JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

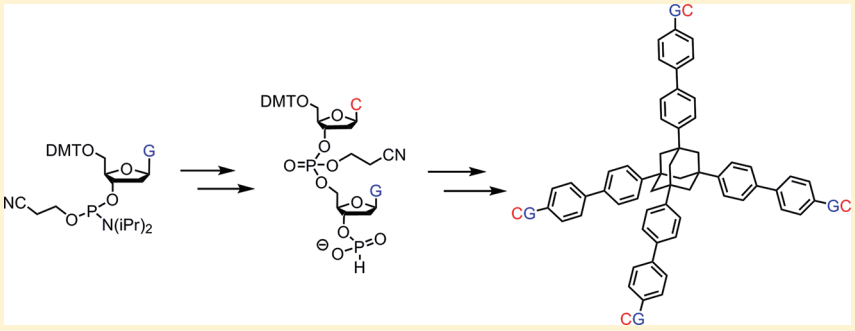
Solution-Phase Synthesis of Branched DNA Hybrids via *H*-Phosphonate Dimers

Arunoday Singh,[‡] Mariyan Tolev,[‡] Christine I. Schilling,[§] Stefan Bräse,[§] Helmut Griesser,[‡] and Clemens Richert*[,‡]

[‡]Institute for Organic Chemistry, University of Stuttgart, 70569 Stuttgart, Germany

[§]DFG-Center for Functionalized Nanostructures (CFN) and Institute of Organic Chemistry, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany

Supporting Information



ABSTRACT: A method for the solution-phase synthesis of branched oligonucleotides with tetrahedral or pseudo-octahedral geometry is described that involves the coupling of 3′-*H*-phosphonates of protected dinucleoside phosphates and organic core molecules. The dimer building blocks are produced by a synthesis that requires no chromatographic purification and that produces the dimer *H*-phosphonates in up to 44% yield in less than three days of laboratory work. A total of seven different branched hybrids were prepared, including a new hybrid of the sequence $(CG)_4$TBA, where TBA stands for tetrakis-(*p*-hydroxybiphenyl)adamantane that assembles into a material from micromolar aqueous solution upon addition of $MgCl_2$.

INTRODUCTION

The first use of an *H*-phosphonate for the assembly of internucleotide bonds dates back to the 1950s.[1] Todd and colleagues used a 5′-*H*-phosphonate of 2′,3′-isopropylideneuridine as a building block and diphenyl phosphorochloridate as activating agent for the solution-phase synthesis of a dinucleoside phosphate. Efficient solid-phase syntheses of oligonucleotides using *H*-phosphonate building blocks were reported in the 1980s,[2,3] producing chains of more than 100 nucleotides in favorable cases. Difficult coupling reactions have been performed repeatedly with *H*-phosphonate monomers,[4] including solid-phase[5] and solution-phase syntheses of oligonucleotides with phosphorothioate linkages.[6] New protocols for solid-phase chain assembly have been published since the breakthrough methodology of Matteucci and colleagues in 1986,[7,8] and the usefulness of *H*-phosphonates for the preparation of nucleoside triphosphates has been demonstrated.[9] However, compared to phosphoramidite-based solid-phase synthesis,[10] the *H*-phosphonate method of chain assembly has found few followers in the field of nucleic acid chemistry.

One reason for this lack of attention may be the potential for side reactions during coupling, such as the formation of branched chains and symmetrical phosphite anhydrides.[11] Another may be the hydrolytic lability of the *H*-phosphonate diesters toward basic aqueous conditions.[12] However, the main reason why *H*-phosphonates have fallen from favor is probably the availability of extremely efficient automated DNA synthesizers for chain assembly using phosphoramidite chemistry that, once established, have outcompeted other methods.

We became interested in the solution-phase synthesis of branched DNA chains with organic branching elements, or "cores", as part of a program to generate nanoporous DNA-based materials. For rigid cores, DNA chains as short as dimers suffice to drive assembly processes that lead to materials.[13] A convergent solution-phase synthesis of such hybrids requires the preparation and handling of dimer building blocks. Since *H*-phosphonates are more stable toward hydrolysis than the corresponding phosphoramidites and thus easier to handle, we decided to study *H*-phosphonates of dimers, using an adaptation of the recently published procedure of Jones et al.[14] for the synthesis of cyclic diguanosine monophosphate (c-di-GMP). The methodology uses inexpensive, commercially available phosphoramidites of nucleosides. It treats *H*-phosphonates both as unreactive, latent phosphates and as readily activable forms of electrophiles that undergo coupling with alcohols. Here, we

Received: December 8, 2011
Published: February 27, 2012

   dx.doi.org/10.1021/jo202508n | *J. Org. Chem.* 2012, 77, 2718–2728

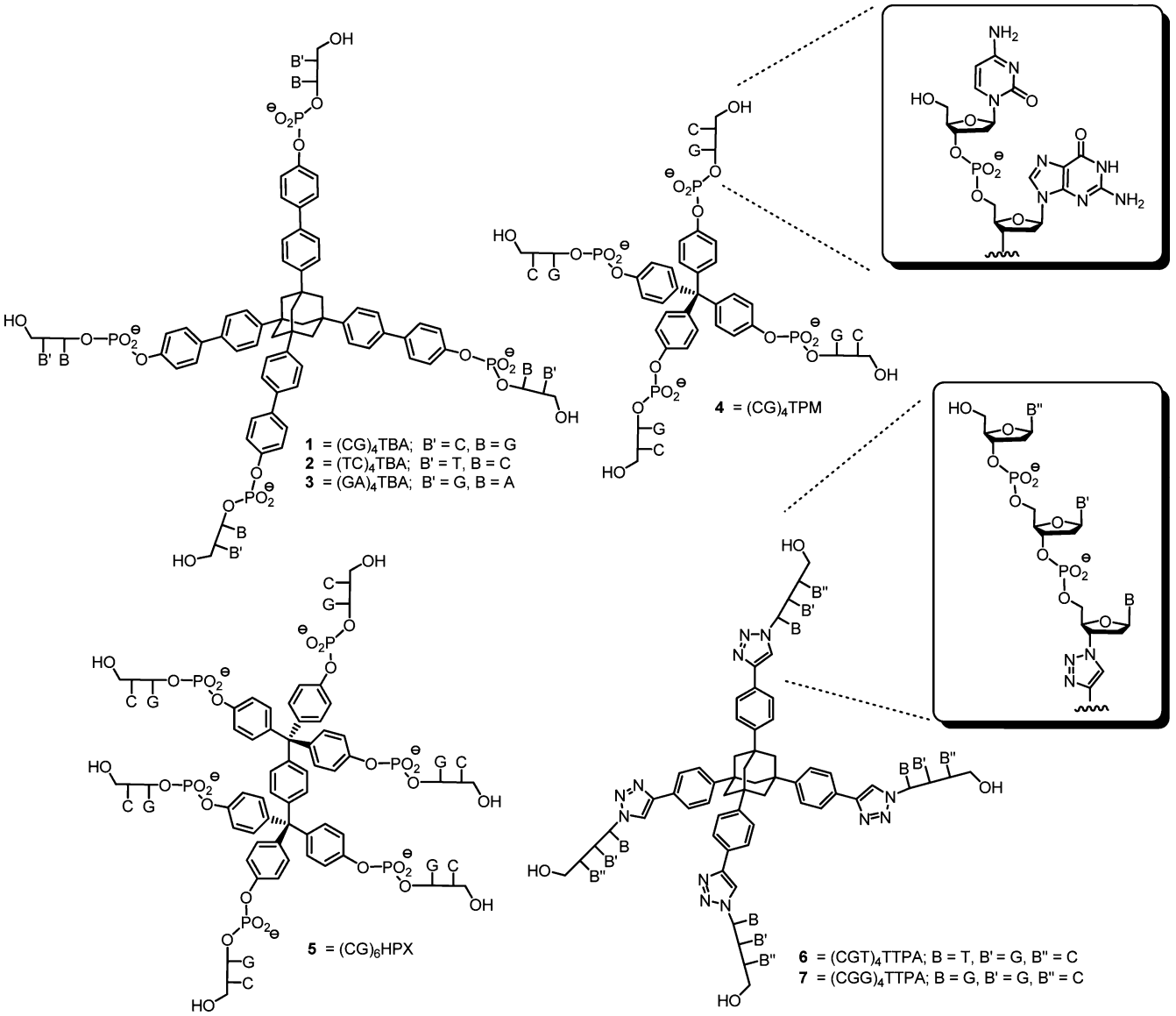


Figure 1. Target molecules of this study.

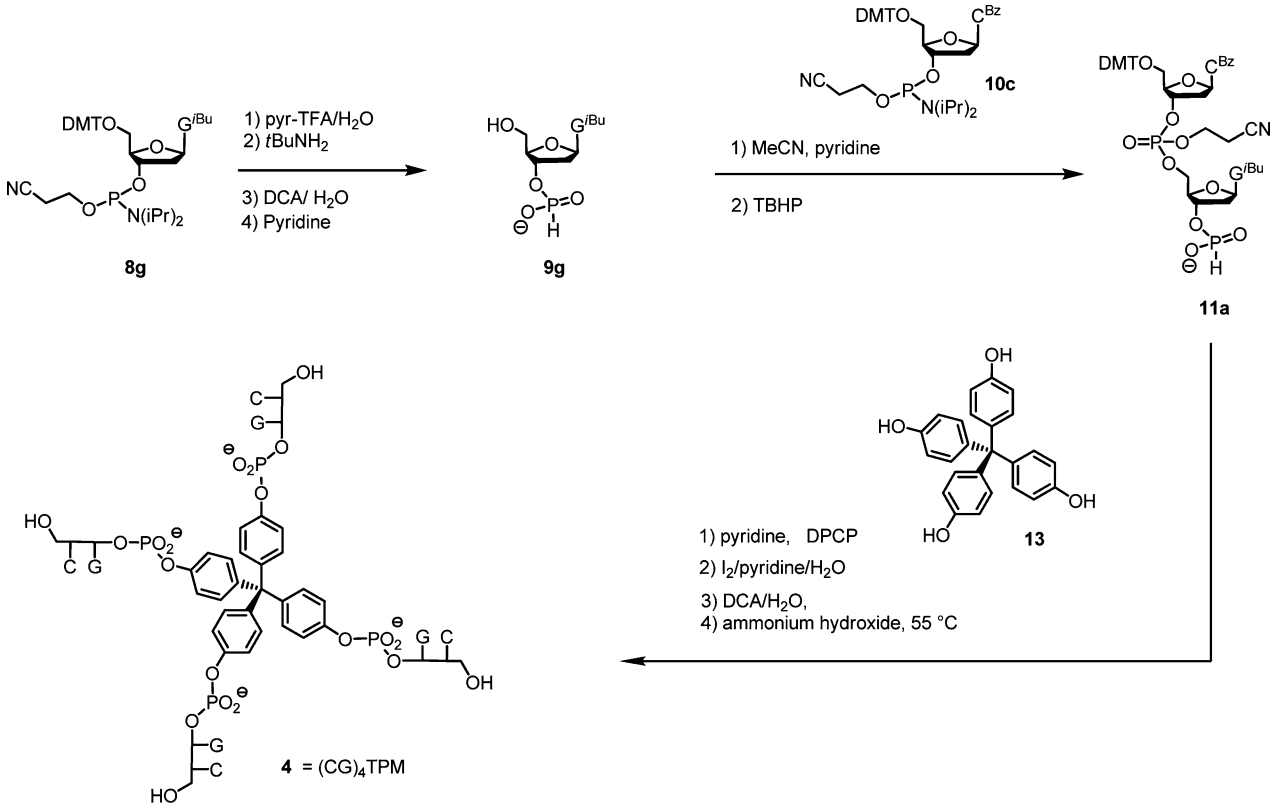
demonstrate that this approach produces branched hybrids with short sticky ends in convenient solution-phase syntheses that involve coupling of *H*-phosphonates dimers to aliphatic or aromatic alcohols. Our study also included the synthesis of a new branched hybrid with trimer nucleoside arms. The methodology presented here is comparable in its yields to that using dimer phosphoramidites, presented in the accompanying publication,[15] but the starting materials are more readily available and the procedure is less time-consuming. The molecules prepared are distantly related to branched oligonucleotides[16] with two DNA chains attached to a branching element and more closely related to oligonucleotide dendrimers.[17] Both branched oligonucleotides and oligonucleotide dendrimers have thus far been prepared via solid-phase syntheses, based on phosphoramidites of nucleosides as building blocks.

RESULTS AND DISCUSSION

Figure 1 shows the target molecules of our study. Four different types of branched oligonucleotides were synthesized. The group of hybrids includes six-arm, pseudo-octahedral hybrid $(CG)_6$HPX, based on hexakis(*p*-hydroxyphenyl)xylene (HPX) as core,[18,19] and three different types of tetrahedral hybrids. The latter were the tetrahedral hybrids $(CG)_4$TPM (**4**), with tetrakis(*p*-hydroxyphenyl)methane (TPM)[13] as branching element, $(CGT)_4$TTPA (**6**) and $(CGG)_4$TTPA (**7**) as more rigid and less charged four-arm hybrids, based on tetrakis(triazoylphenyl)adamantane (TTPA) as core,[20] and $(CG)_4$TBA (**1**), $(TC)_4$TBA (**2**), and $(GA)_4$TBA (**3**) that are oligonucleotide-bearing versions of tetrakis(*p*-hydroxybiphenyl)adamantane (TBA). The new core "TBA" was prepared because of its long and rigid biphenyl linker, designed to keep the DNA arms far apart from each other, reducing the steric crowding and the electrostatic repulsion upon assembly into materials. The sequence 5′-CG-3′ was chosen based on our previous work, showing that this dimer acts as a "sticky end" or "zipper" that is strong enough to induce material formation via multivalent interactions in aqueous solution.[13] The non self-complementary sequences 5′-TC-3′ and 5′-GA-3′ were chosen to access an alternative, binary assembly system. Similar non self-complementary sticky ends were employed by Seeman and co-workers to generate designed DNA crystals.[21] Finally, the trimer sequence 5′-CGG-3′ was included to investigate the influence

dx.doi.org/10.1021/jo202508n | J. Org. Chem. 2012, 77, 2718–2728

Scheme 1. Synthetic Route to Hybrid 4 Using *H*-Phosphonate Chemistry



of a spacer residue (G) on the effect of the "CG-zippers" during the assembly process and to expand the methodology to trimer chains.

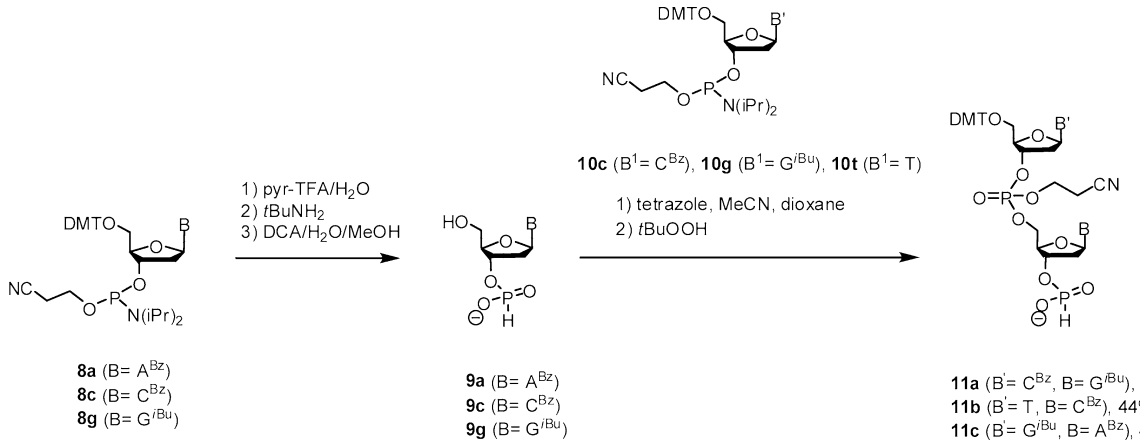
Recently, Jones and co-workers reported the synthesis of cyclic diguanosine monophosphate (c-di-GMP) as a one-pot process in solution, using a combination of phosphoramidite and *H*-phosphonate chemistry.[14] Although other syntheses of cyclic diguanosine monophosphate using *H*-phosphonate chemistry were reported previously,[22] the recent method was particularly attractive, as it uses no chromatographic purification steps up to the level of the linear dimer. The method of Jones et al. employs ribonucleotides instead of deoxyribonucleotides. A ribonucleoside phosphoramidite is hydrolyzed by treatment with pyridinium trifluoroacetate in acetonitrile containing 2 equiv of water, followed by treatment with *tert*-butylamine to remove the cyanoethyl group. Dichloroacetic acid (DCA) is then used for DMT deprotection, followed by addition of 2 equiv of pyridine to generate an *H*-phosphonate with a free 5′-hydroxy group. The subsequent coupling step is induced by the addition of a phosphoramidite to the reaction mixture, followed by oxidation with TBHP. Neither step appeared unsuitable for 2′-deoxynucleosides, and the assembly of the dimer *H*-phosphonate was expected to be easier than that of the dimer 3′-phosphoramidites because the polarity of the product should allow the removal of excess reagents via precipitation. Finally, phosphodiesters are more easily liberated from their cyanoethyl-protected precursors than their methyl-protected counterparts, possibly avoiding complications during the deprotection of phenolic ester linkages.

First, we attempted to synthesize the DNA hybrids in a one-pot procedure, using *H*-phosphonate chemistry. To establish the methodology, hybrid **4** was explored first (Scheme 1). For this, the 5′-DMT-protected dimer *H*-phosphonate **11a** was generated, using an adaptation of the methodology of Jones et al. mentioned above. The coupling to the core was carried out by addition of TPM (**13**) to the dimer *H*-phosphonate **11a** in pyridine, using diphenyl chlorophosphate (DPCP) as condensing agent, followed by oxidation with iodine. Excess iodine was removed by an aqueous washing step with sodium bisulfite solution. The next step (detritylation) was carried out with DCA, followed by removal of the base protecting groups in ammonium hydroxide. The success of the reactions was confirmed by MALDI-TOF mass spectrometry. The mass spectrum gave low intensity peaks but showed the desired product along with a number of side products, including a three-arm hybrid.

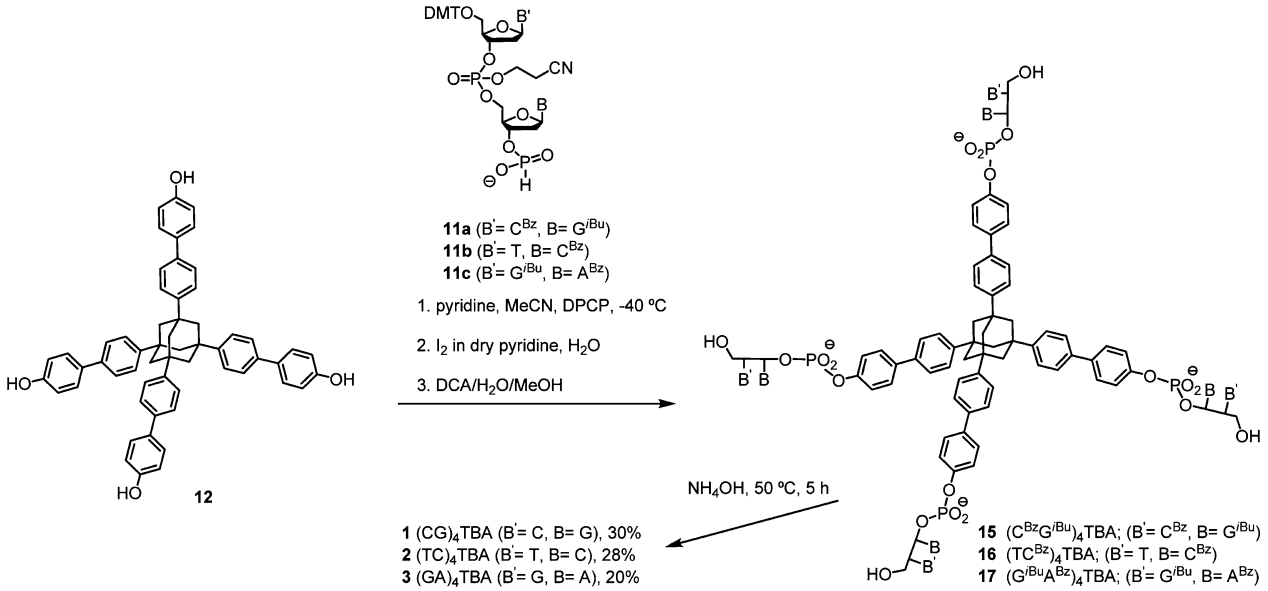
Because the process was a one-pot procedure and did not include any purification steps, the presence of intermediates like **9g** was expected to cause side reactions. Therefore, we synthesized the dimer *H*-phosphonates separately, as shown in Scheme 2, purified them, and then coupled them to the core molecules, followed by oxidation and deprotection. The synthesis started with commercially available phosphoramidites **8a**–**g**, which were hydrolyzed by treatment with pyridinium trifluoroacetate in acetonitrile containing 2 equiv of water, followed by treatment with *tert*-butylamine to remove the cyanoethyl group and detritylation with DCA in dichloromethane in the presence of 10 equiv of water. Quenching with methanol prevented retritylation and afforded crude *H*-phosphonates **9a**–**g**. Residual reagents were removed by precipitating again from a mixture of diethyl ether/ethyl acetate (1:1, v/v), starting from a concentrated solution in dichloromethane. After coevaporation with acetonitrile, **9a**–**g** were obtained in sufficient purity, as judged by ESI MS and ^{31}P NMR. The ^{1}H NMR spectrum showed residual DMT alcohol, which could not be removed completely by reprecipitation, and that proved inconsequential for the subsequent couplings with phosphoramidites **10c**–**t**. The following

dx.doi.org/10.1021/jo202508n | *J. Org. Chem.* 2012, 77, 2718–2728

Scheme 2. Synthesis of *H*-Phosphonates of Protected Dimers



Scheme 3. Synthesis of Branched DNA Hybrids $(CG)_4$TBA (1), $(TC)_4$TBA (2), and $(GA)_4$TBA (3)



three dimer sequences were prepared to access DNA hybrids with self-complementary or non-selfcomplementary arms: 5′-CG-3′, 5′-TC-3′, and 5′-GA-3′.

Coupling with tetrazole as activator in dioxane as solvent followed by oxidation with *tert*-butyl hydroperoxide (TBHP) gave dimers **11a–c**. The dimer *H*-phosphonates were purified by two precipitations from ethyl acetate, starting from solutions in dichloromethane/MeOH (9:1, v/v) to afford **11a–c** in 42–44% overall yield. The ^{1}H NMR spectra showed a purity greater than 90%, and the dimers were then used for coupling to cores, without further purification.

With the dimer *H*-phosphonates in hand, we turned to coupling them to the branching elements or cores to generate branched hybrids. The synthesis of TBA hybrids was performed as shown in Scheme 3. The synthesis of **1** started with the coupling of dimer *H*-phosphonate **11a** to TBA core **12** in a mixture of pyridine and CH_3CN (1:1, v/v), using DPCP as condensing agent.[23] Initial attempts at room temperature did not give satisfactory results, and the MALDI-TOF mass spectrum of the crude reaction mixture, measured after detritylation, showed peaks for products with only two or three DNA arms. Pivaloyl chloride and 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (DMOCP) gave lower yields still and were therefore not tested further. Using DPCP, the reaction temperature was reduced to −40 °C as recommended by Reese and Song for other *H*-phosphonate couplings.[24] Because oxidation with I_2 in pyridine/H_2O led to hydrolysis of the aryl nucleoside *H*-phosphonate diester, the reaction mixture was first treated with I_2 in dry pyridine, followed by addition of water after 1 min.[23] The fully protected crude hybrid (which itself could not be detected in MALDI spectra, but was detectable after removal of the DMT groups) was then treated with dichloroacetic acid in dichloromethane to give 5′-deprotected hybrid **15**, followed by treatment with ammonium hydroxide to yield crude **1**. Purification by reversed-phase HPLC on a C-8 column at 50 °C gave **1** in 30% yield. A large-scale synthesis of $(CG)_4$TBA (12 μmol) was also performed. It gave 10 mg of pure **1** (30% overall yield of isolated pure material).

Using similar methodology, two other hybrids with non-selfcomplementary arms, namely $(TC)_4$TBA (**2**) and

dx.doi.org/10.1021/jo202508n | J. Org. Chem. 2012, 77, 2718–2728

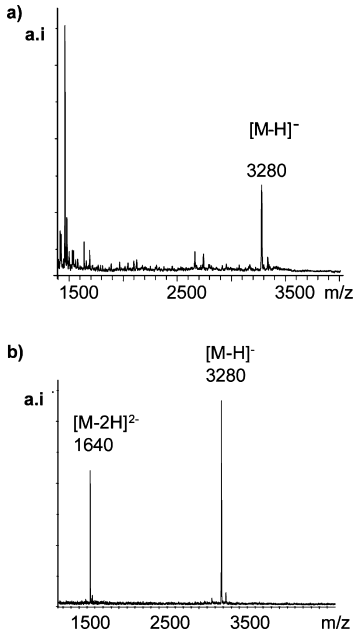


Figure 2. MALDI-TOF mass spectra of $(CG)_4$TBA (**1**): (a) crude product **1** after precipitation from ethyl acetate; (b) after single-stage HPLC purification.

$(GA)_4$TBA (**3**), were synthesized in 28% and 20% yield, respectively. MALDI-TOF spectra of crude and purified **1** are shown in Figure 2. After the acidic step for the removal of DMT groups, approximately 5−10% of the three-arm product was observed, possibly because of incomplete conversion, as only one coupling cycle was employed. The final basic deprotection step to remove the nucleobase and phosphodiester protecting groups caused approximately 10% side product with three DNA arms and one phosphate group. A ^{1}H NMR spectrum of $(GC)_4$TBA (**1**), acquired under denaturing conditions (100 mM NaOH, 60 °C) showed the expected set of sharp signals (Figure 3).

To test the scope of the method, other branched DNA hybrids were synthesized. The first synthesis employed the smaller tetrahedral TPM core (**13**), leading to $(CG)_4$TPM (**4**) in 25% overall yield (Scheme 4). The second hybrid prepared was based on the pseudo-octahedral six-arm HPX core (**14**) and gave $(CG)_6$HPX (**5**) in 20% overall yield. The synthesis of four-arm TPM hybrids proceeded similarly to that of TBA hybrids, but in the case of the six-arm HPX hybrid, slightly larger amounts of side products were observed. After complete deprotection, approximately 10% phosphorylated five-arm product was detected, along with approximately 15−20% five-arm hybrid with a free hydroxy group.[15] All hybrids were successfully purified via one-stage HPLC purification at 50 °C.

We then extended our work to hybrids with trimer DNA arms (Scheme 5). We chose $(CGT)_4$TTPA (**6**) and $(CGG)_4$TTPA (**7**) as targets. Both feature a nucleoside as spacer between the tetrakis(triazolylphenyl)adamantane core and the CG zipper sticky ends. Studies on DNA-mediated crystallization of gold nanoparticles have shown that linkers can be critical for obtaining highly ordered crystallites.[25] The first nucleoside was attached to the acetylenic core via Cu(I)-catalyzed Huisgen cycloaddition.[26] The protocols reported earlier for hybrids with dimer arms[20] were optimized for **22**. Here, 3′-azido-3′-deoxythymidine (AZT, **21t**) was coupled to tetrakis(4-ethynylphenyl)-adamantane (**20**) in the presence of catalytic amounts of $CuSO_4$ and sodium ascorbate in DMSO/water. Using an equimolar ratio of copper and reducing agent led to a reaction time of 1 h, compared to 10 h required for the earlier protocol.[20,19] Further, dropwise addition of a 5% aqueous EDTA solution at the end of the reaction produced **22** as a colorless precipitate that could be readily isolated from all other components of the reaction mixture by centrifugation. Compound **23** was prepared as reported.[19]

Following the protocol developed for **1**−**3**, hybrids **6** and **7** were prepared by coupling the *H*-phosphonate building block of the CG dimer (**11a**) to **22** or **23** with DCPC as coupling agent in a mixture of pyridine/CH_3CN at −40 °C. Higher acetonitrile content led to incomplete solubilization of the starting materials. The subsequent oxidation with TBHP was also performed at −40 °C, as higher temperatures led to partial decomposition. Detritylation with 80% aqueous AcOH and washing with hexanes gave the 5′-deprotected hybrids. The final basic deprotection was induced by treating with concentrated aqueous ammonia, followed by addition of the so-called AMA mixture (ammonium hydroxide/methylamine, 1:1). Adding AMA from the start led to side products with a mass 14 Da higher than that of **6**/**7** that may have been formed by nucleophilic substitution involving methylamine. Pure hybrids were again obtained after a single chromatographic step, employing a gradient of CH_3CN in 10 mM TEAA buffer and a C8 column either at 23 or 70 °C.

We then proceeded to studying the assembly process for the new hybrids **1**, **2**, and **7**. Figure 4 shows association and melting profiles for **1** and control compound **2**, measured at 260 nm. Assays were started at 10 mM NaOH and 95 °C to ensure full denaturation, followed by neutralization at 95 °C. Each cooling and heating cycle was repeated at least twice to ensure reproducibility. At 10 μM hybrid concentration and low salt concentration, hybrid **1** showed a melting point (T_m) of 65 °C, whereas control hybrid **2** with non-selfcomplementary arms did not show any transition (Figure 4a). As expected, addition of NaCl shifted the melting transition for **1** to higher temperatures.

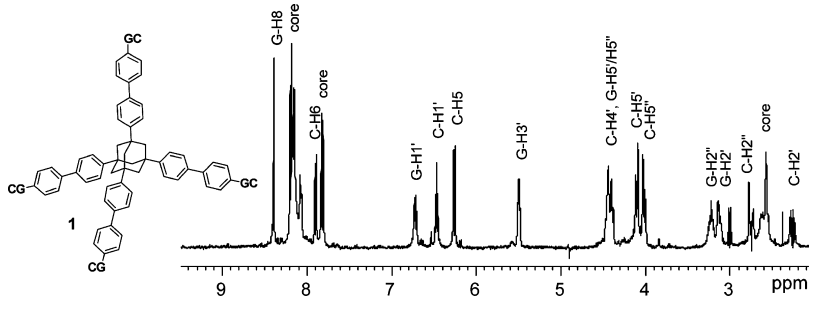
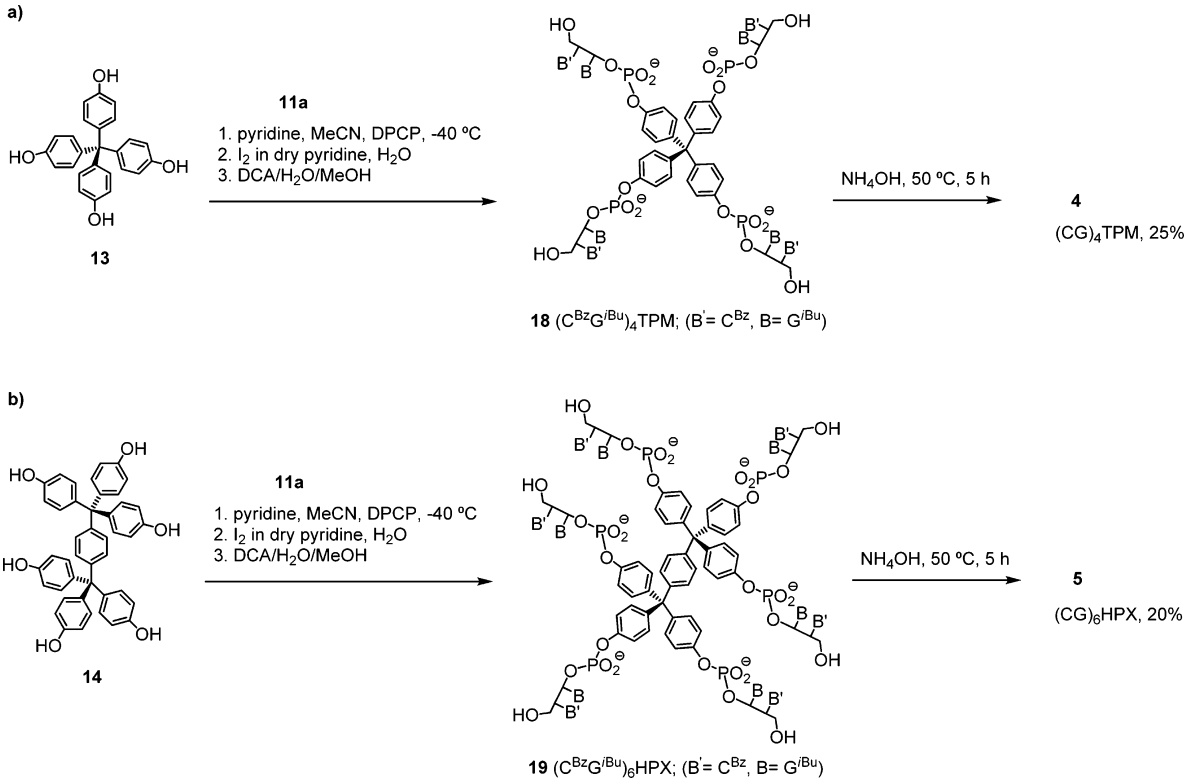


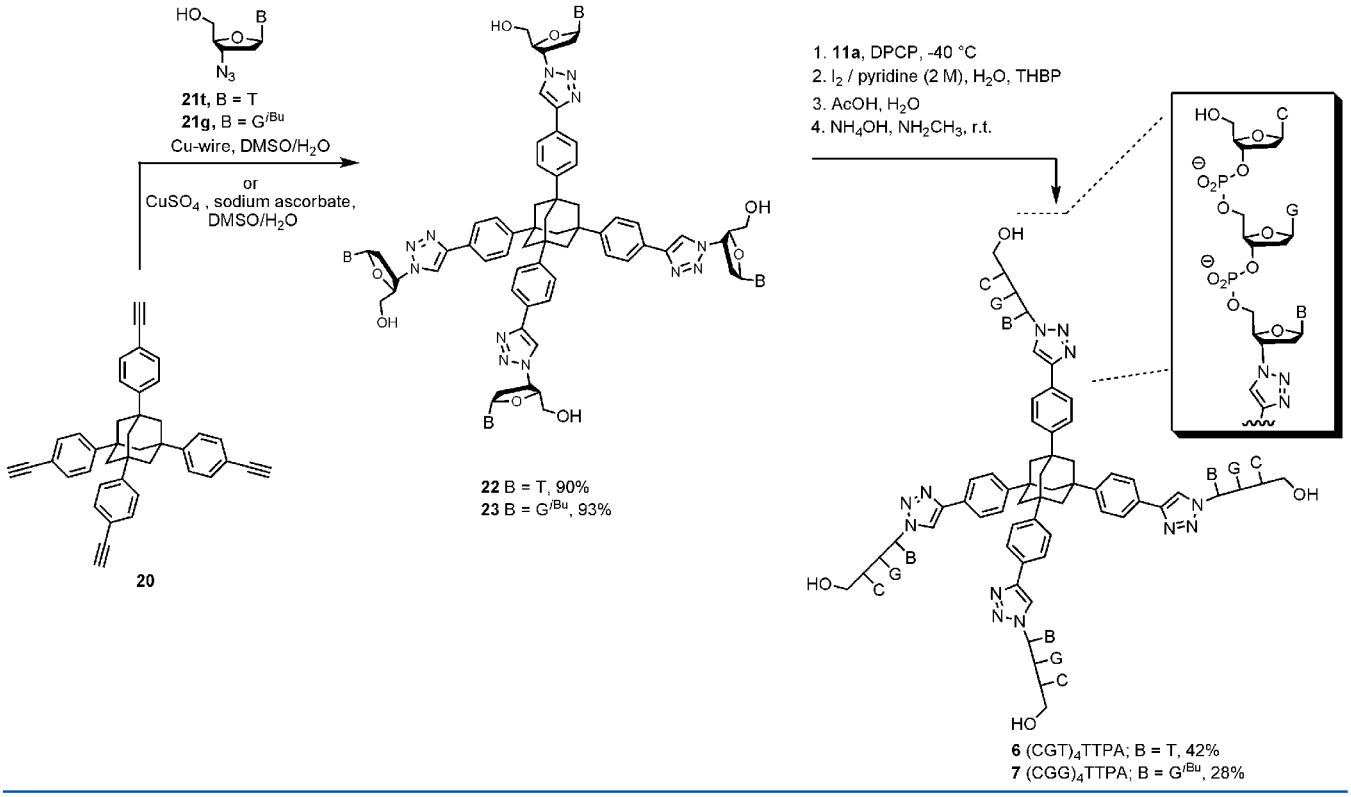
Figure 3. ^{1}H NMR spectrum of purified **1** (0.1 M NaOH/D_2O 9:1, 500 MHz, 60 °C, with solvent suppression).

dx.doi.org/10.1021/jo202508n | J. Org. Chem. 2012, 77, 2718−2728

Scheme 4. Synthesis of the Branched DNA Hybrids (a) $(CG)_4$TPM (4) and (b) $(CG)_6$HPX (5)



Scheme 5. Synthesis of the Branched DNA Hybrids $(CGT)_4$TTPA (6) and $(CGG)_4$TTPA (7)



Since the high temperature baseline could no longer be established, the melting point could not be determined (Figure 4b). After addition of 100 mM $MgCl_2$, a sharp drop in absorption was observed below 25 °C for the solution of **1** (Figure 4c), accompanied by the formation of a visible precipitate. During the subsequent heating, the precipitate melted at a higher temperature (starting at 35 °C) than the temperature of onset of precipitation, as expected for the formation of large assemblies whose kinetics of formation and disassembly are much slower than those of individual duplexes. At 10 mM $MgCl_2$, the onset

dx.doi.org/10.1021/jo202508n | J. Org. Chem. 2012, 77, 2718–2728

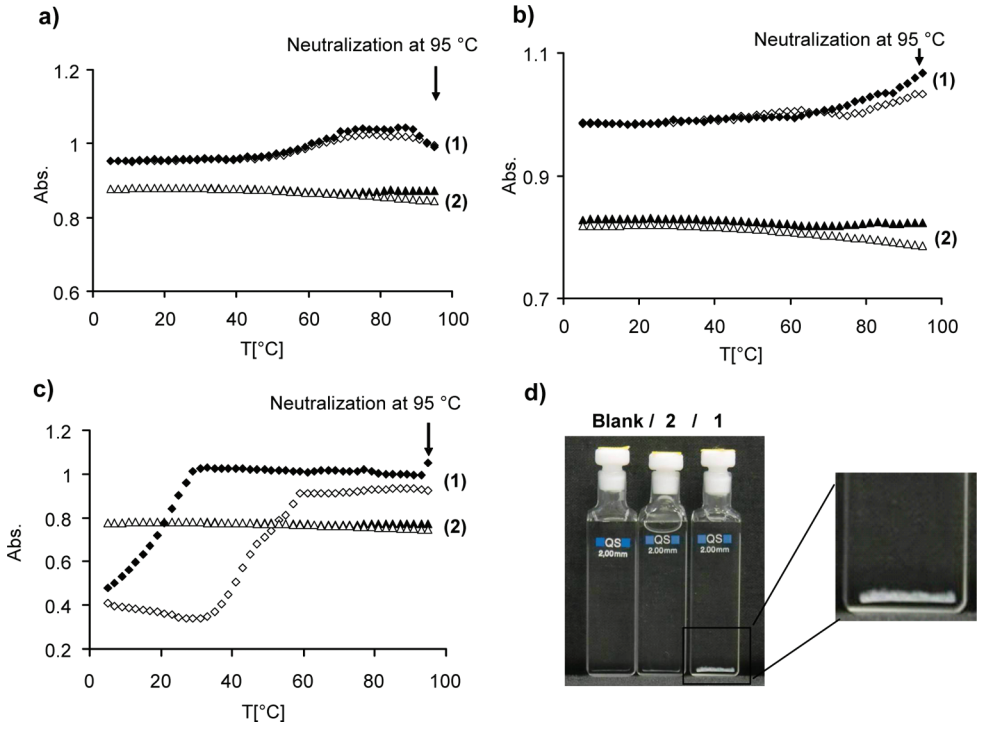


Figure 4. Thermal association and melting profiles of $(CG)_4$TBA (**1**) and $(TC)_4$TBA (**2**) with non-selfcomplementary DNA arms at 10 μM hybrid concentration in 10 mM phosphate buffer, 1.5 mM NaOAc, and cooling or heating rates of 0.5 °C/min. Samples were dissolved by using 10 mM NaOH (100 μL), heated to 95 °C, and neutralized with acetic acid solution (10 mM, 100 μL), followed by dilution to 650 μL through addition of sodium phosphate buffer and water. The first data point after neutralization and dilution is indicated on the graphs. Data from cooling curves are shown with filled symbols, subsequent heating curves are shown with open symbols: (a) curves without additional salt, (b) plus 150 mM NaCl, (c) plus an additional 100 mM $MgCl_2$. The drop of absorbance in (c) was accompanied by formation of a precipitate, as shown in (d) (photograph of cuvettes) for blank buffer, non-self-complementary hybrid **2**, and **1**.

of precipitation for **1** was shifted to lower temperatures (10–12 °C), than the 25–30 °C range found at 100 mM $MgCl_2$ for this compound, and the extent of precipitation was decreased. Again, no melting was observed for **2** under any of the salt conditions tested.

Compared to the other branched hybrids studied thus far,[13,19] compound **1** is the first hybrid for which the earliest signs of duplex formation (Figure 4a) and formation of a macroscopically visible material occur at very different temperatures/salt concentrations. We hypothesize that this is due to slower kinetics, caused by the long and stiff biphenyl linkers that place the CG dimers much further away from each other than in hybrids **4** and **5**.

Compound $(CGG)_4$TTPA (**7**) behaved similarly to $(CG)_4$TTPA that lacks the "linker nucleoside".[19] In the absence of magnesium ions, melting transitions were observed at 25 °C (no NaCl) and 51 °C (150 mM NaCl, Figure 5). Upon addition of $MgCl_2$, even at 85 °C, an off-white material precipitated that did not redissolve when the sample was heated again after the end of the cooling curve. A magnesium concentration of 10 mM sufficed to induce precipitation, but at 100 mM $MgCl_2$ insoluble material formed that did not redissolve upon heating.

■ CONCLUSIONS

In conclusion, we present a convenient route to branched oligonucleotide hybrids with organic cores that can produce 10 mg batches of pure hybrids within days. The methodology may be scaled up to produce larger quantities for material sciences applications.

The results are noteworthy, both on a synthetic level and on the level of the assembly properties of the hybrids. Synthetically,

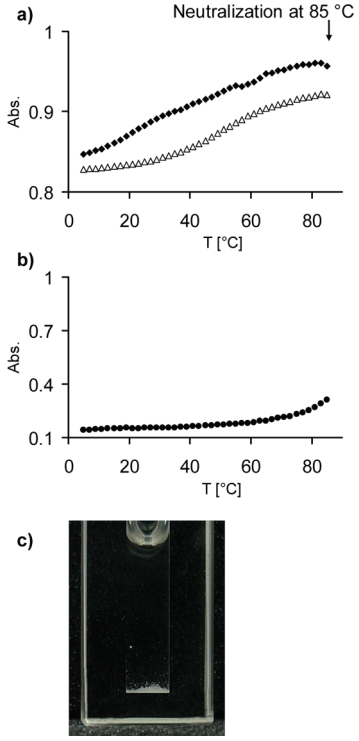


Figure 5. Thermal association curves for $(CGG)_4$TTPA (**7**), at 5 μM hybrid concentration in 10 mM phosphate buffer, pH 7, and NaOAc (1.5 mM), (a) buffer only (♦), +150 mM NaCl (Δ), or (b) plus additional 100 mM $MgCl_2$ (●), (c) photograph of cuvette showing precipitates of hybrid **7** in presence of 100 mM $MgCl_2$.

dx.doi.org/10.1021/jo202508n | J. Org. Chem. 2012, 77, 2718–2728

the *H*-phosphonate route presented here is convenient, as it produces the dimer building blocks quickly and from inexpensive starting materials. The *H*-phosphonate moiety of the 3′-terminal nucleoside remains unreactive throughout the coupling to the 5′-terminal nucleoside and the oxidation, thus avoiding the need for a protecting group. The coupling of dimer *H*-phosphonates with the cores gave satisfactory yields, without repetitive procedures otherwise used to convert residual unreacted phenolic hydroxy groups. The fact that the unprotected phosphodiester linkage between DNA chain and aromatic core does not entirely eliminate the low-level fragmentation for purine-containing sequences[15] further confirms the assumption that these reactions are not induced during deprotection of phosphates, but by another base-induced fragmentation process favored by the dendridic structure. Precipitation produces dimers of sufficient purity to couple them to the cores, thus avoiding a difficult and expensive chromatographic step that would most probably result in the partial loss of cyanoethyl protecting groups.[15]

From the point of view of new materials, the two separate transitions observed for the new, stiff and expanded hybrid $(CG)_4$TBA (**1**) in the assembly experiments are interesting. One transition occurs at low salt concentration (Figure 4a), confirming the strong multivalency underlying the hybridization process (the predicted melting point for the linear duplex of 5′-CG-3′ is −90.8 °C at 10 μM DNA concentration with 150 mM NaCl and 100 mM $MgCl_2$).[27] Only upon addition of $MgCl_2$ did the assemblies precipitate (Figure 4c). It is tempting to speculate that the latter process is caused by a closer packing of duplexes or "condensation", increasing the density and thus driving precipitation. The "two-transition-phenomenon" is in contrast to the properties that the corresponding hybrids with TPM core (**4**) or HPX core (**5**) show (broad transitions at low salt concentration and precipitation accompanying hybridization in the presence of $MgCl_2$).[13,19] We suspect that the stiffer core and the longer, more rigid linker produces more porous assemblies that do not condense readily upon addition of the divalent cation. Thus, the unusual properties observed for hybrids such as **1** further broaden our understanding of this fascinating class of compounds.

EXPERIMENTAL SECTION

General Protocol A (Synthesis of Monomer *H*-Phosphonates). To a solution of commercially available phosphoramidite (500 μmol) in CH_3CN (2.5 mL) and H_2O (18.0 μL, 1.0 mmol, 2.0 equiv) was added pyridinium trifluoroacetate (115.8 mg, 600 μmol, 1.2 equiv). After 5 min, *tert*-butylamine (2.5 mL) was added, and the reaction mixture was stirred for 15 min at room temperature. The mixture was then concentrated in vacuo to a foam. The residue was dissolved in CH_3CN (5.0 mL) and concentrated again to a foam, and this process was repeated one more time. To the residue dissolved in CH_2Cl_2 (6.0 mL) was added H_2O (90 μL, 5.0 mmol, 10 equiv), followed by 6% dichloroacetic acid in CH_2Cl_2 (6.0 mL, 4.4 mmol). After 10 min, the reaction was quenched with CH_3OH (2.0 mL) and concentrated in vacuo to a small volume (500 μL). The crude product was then precipitated by addition of diethyl ether and centrifuged, and the supernatant solution was aspired. The solid was redissolved in CH_2Cl_2/CH_3OH (9/1, v/v, 500 μL), precipitated again by the addition of diethyl ether (3.0 mL), and washed twice with ethyl acetate/diethyl ether (1/1, v/v, 3.0 mL) to give the desired product.

***N*⁶-Benzoyl-2′-deoxyadenosine-3′-yl *H*-Phosphonate (9a).** The reaction was performed following general protocol A, starting from [N^6-benzoyl-5′-*O*-(4,4-dimethoxytrityl)-2′-deoxyadenosine-3′-yl]-(*N*,*N*′-diisopropyl)cyanoethylphosphoramidite (**8a**, 500 mg, 580 μmol) to yield 225 mg (540 μmol, 93%) of **9a** as an off-white solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v); R_f = 0.13; HRMS (ESI-TOF) *m*/*z* calcd for $C_{17}H_{18}N_5O_6P$ $[M - H]^-$ 418.0, *m*/*z* obsd 418.0; ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ = 1.05; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) = 11.28 (brs, 1H), 8.38 (d, *J* = 8.3 Hz, 1H), 8.01 (s, 1H), 7.75 (d, J_{P-H} = 619 Hz, 1H), 7.68−7.10 (m, 5H), 6.23−6.09 (m, 1H), 4.83−4.71 (m, 1H), 4.14−4.05 (m, 1H), 3.69−3.59 (m, 2H), 2.33 − 2.11 (m, 2H).

***N*⁴-Benzoyl-2′-deoxycytidine-3′-yl *H*-Phosphonate (9c).** The reaction was performed following the general protocol A, starting from [N^4-benzoyl-5′-*O*-(4,4-dimethoxytrityl)-2′-deoxycytidine-3′-yl](*N*,*N*′-diisopropyl)cyanoethylphosphoramidite (**8c**, 500 mg, 600 μmol) to yield (212 mg, 530 μmol, 89%) of **9c** as an off-white solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v); R_f = 0.11; HRMS (ESI-TOF) *m*/*z* calcd for $C_{16}H_{18}N_3O_7P$ $[M - H]^-$ 394.0, *m*/*z* obsd 394.0; ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ = 1.05; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) = 11.10 (brs, 1H), 8.38 (d, *J* = 7.6 Hz, 1H), 8.02 (s, 1H), 7.74 (d, J_{P-H} = 616 Hz, 1H), 7.69−7.24 (m, 5H), 6.20−6.10 (m, 1H), 4.86−4.66 (m, 1H), 4.19−4.02 (m, 1H), 3.75−3.57 (m, 2H), 2.28−2.12 (m, 2H).

***N*²-Isobutyryl-2′-deoxyguanosine-3′-yl *H*-Phosphonate (9g).** The reaction was performed following general protocol A, starting from [5′-*O*-(4,4′-dimethoxytrityl)-N^2-isobutyryl-2′-deoxyguanosine-3′-yl]-(*N*,*N*′-diisopropyl)cyanoethylphosphoramidite (**8g**, 500 mg, 590 μmol) to yield (220 mg, 550 μmol, 93%) of **9g** as an off-white solid: TLC (CH_2Cl_2/CH_3OH = 9:1 v/v); R_f = 0.12; HRMS (ESI-TOF) *m*/*z* calcd for $C_{14}H_{20}N_5O_7P$ $[M - H]^-$ 400.1, *m*/*z* obsd 400.1; ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ = 0.77; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) = 12.04 (brs, 1H), 11.82 (brs, 1H), 8.23 (s, 1H), 7.77 (d, J_{P-H} = 623 Hz, 1H), 6.28−6.19 (m, 1H), 4.91−4.80 (m, 1H), 4.09−4.00 (m, 1H), 3.63−3.50 (m, 2H), 2.82−2.59 (m, 3H), 1.12 (d, *J* = 6.8 Hz, 6H).

General Protocol B (Synthesis of Dimer *H*-Phosphonates). Monomer *H*-phosphonate **9a, 9c**, or **9g**, previously dried at 0.1 mbar, was used without further purification. After addition of molecular sieves 3 Å (5 bulbs), **9a**/**9c** or **9g** (500 μmol) was dissolved in dioxane (2.5 mL) by heating to 50 °C. The mixture was allowed to cool to room temperature. Then a solution of previously dried phosphoramidite **10c**/**10 g** or **10t** (600 μmol, 1.2 equiv) in dioxane (2.5 mL) was added, followed by tetrazole solution (0.45 M in acetonitrile, 650 μmol, 1.3 equiv). After 30 min, TLC showed completion of the reaction (CH_2Cl_2/MeOH, 90/10, v/v), and the mixture was cooled to 0 °C. *tert*-Butyl hydroperoxide (TBHP) (1.5 mmol, 3 equiv, 5.5 m in decane, over molecular sieves) was added, and the reaction mixture was allowed to reach room temperature within 15 min. The mixture was concentrated in vacuo, and the resulting foam was dissolved in CH_2Cl_2/CH_3OH (9/1 v/v, 0.5 mL) followed by precipitation with a mixture of diethyl ether and ethyl acetate (3.0 mL, 1/1, v/v). The precipitate was isolated by centrifugation, and the supernatant was aspirated. The solid was redissolved in CH_2Cl_2/CH_3OH (9/1, v/v, 0.5 mL), and precipitated again by the addition of ethyl acetate (3.0 mL). This process was repeated three more times to give the dimer *H*-phosphonates **11a**, **11b**, and **11c** as pale yellow solids.

5′-DMT-C^{Bz}-PO(OCH_2CH_2CN)-G^{iBu}-3′*H*-phosphonate (11a). The reaction was carried out according to general protocol B, starting from **9g** (200 mg, 500 μmol) and **10c** (500 mg, 600 μmol, 1.2 equiv). Precipitation with ethyl acetate afforded 240 mg (210 μmol, 42%) **11a** as a pale yellow solid. The isolated product was used for coupling to the core without any further purification (purity >95%, detected by NMR and mass spectrometry): TLC (CH_2Cl_2/CH_3OH, 9:1 v/v) R_f = 0.12; MS (MALDI-TOF) calcd for $C_{54}H_{56}N_9O_{16}P_2$ $[M - H]^-$ 1148.3, *m*/*z* obsd 1147.1; ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ = −0.10, −2.55, −2.67; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) = 12.02 (brs, 1H), 11.86 (brs, 1H), 11.27 (brs, 1H), 8.25−8.22 (m, 2H), 8.00 (d, *J* = 7.5 Hz, 2H), 7.62 (m, 1H), 7.51 (t, *J* = 7.5 Hz, 2H), 7.40−7.11 (m, 10H), 6.92−6.82 (m, 4H), 6.67 (d, J_{P-H} = 573 Hz, 1H, 6.33−6.22, 6.20−6.10 (2 m, 2H), 5.09−4.95, 4.80−4.69 (2 m, 2H), 4.30−4.11 (m, 4H), 3.71, 3.72 (2 s, 6H), 3.30−3.27 (m, 5H), 2.88 (t, *J* = 5.7 Hz, 2H), 2.78−2.66 (m, 2H), 2.62−2.50 (m, 2H), 1.16−1.03 (m, 6H).

5′-DMT-T-PO(OCH_2CH_2CN)-C^{Bz}-3′*H*-phosphonate (11b). General protocol B was used, starting from **9c** (200 mg, 510 μmol) and **10t** (454 mg, 610 μmol, 1.2 equiv). Yield 235 mg (220 μmol, 44%) of **11b**

dx.doi.org/10.1021/jo202508n | J. Org. Chem. 2012, 77, 2718−2728

as a yellowish solid. The product was used for coupling to the core without further modification and had a purity >95%, as detected by NMR and MS: TLC (CH_2Cl_2/CH_3OH, 9:1, v/v) R_f = 0.10; MS (MALDI-TOF) calcd for $C_{50}H_{51}N_6O_{16}P_2$ $[M - H]^-$ 1053.3 m/z obsd 1052.2; ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ = 0.33, −2.49, −2.70; ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm) = 11.37 (brs, 2H), 8.19−8.10 (m, 1H), 8.00 (d, J = 7.3 Hz, 2H), 7.62 (t, J = 8.1 Hz, 1H), 7.55−7.46 (m, 3H), 7.40−7.10 (m, 10H), 6.92−6.82 (m, 4H), 6.28−6.18, 6.17−6.08 (2 m, 2H), 5.17−5.06, 4.81−4.56 (2 m, 2H), 4.40−4.14 (m, 4H), 3.72, 3.70 (2 s, 6H), 3.31−3.28 (m, 6H), 2.99−2.84 (m, 2H), 1.11 (s, 3H).

5′-DMT-G^{iBu}-PO(OCH$_2$CH$_2$CN)-A^{Bz}-3′*H*-phosphonate (11c). General protocol B was used, starting from **9a** (200 mg, 0.47 mmol) and **10g** (481 mg, 0.57 mmol, 1.2 equiv). Yield 218 mg (39%, 0.19 mmol) of **11c** as pale yellow solid. The dimer building block (purity >95%, as detected by NMR and MS) was used for coupling to the core without further modification: TLC (CH_2Cl_2/CH_3OH, 9:1, v/v); R_f = 0.09; MS (MALDI-TOF) calcd for $C_{55}H_{56}N_{11}O_{15}P_2$ $[M - H]^-$ 1172.3, m/z obsd 1171.3; ^{31}P NMR (DMSO-d_6, 121.5 MHz): δ = 2.02, −2.66, −2.80; ^1H NMR (DMSO-d_6, 300 MHz) δ (ppm) = 12.16, 11.76, 11.20 (3 brs, 3H), 8.47−8.02 (m, 2H), 7.68− 7.61 (m, 2H), 7.52 (t, J = 7.3 Hz, 3H), 7.40−7.15 (m, 11H), 6.95 − 6.82 (m, 4H), 6.37−6.17 (2 m, 2H), 5.04, 4.79 (2 m, 2H), 5.26−5.02, 5.01−4.73 (2 m, 2H), 4.30−4.11 (m, 4H), 3.74, 3.73 (2 s, 6H), 3.42−3.13 (m, 6H), 2.96−2.86 (m, 1H), 2.81 − 2.68 (2 m, 2H), 2.62−2.50 (2 m, 2H), 1.15−1.07 (m, 6H).

Synthesis of Cores. The synthesis of the TPM and the HPX core followed literature protocols.[13,19] The TBA core was obtained from 1,3,5,7-tetrakis(4-methoxybiphen-4-yl)adamantane[28] via demethylation with boron tribromide, as described below.

1,3,5,7-Tetrakis(4′-hydroxy[1,1′-biphenyl]-4-yl)adamantane (TBA) (12). 1,3,5,7-Tetrakis(4-methoxybiphen-4′-yl)adamantane (600 mg 690 μmol, 1.00 equiv) was placed in a Schlenk flask and dissolved in chloroform (75 mL). After being cooled to 0 °C, BBr_3 (1.04 mL, 11.1 mmol, 15.0 equiv) was added dropwise (exothermic reaction). The mixture was stirred for an additional 2 h at this temperature, and allowed to react for another 16 h at room temperature while a precipitate formed. The mixture was treated three times with methanol (3 × 50 mL) and evaporated in vacuo to dryness. Purification via column chromatography (silica gel, 50 g, CH_2Cl_2/methanol 15:1 to 10:1, v/v) yielded 465 mg (84%) of 1,3,5,7-tetrakis(4′-hydroxy[1,1′-biphenyl]-4-yl)adamantane as an off-white solid: R_f = 0.31 (CH_2Cl_2/methanol 15:1, v/v); ^1H NMR (400 MHz, MeOH-d_4) δ = 1.89 (s, 12 H), 6.89 (d, ^3J = 8.5 Hz, 8 H), 7.22 (d, ^3J = 8.3 Hz, 8 H), 7.40−7.42 (m, 16 H) ppm; ^{13}C NMR (100 MHz, MeOH-d_4) δ = 40.1, 48.5, 116.7, 126.7, 127.4, 129.1, 133.7, 139.9, 149.1, 158.0 ppm; IR (DRIFT) $\tilde{\nu}$ = 3437 [br, w, ν_{Ar}(OH)], 3029 [w, ν_{Ar}(CH)], 2925 (w), 2851 [w, ν_{Ad}(CH_2)], 1891 (vw), 1610 (w), 1498 [m, ν_{Ar}(C=C)], 1398 (w), 1355 [w, δ_{Ar}(OH)], 1260 [w, δ_{Ar}(CH)], 1172 (w), 1104 (w), 1003 (w), 819 [m, ν_{Ar}(*p*-subst.)], 786 (w), 704 (w), 583 (w), 532 (w), 456 (w), 418 (w) cm^{-1}; MS (FAB, 3-NBA), m/z 809 (55) $[M + H]^+$, 808 (100) $[M^+]$; HRMS ($C_{58}H_{48}O_4$) calcd 808.3552, obsd 808.3555.

General Protocol C (Synthesis of DNA Hybrids). The core [1,3,5,7-tetrakis(4-hydroxybiphen-4-yl)adamantane (TBA, **12**), tetrakis(4-hydroxyphenyl)methane (TPM, **13**), or 1,4-phenylenebis-[tri(4′-hydroxyphenyl)methane] (HPX, **14**)] was dried for 1 h at 100 °C and 0.001 mbar. The core (5.0 μmol) was then mixed with 5′-*O*-DMT-dimer-*H*-phosphonate (**11a**, **11b**, or **11c**, 3.5 equiv per OH group of core), and the mixture was coevaporated from dry pyridine (2 × 2 mL). After addition of molecular sieve 3 Å (5 beads), a mixture of dry pyridine and CH_3CN (0.5 mL, 2/3, v/v) was added in an argon atmosphere. The reaction mixture was then cooled to −40 °C. Diphenyl chlorophosphate (DPCP) (1.50 equiv) was added, and the mixture was allowed to stir for 30 min at −40 °C. Then a solution of iodine in dry pyridine (2 M, 1.5 equiv to dimer) was added, followed by H_2O (5 equiv) after 1 min. The reaction mixture was slowly allowed to reach room temperature and was stirred for further 30 min. After addition of CH_2Cl_2 (10 mL), the mixture was washed with a solution of aqueous sodium thiosulfate (5 mL, 10%, w/w) and aqueous phosphate buffer (5.0 mL, 0.2 M, pH 7). The aqueous phase was separated and back-extracted with CH_2Cl_2 (5 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was coevaporated twice from toluene and dissolved in a minimal amount of CH_2Cl_2/CH_3OH (95/5, v/v), followed by precipitation with ethyl acetate. After centrifugation (3500 rpm, 5 min) the residue was washed three times with ethyl acetate in an ultrasonic bath for 1 min and centrifuged. The solid obtained was dried at 0.001 mbar and 40 °C to yield the crude fully protected hybrid, which was deprotected without further purification. For this, the protected hybrid was dissolved in CH_2Cl_2 (5 mL) and H_2O (10 equiv), followed by addition of 6% dichloroacetic acid (DCA) in CH_2Cl_2 (5 mL, 3.5 mmol). After 10 min, the reaction was quenched by addition of CH_3OH (2 mL). The solution was then concentrated, and the hybrid was precipitated by addition of diethyl ether/ethyl acetate (3 mL, 1/1, v/v). The precipitate was separated by centrifugation, redissolved in a minimal volume of CH_2Cl_2/CH_3OH (0.5 mL, 9/1, v/v), and precipitated again by addition of ethyl acetate (3 mL). This process was repeated three more times to give the DMT-deprotected hybrid (**15**, **16**, **17**, **18**, or **19**). To remove the cyanoethyl groups and the protecting groups of the nucleobases, the product was treated with ammonium hydroxide (5 mL) for 5 h at 55 °C. Excess ammonia was removed by passing a stream of N_2 over the surface until the sample was odorless. The remaining solution was evaporated to dryness by lyophilization to yield crude hybrid **1**, **2**, **3**, **4**, or **5**. The crude was then purified by reversed-phase HPLC (C8 column) using a gradient of 5−40% CH_3CN in 10 mM TEAA buffer at 55 °C.

(CG)$_4$TBA (1). General protocol C was used, starting from TBA core **12** (10 mg, 12.4 μmol) and **11a** (200 mg, 175 μmol). After complete deprotection, the crude hybrid was dissolved in 10 mM TEAA buffer containing 5% CH_3CN and subjected to HPLC purification (C8 column, 250 × 20 mm), using a gradient of CH_3CN in 10 mM TEAA buffer, 5−30% in 60 min at 55 °C. Hybrid **1** eluted at t_R = 35 min, yield 30%: MS (MALDI-TOF) calcd for $C_{134}H_{144}N_{32}O_{52}P_8$, $[M - H]^-$ 3280, obsd 3280; ^{31}P NMR (122 MHz, NaOH (0.1 M)/D_2O 9:1 v/v) δ −1.05, −5.61; ^1H NMR (500 MHz, NaOH (0.1 M) in D_2O; 9:1 v/v, 60 °C) δ 8.39 (s, 4 × 1H, $H8_G$), 8.20−8.02 (m, 24H, *Ar-H*), 7.89 (d, J = 7.8 Hz, 4 × 1H, $H6_C$), 7.81 (d, J = 9.4 Hz, 6H, *Ar-H*), 6.77−6.69 (m, 4 × 1H, $H1'_G$), 6.50−6.43 (m, 4 × 1H, $H1'_C$), 6.25 (d, J = 8.0 Hz, 4 × 1H, $H5_C$), 5.53−5.46 (m, 4 × 1H, $H3'_G$), 4.51−4.33 (m, 12H, H4′$_C$/H5′/5″$_G$), 4.10 (dd, $J_{5'-4'}$ = 4.4, $J_{5'-5''}$ = 11.6, 4 × 1H, $H5'_C$), 4.01 (dd, $J_{5''-4'}$ = 5.8, $J_{5''-5'}$ = 12.6, 4 × 1H, $H5''_C$), 3.28−3.19 (m, 4 × 1H, $H2'_G$), 3.17−3.09 (m, 4 × 1H, H''_G), 2.67−2.56 (m, 4 × 1H, $H2'_C$), 2.71 (s, 12H, core-*H*), 2.31−2.21 (m, 4 × 1H, $H2''_C$).

(TC)$_4$TBA (2). Following general protocol C, **12** (4 mg, 5 μmol) was reacted with **11b** (73.7 mg, 70 μmol). Crude **2** was taken up in 10 mM TEAA buffer containing 5% CH_3CN, and HPLC purified (C8 column, 250 × 4 mm) using a gradient of CH_3CN in 10 mM TEAA buffer, 5−35% in 60 min at 55 °C. Hybrid **2** eluted at t_R = 37 min, yield 28%; MS (MALDI-TOF) calcd for $C_{134}H_{148}N_{20}O_{56}P_8$ $[M - H]^-$ 3180, obsd 3180.

(GA)$_4$TBA (3). Following general protocol C, **12** (2.0 mg, 2.5 μmol) was reacted with **11c** (41 mg, 35 μmol). The crude was taken up in 10 mM TEAA buffer containing 5% CH_3CN, and HPLC purified (C8 column, 250 × 4 mm), using a gradient of CH_3CN in 10 mM TEAA buffer, 5−35% in 60 min at 55 °C, with elution at t_R = 40 min, yield 20%; MS (MALDI-TOF) calcd for $C_{138}H_{143}N_{39}O_{48}P_8$ $[M - H]^-$ 3361, obsd 3360.

(CG)$_4$TPM (4). General protocol C was used, starting from TPM core **13** (2 mg, 5.2 μmol) and **11a** (83.5 mg, 72.8 μmol, 3.5 equiv per OH group of core). The crude hybrid was dissolved in 10 mM TEAA buffer containing 5% CH_3CN and HPLC purified (C8 column, 250 × 4 mm), using a gradient of CH_3CN in 10 mM TEAA buffer, 5−18% in 45 min at 55 °C. Hybrid **4** eluted at t_R = 16 min, yield 25%; MS (MALDI-TOF) calcd for $C_{101}H_{117}N_{32}O_{52}P_8$ $[M - H]^-$ 2857, obsd 2856.

(CG)$_6$HPX (5). Following general protocol C, HPX core **14** (2 mg, 3.0 μmol) was reacted with **11a** (73.3 mg, 63.8 μmol, 3.5 equiv per OH group of core). The crude hybrid was taken up in 10 mM TEAA

buffer containing 5% CH_3CN and HPLC purified on a C8 column (250 × 4 mm) with a gradient of CH_3CN in 10 mM TEAA buffer, 5–18% in 40 min at 55 °C. Hybrid **5** eluted at t_R = 22 min, yield 20%; MS (MALDI-TOF) calcd for $C_{158}H_{178}N_{48}O_{78}P_{12}$ $[M - H]^-$ 4368, obsd 4367.

Tetrakis[4-(1,3′-deoxythymidin-3′-yl-1,2,3-triazol-4-yl)-phenyl]adamantane (22). The following is a modification of a literature protocol.[20] 3′-Azido-3′-deoxythymidine (**21a**, 52.0 mg, 0.195 mmol) and 1,3,5,7-tetrakis(4-ethinylphenyl)adamantane (**20**) (21.4 mg, 0.039 mmol) were dissolved in DMSO (0.5 mL). A slurry of $CuSO_4 \cdot 5H_2O$ (5.1 mg, 0.020 mmol) and sodium L-(+)-ascorbate (4.4 mg, 0.022 mmol) in water (0.1 mL) was added, and the resulting mixture was stirred at room temperature. To monitor the reaction, a small sample (1 μL) was removed, diluted 200 fold with DMSO, and analyzed by MALDI-TOF-MS. After 1.5 h, the educt was fully converted, and the green solution was poured dropwise into an aqueous solution of EDTA (2 mL, 5%). The precipitate was washed with water (5 × 0.5 mL) and dried over P_4O_{10} at 0.01 mbar, yielding 58 mg (90%, 0.036 mmol) of title compound **22** as a solid. The analytical data agreed with the literature.[20]

Tetrakis[4-(1-*N*²-isobutyryl-2′,3′-dideoxyguanosin-3′-yl-1,2,3-triazol-4-yl)phenyl]adamantane (23). The synthesis followed the protocol given in the literature,[19] starting from **21b** (265 mg, 730 μmol) and **20** (61.0 mg, 110 μmol), and proceeded with an overall yield of 93%.

$(CGT)_4$TTPA (6). The TTPA hybrids **6** and **7** were synthesized using a modification of General Protocol C. To a vacuum-dried flask with **22** (5.30 mg, 3.30 μmol), **11a** (43.1 mg, 37.5 μmol) was added. The mixture was coevaporated from dry pyridine (2 × 200 μL). Then, molecular sieves (3 Å, 5 beads) were added, and the residue was dried for 1 h at 0.001 mbar and 60 °C. After addition of dry pyridine (300 μL) under argon atmosphere, the clear solution was diluted with dry CH_3CN (300 μL). The reaction mixture was cooled to −40 °C, and diphenyl chlorophosphate (19.3 mg, 72.0 μmol) was added. The mixture was stirred for 1 h at this temperature. Then, a mixture of TBHP (5.5 min dodecane, 10 μL) and oxidizer solution for DNA synthesis (0.02 M I_2 in pyridine/H_2O, 90 μL) was added dropwise to the reaction solution, and the solution was stirred at −40 °C for 20 min. Aliquots (4 × 200 μL) of the reaction mixture were added to ethyl acetate (1 mL each), leading to precipitation. The combined slurries were centrifuged, and the residue was washed with ethyl acetate (4 × 300 μL). The solid containing the fully protected hybrid was dissolved in acetic acid (100 μL, 80% aqueous solution), and the mixture was stirred at room temperature. The solution was washed with hexane (8 × 300 μL). After 1 h, the reaction mixture was diluted with water (600 μL) and was washed again with hexane (3 × 200 μL). Residual hexane was removed with a gentle stream of N_2, and the aqueous solution was lyophilized to dryness. The product was then dissolved in saturated aqueous ammonia (28%, 200 μL), and was shaken for 2 h at room temperature. Then, aqueous methylamine (40%, 200 μL) was added, and the reaction mixture was shaken again for another 3 h. Water (400 μL) was added, and the excess of ammonia and methylamine was removed with a gentle stream of N_2, directed onto the surface of the solution. The remaining solution was evaporated to dryness by lyophilization to yield crude hybrid **6**. Samples for assembly studies were purified by reverse phase HPLC (C8 column) using a gradient of CH_3CN in 10 mM TEAA buffer (10% for 5 min, 10–15% in 10 min, 15–18% in 15 min) at 70 °C. Hybrid **6** eluted at t_R = 18 min, yield 42%: MS (MALDI-TOF) calcd for $C_{158}H_{180}N_{52}O_{64}P_8$ $[M - H]^-$ 4077, obsd 4078.

$(CGG)_4$TTPA (7). To **23** (10.1 mg, 5.08 μmol) was added **11a** (70.1 mg, 61.0 μmol). The mixture was coevaporated from pyridine (1 mL) and CH_3CN (1 mL). Molecular sieves (3 Å) were added, followed by drying for 1 h at 0.001 mbar. After addition of pyridine (300 μL) under argon atmosphere, the solution was diluted with CH_3CN (270 μL). The reaction mixture was cooled to −40 °C, and diphenyl chlorophosphate (24.7 mg, 92 μmol) was added. After 1 h, the hybrid was oxidized by addition of a solution of I_2 in pyridine (50 μL, 2 M), and was stirred for another 10 min. Then, water (50 μL) was added, and the reaction mixture was allowed to reach room temperature. After 30 min, the solution was diluted with CH_2Cl_2 (2 mL), and was poured into aqueous $Na_2S_2O_3$ solution (600 μL, 0.1 M). The organic layer was washed with water (2 × 1 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to dryness. The residue was coevaporated from toluene (2 × 2 mL), taken up in a mixture of THF/H_2O (400 μL, 2:1 v/v), and precipitated with ethyl acetate (2 mL). The precipitate was washed with ethyl acetate (4 × 1 mL), and was dried for 30 min at 0.1 mbar. The solid was taken up in acetic acid (80%, 150 μL), and the mixture was stirred at room temperature. The solution was extracted with hexane (8 × 400 μL). After 1 h, the reaction mixture was diluted with water (500 μL) and was washed again with hexane (2 × 400 μL). Residual hexane was removed with a gentle stream of air, directed onto the surface of the solution. After 20 min, the aqueous solution was lyophilized to dryness. The hybrid was then dissolved in saturated aqueous ammonia (28%, 200 μL), and was shaken for 2 h. Then, aqueous methylamine (40%, 200 μL) was added, and the reaction mixture was shaken for 3 h. Water (300 μL) was added, and excess ammonia and methylamine were removed with a gentle stream of air directed onto the surface of the solution. After 60 min, the aqueous solution was lyophilized to dryness. Samples were purified by reversed-phase HPLC (C8 column), using a gradient of CH_3CN in 10 mM TEAA buffer (5–15% in 20 min, 15–40% in 60 min). Hybrid **7** eluted at a retention time (t_R) of 47 min: yield 28%; MS (MALDI-TOF) calcd for $C_{158}H_{175}N_{64}O_{60}P_8^-$ $[M - H]^-$ 4177.0; obsd. 4178.0.

■ ASSOCIATED CONTENT

Supporting Information

Collection of NMR spectra, HPLC chromatograms, and MALDI-TOF mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: 49 (0) 711 685 64311. Fax: 49 (0) 711 685 64321. E-mail: lehrstuhl-2@oc.uni-stuttgart.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Th. Sabirov for contributing to the synthesis of intermediates and C. Gerlach and Dr. B. Claasen for help with the acquisition and interpretation of NMR spectra. This work was supported by DFG (Grant No. RI 1063/13-1 to C.R.) and the Center for Functional Nanostructures at KIT.

■ REFERENCES

(1) Hall, R. H.; Todd, A.; Webb, R. F. *J. Chem. Soc.* **1957**, 3291–3296.

(2) (a) Froehler, B. C.; Matteucci, M. D. *Tetrahedron Lett.* **1986**, *27*, 469–472. (b) Froehler, B. C.; Ng, P. G.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 5399–5407.

(3) (a) Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R. *Tetrahedron Lett.* **1986**, *27*, 4051–4054. (b) Garegg, P. J.; Lindl, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *27*, 4055–4058.

(4) See: (a) Li, H.; Fedorova, O. S.; Trumble, W. R.; Fletcher, T. R.; Czuchajowski, L. *Bioconjugate Chem.* **1997**, *8*, 49–56. (b) Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.-I.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710–12721. (c) An, H.; Wang, T.; Maier, M. A.; Manoharan, M.; Ross, B. S.; Dan Cook, P. *J. Org. Chem.* **2001**, *66*, 2789–2801. (d) Asseline, U.; Cheng, E. *Tetrahedron Lett.* **2001**, *42*, 9005–9010. (e) Zain, R.; Stawinski, J. *J. Org. Chem.* **1996**, *61*, 6617–6622.

(5) (a) Reese, C. B.; Song, Q. L. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1477–1486. (b) Johansson, T.; Stawinski, J. *Tetrahedron* **2004**, *60*, 389–395.

(6) Adamo, I.; Dueymes, C.; Schönberger, A.; Navarro, A.-E.; Meyer, A.; Lange, M.; Imbach, J.-L.; Link, F.; Morva, F.; Vasseur, J.-J. *Eur. J. Org. Chem.* **2006**, 436−448.
(7) Sakatsume, O.; Yamane, H.; Takaku, H.; Yamamoto, N. *Nucleic Acid Res.* **1990**, *18*, 3327−3331.
(8) Ott, G.; Arnold, L.; Smrt, J.; Sobkowski, M.; Limmer, S.; Hofmann, H.-P.; Sprinzl, M. *Nucleosides Nucleotides* **1994**, *13*, 1069−1085.
(9) Sun, Q.; Edathil, J. P.; Wu, R.; Smidansky, E. D.; Cameron, C. E.; Peterson, B. R. *Org. Lett.* **2008**, *10*, 1703−1706.
(10) (a) Caruthers, M. H. *Science* **1985**, *230*, 281−285. (b) Caruthers, M. H. In *Synthesis and Applications of DNA and RNA*; Narang, S. A., Ed.; Academic: Orlando, 1987; pp 47−94. (c) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *22*, 2223−2311.
(11) Blackburn, G. M., Gait, M. J., Loakes, D., Williams, D. M., Eds. *Nucleic Acids in Chemistry and Biology*, 3rd ed.; RSC Publishing: Cambridge, 2006; pp 149−150.
(12) (a) Westheimer, F. H.; Huang, S.; Covitz, F. *J. Am. Chem. Soc.* **1988**, *110*, 181−185. (b) Reese, C. B. *Tetrahedron* **2002**, *58*, 8893−8920.
(13) Meng, M.; Ahlborn, C.; Bauer, M.; Plietzsch, O.; Soomro, S. A.; Singh, A.; Muller, T.; Wenzel, W.; Bräse, S.; Richert, C. *ChemBioChem* **2009**, *10*, 1335−1339.
(14) Gaffney, B. L.; Veliath, E.; Zhao, J.; Jones, R. A. *Org. Lett.* **2010**, *12*, 3269−3271.
(15) Griesser, H.; Tolev, M.; Singh, A.; Sabirov, T.; Gerlach, C.; Richert, C. *J. Org. Chem.* **2012**, *77*, DOI: 10.1021/jo202505h.
(16) (a) Hudson, R. H. E.; Uddin, A. H.; Damha, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 12470−12477. (b) Vargas-Baca, I.; Mitra, D.; Zulyniak, H. J.; Banerjee, J.; Sleiman, H. F. *Angew. Chem., Int. Ed.* **2001**, *40*, 4629−4632.
(17) (a) Hudson, R. H. E.; Damha, M. J. *J. Am. Chem. Soc.* **1993**, *115*, 2119−2124. (b) Hudson, R. H. E.; Robidoux, S.; Damha, M. J. *Tetrahedron Lett.* **1998**, *39*, 1299−1302. (c) Shchepinov, M. S.; Mir, K. U.; Elder, J. K.; Frank-Kamenetskii, M. D.; Southern, E. M. *Nucleic Acids Res.* **1999**, *27*, 3035−3041. (d) Shchepinov, M. S.; Udalova, I. A.; Bridgman, A. J.; Southern, E. M. *Nucleic Acids Res.* **1997**, *25*, 4447−4454.
(18) Hatano, T.; Kato, T. *Tetrahedron* **2008**, *64*, 8368−8380.
(19) Singh, A.; Tolev, M.; Meng, M.; Klenin, K.; Plietzsch, O.; Schilling, C. I.; Muller, T.; Nieger, M.; Bräse, S.; Wenzel, W.; Richert, C. *Angew. Chem., Int. Ed.* **2011**, *50*, 3227−3231.
(20) Plietzsch, O.; Schilling, C. I.; Tolev, M.; Nieger, M.; Richert, C.; Muller, T.; Bräse, S. *Org. Biomol. Chem.* **2009**, *7*, 4734−4743.
(21) Zheng, J.; Birktoft, J. J.; Chen, Y.; Wang, T.; Sha, R.; Constantinou, P. E.; Ginnell, S. L.; Mao, C.; Seeman, N. C. *Nature* **2009**, *461*, 74−77.
(22) (a) Zang, Z.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **2004**, *126*, 16700−16701. (b) Zeng, F.; Jones, R. A. *Nucleosides Nucleotides* **1996**, *15*, 1679−1686. (c) Yan, H.; Aguilar, A. L. *Nucleosides Nucleotides Nucl. Acids* **2007**, *26*, 189−204.
(23) Cieślak, J.; Jankowska, J.; Sobkowski, M.; Wenska, M.; Stawiński, J.; Kraszewski, A. *J. Chem. Soc., Perkin Trans.* **2002**, 31−37.
(24) Reese, C. B.; Song, Q. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2787−2792.
(25) (a) Nykypanchuk, D.; Maye, M. M.; van der Lelie, D.; Gang, O. *Nature* **2008**, *451*, 549−552. (b) Park, S. Y.; Lytton-Jean, A. K. R.; Lee, B.; Weigand, S.; Schatz, G. C.; Mirkin, C. A. *Nature* **2008**, *451*, 553−556. (c) Macfarlane, R. J.; Lee, B.; Jones, M. R.; Harris, N.; Schatz, G. C.; Mirkin, C. A. *Science* **2011**, *334*, 204−208.
(26) (a) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *66*, 3057−3064. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599.
(27) Calculated using Hyther by Peyret, N.; Santa Lucia, J., Jr., Wayne State University.
(28) Schilling, C. I.; Plietzsch, O.; Nieger, M.; Muller, T.; Bräse, S. *Eur. J. Org. Chem.* **2011**, 1743−1754.