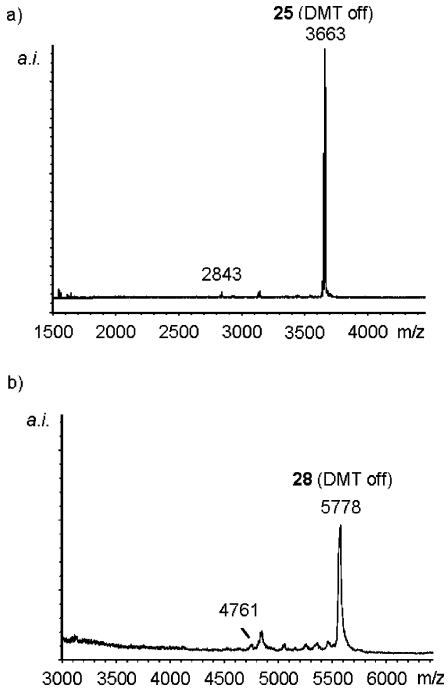


Figure 3. MALDI-TOF mass spectra of crudes of protected hybrids after removal of the DMT groups with trichloroacetic acid (TCA, 3% in CH_2Cl_2 for 30 s, followed by addition of methanol). (a) Four-arm hybrid **25** and b) six-arm hybrid **28**. In each spectrum, peaks of residual side products lacking one oligonucleotide chain are labeled, with m/z = 2843 for $(C^{Bz}G^{iBu})_3$TPM, and m/z = 4761 for $(C^{Bz}G^{iBu})_5$HPX.

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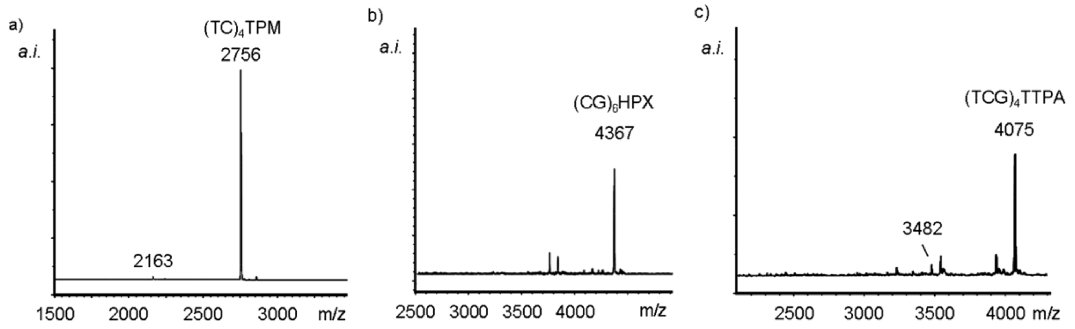

Figure 4. MALDI-TOF mass spectra of (a) crude $(TC)_4$TPM (**3**), (b) crude $(CG)_6$HPX (**1**), and (c) crude $(TCG)_4$TTPA (**34**).

reagent also gave higher yields than 2-carbamoyl-2-cyano-ethylene-1,1-dithiolate, a reagent developed specifically for the demethylation step.[28] A solution in THF/water with diisopropylethylamine (DIEA) as base gave the best results. Subsequently, the DMT groups were removed by treatment with acidic cation-exchange resin in a mixture of water and ethyl acetate. Finally, the base protecting groups were cleaved using ammonia and methylamine. For purine-free **26**, the demethylation step was also performed with thionaphthol in THF, but with triethylamine as base, followed by detritylation with 80% acetic acid. The final deprotection step proceeded in high yield with just aqueous ammonia. Hybrid **3** was obtained in sufficiently pure form for assembly studies, without chromatography, after precipitation from water/ethanol (Figures 4a and 5).

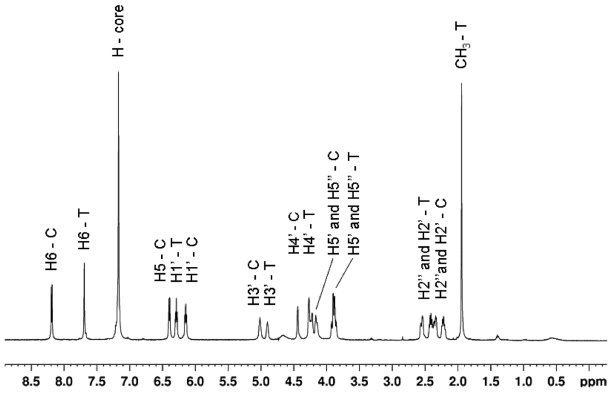

Figure 5. ^{1}H NMR spectrum of $(TC)_4$TPM (**3**) in D_2O at 40 °C (500 MHz). Assignments, based on 2D spectra, are given above the peaks.

As expected, on the basis of statistics, establishing a high-yielding synthesis for six-arm hybrid $(CG)_6$HPX was more difficult than for the TPM hybrids (Scheme 3). High-yielding coupling to all six phenolic hydroxy groups of HPX was achieved when the strategy described above was applied (finely dispersed core, "inverse" temperature regime, two coupling cycles) for each of the different CG dimers employed (**20**, **23**, and **24**). With fully protected hybrids **28**–**30**, a range of conditions was tested in order to optimize the deprotection to hybrid **1**. For this, crudes were again analyzed by MALDI-TOF mass spectrometry, and relative peak heights were compared without an attempt to compensate for differences in desorption and ionization efficiency. Since longer sequences fly more poorly,[29] the intensity of product peaks should underrepresent the true concentration of the fully assembled hybrids, compared to the failure sequences. As with the TPM hybrids, sufficient MALDI signal was observed only after removal of DMT groups.

For **28** with its conventional protection of 5′-termini and phosphodiesters, crudes of **1** contained 25–30% of the five-arm product with a phosphoryl group on one of the phenolic arms, when thionaphthol was used first, followed by removal of base protecting groups with NH_4OH and methylamine (a mixture commonly referred to as "AMA") and subsequent detritylation. When the DMT groups were removed first, significantly more truncation products were observed in MALDI spectra of crude **1**, with up to 10% four-arm products and up to 40% five-arm fragments. A similar result (35% terminally phosphorylated five-arm side product) was obtained when silyl-protected **29** was demethylated with thionaphthol and DIEA, followed by desilylation with TBAF and deacylation of the bases with AMA. Changing the order of desilylation with TBAF and deacylation

Table 1. Yield and Composition of Crudes and Isolated Yields of Purified Hybrids

entry no.	compd	yield of crude (%)	product ratio by MS *Pr*/*phosph*/−*arm*[a]	product ratio by HPLC *Pr*/*phosph*/−*arm*[b]	yield UV[c]/grav[d] (%)
1	$(CG)_6$HPX (**1**) from **28**	98	72:25:3	62:27:11	13/n.d.
2	$(CG)_6$HPX (**1**) from **29**	95	57:35:8		−/−
3	$(CG)_6$HPX (**1**) from **30**	88	74:12:14	72:13:15	25/50[e]
4	$(CG)_4$TPM (**2**)	94	60:29:11	61:28:11	18/n.d.
5	$(TC)_4$TPM (**3**)	87	96:<1:4	94:<1:6	40/61[f]
6	$(GA)_4$TPM (**4**)	94	35:59:6	51:40:9	5/n.d.
7	$(TCG)_4$TTPA (**34**)	quant	88:4:8		−/24[e]

[a]Ratio of product (*Pr*) to phosphorylated side product lacking one DNA arm (*phosph*) and hybrid lacking one arm (−arm), as determined by MALDI-TOF MS. Also compare Scheme 4 for the structure of fragments formed during deprotection. [b]Ratio of product and fragments, as determined by HPLC, corrected for differences in extinction coefficient. [c]Yield of hybrid determined by UV absorption at λ = 260 nm. [d]Yield of purified, isolated hybrid, determined gravimetrically. [e]Purified by HPLC. [f]Purified by precipitation.

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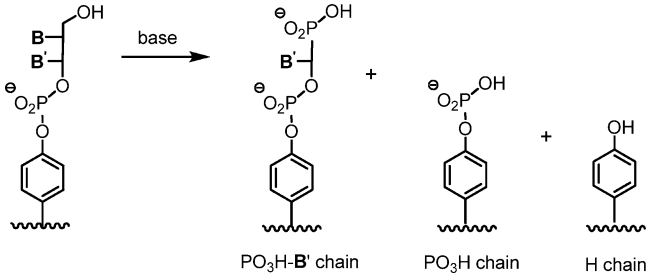
with AMA did not affect the compositions of crudes significantly.

Since the latter route avoids acidic steps, this, again, confirmed that the detritylation step is not the cause of the presumed strand cleavage reaction producing the phosphorylated five-arm product. In order to set the phenolic phosphodiester free early, **30** with its cyanoethyl protecting groups at the proximal linkages was first treated with tertiary amine, followed by demethylation of the distal methyl triesters, detritylation and final removal of the base protecting groups with AMA. Removal of methyl and cyanoethyl protecting groups was induced by a one-pot procedure, starting with a solution of DIEA, to which thionaphthol was added after 1 h. The four-step protocol gave less phosphorylated five-arm product, but some 14% five-arm hybrid with free phenolic hydroxy group, possibly due to incomplete coupling early on (Figure 4b).

Table 1 gives an overview of the results of the syntheses with the different hybrids. The comparison of ratios between full chain products and fragments determined by HPLC validate the results obtained by MALDI-TOF mass spectrometry. Only for $(GA)_4$TPM (**4**) did MALDI analysis show considerably less favorable product composition, most probably due to depurination during ionization and/or desorption in the MALDI, overlaying the fragmentation occurring during deprotection in solution. It is interesting to note that UV-determined yields are lower than those determined gravimetrically. This may be due, at least in part, to hypochromicity, caused by assembly (UV measurement), encapsulated water (gravimetry), and possibly incomplete dissolution of hybrids in stock solutions for absorption measurements.

Scheme 4. Base-Induced Fragmentation of Side Chains of DNA Hybrids



We suspected that the protected hybrids show an increased propensity to undergo an elimination reaction that leads to strand cleavage, with the phosphoryl group remaining on the phenolic core. Scheme 4 shows possible side products resulting from cleavage of phosphodiester linkages. In order to better understand the side products observed in the crudes of hybrids, we studied the resistance of hybrid **3** toward a number of different bases (Table 2, hybrid **1** gave similar results). Under conventional deprotection conditions (NH_4OH), little fragmentation was found (entries 1–3). The same was true for exposure to DBU, a base commonly used to induce β-eliminations (entries 4 and 5). When the hybrid was exposed to methylamine plus NH_4OH (AMA) at 60 °C, strand cleavage did occur, with a phosphorylated phenolic or aliphatic alcohol as the most common terminal group of the chain suffering cleavage. Exposure to potassium hydroxide in the heat gave the free phenolic chain, however, as expected for hydrolysis of a mixed alkyl/aryl ester, where the phenolate is the best leaving group.

Next, phenyl 2′-deoxyguanosine-3′-monophosphate was prepared as a model compound that contains the phenolic ester moiety but lacks the dendrimeric structure of hybrids (Scheme S2, Supporting Information). Using phosphoramidite **6** and phenol, the methyl phosphite triester intermediate was synthesized, mimicking fully protected hybrids with a 3′-terminal guanosine residue, such as **28**. When this intermediate was demethylated by treatment with thionaphthol, detritylated with TCA, and finally base-deprotected with AMA, the crude did not show signs of chain cleavage or other kinds of fragmentation (Figure S50, Supporting Information). Together, these results suggest that the lability toward basic deprotection under forced basic conditions is caused by the dendritic structure, not by the fact that the linkage to the core is a phenolic ester. The results also suggest that more labile protecting groups for the exocyclic amines of the nucleobases could reduce what remains a low-level side reaction for purine-containing hybrids.

Finally, we performed a hybrid synthesis starting from $(G^{iBu})_4$TTPA (**33**) as core, where TPPA stands for tetrakis-(triazolylphenyl)adamantane. Adamantane-based DNA hybrids with a triazole linkage to the 3′-terminal nucleoside were recently shown to assemble into materials from micromolar aqueous solutions at temperatures as high as 95 °C.[18] Starting from **33**, a hybrid with four trimer arms was assembled by coupling it to dimer phosphoramidite **22**, followed by oxidation and three-stage deprotection. Figure 4c shows the MALDI spectrum of the crude, confirming that the methodology established for TPM and HPX cores is also suitable for chain extension on cores terminating in (less reactive) aliphatic

Table 2. Extent of Fragmentation of DNA Hybrid $(TC)_4$TPM (3) under Different Basic Conditions

entry	base	time (d)	T (°C)	PO_3H-**C**-$(\mathbf{TC})_3$TPM[a] (%)	PO_3H-$(\mathbf{TC})_3$TPM[a] (%)	H-$(\mathbf{TC})_3$TPM[a] (%)
1	–	–	20	–	1	2
2	NH_4OH[b]	3	60	–	2	5
3	NH_4OH[b]	10	60	4	8	10
4	DBU[c]	1	20	–	1	3
5	DBU[c]	4	60	1	6	8
6	AMA[d]	3	20	–	1	4
7	AMA[d]	4	60	28	37	3
8	KOH[e]	1	20	–	2	7
9	KOH[e]	1	60	1	approx 10–20	65

[a]As determined by MALDI-TOF MS, based on and relative peak heights, not corrected for differences in desorption and ionization efficiency. [b]NH_4OH 25% aqueous ammonia. [c]DBU/H_2O (1:4, *v*/*v*). [d]AMA = NH_4OH/NH_2Me (1:1, *v*/*v*). [e]KOH, 2 M aqueous solution.

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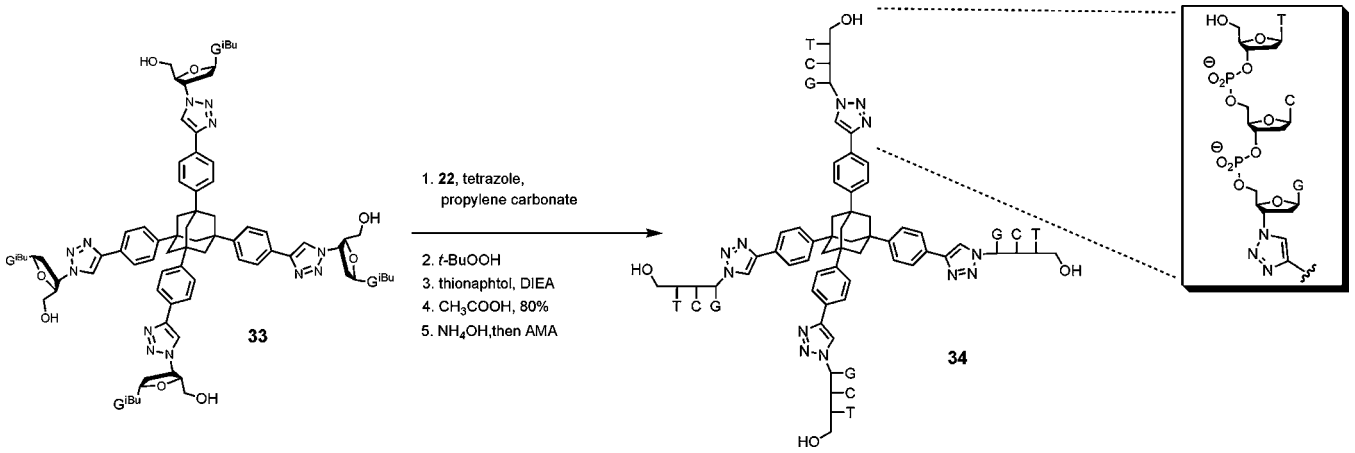
Scheme 5. Synthesis of Adamantane-Based Hybrid 34 with Four Trimer Arms



alcohols. As is common for branched oligonucleotides with a strong propensity to assemble, conventional HPLC conditions gave modest yields (24%). We are currently establishing chromatography under denaturing conditions to overcome this limitation. Still, one-stage HPLC purification gave spectroscopically pure **34** (Figure S37 and S49, Supporting Information).

With **34** in hand, we studied the assembly properties of this hybrid, whose dangling terminal nucleotide was designed to temper the very strong propensity of $(CG)_4$TTPA to form materials.[18] Figure 6 shows the binding curves, acquired under UV-melting curve conditions. Under low-salt conditions, a melting point of 18 °C was found. Thus, the reduction in melting point compared to $(CG)_4$TTPA, which shows a Tm of 61 °C at low salt[18] is very significant. Addition of NaCl (150 mM) increased this value to 35 °C. The strong dependence of the Tm on the salt concentration is expected for tightly packed three-dimensional lattices.[17] Further addition of $MgCl_2$ (10 mM) led to precipitation. At this magnesium concentration, the precipitate redissolved upon heating. At 100 mM $MgCl_2$ the material resisted thermal denaturation, even at 85 °C (Figure 6b). The material is porous enough to take up approximately 2 equiv of ethidium bromide per hybrid (Figure S51, Supporting Information), with strong fluorescence of the intercalator under UV light.

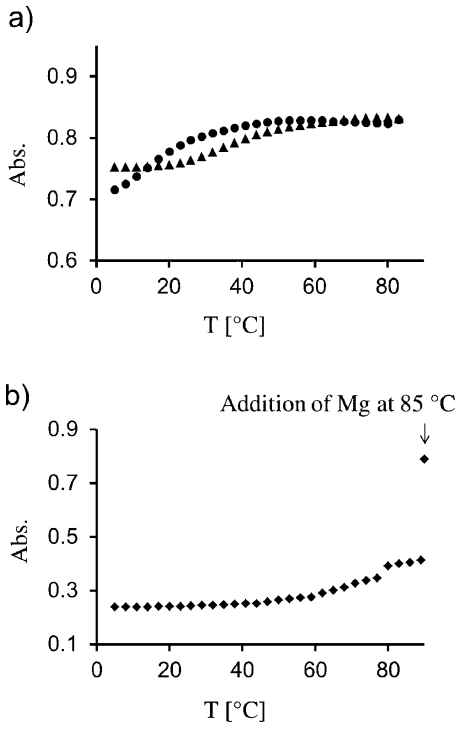


Figure 6. Assembly of $(TCG)_4$TTPA (**34**), as monitored by UV absorbance of 5 μM solutions in phosphate buffer (10 mM, pH 7) at λ_{det} = 260 nm and a cooling rate of 0.5 °C/min: (a) filled circles, buffer only; filled triangles, buffer plus 150 mM NaCl; (b) buffer plus 150 mM NaCl, plus 100 mM $MgCl_2$. Note the different expansion of the *y*-axis. The massive drop in absorbance is caused by precipitation. See Figure S51 in the Supporting Information for photographs of precipitates.

CONCLUSIONS

Here we present a methodology for solution-phase synthesis of branched oligonucleotide hybrids with up to three nucleosides per oligonucleotide chain. The methodology allows for the scalable preparation of compounds that assemble into a new type of material, based on programmable base-pairing interactions. The results show that high yields can be obtained for such hybrids, despite the congested steric situation, the lability of phenolic esters, low solubility of the cores, and the need to grow several chains simultaneously in dendrimer-like fashion. On the level of molecular recognition and material design, the properties of $(TCG)_4$TTPA (**34**) with its dangling 5′-terminal nucleotide demonstrate how the sequence and length of the DNA chain can be used to fine-tune the assembly process.

The phenolic nature of the linkages to the cores was initially considered the most problematic feature of the design. Certain aryl diesters of nucleotides are "active esters" that undergo rapid reactions with nucleophiles in aqueous solution, at least when nucleophile and ester are aligned on a template.[30] Classical DNA syntheses, such as the "phosphotriester method", employ chlorophenyl groups as protecting groups for the backbone linkages.[31] Other oligonucleotide constructs with phenolic ester linkages did exist prior to our study, though,[19] confirming that such esters *can* survive the deprotection conditions of conventional solid-phase DNA synthesis. Our experiments on the lability of hybrids (Table 2 and Figure S50, Supporting Information) indicate that the low-level fragmentation observed in crudes of purine-containing sequences (Figure 4) is not the consequence of the leaving group qualities of the phenolates. Rather, it appears that the

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more crowded steric situation in the (partially protected) hybrids amplifies an intrinsic lability of the DNA chain. Due to their greater steric demand, purines appear to favor this fragmentation more than pyrimidines. A proper choice of protecting groups and of the order or steps minimizes the fragmentation (Scheme 3). More extended cores and more labile protecting groups on the nucleobases may all but eliminate it under optimized conditions.

The method presented here employs no more than 4 equiv of dimer phosphoramidites, overcomes the low solubility of cores, and simplifies purification due to the block coupling strategy. These advances also facilitated implementation of an alternative approach, based on *H*-phosphonates, that is presented in the accompanying paper.[32] Irrespective of what type of building block is being used, syntheses that avoid solid supports should facilitate scale-up beyond the 10 mg batches prepared in this work, opening avenues to larger quantities of new chiral materials, assembled via programmable DNA:DNA interactions.

EXPERIMENTAL SECTION

N^4-Benzoyl-5′-*O*-*tert*-butyldimethylsilyl-3′-*O*-[(*N*,*N*-diisopropylamino)methoxyphosphino]-2′-deoxycytidine (8). N^4-Benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxycytidine[33] (940 mg, 2.11 mmol), previously dried at 0.001 mbar and 50 °C for 4 h, was suspended in acetonitrile (5 mL) at room temperature. After addition of molecular sieves (3 Å, 10 beads), DIEA (0.75 mL, 4.28 mmol), and chloro-*N*,*N*-diisopropylmethylphosphoramidite (0.45 mL, 2.35 mmol), the clear yellowish solution was shaken at room temperature for 4 h. The solution was then poured into a mixture of ethyl acetate (30 mL) and $NaHCO_3$ (30 mL, saturated aqueous solution) at 0 °C with vigorous stirring. The aqueous layer was separated and extracted twice with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude was purified by flash chromatography (15 g silica, pretreated with hexane/ethyl acetate/triethylamine 74:25:1 v/v/v; eluted with a step gradient of 25−50% ethyl acetate). Product-containing fractions were pooled and concentrated in vacuo, followed by recrystallization from pentane to yield 800 mg (1.32 mmol, 63%) of the title compound (**8**, mixture of diastereomers) as colorless needles: mp = 120−121 °C; TLC (hexane/ethyl acetate/triethylamine 49:50:1 v/v/v) R_f = 0.31/0.37; ^{31}P NMR (122 MHz, CD_3CN) δ 148.98, 148.54 (ratio =53:47); ^{1}H NMR (300 MHz, CD_3CN) δ 9.13 (brs, 1H), 8.34 and 8.31 (2d, J = 7.5 Hz, 1H), 7.96 (m, 2H), 7.63 (m, 1H), 7.52 (m, 2H), 7.38 (br, 1H), 6.15 (t, J = 6.1 Hz, 1H), 4.56−4.46 (m, 1H), 4.16−4.10 (m, 1H), 3.98−3.80 (m, 2H), 3.67−3.54 (m, 2H), 3.36 (2 d, J = 13.2 Hz, 3H), 2.64−2.51 (m,1H), 2.25−2.13 (m, 1H), 1.17 (d, J = 6.8 Hz, 12H), 0.93 and 0.92 (2 s, 9H), 0.13 (2 s, 3H), 0.12 (2 s, 3H).

General Protocol A (Synthesis of 3′-PAC-protected Dimers). The protocol is a modification of that given in ref 23. To a solution of the 3′-phenoxyacetyl-2′-deoxynucleoside (0.2 M, previously coevaporated from toluene, 2 × 10 mL and dried at 0.001 mbar, 50 °C) in dioxane or CH_3CN were added molecular sieves (3 Å, 10 beads), the (*N*,*N*-diisopropylamino)methoxyphosphoramidite of a 5′-DMT-protected 2′-deoxynucleoside (1.1 equiv, previously dried at 0.001 mbar, 40 °C), and 1-*H*-tetrazole (1.1 equiv of a 0.45 M solution in CH_3CN). The reaction mixture was shaken at this temperature for 20 h with diisopropylammonium tetrazolide (DIPAT) beginning to precipitate after approximately 30 min. The mixture was cooled to 0 °C, and *tert*-butyl hydroperoxide (2.7 equiv of a 5.5 M solution in decane) was added. After the mixture was stired at 0 °C for 15 min, ethyl acetate (50 mL) was added, and the solution was washed with phosphate buffer (25 mL, 0.2 M, pH 7). The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated in vacuo. The resulting crude was purified by chromatography using silica (50 g, pretreated with CH_2Cl_2/NEt_3 0.25%) and a step gradient of 0−5% CH_3OH in CH_2Cl_2. Product-containing fractions were combined and dried in vacuo (40 °C, 0.001 mbar) to yield the 3′-PAC-protected dimer nucleotide as colorless or slightly colored solid.

5′-DMT-C^{Bz}-PO(OMe)-G^{iBu}-3′-PAC (12). The reaction was performed following general protocol A, starting from N^2-isobutyryl-3′-*O*-phenoxyacetyl-2′-deoxyguanosine (**9**, 640 mg, 1.36 mmol) and [N^4-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine-3′-yl]-(*N*,*N*′-diisopropyl)methylphosphoramidite (**5**, 1.19 g, 1.5 mmol) in dioxane (7 mL). Yield 1.29 g of **12** (1.09 mmol, 80%) as a pale yellow foam: TLC (CH_2Cl_2/CH_3OH = 9:1 *v*/v) R_f = 0.38; ^{31}P NMR (203 MHz, DMSO-d_6) δ −0.94, −1.10 (ratio = 56:44); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 1H), 11.61 and 11.60 (2 brs, 1H), 11.27 (m, 1H), 8.23, 8.22 (2 s, 1H), 8.14 (d, J = 7.5 Hz, 1H), 8.00 (d, J = 7.6 Hz, 2H), 7.63 (m, 1H), 7.52 (t, J = 7.6 Hz, 2H), 7.40−7.19 (m, 12H), 7.00−6.85 (m, 7H), 6.25 and 6.13 (2 m, 2H), 5.45 and 4.95 (2 m, 2H), 4.86, 4.85 (2 s, 2H), 4.33−4.19 (m, 4H), 3.74, 3.72 (2 s, 6H), 3.61, 3.58 (2 d, J = 11.4 Hz, 3H), 3.31−3.26 (m, 2H), 2.96−2.80 (m, 1H), 2.76 (sept, J = 7.0 Hz, 1H), 2.70−2.15 (m, 3H), 1.11 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.1, 168.4, 167.3 163.2, 158.1, 157.5, 154.7, 148.5, 148.2, 144.5, 144.3, 137.3, 135.3,135.2, 133.0, 132.8, 130.0, 129.7, 129.4, 128.4, 127.9, 127.7, 126.8, 121.3, 120.4, 120.1, 114.5, 113.2; 96.1, 86.1, 85.8, 84.1, 83.2, 82.2, 77.6, 75.2, 66.9, 64.6, 62.4, 54.9, 54.5, 54.4, 39.0, 36.0, 35.4, 18.8; HRMS (ESI-TOF) m/z calcd for $C_{60}H_{61}N_8O_{16}P$ [M + Na]$^+$ 1203.384, m/z obsd 1203.383.

5′-DMT-G^{iBu}-PO(OMe)-A^{Bz}-3′-PAC (13). The reaction was performed following general protocol A, starting from N^6-benzoyl-3′-*O*-phenoxyacetyl-2′-deoxyadenosine (**11**, 735 mg, 1.5 mmol) and [5′-*O*-(4,4′-dimethoxytrityl)-*N*2-isobutyryl-2′-deoxyguanosine-3′-yl]-(*N*,*N*′-diisopropyl)methylphosphoramidite (**6**, 1.32 g, 1.65 mmol) in dioxane (7 mL). Yield 1.30 g of **13** (1.08 mmol, 72%) as a colorless foam: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.43; ^{31}P NMR (122 MHz, DMSO-d_6) δ −0.96, −1.21 (ratio =44:56); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.05 (s, 1H), 11.61 and 11.60 (2 s, 1H), 11.22, 11.20 (2 s, 1H), 8.75, 8.73, 8.67 and 8.66, (4 s, 2H), 8.10 and 8.09 (2s, 1H), 8.01 (t, J = 7.1 Hz, 2H), 7.65 (t, J = 7.4 Hz, 1H), 7.54 (m, 2H), 7.32−7.27 (m, 4H), 7.23−7.15 (m, 7H), 6.98−6.95 (m, 3H), 6.81−6.77 (m, 4H), 6.52 and 6.21 (2 m, 2H), 5.58 and 5.00 (2 m, 2H), 4.88 and 4.87 (2 s, 2H), 4.36−4.26 (m, 3H), 4.14 (m, 1H), 3.70 and 3.69 (2 s, 6H), 3.59and 3.58 (2d, J = 11.2 Hz, 3H), 3.25−3.12 (m, 3H), 2.92 (m, 1H), 2.73 (sept, J = 7.0 Hz, 1H), 2.69−2.58 (m, 2H), 1.11 (d, J = 7.0 Hz, 6H); ^{13}C-NMR (126 MHz, DMSO-d_6) δ 180.0, 168.5, 165.6, 158.0, 157.5, 154.8, 151.9, 151.7, 150.5, 148.6, 148.0, 144.6, 143.0, 137.3, 135.3, 133.2, 132.5, 129.7, 129.6, 129.5, 128.5, 128.4, 127.7, 127.6, 126.7, 125.8, 121.3, 120.5, 120.4, 114.5, 113.1; 85.7, 84.1, 83.8, 82.7, 82.3, 78.1, 74.6, 66.7, 64.6, 63.3, 54.9, 54.4, 54.3, 36.6, 35.4, 34.8, 18.8; HRMS (ESI-TOF) m/z calcd for $C_{65}H_{65}N_{10}O_{15}P$ [M + Na]$^+$ 1227.395, m/z obsd 1227.395.

5′-DMT-T-PO(OMe)-C^{Bz}-3′-PAC (14). The reaction was performed following the general protocol A, starting from N^4-benzoyl-3′-*O*-phenoxyacetyl-2′-deoxycytidine (**10**, 700 mg, 1.5 mmol) and [5′-*O*-(4,4′-dimethoxytrityl)thymidine-3′-yl](*N*,*N*′-diisopropyl)-methylphosphoramidite (**7**, 1.17 g, 1.65 mmol) in dioxane (8 mL). Yield 1.15 g of **14** (1.06 mmol, 71%) as a colorless foam: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.64; ^{31}P NMR (122 MHz, DMSO-d_6) δ −0.84, −0.98 (ratio = 57:43); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.38 and 11.29 (2 brs, 2H), 8.17 (2d, J = 7.8 Hz, 1H), 8.00 (d, J = 7.8 Hz, 2H), 7.63 (m, 1H), 7.53−7.49 (m, 3H), 7.39−7.19 (m, 12H), 6.98−6.94 (m, 3H), 6.89−6.86 (m, 4H), 6.22 and 6.16 (2 m, 2H), 5.34 and 5.04 (2 m, 2H), 4.86 (m, 2H), 4.34−4.25 (m, 3H), 4.17 (m, 1H), 3.72 and 3.71 (2 s, 6H), 3.68 and 3.63 (2d, J = 11.2 Hz, 3H), 3.29−3.23 (m, 2H), 2.60−2.34 (m, 4H), 1.45 (2 s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.3, 167.2, 163.5, 163.2, 158.1, 157.4, 154.2, 150.2, 144.9, 144.4, 135.5, 135.1, 135.0, 132.9, 132.7, 129.6, 129.4, 128. 4, 128.3, 127.8, 127.5, 126.7, 121.2, 114.4, 113.1, 109.8, 96.3, 86.8, 86.0, 83.7, 83.2, 82.4, 77.6, 64.3, 66.8, 64.4, 63.0, 54.9, 54.5, 54.4, 37.3, 37.0, 11.6; HRMS (ESI-TOF) m/z calcd for $C_{56}H_{56}N_5O_{16}P$ [M + Na]$^+$ 1108.335, m/z obsd 1108.335.

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5′-TBDMS-C^{Bz}-PO(OMe)-G^{iBu}-3′-PAC (15). The reaction was performed following general protocol A, starting from N^2-isobutyryl-3′-*O*-phenoxyacetyl-2′-deoxyguanosine (**9**, 500 mg, 1.06 mmol) and [N^4-benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine-3′-yl]-(*N*,*N*′-diisopropyl)methylphosphoramidite (**8**, 705 mg, 1.16 mmol; previously prepared via phosphitylation from the 3′-unprotected precursor as detailed in the Supporting Information) in CH_3CN (5 mL). Yield 800 mg of **15** (0.81 mmol, 76%) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.55; ^{31}P NMR (122 MHz, DMSO-d_6) δ −1.10, −1.13 (ratio = 54:46); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.09 (s, 1H), 11.64 (s, 1H), 11.27 and 11.26 (2 s, 1H), 8.26 and 8.25 (2 s, 1H), 8.21 and 8.19 (2 d, J = 7.5 Hz, 1H), 8.01 (d, J = 7.7 Hz, 2H), 7.63 (t, J = 7.5 Hz, 1H), 7.51 (t, J = 7.6 Hz, 2H), 7.37 (d, J = 7.4 Hz, 1H), 7.30 (m, 2H), 6.97 (m, 3H), 6.27 (2dd, J = 5.9 Hz, J = 8.6 Hz, 1H), 6.13 (t, J = 6.4 Hz, 1H), 5.48 and 4.86 (2 m, 2H), 4.86 (s, 2H), 4.35−4.19 (m, 4H), 3.80−3.70 (m, 2H), 3.67 (d, J = 11.3 Hz, 3H), 3.00 (m, 1H), 2.76 (sept, J = 6.9 Hz, 1H), 2.69, 2.60, 2.24 (3 m, 3H), 1.12, 1.11 (2 d, J = 6.9 Hz, 6H), 0.83, 0.82 (2 s, 9H), 0.05, 0.04 (2 s, 3H), 0.03, 0.02 (2 s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.1, 168.4, 167.3, 163.2, 157.5, 154.8, 154.2, 148.5, 148.2; 144.3, 137.5, 133.0, 132.8, 129.5, 128.4, 121.3, 120.5, 120.4, 114.6; 96.0, 86.4, 85.6, 83.2, 82.2, 77.7, 74.6, 67.0, 64.6, 62.3, 54.5, 54.4, 39.4, 35.5, 34.8, 25.6, 18.8, 17.8, −5.7, −5.8; HRMS (ESI-TOF) m/z calcd for $C_{45}H_{57}N_8O_{14}PSi$ [M + H]$^+$ 993.357, m/z obsd 993.358.

General Protocol B (Cleavage of the 3′-PAC Protecting Group of Dimers). The protocol is a modification of that given in ref 23. To a solution of the 3′-phenoxyacetyl protected dimer (0.1 M) in CH_3OH (and 20% CH_2Cl_2 in case of poorly soluble educt) was added ammonia (7 M solution in CH_3OH, 2 equiv), and the mixture was stirred at 20 °C. After TLC analysis (CH_2Cl_2/CH_3OH = 9:1 v/v) showed complete conversion (30−60 min), excess ammonia was removed with a gentle stream of nitrogen, and the solution was concentrated in vacuo at 20 °C. The resulting crude was purified by chromatography, using silica (20 g, pretreated with CH_2Cl_2/NEt_3 0.25%) and a step gradient of 0−10% CH_3OH in CH_2Cl_2. Product-containing fractions were combined, concentrated, and dried in vacuo (40 °C, < 0.001 mbar) to yield the 3′-OH dimer nucleotide as a colorless solid.

5′-DMT-C^{Bz}-PO(OMe)-G^{iBu} (16). The reaction was performed following the general protocol B, starting from **12** (1.2 g, 1.02 mmol) and ammonia (0.3 mL, 7 M solution in CH_3OH) in CH_3OH (8 mL) and CH_2Cl_2 (2 mL) for 45 min. Yield 900 mg of **16** (0.86 mmol, 84%) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.27; ^{31}P NMR (203 MHz, DMSO-d_6) δ −0.82, −0.98 (ratio = 55:45); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.06 (brs, 1H), 11.62 (brs, 1H), 11.30 (brs, 1H), 8.18 and 8.17 (2 s, 1H), 8.16 and 8.15 (2 d, J = 7.6 Hz, 1H), 8.00 (d, J = 7.7 Hz, 2H), 7.63 (m, 1H), 7.52 (t, J = 7.7 Hz, 2H), 7.39−7.20 (m, 10H), 6.90−6.87 (m, 4H), 6.24 and 6.14 (2 m, 2H), 5.54 (d, J = 4.1 Hz, 1H), 4.95 and 4.41 (2 m, 2H), 4.27−4.09 (m, 3H), 4.01 (m, 1H), 3.73 and 3.72 (2 s, 6H), 3.63 and 3.58 (2 d, J = 11.5 Hz, 3H), 3.31−3.28 (m, 2H), 2.75 (sept, J = 7.0 Hz, 1H), 2.70−2.61 (2 m, 2H), 2.43−2.30 (2 m, 2H), 1.11 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.0, 163.1, 158.1, 154.7, 148.3, 148.0, 144.3, 137.4, 135.1, 135.0, 133.1, 132.7, 129.7, 128.4, 127.9, 127.7, 126.8, 120.3, 113.2, 86.3, 86.1, 84.8, 84.0, 83.1, 76.7, 70.1, 67.3, 62.4, 55.0, 54.4, 54.3, 45.6, 38.8, 34.8, 18.8; HRMS (ESI-TOF) m/z calcd for $C_{52}H_{55}N_8O_{14}P$ [M + Na]$^+$ 1069.347, m/z obsd 1069.347.

5′-DMT-G^{iBu}-PO(OMe)-A^{Bz} (17). The reaction was performed following the general protocol B, starting from **13** (1.15 g, 1.02 mmol) and ammonia (0.27 mL, 7 M solution in CH_3OH) in CH_3OH (8 mL) and CH_2Cl_2 (2 mL) for 60 min. Yield 820 mg of **17** (0.77 mmol, 81%) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.25, 0.27; ^{31}P NMR (122 MHz, DMSO-d_6) δ −0.87, −1.09 (ratio = 55:45); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.05 (s, 1H), 11.62, 11.61 (2 s, 1H), 11.19 and 11.17 (2 s, 1H), 8.72, 8.70, 8.63, and 8.62 (4s, 2H), 8.11 and 8.10 (2s, 1H), 8.02 (t, J = 7.3 Hz, 2H), 7.65 (m, 1H), 7.54 (m, 2H), 7.30 (d, J = 7.6 Hz, 2H), 7.24−7.16 (m, 7H), 6.82−6.77 (m, 4H), 6.50 and 6.22 (2 m, 2H), 5.59 (2 d, J = 4.8 Hz, 1H), 5.00 and 4.52 (2 m, 2H), 4.29−4.13 (m, 3H), 4.05 (m, 1H), 3.70 (s, 6H), 3.60, 3.58 (2d, J = 11.2 Hz, 3H), 3.23 and 3.15 (2 m, 2H), 2.98−2.87 (m, 2H), 2.74 (sept, J = 6.9 Hz, 1H), 2.57 and 2.43 (2 m, 2H), 1.11 (d, J = 6.9 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.0, 158.0, 154.8, 150.4, 148.6, 144.6, 143.1, 135.2, 129.7, 129.6, 128.5, 127.7, 127.6, 113.1, 85.7, 84.8, 84.0, 83.7, 82.7, 77.9, 70.1, 67.3, 63.4, 54.9, 54.4, 38.4, 36.7, 34.8, 18.8; HRMS (ESI-TOF) m/z calcd for $C_{53}H_{55}N_{10}O_{13}P$ [M + Na]$^+$ 1093.358, m/z obsd 1093.358.

5′-DMT-T-PO(OMe)-C^{Bz} (18). The reaction was performed following the General Protocol B, starting from **14** (1.1 g, 1.01 mmol) and ammonia (0.3 mL, 7 M solution in CH_3OH) in CH_3OH (8 mL) and CH_2Cl_2 (2 mL). The reaction was complete after 30 min. Yield 920 mg of **18** (0.96 mmol, 95%) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.46; ^{31}P NMR (122 MHz, DMSO-d_6) δ −0.73, −0.84 (ratio = 57:43); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.36 and 11.24 (2 brs, 2H), 8.14 (2d, J = 7.7 Hz, 1H), 8.00 (d, J = 7.7 Hz, 2H), 7.62 (m, 1H), 7.52−7.47 (m, 3H), 7.38−7.20 (m, 10H), 6.89−6.86 (m, 4H), 6.22 and 6.15 (2 m, 2H), 5.50 (d, J = 4.4 Hz, 1H), 5.05 (m, 1H), 4.28−4.16 (m, 4H), 4.03 (m, 1H), 3.72 and 3.71 (2 s, 6H), 3.69 and 3.65 (2d, J = 11.2 Hz, 3H), 3.31−3.23 (m, 2H), 2.54−2.47 (m, 2H), 2.36−2.31 (m, 1H), 2.15−2.08 (m, 1H), 1.46 and 1.45 (2 s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.4, 163.6, 163.5, 158.2, 154.2, 150.3, 144.6, 144.5, 135.6, 135.2, 135.1, 133.1, 132.7, 129.7, 128. 4, 127.9, 127.6, 126.8, 113.2, 109.8, 96.2, 86.4, 86.1, 85.0, 83.7, 83.3, 77.6, 69.7, 67.2, 63.1, 55.0, 54.5, 54.4, 40.0, 37.4, 11.7; HRMS (ESI-TOF) m/z calcd for $C_{48}H_{50}N_5O_{14}P$ [M + Na]$^+$ 974.298, m/z obsd 974.298.

5′-TBDMS-C^{Bz}-PO(OMe)-G^{iBu} (19). The reaction was performed following the General Protocol B, starting from **15** (700 mg, 0.7 mmol) and ammonia in CH_3OH (0.22 mL, 7 M solution) in CH_3OH (7 mL) with a reaction time of 30 min. Yield 490 mg of **19** (0.57 mmol, 81%) as a colorless foam: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.33; ^{31}P NMR (122 MHz, DMSO-d_6) δ −0.94, −1.03 (ratio =55:45); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.07 (brs, 1H), 11.63 (brs, 1H), 11.26 (brs, 1H), 8.21 (m, 2H), 8.00 (d, J = 7.6 Hz, 2H), 7.63 (m, 1H), 7.51 (t, J = 7.7 Hz, 2H), 7.37 (d, J = 7.5 Hz, 1H), 6.26 (t, J = 6.7 Hz, 1H), 6.13 (q, J = 6.4 Hz, 1H), 5.53 (br, 1H), 4.86 and 4.43 (2 m, 2H), 4.28−4.13 (m, 3H), 4.03 (m, 1H), 3.80−3.70 (m, 2H), 3.69 and 3.68 (2 d, J = 11.4 Hz, 3H), 2.76 (sept, J = 7.0 Hz, 1H), 2.72−2.63 (2 m, 2H), 2.36−2.22 (2 m, 2H), 1.11 (m, 6H), 0.83 and 0.82 (2 s, 9H), 0.05 and 0.04 (2 s, 3H), 0.03 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.1, 167.3, 163.2, 154.8, 154.2, 148.3, 148.0, 144.3, 137.5, 133.0, 132.8, 128.4, 120.3, 96.0, 86.4, 85.6, 84.9, 83.1, 77.5, 70.1, 67.4, 62.3, 54.4, 54.3, 39.3, 38.8, 34.8, 25.6, 18.8, 17.8, −5.7, −5.8; HRMS (ESI-TOF) m/z calcd for $C_{37}H_{51}N_8O_{12}PSi$ [M + H]$^+$ 859.321, m/z obsd 859.321.

General Protocol C (Synthesis of 3′-Phosphoramidites of Dimers). The protocol is a modification of that given in ref 21. The 5′-*O*-protected dinucleotide previously coevaporated from CH_3CN (10 mL) and dried (<0.001 mbar, 50 °C) was suspended in CH_3CN (0.2 M) at room temperature. After addition of molecular sieves (3 Å, 10 beads), diisopropylethylamine (DIEA, 2 equiv), and chloro-*N*,*N*-diisopropylmethylphosphoramidite or chloro-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (1.1 equiv), the slurry turned into a clear, yellowish solution within a few minutes and was shaken at 20 °C for 3 h. The reaction mixture was then poured into a vigorously stirred mixture of ethyl acetate (20 mL) and $NaHCO_3$ (20 mL, saturated aqueous solution) at 0 °C. The aqueous layer was separated and extracted twice with ethyl acetate (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated in vacuo. The resulting crude was purified by flash chromatography, using silica (3 g, pretreated with ethyl acetate with 2% triethylamine) and a step gradient of CH_3CN (0−30%) in ethyl acetate with 1% triethylamine. Product-containing fractions were combined and concentrated in vacuo, followed by precipitation (twice) from ethyl acetate (0.5 mL) treated with hexane (4 mL). The precipitate was separated by centrifugation (3500 rpm, 5 min) and dried in vacuo (<0.001 mbar, 40 °C) to yield the 5′ protected dinucleotide phosphoramidite as a colorless solid.

5′-DMT-C^{Bz}-PO(OMe)-G^{iBu}-3′-PN(iPr)$_2$(OMe) (20). The reaction was performed following general protocol C, starting from **16** (750 mg, 0.72 mmol), DIEA (185 mg, 1.44 mmol), and chloro-*N*,*N*-

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diisopropylmethylphosphoramidite (155 μL, 0.8 mmol) in CH_3CN (3.5 mL). Yield 650 mg of **20** (0.54 mmol, 75%) as a colorless solid: TLC (ethyl acetate/CH_3CN/NEt_3 = 7:3:0.1 v/v) R_f = 0.34; ^{31}P NMR (122 MHz, CD_3CN) δ 149.12, 149.02, 148,85 (*P*(III)), −1.05, −1.10, −1.20, −1.25 (*P*(V)); ^1H NMR (300 MHz, CD_3CN) δ 10.25 (br, 1H), 8.20−7.94 (m, 3H), 7.76 (m, 1H), 7.64 (m, 1H), 7.53 (t, *J* = 7.7 Hz, 2H), 7.42−7.15 (m, 10H), 6.88−6.83 (m, 4H), 6.23−6.02 (m, 2H), 5.07 and 4.95 (2 m, 1H), 4.66 (m, 1H), 4.53−4.21 (m, 4H), 3.76, 3.75, and 3.73 (3 s, 6H), 3.73, 3.72, 3.68, and 3.67 (4d, *J* = 11.1 Hz, 3H), 3.65−3.56 (m, 2H), 3.38 and 3.35 (2 d, *J* = 13.3, 3H), 3.30−2.20 (m, 7H), 1.22−1.06 (m, 18H); HRMS (ESI-TOF) *m*/*z* calcd for $C_{59}H_{71}N_9O_{15}P_2$ $[M + H]^+$ 1208.462, *m*/*z* obsd 1208.460.

5′-DMT-G^{iBu}-PO(OMe)-A^{Bz}-3′-PN(iPr)$_2$(OMe) (21). The reaction was performed following general protocol C, starting from **17** (535 mg, 0.5 mmol), DIEA (130 mg, 1 mmol), and chloro-*N*,*N*-diisopropylmethylphosphoramidite (106 μL, 0.55 mmol) in CH_3CN (2.5 mL). Yield 415 mg of **21** (0.34 mmol, 68%) as a colorless foam: TLC (ethyl acetate/CH_3CN/NEt_3 = 7:3:0.1 v/v) R_f = 0.29; ^{31}P NMR (122 MHz, CD_3CN) δ 149.36, 149.34, 149.06, 149,01 (*P*(III)), −0.80, −0.84, −0.98, −1.01 (*P*(V)); ^1H NMR (500 MHz, CD_3CN) δ 9.95 (br), 8.66 (m, 1H), 8.29 (s, 1H); 7.96−7.91 (m, 2H), 7.76 and 7.75 (2 s, 1H), 7.61 (m, 1H), 7.52−7.46 (m, 2H), 7.31 (m, 2H), 7.21−7.15 (m, 7H), 6.76−6.73 (m, 4H), 6.43 (m, 1H), 6.07 and 6.03 (2 m, 1H), 5.20 and 5.11 (m, 1H), 4.96−4.82 (m, 1H), 4.32−4.21 (m, 3H), 4.09 (m, 1H), 3.72 and 3.71 (2 s, 6H), 3.67, 3.66, 3.58, and 3.57 (4 d, *J* = 11.3 Hz, 3H), 3.65−3.60 (m, 2H), 3.39 and 3.37 (2 d, *J* =13.3, 3H), 3.29−3.02, 2.92−2.78, and 2.68−2.39 (m, 7H), 1.22−1.09 (m, 18H); HRMS (ESI-TOF) *m*/*z* calcd for $C_{60}H_{71}N_{11}O_{14}P_2$ $[M + Na]^+$ 1254.455, *m*/*z* obsd 1254.455.

5′-DMT-T-PO(OMe)-C^{Bz}-3′-PN(iPr)$_2$(OMe) (22). The reaction was performed following general protocol C, starting from **18** (475 mg, 0.5 mmol), DIEA (130 mg, 1 mmol) and chloro-*N*,*N*-diisopropylmethylphosphoramidite (106 μL, 0.55 mmol) in CH_3CN (2.5 mL). Chromatography was performed using ethyl acetate with triethylamine (1%) as eluant. Yield 440 mg of **22** (0.4 mmol, 80%) as a colorless solid: TLC (ethyl acetate/NEt_3 = 10:0.1 v/v) R_f = 0.30; ^{31}P NMR (122 MHz, CD_3CN) δ 149.52, 149.15, (*P*(III)), −0.89, −0.92, −0.93, −0.96 (*P*(V)); ^1H NMR (500 MHz, CD_3CN) δ 9.10 (br, 2H), 8.08 and 8.03 (2d, *J* = 7.6 Hz, 1H), 7.94 (d, *J* = 7.4 Hz, 2H), 7.63 (m, 1H), 7.51(m, 2H), 7.43−7.21 (m, 11H), 6.87−6.84 (m, 4H), 6.25 and 6.12 (2 m, 2H), 5.10, 4.47 (2 m, 2H), 4.31−4.20 (m, 4H), 3.74 (2 s, 6H), 3.72−3.68 (m, 3H), 3.63−3.56 (m, 2H), 3.37−3.01 (m, 5H), 2.61−2.52 (m, 2H), 2.48−2.41 (m, 1H); 2.23−2.18 (m, 1H), 1.45 and 1.44 (2 s, 3H), 1.17−1.15 (m, 12H); HRMS (ESI-TOF) *m*/*z* calcd for $C_{55}H_{66}N_6O_{15}P_2$ $[M + Na]^+$ 1135.395, *m*/*z* obsd 1135.396.

5′-TBDMS-C^{Bz}-PO(OMe)-G^{iBu}-3′-PN(iPr)$_2$(OMe) (23). The reaction was performed following general protocol C, starting from **19** (430 mg, 0.50 mmol), DIEA (130 mg, 1 mmol), and chloro-*N*,*N*-diisopropylmethylphosphoramidite (106 μL, 0.55 mmol) in CH_3CN (2.5 mL). Yield: 250 mg of **23** (0.25 mmol, 50%) as a colorless solid: TLC (ethyl acetate/CH_3CN/NEt_3 = 7:3:0.1 v/v) R_f = 0.40; ^{31}P NMR (122 MHz, CD_3CN) δ 149.21, 149.18, 148.93 (*P*(III)), −1.32, −1.37, −1.39 (*P*(V)); ^1H NMR (300 MHz, CD_3CN) δ 12.09, 10.53, and 9.70 (3 brs, 2H), 8.21−8.15 (m, 1H), 7.99 (m, 2H), 7.80 and 7.79 (2 d, *J* = 6.8 Hz, 1H), 7.63 (m, 1H), 7.53 (m, 2H), 7.44 and 7.38 (2 br, 1H), 6.26−6.08 (m, 2H), 4.98, 4.88, and 4.69 (3 m, 2H), 4.53−4.19 (m, 4H), 3.78, 3.77, 3.72, and 3.71 (4d, *J* = 11.4 Hz, 3H), 3.72−3.55 (m, 2H), 3.40, 3.39, 3.38, and 3.37 (4 d, *J* = 13.3, 3H), 3.04−2.13 (m, 7H), 1.22−1.16 (m, 18H), 0.86 (s, 9H), 0.08−0.05 (m, 6H); HRMS (ESI-TOF) *m*/*z* calcd for $C_{44}H_{67}N_9O_{13}P_2Si$ $[M + H]^+$ 1020.418, *m*/*z* obsd 1020.417.

5′-DMT-C^{Bz}-PO(OMe)-G^{iBu}-3′-PN(iPr)$_2$(OCET) (24). The reaction was performed following general protocol C, starting from **16** (550 mg, 0.52 mmol), DIEA (140 mg, 1.08 mmol), and chloro-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (130 μL, 0.58 mmol) in CH_3CN (3 mL). Yield 340 mg of **24** (0.27 mmol, 52%) as a colorless solid: TLC (ethyl acetate/CH_3CN/NEt_3 = 7:3:0.1 v/v) R_f = 0.43; ^{31}P NMR (122 MHz, CD_3CN) δ 148.56, 148.37, 148.33 148.08 (*P*(III)), −1.26, −1.27, −1.29 −1.33 (*P*(V)); ^1H NMR (300 MHz, CD_3CN) δ 11.90 and 10.42 (2 brs, 2H), 8.12−7.95 (m, 3H), 7.76 (m, 1H), 7.63 (m, 1H), 7.52 (m, 2H), 7.42−7.16 (m, 10H), 6.89−6.84 (m, 4H), 6.26−6.02 (m, 2H), 5.11−4.68, (m, 1H), 4.52−4.21 (m, 4H), 3.91−3.75 (m, 2H), 3.76, 3.75, and 3.73 (3 s, 6H), 3.73−3.58, (m, 5H), 3.35−2.20 (m, 9H), 1.23−1.06 (m, 18H); HRMS (ESI-TOF) *m*/*z* calcd for $C_{61}H_{72}N_{10}O_{15}P_2$ $[M + Na]^+$ 1269.455, *m*/*z* obsd 1269.455.

General Protocol D (Synthesis of DNA Hybrids from Cores and Dimer Phosphoramidites). The core (200 mg of tetrakis(4-hydroxyphenyl)methane[17] or of 1,4-phenylenebis[tris(4′-hydroxyphenyl)methane])[18] was dissolved in dioxane (10 mL) by heating, and the solution then immediately frozen by cooling with liquid nitrogen, followed by drying in vacuo (0.001 mbar) in a process resembling lyophilization. The resulting voluminous material was further dried in vacuo (<0.001 mbar) at 130 °C for 2 h. The core (5−10 μmol) and the 3′-phosphoramidite of the 5′-protected dinucleoside phosphate (**20**, **21**, **22**, **23**, or **24**, 1.5−3 equiv per OH group of the core) were transferred to a 5 mL vial, and the mixture was dried in vacuo at 40 °C for 1 h. The flask was flushed with argon and sealed with a septum, after addition of molecular sieves (3 Å, 5 beads). Dioxane (freshly distilled over sodium/benzophenone) and 1*H*-tetrazole (0.45 M solution in CH_3CN, 2−3 equiv for each OH group of the core) was added at room temperature, and the slurry was put in an ultrasonic bath for 2 min at 20 °C until the core was fully dissolved. The reaction mixture was kept at 20 °C for 30 min and then at 5 °C for 3 h. (The reaction was usually monitored by drawing samples, oxidizing with *tert*-butylhydroperoxide in CH_2Cl_2 and precipitation with hexane. After the DMT groups were removed with trichloroacetic acid (3% TCA in CH_2Cl_2) and CH_3OH, the analytical samples were analyzed by MALDI-TOF MS.) After 3 h, *tert*-butyl hydroperoxide (5.5 M solution in decane, 5 equiv for each OH group of the core) was added, and the mixture was again kept at 5 °C for 15 min. The solution was then diluted with CH_2Cl_2 (20 mL) and washed with phosphate buffer (15 mL, 0.2 M, pH 7). The aqueous phase was back-extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were dried over $NaSO_4$, filtered, and concentrated in vacuo. To remove excess dimer, the residue was dissolved in CH_2Cl_2 (0.5 mL) and precipitated with hexane (3 mL). The precipitate was treated three times with ethyl acetate and 0−20% hexane (3 × 3 mL) in an ultrasonic bath for 1 min and separated by centrifugation. In isolated cases, it was purified by chromatography using silica (5 g, pretreated with CH_2Cl_2/NEt_3 0.2%) and a step gradient of CH_3OH in CH_2Cl_2. The resulting product was dried (<0.001 mbar, 40 °C) to yield the protected hybrid as a colorless or a pale yellow solid. The completeness of the reaction was checked again by MALDI-TOF MS after DMT deprotection in the analytical sample. In the case of incomplete conversion (<90% of the last phenole of the core remained unreacted), the hybrid was coupled again, as described above, using the 3′-phosphoramidite of 5′-protected dinucleotide (0.5 equiv per OH group of the starting amount of the core), 1*H*-tetrazole (0.5−1 equiv), and *tert*-butyl hydroperoxide (3 equiv) for the oxidation. After workup, as described above, the resulting product was dried (<0.001 mbar, 40 °C) to yield the protected DNA hybrid (>98% of all OH groups reacted) as a colorless solid.

[5′-DMT-C^{Bz}-PO(OMe)-G^{iBu}-3′-PO(OMe)]$_4$TPM (25). The reaction was performed following general protocol D, starting from TPM (3.8 mg, 10 μmol) and **20** (145 mg, 120 μmol, 3 equiv per OH group) in dioxane (0.7 mL), and 1*H*-tetrazole (0.27 mL of a 0.45 M solution in CH_3CN, 120 μmol) and then *tert*-butyl hydroperoxide (36 μL of a 5.5 M solution in decane, 200 μmol). For removal of excess dimer, ethyl acetate/hexane (9:1 *v*/*v*) was used. Yield 55 mg of crude **25** (quant). MALDI-TOF MS showed 80−85% conversion of the fourth phenolic OH group. In a second coupling cycle, the hybrid was reacted again with **20** (24 mg, 20 μmol, 0.5 equiv/OH) in dioxane (0.7 mL) and 1*H*-tetrazole (45 μL, 0.45 M solution in CH_3CN, 20 μmol) and then *tert*-butyl hydroperoxide (22 μL of a 5.5 M solution in decane, 120 μmol). The crude was washed with ethyl acetate (3 × 3 mL), followed by dissolving in CH_2Cl_2 (0.1 mL) and precipitation with ethyl acetate. Yield 44 mg of **25** (9.0 μmol, 90%), after centrifugation, as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.43; ^{31}P NMR (203 MHz, CD_2Cl_2) δ −1.51, −1.67 ($POCH_2$), −6.18, −6.35 (*P*OAr); ^1H NMR (500 MHz, CD_2Cl_2) δ 12.13 (br, 4H), 10.70 (br,

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4H), 9.52 (br, 4H), 8.12−7.77 (m, 4 × 4H), 7.71−6.90 (m, 4 × 17H), 6.75 (m, 4 × 4H), 6.25−5.91 (m, 4 × 2H), 5.37 and 5.04−4.80 (2 m, 4 × 2H), 4.63−4.00 (m, 4× 4H), 3.85−3.50 (m, 4 × 12H), 3.40−2.10 (m, 4 × 7H), 1.15−0.85 (m, 4 × 6H); MALDI-TOF MS (DMT off) calcd for $C_{153}H_{172}N_{32}O_{60}P_8$ $[M - H]^-$ 3665, obsd 3663 plus 2843 (1%, $[(CG)_3TPM - H]^-$).

[5′-DMT-T-PO(OMe)-C^{Bz}-3′-PO(OMe)]$_4$TPM (26). The reaction was performed following general protocol D, starting from TPM (3.8 mg, 10.0 μmol) and **22** (133 mg, 120 μmol, 3 equiv/OH) in dioxane (0.7 mL), using 1*H*-tetrazole (0.27 mL of a 0.45 M solution in CH_3CN, 120 μmol) and then *tert*-butyl hydroperoxide (36 μL of a 5.5 M solution in decane, 200 μmol). Excess dimer was removed by chromatography, using silica (5 g, pretreated with CH_2Cl_2/NEt_3 0.2%) and a step gradient of 0−5% CH_3OH in CH_2Cl_2. Product-containing fractions were combined and concentrated in vacuo, followed by dissolving in CH_2Cl_2 (0.2 mL) and precipitation with hexane (3 mL). Centrifugation gave 38 mg of **26** (8.5 μmol, 85%) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.49; ^{31}P NMR (122 MHz, CD_2Cl_2) δ −0.61, −0.71, −0.96, −1.00 (*P*OCH$_2$), −6.15, −6.23, −6.31, −6.73 (*P*OAr); ^{1}H NMR (500 MHz, CD_2Cl_2) δ 10.24−9.90 (m, 4 × 2H), 8.11−7.91 (m, 4 × 3H), 7.61−7.37 (m, 4 × 7H), 7.33−7.13 (m, 4 × 11H), 6.86−6.81 (m, 4 × 4H), 6.34 and 6.19 (2 m, 4 × 2H), 5.20−5.10 (m, 4 × 2H), 4.43−4.18 (m, 4 × 4H), 3.84 (m, 4 × 3H), 3.75, (brs, 4 × 6H), 3.73−3.64 (m, 4 × 3H), 3.48−3.34 (m, 4 × 2H), 2.82, 2.62, 2.39, and 2.21 (4 m, 4 × 4H), 1.41 (brs, 4 × 3H); MALDI-TOF MS (DMT off) calcd for $C_{137}H_{152}N_{20}O_{60}P_8$ $[M - H]^-$ 3284, obsd 3281 plus 2556 (3%, $[(TC)_3TPM - H]^-$).

[5′-DMT-G^{iBu}-PO(OMe)-A^{Bz}-3′-PO(OMe)]$_4$TPM (27). The reaction was performed following general protocol D, starting from TPM (3.8 mg, 10.0 μmol) and **21** (148 mg, 120 μmol, 3 equiv/OH group) in dioxane (0.7 mL), and 1*H*-tetrazole (0.27 mL of a 0.45 M solution in CH_3CN, 120 μmol) and then *tert*-butyl hydroperoxide (36 μL of a 5.5 M solution in decane, 200 μmol). To remove excess dimer, the crude chromatographed on silica (5 g, pretreated with CH_2Cl_2/NEt_3 0.2%) with a step gradient of 0−8% CH_3OH in CH_2Cl_2. Product-containing fractions were combined and concentrated in vacuo, followed by dissolving in CH_2Cl_2 (0.2 mL) and precipitation with hexane (3 mL). Centrifugation gave50 mg of **27** (10 μmol, quant) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.44; ^{31}P NMR (203 MHz, CD_2Cl_2) δ −0.47, −0.75, −1.08, −1.17 (*P*OCH$_2$), −6.32, −6.58, −7.60 (*P*OAr); ^{1}H NMR (500 MHz, CD_2Cl_2) δ 12.03 (br, 4 × 1H), 10.93−10.30 (m, 4 × 1H), 9.7−9.23 (m, 4 × 1H), 8.66 and 8.30, (2 m, 4 × 2H), 7.94 (m, 4 × 2H), 7.70 (m, 4 × 1H), 7.59−7.05 (m, 4 × 16H), 6.80−6.73 (m, 4 × 4H), 6.50−6.07 (m, 4 × 2H), 5.01−4.75 (m, 4 × 2H), 4.49−4.16 (m, 4 × 4H), 3.88 (m, 4 × 3H), 3.73 and 3.72 (2s, 4 × 6H), 3.66 and 3.58 (2d, *J* = 11.3 Hz, 4 × 3H), 3.32−3.20 (m, 4 × 2H), 2.83−2.00 (m, 4 × 5H), 1.13−0.99 (m, 4 × 6H); MALDI-TOF MS (DMT off) calcd for $C_{157}H_{172}N_{40}O_{56}P_8$ $[M - H]^-$ 3761, obsd 3759 plus 2916 (4%, $[(GA)_3TPM - H]^-$).

[5′-DMT-C^{Bz}-PO(OMe)-G^{iBu}-3′-PO(OMe)]$_6$HPX (28). The reaction was performed following General Protocol D, starting from HPX (7.0 mg, 10.6 μmol) and **20** (115 mg, 95 μmol, 1.5 equiv/OH group) in dioxane (0.7 mL) and 1*H*-tetrazole (0.21 mL of a 0.45 M solution in CH_3CN, 95 μmol) and then *tert*-butyl hydroperoxide (58 μL of a 5.5 M solution in decane, 320 μmol). The crude was treated with ethyl acetate (3 × 3 mL). Yield 73 mg of **28** (9.9 μmol, 93%); MALDI-TOF MS showed 70−75% conversion of the fifth phenolic OH group. In a second cycle the hybrid was coupled again with **20** (36 mg, 30 μmol, 0.5 equiv per OH group) in dioxane (0.7 mL), 1*H*-tetrazole (67 μL of a 0.45 M solution in CH_3CN, 30 μmol), and then *tert*-butyl hydroperoxide (33 μL of a 5.5 M solution in decane, 180 μmol). The resulting crude was treated with ethyl acetate (2 × 3 mL), dissolved in CH_2Cl_2 (0.3 mL) and precipitated with ethyl acetate (3 mL). Centrifugation gave 64 mg of **28** (82%, 8.7 μmol) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.37; ^{31}P NMR (122 MHz, CD_2Cl_2) δ −1.45, −1.51, −1.72 (*P*OCH$_2$), −6.16, −6.27, −6.42 (*P*OAr); ^{1}H NMR (500 MHz, CD_2Cl_2) δ 12.25 (m, 6H), 11.03−10.70 (m, 6H), 10.30−9.30 (m, 6H), 8.17−7.88 (m, 6 × 3H), 7.80−6.93 (m, 112H), 6.89−6.76 (m, 6 × 4H), 6.30−6.04 (m, 6 × 2H), 5.46 and 5.10−4.92 (2 m, 6 × 2H), 4.70−4.10 (m, 6 × 4H), 3.88−3.55 (m, 6 × 12H), 3.48−1.90 (m, 6 × 7H), 1.20−0.95 (m, 6 × 6H); MALDI-TOF MS (DMT off) calcd for $C_{236}H_{262}N_{48}O_{90}P_{12}$ $[M - H]^-$ 5580, obsd 5578 plus 4761 (3%, $[(CG)_5HPX - H]^-$).

[5′-TBDMS-C^{Bz}-PO(OMe)-G^{iBu}-3′-PO(OMe)]$_6$HPX (29). The reaction was performed following general protocol D, starting from HPX (3.3 mg, 5.0 μmol) and **23** (61 mg, 60 μmol, 2.0 equiv per OH group) in dioxane (0.4 mL) and 1*H*-tetrazole (0.14 mL of a 0.45 M solution in CH_3CN, 60 μmol) and then *tert*-butyl hydroperoxide (27 μL of a 5.5 M solution in decane, 150 μmol). The crude was treated with ethyl acetate (3 × 3 mL) to give 26 mg of **29** (4.2 μmol, 83%) as a colorless solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.27; ^{31}P NMR (122 MHz, CD_2Cl_2) δ −1.73 (*P*OCH$_2$), −6.23, −6.41 (*P*OAr); ^{1}H NMR (300 MHz, CD_2Cl_2) δ 12.45−12.16 (m, 6H), 11.37−10.82 (m, 6H), 10.30−9.48 (m, 6H), 8.22−8.13 (m, 6H), 8.09−7.93 (m, 6 × 3H), 7.84−7.42 (m, 6 × 5H), 7.28−7.00 (m, 28H), 6.32−6.13 (m, 6 × 2H), 5.84−5.43 and 5.04−4.83 (m, 6 × 2H), 4.73−4.13 (m, 6 × 4H), 3.87 and 3.77 (2 m, 6 × 6H), 3.72−3.58 (m, 6 × 2H), 3.29, 2.86−3.15 and 2.09 (3 m, 6 × 5H), 1.22−1.08 (m, 6 × 6H), 0.85 (s, 6 × 9H), 0.07 and 0.06 (2s 6 × 6H); MALDI-TOF MS calcd for $C_{272}H_{346}N_{48}O_{90}P_{12}Si_6$ $[M - H]^-$ 6266, obsd 6268 plus 5330 (6%, $[(CG)_5HPX - H]^-$.

[5′-DMT-C^{Bz}-PO(OMe)-G^{iBu}-3′-PO(OCET)]$_6$HPX (30). The reaction was performed following general protocol D, starting from HPX (3.3 mg, 5.0 μmol) and **24** (112 mg, 90 μmol, 3 equiv per OH group) in dioxane (0.4 mL) and 1*H*-tetrazole (0.2 mL of a 0.45 M solution in CH_3CN, 90 μmol) and then *tert*-butyl hydroperoxide (27 μL of a 5.5 M solution in decane, 150 μmol). The crude was washed with ethyl acetate (3 × 3 mL) to give 33 mg of **30** (4.3 μmol, 85%). The hybrid was treated again with **24** (37 mg, 30 μmol, 1 equiv per OH group) in dioxane (0.4 mL), 1*H*-tetrazole (67 μL of a 0.45 M solution in CH_3CN, 30 μmol), and then *tert*-butyl hydroperoxide (27 μL of a 5.5 M solution in decane, 150 μmol). The crude was again washed with ethyl acetate (3 × 3 mL). Yield 31 mg **30** (82%, 4.1 μmol) of a pale yellow solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v) R_f = 0.35; ^{31}P NMR (122 MHz, CD_2Cl_2) δ −1.46, −1.84 (*P*OCH$_2$), −7.84, −8.07, −8.17 (*P*OAr); ^{1}H NMR (300 MHz, CD_2Cl_2) δ 12.32−12.10 (m, 6H), 11.02−10.60 (m, 6H), 10.30−9.40 (m, 6H), 8.16−7.88 (m, 6 × 3H), 7.80−6.97 (m, 112H), 6.91−6.78 (m, 6 × 4H), 6.28−6.06 (m, 6 × 2H), 5.50 and 5.10−4.92 (2 m, 6 × 2H), 4.70−4.19 (m, 6 × 6H), 3.82−3.57 (m, 6 × 9H), 3.48−2.15 (m, 6 × 9H), 1.20−0.95 (m, 6 × 6H).

(CG)$_4$TPM (2). (*Caution*: The 1-thionaphthol used for cleavage of the POMe groups is toxic. The resulting waste should be oxidized with an aqueous $KMnO_4$ solution.) To a solution of protected hybrid $(CG)_4$TPM (**25**, 12 mg, 2.5 μmol) in THF/H_2O (0.15 mL, 2:1 v/v) was added a solution of diisopropylethylamine (DIEA, 25 mg, 180 μmol) and 1-thionaphthol (0.07 mL, 500 μmol) in THF (0.2 mL), and the clear solution was stirred at 20 °C for 2 h. Ethyl acetate (0.5 mL) and hexane (3 mL) were added, and the precipitate was separated by centrifugation (3500 rpm, 5 min). The resulting viscous oil was treated with ethyl acetate (3 × 3 mL) and again isolated by centrifugation (3500 rpm, 5 min). The resulting pale yellow solid was dissolved in H_2O (2 mL) and dried by freeze-drying to yield 12.0 mg of (5′-DMT-C^{Bz}G^{iBu})$_4$TPM as a pale yellow solid. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.66. For removal of the DMT groups, the solid was taken up in water (3 mL). After addition of ethyl acetate (2 mL) and a weakly acidic cation exchange resin DOWEX MAC3 (70 mg wet, approximately 250 μmol H^+ according the specification of suppliers), and the mixture was shaken for 12 h at 20 °C. Ethyl acetate was aspired, and the reaction mixture was washed with ethyl acetate (3 × 2 mL). The resin was filtered off and washed with THF/H_2O (3 × 1 mL, 1:1 v/v). After addition of NH_4OH (1 M, 50 μL), THF was removed in vacuo, and the remaining solution was evaporated to dryness by lyophilization to yield 9.0 mg of (C^{Bz}G^{iBu})$_4$TPM as a colorless solid. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.65. For removal of the base protecting groups, the solid was treated with NH_4OH (250 μL, 25% aqueous solution) at 5 °C for 1 h and at room temperature for 2 h. After addition of $MeNH_2$ (250 μL, 40% aqueous solution), the reaction mixture was again stored at 5 °C for 1 h and at room temperature for 2

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h. Excess ammonia and methylamine were removed with a gentle stream of nitrogen, and the remaining solution was evaporated to dryness by lyophilization. The solid was treated twice with CH_2Cl_2/ethyl acetate (3 mL, 1:2 v/v) in an ultrasonic bath to yield 7.0 mg (approximately 94%, 2.3 μmol crude) of the deprotected hybrid **2** after centrifugation (3000 rpm, 5 min) as a colorless solid. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.43. The crude was purified by chromatography using a cartridge (Chromabond C18ec) and a manual step gradient of 5−30% CH_3CN in 10 mM TEAA buffer, with elution at 10−15% CH_3CN, followed by HPLC chromatography, using a C8 column and a gradient of MeCN in 10 mM TEAA buffer (5% for 5 min, 5−8% in 5 min, 8−18% in 55 min) at 55 °C. Hybrid **2** eluted at t_R = 18.2 min, yield 18%. MALDI-TOF MS calcd for $C_{101}H_{116}N_{32}O_{52}P_8$ $[M - H]^-$ 2857, obsd 2856, reinjection gave HPLC t_R = 19.1 min (95% of integration).

$(TC)_4$TPM (3). To a solution of protected $(TC)_4$TPM hybrid **26** (8.4 mg, 1.87 μmol) in THF (0.1 mL) and H_2O (0.05 mL) was added a solution of triethylamine (0.1 mL, 0.72 mmol) and 1-thionaphthol (0.07 mL, 500 μmol) in THF (0.1 mL), and the mixture was stirred at 20 °C for 1 h. The reaction mixture was diluted with H_2O (5 mL) and extracted twice with ethyl acetate (2 × 10 mL). The aqueous phase was concentrated in vacuo, and the residual viscous oil was treated twice with ethyl acetate/hexane (2 × 4 mL, 1:1 v/v). After centrifugation (3500 rpm, 5 min), the residue was dissolved in water (2 mL) and evaporated to dryness by lyophilization to yield 7.7 mg of $(5'\text{-DMT-TC}^{Bz})_4$TPM as a colorless solid. For removal of the DMT groups, the solid was stirred with acetic acid (1 mL, 80% in water) at 20 °C for 1 h. The reaction mixture was diluted with H_2O (4 mL) and evaporated to dryness by lyophilization. The residual crude was washed with ethyl acetate (2 × 3 mL), followed by centrifugation (3500 rpm, 5 min). NH_4OH (1M, 50 μL) was added, and the solvent was evaporated by lyophilization to yield 6.0 mg of $(TC^{Bz})_4$TPM as a colorless solid. TLC (*i*-PrOH/H_2O/NH_3 (25%) 7:2:1 *v/v/v*) R_f = 0.68. For removal of the benzoyl protecting groups, the solid was treated with aqueous ammonia (1 mL, 25%) at 20 °C for 3 h. Excess ammonia was removed with a gentle stream of nitrogen, and the remaining solution was evaporated to dryness by lyophilization, followed by washing in an ultrasonic bath with CH_2Cl_2/ethyl acetate (2 mL, 1:1 v/v) to yield 4.7 mg ("1.62 μmol, 87%", from **26**) of the deprotected hybrid **3** after centrifugation (3000 rpm, 5 min) as a colorless solid . TLC (*n*-PrOH/H_2O/NH_3 (25%) 55:10:35 v/v/v) R_f = 0.50; A sample of the crude hybrid **3** was purified by HPLC chromatography, using a C18 phase and a gradient of MeCN in 0.1 M TEAA buffer (0% for 3 min; 0−5% in 10 min and 5−15% in 50 min) at 60 °C. Hybrid **3** eluted at t_R = 32.6 min, yield 40%; MS MALDI-TOF MS of **3** calcd for $C_{101}H_{120}N_{20}O_{56}P_8$ $[M - H]^-$ 2757, obsd 2756. The residual crude (4.5 mg) was then solved in water (0.5 mL) and precipitated by vapor diffusion of ethanol for 5 days. The precipitate was separated by centrifugation and washed with ethanol (1 mL) to yield 3.3 mg (1.14 μmol, 61%; 28% from **10**) of a colorless but amorphous solid: ^{31}P NMR (203 MHz, D_2O) δ −0.92, ($POCH_2$), −5.88, (*P*OAr); ^{1}H NMR (500 MHz, D_2O, suppression of the excess solvent peak was achieved by presaturation during the recycle delay,[34] 40 °C) δ 8.14 (d, *J* = 7.9 Hz, 4 × 1H, *H6-C*), 7.69 (s, 4 × 1H, *H6-T*); 7.16 (brs, 4 × 4H, Ar-H_{core}), 6.36 (d, *J* = 7.9 Hz, 4 × 1H, *H5-C*), 6.29 (dd, *J* = 6.4 Hz, *J* = 7.5 Hz, 4 × 1H, *H1′-T*), 6.16 (dd, *J* = 6.0 Hz, *J* = 7.7 Hz, 4 × 1H, *H1′-C*), 5.01 (m, 4 × 1H, *H3′-C*), 4.90 (m, 4 × 1H, *H3′-T*), 4.43 (m, 4 × 1H, *H4′-C*), 4.27 (m, 4 × 1H, *H4′-T*), 4.23 (m, 4 × 1H, *H5′-C*), 4.15 (m, 4 × 1H, *H5″-C*), 3.91 (dd, $J_{5'-4'}$ = 3.5 Hz, $J_{5'-5''}$ = 12.5 Hz, 4 × 1H, *H5′-T*), 3.87 (dd, $J_{5''-4'}$ = 4.6 Hz, $J_{5''-5'}$ = 12.5 Hz, 4 × 1H, *H5″-T*), 2.55 (m, 4 × 1H, *H2′-T*), 2.41 (m, 4 × 1H, *H2″-T*), 2.35 (m, 4 × 1H, *H2′-C*), 2.22 (m, 4 × 1H, *H2″-C*), 1.94 (m, 4 × 3H, CH_3-*T*).

$(GA)_4$TPM (4). The cleavage of the fully protected $(GA)_4$TPM (**27**) was performed as described for $(CG)_4$TPM (**2**). Starting from **27** (10.3 mg, 2.07 μmol), treatment with 1-thionaphthol (0.07 mL, 500 μmol) and DIEA (25 mg, 180 μmol) in THF/H_2O (0.35 mL, 6:1 v/v) yielded 10.2 mg of $(5'\text{-DMT-G}^{iBu}\text{A}^{Bz})_4$TPM as a pale yellow solid. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.72. After removal of the DMT groups with the weakly acidic cation-exchange resin DOWEX MAC3 (70 mg wet, approximately 250 μmol H^+ according the specification of suppliers), 6.8 mg of $(G^{iBu}A^{Bz})_4$TPM was obtained as a colorless solid. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.70. Removal of the base protecting groups with NH_4OH (250 μL, 25% aqueous solution) and $MeNH_2$ (250 μL, 40% aqueous solution) yielded 6.0 mg of the title hybrid **4** (1.94 μmol, 94%) as a colorless solid; TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.5. The crude was purified by chromatography using a cartridge (Chromabond C18ec) and a manual step gradient of 5−30% CH_3CN in 10 mM TEAA buffer, with elution at 10−15% CH_3CN, followed by HPLC chromatography, using a C8 column and a gradient of MeCN in 10 mM TEAA buffer (5% for 5 min, 5−8% in 5 min, 8−18% in 55 min) at 55 °C. Hybrid **4** eluted at t_R = 23.7 min, yield 5% (2% from **11**); MALDI-TOF MS calcd for $C_{105}H_{116}N_{32}O_{52}P_8$ $[M - H]^-$ 2953, obsd 2953; reinjection gave HPLC t_R = 32.3 (90%).

$(CG)_6$HPX (1) from 28. To a solution of protected hybrid **28** (17.3 mg, 2.34 μmol) in THF (0.3 mL) and H_2O (0.05 mL) were added a mixture of DIEA (25 mg, 180 μmol) and 1-thionaphthol (0.07 mL, 500 μmol) in THF (0.2 mL). The clear solution was stirred at 20 °C for 2 h. Ethyl acetate (0.5 mL) and hexane (3 mL) were added, and the precipitate was separated by centrifugation (3500 rpm, 5 min). The resulting viscous oil was treated with ethyl acetate (3 × 3 mL) and isolated by centrifugation (3500 rpm, 5 min). The residue was dissolved in H_2O (2 mL) and dried by lyophilization to yield 19.8 mg of $(5'\text{-DMT-C}^{Bz}\text{G}^{iBu})_6$HPX as a colorless foam. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 *v/v/v*) R_f = 0.61. For removal of the base protecting groups, the solid was treated with NH_4OH (250 μL, 25% aqueous solution) at 5 °C for 1 h and at room temperature for 2 h. After addition of $MeNH_2$ (250 μL, 40% aqueous solution), the reaction mixture was again stored at 5 °C for 1 h and at room temperature for 2 h. Excess ammonia and methylamine were removed with a gentle stream of nitrogen, and the remaining solution was evaporated to dryness by lyophilization. The solid was treated twice in an ultrasonic bath with CH_2Cl_2/ethyl acetate (3 mL, 1:1 v/v) to yield 15.0 mg of **32** after centrifugation (3000 rpm, 5 min) as a colorless solid: TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 *v/v/v*) R_f = 057. Finally, for removal of the DMT groups, the product was taken up in H_2O (5 mL) at room temperature. After addition of ethyl acetate (3 mL) and weakly acidic cation exchange resin DOWEX MAC3 (70 mg, approximately 250 μmol H^+ according the specification of suppliers), the mixture was shaken at 20 °C for 12 h. The organic layer was aspired, and the aqueous layer was washed with ethyl acetate (2 × 2 mL). The resin was filtered off and washed three times with H_2O (3 *x* 1 mL). NH_4OH (1 M, 50 μL) was added, and the solution was evaporated to dryness by lyophilization. The resulting residue was treated twice with CH_2Cl_2/ethanol (1:1, 2 × 2 mL) to yield 10.5 mg crude **1** as a colorless solid after centrifugation (3500 rpm, 5 min). TLC (*n*-PrOH/H_2O/NH_3 (25%) 55:10:35 v/v/v) R_f = 0.24. The crude was purified by HPLC, using a C8 column and a gradient of MeCN in 10 mM TEAA buffer (5% for 5 min, 5−8% in 5 min, 8−18% in 55 min) at 55 °C. Hybrid **1** eluted at t_R = 28.4 min: yield 13%; MALDI-TOF MS calcd for $C_{158}H_{178}N_{48}O_{78}P_{12}$ $[M - H]^-$ 4367, obsd 4367.

$(CG)_6$HPX (1) from 29. Protected hybrid **29** (2.0 mg, 0.32 μmol) was treated with DIEA (25 mg, 180 μmol) and 1-thionaphthol (0.07 mL, 500 μmol) in THF (0.5 mL) and H_2O (0.05 mL) as described for compound **28** to yield 2.0 mg of $(5'\text{-TBDMS-C}^{Bz}\text{G}^{iBu})_6$HPX as a pale yellow solid. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.66. The resulting solid was taken up in THF (100 μL) and H_2O (50 μL) at 20 °C, and the clear solution was treated with TBAF (30 μL, 30 μmol, 1 M solution in THF) for 12 h. After addition of H_2O (1 mL) and NH_4OH (0.1 mL, 25%), THF was removed with a gentle stream of nitrogen, and the remaining solution was evaporated to dryness by lyophilization. The resulting residue was washed twice with ethyl acetate (2 × 3 mL) to yield 2 mg of **31** after centrifugation (3000 rpm, 5 min) as a colorless solid: TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.62. Finally, hybrid **31** was successively treated with NH_4OH (250 μL, 25% aqueous solution) and $MeNH_2$ (250 μL, 40% aqueous solution) at 5 °C for 1 h and at room temperature for 2 h, as described above. After excess ammonia and methylamine were

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removed with a gentle stream of nitrogen, and the remaining solution was evaporated to dryness by lyophilization. The crude was taken up in H_2O/THF (1 mL, 1:1 v/v) and treated with DOWEX MAC3 cation exchange resin (50 mg) for 5 min. The resin was filtered off and washed with water (2 × 1 mL). NH_4OH (0.1 mL, 25%) was added to the filtrate, and the excess ammonia was again removed with a gentle stream of nitrogen. The remaining solution was evaporated to dryness by lyophilization, and the solid was treated twice in an ultrasonic bath with CH_2Cl_2/ethyl acetate (3 mL, 1:1 v/v) to yield, after centrifugation, 1.0 mg (0.2 μmol, approximately 95%) of **1** as a colorless solid: TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.25; MALDI-TOF MS calcd for $C_{158}H_{178}N_{48}O_{78}P_{12}$ $[M - H]^-$ 4367, obsd 4363.

(CG)$_6$HPX (1) from 30. To a solution of protected hybrid **30** (17 mg, 2.23 μmol) in THF (0.6 mL) and H_2O (0.1 mL) was added DIEA 25 mg (180 μmol), and the mixture was shaken for 1 h at 20 °C. Then, 1-thionaphthol (0.07 mL, 500 μmol) was added, and the solution was again shaken for 1 h at 20 °C. Ethyl acetate (0.5 mL) and hexane (3 mL) was added, and the precipitate was separated by centrifugation (3500 rpm, 5 min). The resulting viscous oil was treated with ethyl acetate (3 × 3 mL) and isolated by centrifugation (3500 rpm, 5 min). The resulting solid was dissolved in H_2O (2 mL) and dried by lyophilization to yield 20.0 mg of (5′-DMT-C^{Bz}G^{iBu})$_6$HPX as colorless foam. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35 v/v/v) R_f = 0.65. For DMT removal, the solid was taken up in water (3 mL) at 20 °C. After addition of ethyl acetate (2 mL) and cation-exchange resin DOWEX MAC3 (70 mg, approximately 250 μmol H^+), the mixture was shaken at 20 °C for 12 h. The resin was filtered off and washed with THF/H_2O (1:1, 3 × 1 mL). The organic layer of the filtrate was aspirated, and the aqueous layer was washed three times with ethyl acetate (3 × 1 mL). After addition of NH_4OH (50 μL, 1 M), the THF was removed in vacuo, and the remaining aqueous solution was evaporated to dryness by lyophilization to yield 11 mg of **31** as a colorless solid. TLC (*n*-PrOH/H_2O/NH_4OH (25%) 55:10:35): R_f = 0.63. Finally, hybrid **31** was treated with NH_4OH (250 μL, 25% aqueous solution) and $MeNH_2$ (250 μL, 40% aqueous solution), as described above for conversion of **28** to **1**. The resulting solid was treated twice with CH_2Cl_2/ ethanol (1:1, 2 × 2 mL) to yield 9.0 mg (2 μmol, approximately 88%) of the title hybrid **1**, after centrifugation (3500 rpm, 5 min), as a colorless solid. TLC (*n*-PrOH/H_2O/NH_3 (25%) 55:10:35 v/v/v) R_f = 0.24. A sample of the crude was then purified by HPLC, using a C8 column and a gradient of MeCN in 10 mM TEAA buffer (5% for 5 min, 5−8% in 5 min, 8−18% in 55 min) at 55 °C. Hybrid **1** eluted at t_R = 29.2 min, yield 25%. The residual crude (8 mg) was then washed with methanol (2 × 1 mL), and a portion (2.6 mg, 0.57 μmol) was purified by HPLC, using a semipreparative Nucleosil C8 column and a gradient of MeCN in 10 mM TEAA buffer (5% for 5 min, 5−10% in 5 min, 10−17% in 50 min) to yield 1.3 mg (0.28 μmol, 50%) of **1**: ^{31}P NMR (122 MHz, CD_3CN/D_2O 2:1 v/v) δ −1.0, (*P*OCH$_2$), −5.34, (*P*OAr); ^{1}H NMR (500 MHz, NaOH (0.1 M)/D_2O 9:1 *v/v*, suppression of solvent peak with the WATERGATE gradient pulse sequence,[34] 90 °C) δ 8.28 (s, 6 × 1H, *H8-G*), 7.79 (d, *J* = 7.9 Hz, 6 × 1H, *H6−C*), 7.46−7.40 (m, 28H, Ar-*H$_{core}$*), 6.62 (m, 6 × 1H, *H1′-G*), 6.44 (m, 6 × 1H, *H1′-C*), 6.14 (d, *J* = 7.9 Hz, 6 × 1H, *H5−C*), 5.46 (m, 6 × 1H, *H3′-G*), 3.99 (dd, $J_{5'-4'}$ = 2.6 Hz, $J_{5'-5''}$ = 11.9 Hz, 6 × 1H, *H5′-C*), 3.92 (dd, $J_{5''-4'}$ = 4.6 Hz, $J_{5''-5'}$ = 11.9 Hz, 6 × 1H, *H5″-C*), 3.13 (m, 6 × 1H, *H2′-G*), 3.03 (m, 6 × 1H, *H2″-G*), 2.74 (m, 6 × 1H, *H2′-C*), 2.24 (m, 6 × 1H, *H2″-C*); MALDI-TOF MS calcd for $C_{158}H_{178}N_{48}O_{78}P_{12}$ $[M - H]^-$ 4367, obsd 4365.

(TCG)$_4$TTPA (34). To a sample of (G)$_4$TTPA (**33**, 5.8 mg, 2.9 μmol, previously coevaporated from CH_3CN, 3 × 1 mL) was added phosphoramidite **22** (19.5 mg, 17.5 μmol), and the mixture was dried at <0.001 mbar and 60 °C for 1.5 h. After addition of molecular sieves (3 Å, 5 beads), the flask was flushed with argon and sealed with a septum. Propylenecarbonate (500 μL) and THF (100 μL) were added, and the suspension was heated to 55 °C. After addition of 1*H*-tetrazole (0.1 mL of a 0.45 M solution in CH_3CN, 45 μmol), the reaction mixture was stirred for 4 h at 55 °C. The heating bath was removed, *tert*-butyl hydroperoxide (50 μL of a 5.5 M solution in decane, 275 μmol) was added, and the mixture was stirred for 15 min at room temperature. Finally, the product was precipitated by slowly adding the solution to ethyl acetate (1 mL). The resulting solid was filtered off, washed with ethyl acetate (6 × 1 mL), and dried in vacuo to yield 7.8 mg of crude. For MALDI-TOF MS analysis, an analytical sample was fully deprotected. When the spectrum indicated an incomplete conversion of the core, the product was coupled again to **22** (32.8 mg, 29.5 μmol) in propylencarbonate (300 μL) and THF (100 μL), 1-*H*-tetrazole (0.1 mL of a 0.45 M solution in CH_3CN, 45 μmol) and *tert*-butyl hydroperoxide (60 μL of a 5.5 M solution in decane, 330 μmol), as described above. The product was then precipitated again from ethyl acetate (1 mL). The solid was filtered off, washed with ethyl acetate (6 × 1 mL), and dried in vacuo, to yield 14.4 mg (2.36 mmol, 81%) of protected hybrid **34**. To a solution of protected hybrid **34** (10.5 mg, 1.72 μmol) in THF (0.13 mL) and H_2O (0.07 mL) was added a mixture of DIEA (25 μL) and 1-thionaphthol (50 μL) in THF (1.25 mL). The clear solution was shaken at 20 °C for 2.5 h. Ethyl acetate (0.5 mL) and hexane (2 mL) was added, the aqueous layer was separated and washed with hexane/ethyl acetate (3 × 1 mL, 4:1 v/v). After addition of water (200 μL), the mixture was evaporated to dryness by lyophilization. For removal of the DMT groups, acetic acid (0.2 mL, 80% in water) was added at 20 °C, and the solution was stirred for 1 h, while the mixture was washed six times with hexane (6 × 1 mL) during this time. The reaction mixture was then diluted with H_2O (0.6 mL) and evaporated to dryness by lyophilization. The resulting solid was treated with NH_4OH (250 μL, 25% aqueous solution) at room temperature for 2 h. After addition of $MeNH_2$ (250 μL, 40% aqueous solution), the reaction mixture was again reacted at room temperature for 2 h. Excess ammonia and methylamine were removed with a gentle stream of nitrogen, and the remaining solution was evaporated to dryness by lyophilization to yield 8.0 mg of crude **34** as a colorless solid. The crude was purified by HPLC, using a Nucleosil C8 column and a gradient of MeCN in 10 mM TEAA buffer (10% for 5 min, 10−15% in 10 min, 15−18% in 15 min and 18−22% in 30 min) at 20 °C. Hybrid **34** eluted at t_R = 46.0 min, yield 24%: ^{31}P NMR (202 MHz, D_2O, 40 °C) δ −0.72; ^{1}H NMR (500 MHz, D_2O, suppression of solvent peak by presaturation,[34] 40 °C) δ 8.50 (s, 4 × 1H, *H5-triazole*), 7.92 (s, 4 × 1H, *H8-G*), 7.72 (d, *J* = 6.5 Hz, 4 × 2H, *Ar-H*), 7.49 (d, *J* = 7.4 Hz, 4 × 1H, *H6-C*), 7.31 (s, 4 × 1H, *H6-T*), 7.18 (bs, 4 × 2H, *Ar-H*), 6.32 (bs, 4 × 1H, *H1′-G*), 5.92 (m, 4 × 2H, *H1′-C, H1′-T*), 5.75 (d, *J* = 7.4 Hz, 4 × 1H, *H5-C*), 5.66 (bs, 4 × 1H, *H3′-G*), 4.67 (m, 4 × 1H, *H3′-C*), 4.60 (m, 4 × 1H, *H3′-T*), 4.47 (suppression of *H4′-G*), 4.04 (m, 4 × 2H, *H5′-G and H5″-G*), 3.98 (m, 4 × 1H, *H4′-C*), 3.92 (m, 4 × 1H, *H4′-T*), 3.83 (m, 4 × 2H, *H5′-C and H5″-C*), 3.60 (m, 4 × 2H, *H5′-T and H5″-T*), 3.03 (m, 4 × 2H, *H2′-G, H2″-G*), 2.27 (m, 4 × 2H, *H2′-C, H2′-T*), 2.08 (m, 4 × 1H, *H2″-T*), 1.89 (m, 4 × 1H, *H2″-C*), 1.58 (s, 4 × 3H, *CH$_3$*), 1.55 (brs, 12H, *ad-H*); MS (MALDI-TOF) calcd for $C_{158}H_{179}N_{52}O_{64}P_8^-$ 4077 $[M - H]^-$, obsd 4075.

Phenyl 2′-Deoxyguanosine-3′-monophosphate. This model compound was synthesized purely to study basic deprotection of a phenolic phosphodiester via MALDI-TOF MS. To a solution of 3′-phosphoramidite **6** (280 mg, 0.35 mmol) and phenol (30.0 mg, 0.32 mmol) in dioxane (1 mL) were added molecular sieves (3 Å, 5 beads). After addition of 1*H*-tetrazole (0.71 mL, 0.45 M solution in CH_3CN), the mixture was put in an ultrasonic bath for 2 min at 20 °C. The reaction mixture was kept at 20 °C for 5 min and then in a refrigerator at 5 °C for 3 h. Then, *tert*-butyl hydroperoxide (0.2 mL, 5.5 M solution in decane) was added, and the mixture was kept at 5 °C again for 15 min. The solution was diluted with CH_2Cl_2 (20 mL) and washed with phosphate buffer (15 mL, 0.2 M, pH 7). The aqueous phase was back-extracted with CH_2Cl_2 (10 mL), and the combined organic layers were dried over $NaSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in ethyl acetate (1 mL) and precipitated with hexane (4 mL). The mixture was centrifuged, and the supernatant solution was discarded. This process was repeated, and the resulting solid was purified by chromatography using silica (10 g) and a step gradient of 2-propanol (0−4%) in ethyl acetate. Product-containing fractions were combined, concentrated, and dried in vacuo to yield 220 mg (0.27 mmol, 85%) of methyl phenyl N^2-isobutyryl-2′-deoxy-5′-*O*-dimethox-

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ytritylguanosine-3′-monophosphate: TLC (ethyl acetate/2-propanol = 95:5 v/v) R_f = 0.16, 0.25; ^{31}P NMR (122 MHz, CD_2Cl_2) δ −5.65, −5.69 (*P*OAr); ^{1}H NMR (300 MHz, CD_2Cl_2) δ 11.84 (brs, 1H), 8.83 and 8.71 (2s, 1H), 7.75 and 7.73 (2s, 1H), 7.38−7.05 (m, 14H), 6.79−6.72 (m, 4H), 6.16 and 6.11 (2t, J = 6.1 Hz, 1H), 5.88 and 5.79 (2 m, 1H), 4.22 (m, 1H), 3.84 and 3.75 (2d, J = 11.4 Hz, 3H), 3.75 and 3.74 (2s, 6H), 3.39, (m, 1H), 3.26−3.10 (m, 2H), 2.72−2.54 (m, 1H), 2.27−2.09 (m, 1H), 1.11−0.96 (m, 6H); MALDI-TOF MS (DMT off) calcd for $C_{21}H_{26}N_5O_8P$ $[M - H]^-$ 506, obsd 506. To a solution of methyl phenyl N^2-isobutyryl-2′-deoxy-5′-*O*-dimethoxytritylguanosine-3′-monophosphate (10 mg, 12.3 μmol) in THF (0.1 mL) and H_2O (0.05 mL) was added a solution of *N*,*N*-diisopropylethylamine (DIEA, 13 mg, 0.1 mmol) and 1-thionaphthol (0.07 mL, 0.5 mmol) in THF (0.1 mL). The mixture was stirred at 20 °C for 15 min. A mixture of ethyl acetate (0.5 mL) and hexane (3 mL) was added, and the precipitate was isolated by centrifugation (3500 rpm, 5 min). This process was repeated twice, and the residual viscous oil was dried in vacuo. For removal of the DMT group, the product was dissolved in CH_2Cl_2 (0.2 mL) and was treated with trichloroacetic acid (0.1 mL, 3% TCA in CH_2Cl_2) for 30 s at room temperature, followed by addition of CH_3OH. After 30 s, hexane (3 mL) was added, and the precipitate was isolated by centrifugation. The detritylation procedure was repeated once. [MALDI-TOF MS calcd for $C_{20}H_{24}N_5O_8P$ $[M - H]^-$ 492, obsd 493.] For the base-induced removal of the isobutyryl protecting group (compare Figure S50, Supporting Information), the solid was treated with a mixture of NH_4OH (150 μL, 25 aqueous solution) and $MeNH_2$ (150 μL, 40% aqueous solution) at 5 °C for 2 h and at room temperature for 2 h. Excess ammonia and methylamine were removed with a gentle stream of nitrogen, and the remaining solution was evaporated to dryness by lyophilization to yield 4.9 mg (93%, 11.6 μmol) of phenyl 2′-deoxyguanosine-3′-monophosphate as a colorless solid. MALDI-TOF MS calcd for $C_{16}H_{18}N_5O_7P$ $[M - H]^-$ 422, obsd 422.

NMR of Hybrids. For the assignment of the NMR spectra of hybrids, samples were taken up in 99.9% D_2O (200 μL) and transferred to a Bruker Match System NMR tube to give a 3 mM solution. Two-dimensional spectra were recorded using a Bruker Avance 500 MHz spectrometer at 313 K. Suppression of the excess solvent peak was achieved by presaturation during the recycle delay. The repetition delay was set to 2 s. ROESY spectra were acquired at a mixing time of 300 ms, and TOCSY spectra at a mixing time of 60 ms. All two-dimensional spectra were recorded with 256 increments in F1 and 2048 in F2, at 8 scans per increment and were processed with zero filling to 512 data points in F1. Assignment of H1′ and H6 protons of the nucleotides was based on conventional assignment strategies for DNA.[35] The starting point for the assignment of thymidine residues was the resonance of the methyl group, which was identified on the basis of the ROESY crosspeak to its H6 neighbor. The deoxycytidine residues were identified based on the ROESY crosspeak of H5 to H6. Starting from the H1′ resonance of each nucleotide, chemical shifts of the deoxyribose protons were assigned based on TOCSY crosspeaks and were confirmed via the ROESY spectrum. The resonances of the core were assigned based on chemical shifts and coupling constants.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of nucleoside derivatives, NMR spectra, HPLC chromatograms, MALDI-TOF mass spectra, and photographs of material formed by **34** prior to and after addition of ethidium bromide. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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