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A Journal of
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Accepted Article

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To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201700686

Link to VoR: http://dx.doi.org/10.1002/ejoc.201700686

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10.1002/ejoc.201700686

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Solution-Phase Synthesis of Branched Oligonucleotides with up to 32 Nucleotides and the Reversible Formation of Materials

Alexander Schwenger, Nicholas Birchall, and Clemens Richert*

Abstract: A linear solution-phase synthesis of branched oligonucleotides with adamantane as core has been developed. The method uses conventional phosphoramidites only, achieves chain assembly without chromatography of intermediates, and overcomes the low reactivity of adamantane-1,3,5,7-tetraol as core. The assembly of four-arm hybrids with up to 32 nucleotides total was performed, with monodisperse products of up to 10 kDa in size. Overall yields of 20 % over 19 steps (hexamer arms) and 11 % over 25 steps (octamer arms) of HPLC-purified compounds were obtained. The adamatane-based hybrids show more DNA-dominated assembly properties than their brethrens with larger lipophilic cores. Reversible formation of macroscopic amounts of materials through hybridization was achieved, both for self-complementary systems and two-hybrid systems with two non-selfcomplementary DNA sequences.

Introduction

Synthetic oligodeoxynucleotides have become important building blocks in nanotechnology.[1-3] A number of functional nanomaterials have been prepared from modified, rather than natural oligonucleotides, in order to benefit from structural traits not found in linear DNA.[4-6] To be useful for applications in the field of materials, macroscopic quantities of oligonucleotides are called for. To produce such quantities is a challenge for nucleic acid chemists.

For linear oligonucleotides, several modes of synthesis have been described.[7] Initially, solution-phases syntheses were used exclusively, based on phosphodiester or phosphotriester intermediates,[8-11] combined with enzymatic ligation to assemble the final sequence.[12] With the advent of solid-phase synthesis,[13-15] these labour-intensive approaches were all but abandoned. Three methods were optimized to obtain oligodeoxynucleotides on a solid support, namely the phosphotriester method,[16] the *H*-phosphonate method,[17] and the phosphoramidite or phosphite triester method.[18,19] Optimization of the phosphoramidite method continues,[20,21] including approaches to reduce protecting group usage.[22,23] Still, solid-phase chain assembly dominates the field.

Large-scale solid-phases syntheses are costly and producing bulk quantities of oligonucleotides via this approach is problematic for applications other than medicinal chemistry or nanotechnology. As a consequence, new methods for solution-phase synthesis that avoid or reduce the high cost and limited scalability of solid-phase syntheses[24] are being developed.[25-27] Further, there is active research on new approaches for preparing trimer building blocks, where part of the chain assembly is performed in solution.[28-30] Although branched intermediates with DNA chains linked to soluble supports have been reported, the largest products of such syntheses reported in the recent literature contain no more than 5-9 nucleotides total,[25-27] demonstrating how difficult it is to prepare large oligonucleotides without immobilization.

Our interest in DNA-based materials has focused on branched oligonucleotides that form nanoporous three-dimensional networks through hybridization. This is an active area of research,[31] in which compounds prepared by solid-phase methods[32] are also used for subsequent enzymatic steps.[33] We had observed that, due to their ability to undergo multivalent hybridization, synthetic tetrahedral DNA hybrids consisting of an organic core and four DNA arms can form materials in dilute aqueous solution. Sequences as short as CG dimers can act as "zippers" and bring about the assembly process at a high concentration of divalent cations.[34] We then found that the propensity to form materials in aqueous solution increases when constructs with six arms and rigid cores are used.[35] But, when the number of arms was increased to eight and the size of the rigid and lipophilic core increased, the sequence dependence of the assembly process was lost, indicating that it was no longer dominated by Watson-Crick base pairing.[36] This prompted us to shift our attention to branched DNA hybrids with a smaller, entirely aliphatic core, hoping to build materials with larger pore size without losing the ability to steer the assembly process through choice of the sequence and length of the DNA arms.

Here we report the solution-phase synthesis of DNA hybrids based on adamantane-1,3,5,7-tetraol. The synthesis of these hybrids was performed entirely in solution, based on inexpensive, commercial phosphoramidite building blocks. Hybrids with up to 32 nucleotides were obtained in 200 mg batches with no more than a single chromatographic step at the very end of the syntheses. The resulting hybrids were found to form three-dimensional networks reversibly, both when a single type of hybrid with self-complementary DNA arms was used and when a combination of two hybrids with sequences that are complementary to each other were used.

Results and Discussion

As shown in Figure 1, the group of hybrids to be prepared included compounds with dimer arms on a phenolic core (**1**), homooligomer arms on an aliphatic core (**2**, **3**), mixed-sequence arms on the same core (**4**), and self-complementary dimer, tetramer, hexamer, and octamer arms on the aliphatic

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adamantane core (**5**-**8**). Both self-complementary and non-self-complementary sequences were chosen to allow for modulation of hybridization strength in processes forming three-dimensional networks.

The challenges in preparing longer DNA hybrids in solution are significant, if one wants to start from inexpensive, commercial phosphoramidites that have been optimized for solid-phase synthesis. One challenge is to handle richly-functionalized, labile biomacromolecules. The intermediates of solution-phase syntheses are polar mixtures of diastereomers that readily lose their cyanoethyl protecting groups, all but precluding conventional chromatography on supports like silica. Further, simultaneous couplings at the termini of all arms of the branched constructs call for high-yielding reactions, a problem known from dendrimer chemistry.[37,38] Finally, the purification of the final products can be challenging, as increasingly complex mixtures are being formed that have the ability to assemble into higher order structures, even in protected state.

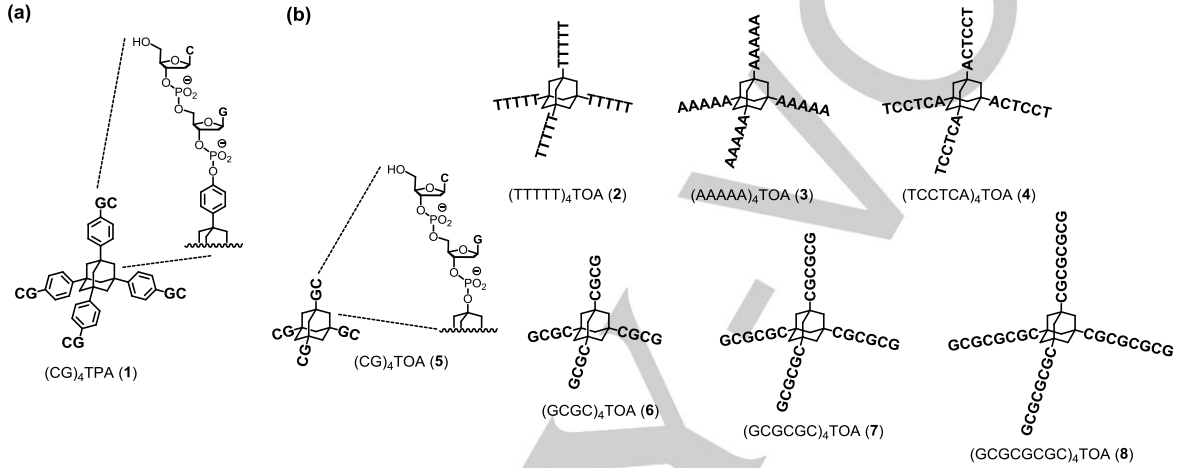
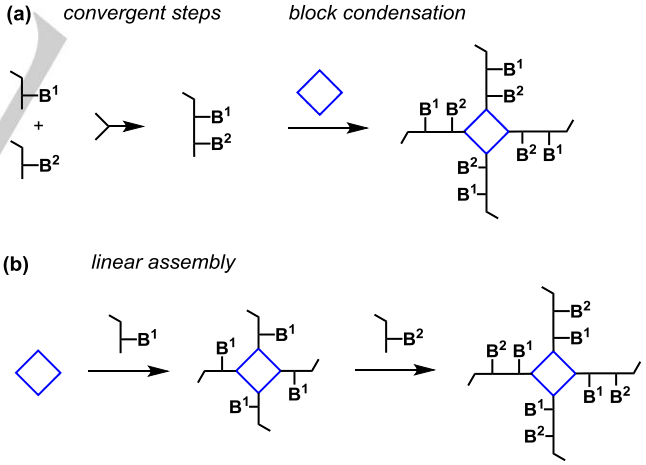


Figure 1. Structures of target molecules; a) with tetrakis(*p*-hydroxyphenyl)adamantane core (TPA), and b) with adamantanetetraol core (TOA).

Since our earlier work had shown that large branching elements or "cores" can interfere with rule-based self-assembly,[36] we focused on hybrids with small cores. The first target molecule contained a tetrakis(*p*-hydroxyphenyl)adamantane (TPA) core, which is considerably smaller than those studied earlier,[35,36] but features the phenols as reactive nucleophiles, for which much of our earlier solution-phase work had been optimized.[39]

At the beginning of our study, the favored assembly method was block condensation of the core with CG dimer "zipper" arms via *H*-phosphonate chemistry (Scheme 1a). The next step in shrinking the core led to 1,3,5,7-adamantanetetraol, often referred to as 1,3,5,7-tetrahydroxyadamantane (TOA) in our laboratory. Here, the hydroxy groups to which the DNA arms were to be attached were tertiary, aliphatic alcohols, which are much less nucleophilic and considerably more sterically hindered than *para*-substituted phenols.

The syntheses of adamantane tetraol (**9**)[40] and tetrakis(4-hydroxyphenyl)adamantane (**10**, TPA) started from 1,3,5,7-tetrabromoadamantane, which is accessible from adamatane itself via four-fold bromination.[40,41] The tetrabromide was then hydrolyzed to **9**,[40,42] whose four-fold Friedel–Crafts alkylation with anisole, catalyzed by TfOH, and subsequent ether cleavage with BBr_3 furnished **10** in 32% overall yield (Scheme 2).



Scheme 1. Synthetic strategies for the assembly of branched oligonucleotide hybrids with organic cores.

With the two cores in hand, we then synthesized DNA hybrids. We initially attempted to prepare both **1** and **5** using our solution phase *H*-phosphonate method.[36,39] Preparation of dimer building block **11**,[39] proceeded uneventfully. Couplings involved 3 equiv of the dimer *H*-phosphonate per alcohol of the cores, diphenyl chlorophosphate (DPCP) as condensing agent, and a solvent mixture of pyridine/CH_3CN (4:1) at -40 °C,

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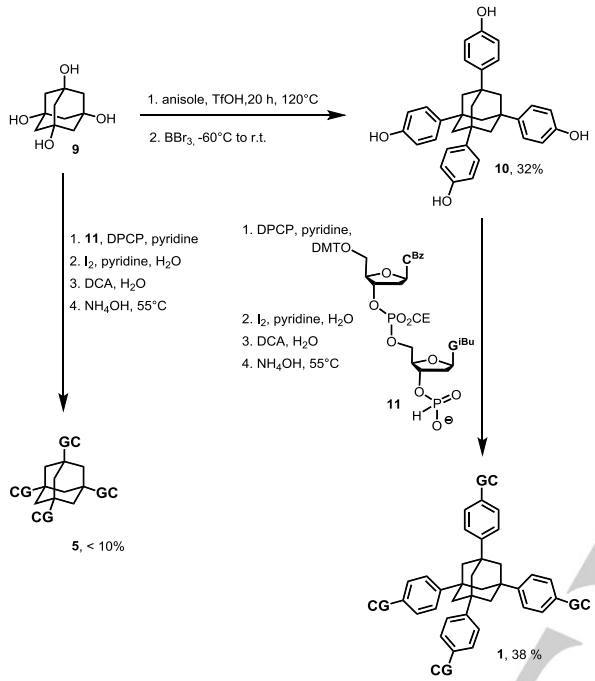
followed by oxidation with aqueous iodine in pyridine, aqueous workup, detritylation and hydrolysis/aminolysis.[39] Hybrid **1** with its phenolic core was thus obtained in 38% overall yield after cartridge purification. The chromatogram of the crude showed an intense peak for the product, together with the expected side-products, including the symmetrical pyrophosphate formed by dimerization of the *H*-phosphonate (Figure 2).



Scheme 2.

Using the same methodology, hybrid **5** was obtained in less than 10% yield, and its chromatographic purification was difficult. Figure 2b shows the HPLC chromatogram of the crude. In addition to other side products, incomplete coupling products with one, two or three DNA arms were detected, even after long reaction times. This suggested that the tertiary alcohols of **9** are too unreactive for coupling to *H*-phosphonate **11**.

We assumed that phosphoramidites would be more reactive. Further, we suspected that the reactivity of monomers would be higher than that of dimers. To avoid the costly methyl-protected monomers used earlier,[43] we limited ourselves to the more common cyanoethyl phosphoramidites and used linear syntheses (Scheme 1b). For this, one of the four monomer phosphoramidites **12a**-**t** was coupled directly to the core.

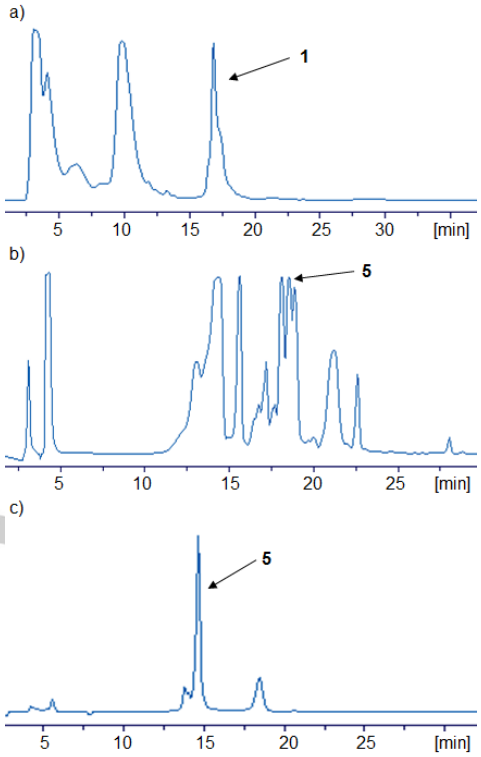


Figure 2. Representative RP-HPLC traces of crude products from the synthesis of: (a) **1** via *H*-phosphonate coupling, (b) **5** via *H*-phosphonate coupling, and (c) **5** via linear assembly with phosphoramidite monomers. Product peaks are labeled with arrows.

One problem we encountered was solubility. Both acetonitrile and dioxane as solvent gave almost no coupling because the core did not dissolve well enough. Addition of dry DMF gave a homogeneous solution and near-quantitative coupling. In the optimized method, all other components of the reaction mixture were dissolved in DMF and the tetrazole solution (0.45 M in CH_3CN) was added last. The reaction mixture was shaken at room temperature for 30 min and then stored overnight at 5 °C. The subsequent oxidation used *t*-BuOOH. After aqueous workup, the fully protected DNA hybrids were isolated by precipitation with MTBE, starting from a concentrated CH_2Cl_2 solution. Subsequent washing of the solid with methanol was important for obtaining pure hybrids. The solubility in MeOH is higher for pyrimidine hybrids, though, so that excessive washing can lead to loss of material. Detritylation with dichloroacetic acid in CH_2Cl_2 was followed by quenching with MeOH. Concentrating *in vacuo* and precipitations with MTBE then gave mononucleotide hybrids **13a**-**t** in near-quantitative yield (purines) or satisfactory yields (pyrimidines), without a need for chromatography (Table 1).

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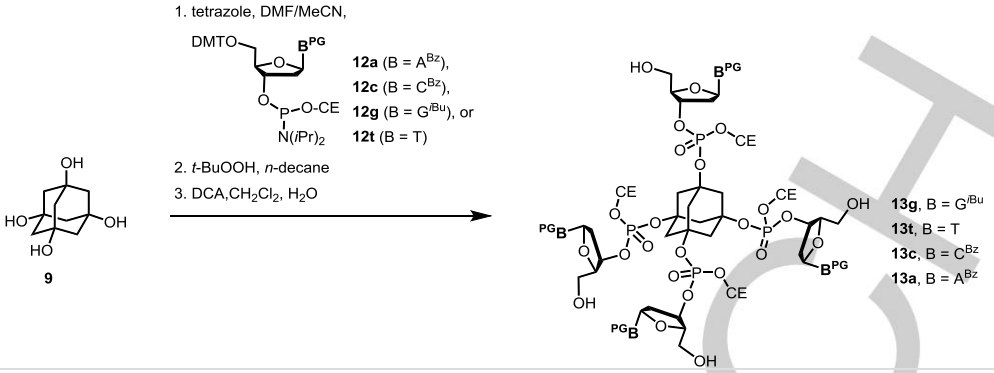


Table 1. Synthesis of DNA hybrids **13a**-**t**, starting from **9** and monomer phosphoramidites.

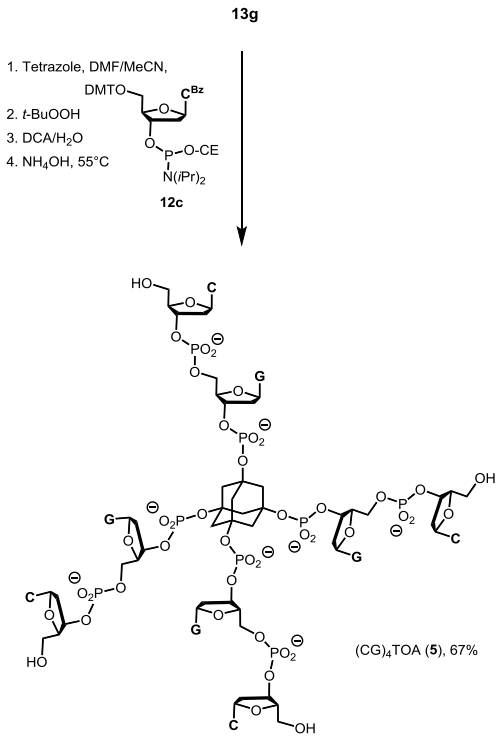
Monomer [a]	Product	Equiv. **12**	Equiv tetrazole	Ratio DMF/CH_3CN	time (h)	Yield of crude (%)[a]
12g	**13g**	7	1.5	1:1.9	19	50
12g	**13g**	8	1.2	1:1.7	48	95
12t	**13t**	7	1.5	1:2.3	22	65
12t	**13t**	6.5	1.5	1:1.4	21	68
12c	**13c**	7	1.5	1:1.2	25	54
12c	**13c**	8	1.5	1:1.5	44	61
12a	**13a**	8	1.5	1:1.3	24	78
12a	**13a**	8	1.5	1:1.1	24	quant.

[a] See Figures S3-6 (Supporting Information) for typical NMR spectra.

The preferred protocol that emerged for the first coupling to **9** involves 2 equivalents phosphoramidite per hydroxy group, 1.2-1.5 equivalents of activator and a reaction time of 12-48 h, depending on the phosphoramidite (Table 1). Just 1.5-1.75 equiv phosphoramidite per OH group gave an average yield of 62 %. More than 1.5 equiv of tetrazole over phosphoramidites led to significant amounts of 'overreacted' products, containing more than four nucleotides, most probably due to partial loss of DMT groups and subsequent coupling to the exposed 5'-alcohols. Long coupling times increase the likelihood of detritylation, but are also important for achieving near-quantitative conversion. In many cases where near-quantitative coupling occurred, visible crystals of diisopropylammonium tetrazolide (DIPAT) appeared after 7 h (**13a**) or 24 h (**13g**), helping to visually confirm successful couplings under our reaction conditions.

Neither of the mononucleotide hybrids (**13a**-**t**) required further purification for use in the next coupling cycle. When a sample of **13g**, featuring the most stable acyl protecting group among the nucleobases, was fully deprotected with NH_4OH and analyzed via MALDI-TOF and HPLC, a purity of approx. 90% was found (Figures S13 and S32, SI). A large-scale synthesis with 300 mg of **9** and 10 g of **12g** gave 2.8 g of **13g**, a yield of 95%.

With hybrids **13a**-**t** in hand, we proceeded to synthesizing hybrid **5** with CG dimer arms (Scheme 3). For this, detritylated hybrid **13g** was reacted with phosphoramidite **12c**. Two equiv of the phosphoramidite and three equiv tetrazole per arm in CH_3CN/DMF (1:1.2) for one day gave crude **5** in 66% yield after coupling, oxidation and detritylation. With 70% DMF in the coupling mixture, the yield dropped by 30%. But, the yield increased to 81% when 2.25 equiv phosphoramidate and 2.9 equiv tetrazole were used, the DMF content was lowered to 37% and the coupling time was increased to 2 d.



Scheme 3.

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Deprotection with aqueous ammonia then gave a crude in which **5** as the dominant component (Figure 2c), and HPLC purification led to the pure hybrid in 67% overall yield for the four-step sequence of coupling, oxidation, detritylation, and basic deprotection. So, compared to the synthesis via *H*-phosphonate dimer **11** (Figure 2b), a much higher yield resulted.

Encouraged by these results, we then prepared homooligomers **2** and **3**. In the first part of Table 2 (entries 1-7), selected results for thymidine-containing oligomer **2** are shown. For each step but the last, two different coupling conditions are shown to highlight how changes in the reaction conditions affect the yield of crude of intermediates **15**-**17**. Overall, it again emerged that the excess of phosphoramidite and tetrazole, the DMF content, and the reaction time are important factors for achieving high yields. For the last coupling cycle, producing the protected precursor of **2**, we used 11 equiv, which is still less than the 15-25 equiv typically used for solid-phase DNA syntheses on conventional DNA synthesizers. After full deprotection and HPLC, **2** was obtained in 54% yield or 86% per DNA arm for the last coupling cycle.

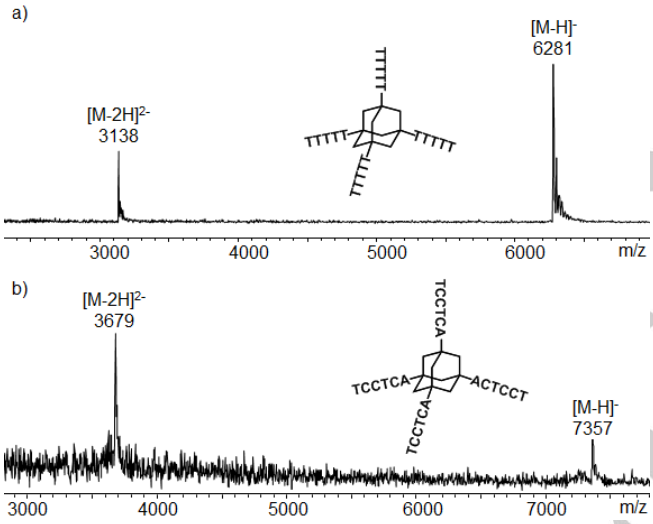

Figure 3. MALDI-TOF mass spectra of (a) pentamer hybrid **2** and (b) hexamer hybrid **4**.

Figure 3a shows a mass spectrum of the pentamer hybrid. The overall yield of **2**, with its 20 nucleotides, was 10% (56% per arm) over 16 steps. Hybrid d(A)$_5$ (**3**) was prepared similarly, as detailed in Table S1 (Supporting Information), and was obtained in 26% overall yield after HPLC purification. The higher yield may again be due to solubility and thus greater ease in isolating intermediates through precipitation.

We then turned to mixed sequence hybrids. First, hexamer **4** was prepared, which contains three of the four different nucleobases (A/C/T). Starting from **13a**, dimer intermediate **21** was obtained in 90-98 % yield (first coupling cycle with **12c** in Table 2, entries No. 8-9). Through increasing the amount of the phosphoramidite by 0.25 equiv/arm with every extension cycle, lowering the activator content to 1.2 equiv over the phosphoramidite, and adjusting the DMF content, yields of 82-90% for the four-fold extension were obtained on every stage of chain extension process (intermediates **22**-**24**). A good balance of the amount of tetrazole, the reaction time and the DMF content was obtained with the conditions shown in entries 13-15 of Table 2. After full deprotection, hexamer hybrid **4** was isolated in 84 % yield for the last extension cycle and 15% (62% per arm) overall yield for the 19 step route.

Finally, we undertook the synthesis of hybrids **6**, **7**, and **8** with self-complementary DNA arms. This synthesis was considered the most difficult, not just because up to 32 nucleotides had to be appended to the core, but also because the propensity of the *all*-CG sequences to self-assemble is high, and even protected intermediates have the ability to form large base-paired structures, complicating synthesis and purification. The last section of Table 2 (entries 16-22) gives an overview of the coupling conditions and yields.

The coupling to dimer hybrid **25** was carried out with 7.5 equiv of **12g**, 1.4 equiv tetrazole, and a DMF/CH_3CN mixture of 1:1.2, yielding hybrid **25** in near-quantitative yield. For the coupling cycle producing trimer hybrid **26**, the equivalents of **12c** were increased, and the reaction time extended, resulting in a yield of 75% (93% per arm). The coupling rounds leading to **27** and **28** were carried out similarly, but with a coupling time of 2 d. Again, the pyrimidine coupling was slightly lower yielding. The sixth coupling cycle used 11 equiv of **12g** and slightly more DMF in the solvent mixture to reduce self-assembly and to obtain a homogeneous solution. The seventh and eighth extension steps were carried out with 1.2 equiv of tetrazole to avoid loss of DMT groups. Crude heptamer **30** was obtained in a yield of 90%, and the subsequent coupling used a 1:1 mixture of DMF/CH_3CN to ensure a homogeneous solution. Conventional deprotection with hot aqueous ammonia gave tetramer hybrid $(GCGC)_4TOA$ (**6**) in 55% from **26** and 25 % overall yield for 13 steps after HPLC purification. Figure 4 shows chromatogram and MALDI-TOF mass spectrum of this hybrid.

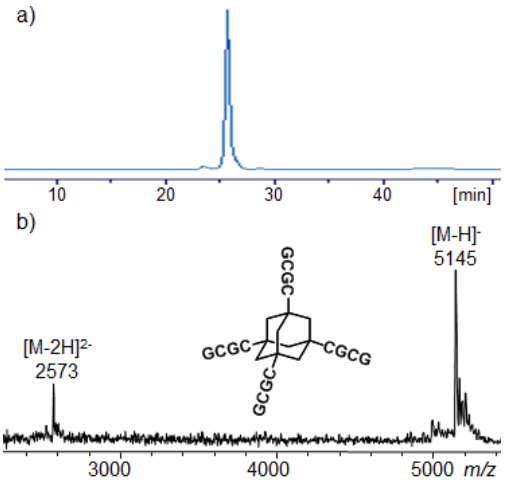

Figure 4. Analytical data for self-complementary tetramer hybrid $(GCGC)_4TOA$ (**6**). (a) HPLC trace of purified product (C18-phase, gradient of 1-25% CH_3CN in 10 mM TEAA buffer, at 55 °C column temperature, λ_{det} 260 nm). (b) MALDI-TOF mass spectrum after HPLC purification.

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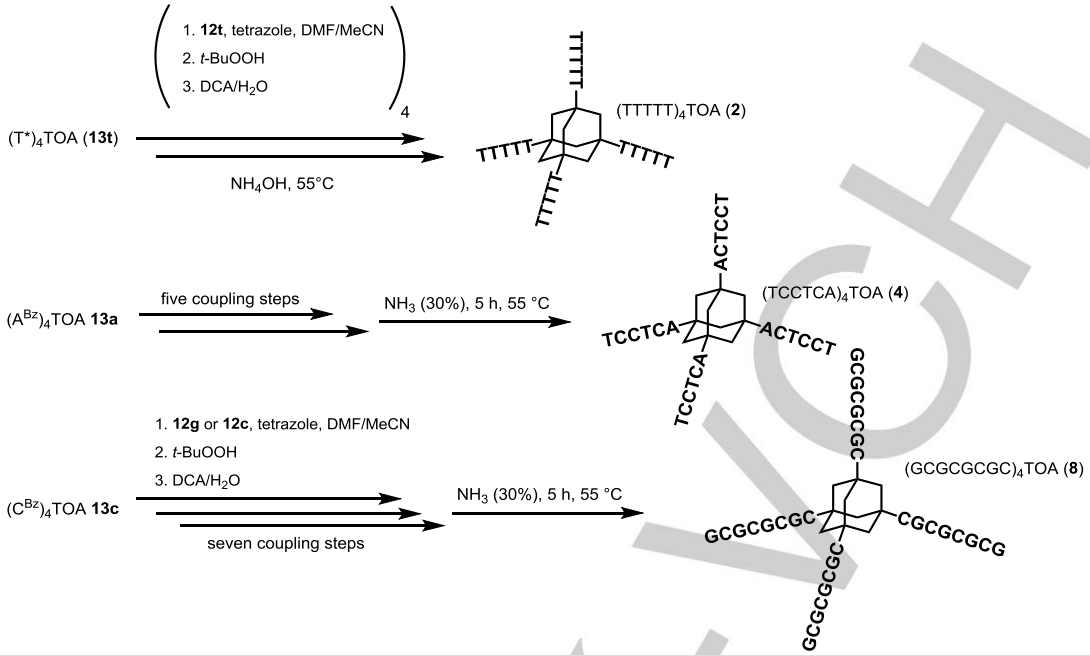

Table 2. Results of the synthesis of $(TTTTT)_4TOA$ (**2**), $(TCCTCA)_4TOA$ (**4**), and $(GCGCGCGC)_4TOA$ (**8**) via linear, phosphoramidite-based chain assembly.

Entry No.	Educt	Monomer	Product[a]	Equiv **12**	Equiv tetrazole	Ratio DMF/CH_3CN	time (h)	Yield of crude (%)
1	**13t**	**12t**	$(T^*T^*)_4TOA$ (**15**)	7	1.5	1:1.6	22	50
2	**13t**	**12t**	$(T^*T^*)_4TOA$ (**15**)	8	1.5	1:1.3	23	73
3	**15**	**12t**	$(T^*T^*T^*)_4TOA$ (**16**)	8	1.3	1.7:1	32	72
4	**15**	**12t**	$(T^*T^*T^*)_4TOA$ (**16**)	9	1.3	1:1.2	47	91
5	**16**	**12t**	$(T^*T^*T^*T^*)_4TOA$ (**17**)	9	1.3	1:1.2	50	23
6	**16**	**12t**	$(T^*T^*T^*T^*)_4TOA$ (**17**)	10	1.3	1:1.3	48	57
7	**17**	**12t**	$(T^*T^*T^*T^*T^*)_4TOA$ (**2p**)[b]	11	1.3	1:1.3	48	55
			$(TTTTT)_4TOA$ (**2**)					38[c]
8	**13a**	**12c**	$(C^{Bz}A^{Bz})_4TOA$ (**21**)	9	1.3	1:1	72	90
9	**13a**	**12c**	$(C^{Bz}A^{Bz})_4TOA$ (**21**)	8	1.2	1:1.4	48	98
10	**21**	**12t**	$(T^*C^{Bz}A^{Bz})_4TOA$ (**22**)	9	1.2	1:1.4	48	87
11	**21**	**12t**	$(T^*C^{Bz}A^{Bz})_4TOA$ (**22**)	10	1.3	1:1.3	39	90
12	**22**	**12c**	$(C^{Bz}T^*C^{Bz}A^{Bz})_4TOA$ (**23**)	10	1.1	1:1.4	69	78
13	**22**	**12c**	$(C^{Bz}T^*C^{Bz}A^{Bz})_4TOA$ (**23**)	10	1.2	1:1.4	48	89
14	**23**	**12c**	$(C^{Bz}C^{Bz}T^*C^{Bz}A^{Bz})_4TOA^*$ (**24**)	11	1.2	1:1.4	48	82
15	**24**	**12t**	$(T^*C^{Bz}C^{Bz}T^*C^{Bz}A^{Bz})_4TOA^*$ (**4p**)[b]	12	1.2	1:1.4	48	84
			$(TCCTCA)_4TOA$ (**4**)					23[c]
16	**13c**	**12g**	$(G^{ibu}C^{Bz})_4TOA$ (**25**)	7.5	1.4	1:1.2	26	99
			$(GC)_4TOA$ (**25d**)[d]					64[c]
17	**25**	**12c**	$(C^{Bz}G^{ibu}C^{Bz})_4TOA$ (**26**)	8	1.3	1:1.2	44	75
18	**26**	**12g**	$(G^{ibu}C^{Bz}G^{ibu}C^{Bz})_4TOA$ (**27**)	9	1.3	1:1.2	48	82
			$(GCGC)_4TOA$ (**6**)					55[c]
19	**27**	**12c**	$(C^{Bz}G^{ibu}C^{Bz}G^{ibu}C^{Bz})_4TOA$ (**28**)	10	1.3	1:1.2	48	70
20	**28**	**12g**	$(G^{ibu}C^{Bz}G^{ibu}C^{Bz}G^{ibu}C^{Bz})_4TOA$ (**29**)	11	1.3	1:1.1	48	85
			$(GCGCGC)_4TOA$ (**7**)					77[c]
21	**29**	**12c**	$(C^{Bz}G^{ibu}C^{Bz}G^{ibu}C^{Bz}G^{ibu}C^{Bz})_4TOA$ (**30**)	12	1.2	1:1.4	48	90
22	**30**	**12g**	$(G^{ibu}C^{Bz}G^{ibu}C^{Bz}G^{ibu}C^{Bz}G^{ibu}C^{Bz})_4TOA$ (**8p**)[b]	13	1.2	1:1	48	80
			$(GCGCGCGC)_4TOA$ (**8**)					57[c]

[a] Asterisk denotes thymidine nucleotide with protected backbone; Protecting group-bearing nucleobase and with protected backbone. [b] The letter 'p' denotes protected hybrid. [c] Yield of fully deprotected hybrid after HPLC purification. [d] The letter 'd' denotes fully deprotected hybrid.

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For hexamer hybrid **7**, the yield of protected crude was 85% (96% coupling yield per arm) and 20% for the overall chain assembly process. The protected form of octamer hybrid **8** was obtained in 16% after 24 steps, and fully deprotected **8** with its 32 nucleotides in 11% yield after HPLC. Figure 5 shows the HPLC chromatogram, a MALDI-TOF mass spectrum and an NMR spectrum of **8**. The NMR spectrum was acquired at elevated temperature, as the strong propensity for self-assembly manifested itself in the room temperature spectrum through broad signals.

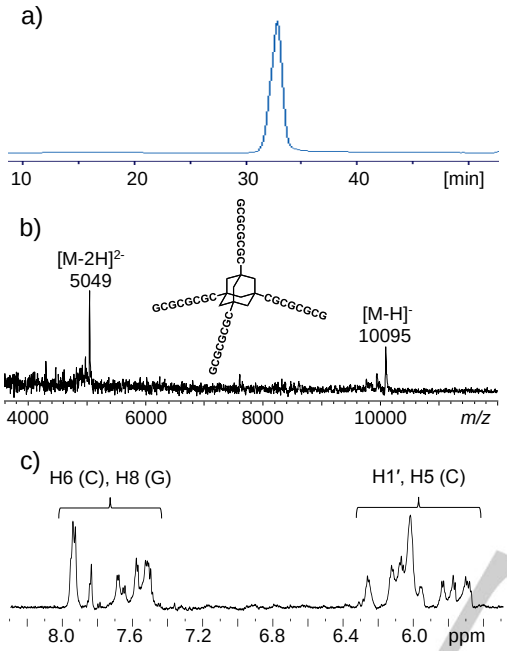

Figure 5. Analytical data for purified octamer hybrid $(GCGCGCGC)_4TOA$ (**8**). (a) HPLC chromatogram (same conditions as in Figure 4a). (b) MALDI-TOF mass spectrum after single-stage HPLC, and (c) low-field region of ^1H NMR spectrum of **8** (0.02 mM solution in D_2O, 700 MHz, 70°C).

The assembly of molecules or particles with multiple DNA strands on their surface through Watson-Crick base pairing depends on the structure of the non-DNA part.[36,44] We expected the TOA hybrids to show hybridization properties that are more strongly dominated by the DNA strands than the hybrids with large hydrophobic cores reported recently.[36] Several DNA hybrids with aromatic cores have been found to form nanoporous material irreversibly upon addition of magnesium salts.[34,35,39] The deprotected version of dimer hybrid **25** $(GC)_4TOA$ (**25d**) showed no melting transition in buffered solution containing 150 mM NaCl. Upon addition of $MgCl_2$, a very modest amount of a precipitate was formed below 10 °C, which fully re-dissolved at room temperature (see Figure S53c of the SI).

Figure 6 shows UV-melting curves for the *all*-GC hybrids **6**, **7** and **8**. In each case, the sample was fully denatured with NaOH at 90 °C, followed by neutralization with acetic acid, addition of triethylammonium actetate buffer, NaCl and $MgCl_2$. Upon cooling and subsequent re-heating, only weak transitions were observed for tetramer hybrid **6** (Figure 5a). Hexamer hybrid **7** gave stronger and more cooperative UV-transitions, with clear hysteresis between cooling and heating curves, and formal T_m values of 35 and 57 °C. This suggested that large structures were forming that were slow to dissociate on the time scale of the experiment. Further, a well visible precipitate formed upon cooling, which all but fully dissolved upon re-heating. For hybrid **8** with its strongly pairing octamer arms, sharp and cooperative transitions were measured, with formal melting points of 45 and 63 °C. Cooling was accompanied by an apparent hypochromicity of 50%, which was again the result of decreased absorbance base stacking, loss of soluble material through precipitation, and scattering of light by the now turbid mixture. Also, the material fully redissolved upon heating, despite the high concentration of magnesium ions in the solution The melting point of the linear control duplex $(GCGCGCGC)_2$ is 59 °C under the same buffer conditions (Figure S62, Supporting Information).

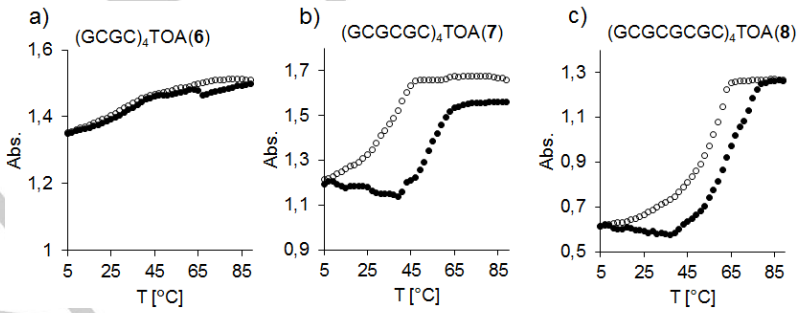

Figure 6. UV-Monitored assembly and disassembly results for self-complementary DNA hybrids **6**, **7** and **8**, measured at 10, 8 or, 5 μM hybrid concentration, respectively, in 10 mM TEAA buffer, pH 7.0, 150 mM NaCl and 100 mM $MgCl_2$, detected at 260 nm. Cooling curves (open circles) and heating curves (filled circles) were acquired at 0.5 °C/min. For additional melting curve data, including curves acquired under other salt conditions, see Figure S54-S58 in the Supporting Information.

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Reversible formation of a material was also observed for the mixture of the homooligomer hybrids **2** and **3**. Figure 7 shows the UV profiles, together with photographs of cuvettes containing concentrated solutions of the hybrids with $(dT)_5$ and $(dA)_5$ arms on the TOA core. In the presence of $MgCl_2$, a voluminous precipitate formed upon cooling. Still, even here the solid fully re-dissolved upon warming to room temperature (25 °C).

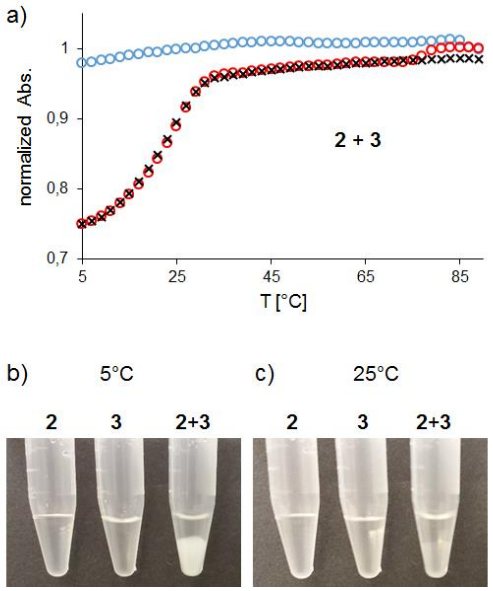

Figure 7. Reversible formation of a material from hybrids with homopolymer sequences. (a) Thermal association and melting profiles of a solution containing a mixture of $(TTTTT)_4$TOA (**2**) and $(AAAAA)_4$TOA (**3**) at 2 µM hybrid concentration and cooling/heating rates of 0.5 °C/min (10 mM TEAA buffer, pH 7, 150 mM NaCl). Blue symbols: cooling and heating curve in magnesium-free buffer; black and red symbols: buffer plus 100 mM $MgCl_2$ (cooling: black crosses, heating: red circles). Cooling and heating curves were measured immediately after each other. (b) Photographs of 2 mM hybrid solutions at 5 °C, after annealing from 85 °C and storing at 5 °C for 5 h. The compound numbers of hybrids in each sample are noted above the tubes. (c) Same samples as in (b), but after 1 h at 25 °C. For additional control experiments performed under melting curve conditions (2 µM hybrid concentration), see Figures S59-S61 of the Supporting Information.

Finally, we performed exploratory experiments on the uptake of small molecules into the material formed by TOA hybrids with self-complementary DNA arms. For this, 300 µM solutions of **5**, **7**, or **8** in buffer solution were treated with $MgCl_2$ and assembly of materials was induced by heating to 85 °C, followed by cooling to 4 °C over the time course of 12 h. The materials thus formed (Figure 8a) were washed with assembly buffer and then treated with a 3 mM solution of ethidium bromide in water. Either of the hybrids assembled with similar efficiency (Table 3), but the amount of intercalator taken up post-assembly from the aqueous solution mirrored the length of the DNA arms, with **8** taking up close to the eight equivalents expected for intercalation between every other base pair (nearest neighbor exclusion principle). Further, the ethidium bromide guest was brightly fluorescent (Figure 8d), strongly suggesting that the chromophores were indeed intercalated and not aggregated. Together this data suggest that the biohybrid materials are nanoporous, as they can take up small molecules efficiently, and in doing so can produce three-dimensional lattices with photoactive chromophores.

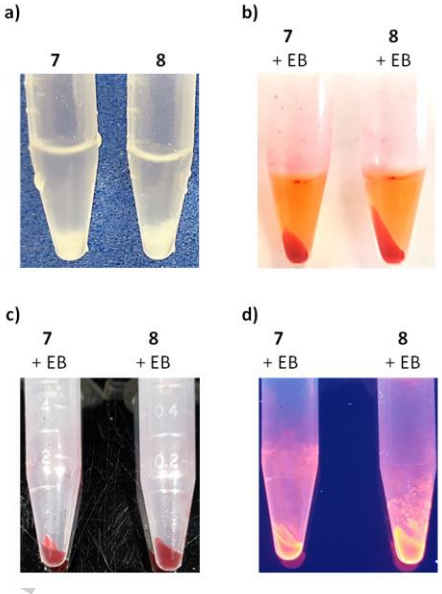

Figure 8. Photographs of samples of hybrids **7** and **8** prior to and after uptake of ethidium bromide (EB) from aqueous solution. a) Materials formed from aqueous solution at a 0.3 mM concentration of either hybrid in 10 mM TEAA buffer, 150 mM NaCl, 100 mM $MgCl_2$, after annealing from 85 to 4°C in 12 h, viewed at ambient light. b) The same samples after aspirating the mother liquor, and adding a 3 mM solution of ethidium bromide in water. c) The hybrid material after aspirating the EB solution. d) Picture of the same samples as in c) under UV-illumination (254-365 nm). See the Supporting Information for further experimental details.

Table 3. Encapsulation of ethidium bromide into hybrid material formed by **5**, **7** or **8**.

Hybrid	Efficiency of material formation (%)[a]	Molar ratio hybrid/guest in material [b]
$(CG)_4$TOA (**5**)	90	1 : 1.9
$(GCGCGC)_4$TOA (**7**)	87	1 : 7.8
$(GCGCGCGC)_4$TOA (**8**)	86	1 : 8.9

[a] Percentage of hybrid that formed a solid from a 300 µM solution of the hybrid in buffer (10 mM TEAA, 150 mM NaCl) containing 100 mM $MgCl_2$, after annealing from 85 to 4°C in 12 h. [b] Uptake from a 3 mM solution of ethidium bromide (EB), as determined by UV/Vis-spectrophotometry.

Conclusions

Synthetically, our results show that solution-phase syntheses of branched oligonucleotide hybrids with arms long enough for stable duplex formation, are feasible, even if four strands are assembled in parallel. Unlike solid-phase synthesis, our optimized protocol uses long reaction times and a modest excess of phosphoramidites of approx. 1.5-3 equiv per chain. Handling loss, poor solubility, and 'overcoupling' caused by detritylation are the most significant challenges during chain extension that can be overcome by a proper choice of solvents and amount of activator. Specifically, we have found that increasing the amount of phosphoramidite by 0.25 equivalents

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after each coupling cycle, increasing the DMF content in the coupling mixture up to 50% for long sequences, and reducing the tetrazole concentration to no more than 1.2 equiv over the phosphoramidite for long coupling times, all help to achieve high yields. With this, branched oligonucleotides with up to 32 nucleotides total are obtained without chromatography during chain assembly in an easy-to-scale up procedure that avoids supports. The results show that a wide range of different sequences can be produced with minimal set-up costs, as no dimer building blocks have to be prepared.

With the adamantane tetraol core, branched oligonucleotides behave more DNA-like than the known hybrids with more lipophilic cores. Even with long DNA sequences as arms, the TOA hybrids assemble into materials reversibly, without being trapped in kinetically blocked, insoluble or irreversible assemblies. These properties bode well for the formulation of biomacromolecules in the three-dimensional networks thus formed. Studies on new inclusion materials are under way in our laboratories.

Experimental Section

General. All HPLC purifications were performed on a Nucleosil C_{18} column (5 µm, 250 x 4.6 mm) from Macherey-Nagel (Düren, Germany). A mixture of CH_3CN and triethylammonium acetate buffer (TEAA, 0.01 M, pH 7) was used as eluant, with a flow rate of 1 mL/min and a column temperature of 55 °C and detection at 260 nm. MALDI-TOF mass spectra were measured in the linear negative mode with a matrix/comatrix mixture of 2,4-6-trihydroxyacetophenone/diammonium citrate. UV-melting curves were measured at pH 7, with a λ_{det} of 260 nm and a cooling/heating rate of 0.5°C/min.

1,3,5,7-Tetrakis(4-hydroxyphenyl)adamantane (**10**). A solution of BBr_3 (317 µL, 0.32 mmol) in CH_2Cl_2 (3 mL) was cooled to -60°C, and a solution of 1,3,5,7-tetrakis(4-methoxyphenyl)adamantane[40] (14.8 mg, 26 µmol) in CH_2Cl_2 (1 mL) was added dropwise. The mixture was stirred for 3 h and then allowed to warm to 25 °C, followed by stirring for 19 h. The mixture was then treated with CH_3OH (10 ml), followed by drying *in vacuo*. This step was repeated twice. The crude product was washed with CH_2Cl_2 (5 mL), and the residue was dried *in vacuo*. Purification via column chromatography (silica gel, 10 g, CH_2Cl_2/MeOH, 10/1, *v/v*) yielded 11 mg (21 µmol, 81%) of the title compound (**10**) as an off-white solid: R_f = 0.48 (CH_2Cl_2/MeOH 10/1, *v/v*); ^{1}H NMR (300 MHz, CD_3OD) δ = 1.97 (s, 12 H), 6.74 (d, ^{3}J = 8.7 Hz, 8 H), 7.27 (d, ^{3}J = Hz, 8.7, 8 H); ^{13}C NMR (75.5 MHz, CD_3OD) δ = 156.3, 142.5, 127.0, 115.9, 39.8; HRMS (ESI-TOF) *m/z* calcd for $C_{34}H_{32}O_4$ 503.2223, obsd $[M-H]^-$ 503.2217.

(CG)$_4$TPA (**1**) To assemble **1** via the *H*-phosphonate block condensation method,[36,39] a mixture of **10** (26 µmol, 1 equiv), *H*-phosphonate dimer **11** (414 mg, 0.36 mmol, 14 equiv) and molecular sieves (3 Å, seven beads) was dried for 2 h at 45 °C and 0.001 mbar, followed by flushing with argon. A solution of pyridine in CH_3CN (3 mL, 4:1, *v/v*) was added, and the mixture was cooled to -40 °C. Then, diphenyl chlorophosphate (112 µL, 0.54 mmol) was added, and the mixture was stirred at -40 °C for 120 min. A solution of iodine in pyridine (541 µL, 1 M) was added, followed after 1 min by H_2O (32 µL, 1.8 mmol). The mixture was stirred for 10 min at -40 °C and then for 30 min at room temperature. After addition of CH_2Cl_2 (15 mL), the solution was washed with aqueous sodium thiosulfate (8 mL, 10%, *w/w*) and phosphate buffer (7 mL, 0.2 M, pH 7). The aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were concentrated *in vacuo*, the residue was coevaporated three times from toluene and redissolved in CH_2Cl_2 (5 mL), followed by precipitation with MTBE (45 mL) and isolation by centrifugation. This precipitation was repeated twic, followed by dissolving in CH_2Cl_2 (5 mL). The supernatant was separated from any remaining solid, which was washed with CH_2Cl_2 (2× 2 mL). Evaporation yielded a crude that was used for deprotection. The protected hybrid was dissolved in CH_2Cl_2 (20 mL) with H_2O (30 µL, 1.7 mmol), followed by addition of dichloroacetic acid (DCA, 6% in CH_2Cl_2, 26 mL). After 15 min, methanol (15 mL) was added. The solution was then concentrated, and the detritylated hybrid was isolated by precipitation with MTBE (30 mL) and centrifugation, redissolving in CH_2Cl_2/CH_3OH (5 mL, 3/1, *v/v*) and precipitation with MTBE (30 mL). This process was repeated twice. Remaining protecting groups were removed by treating with ammonium hydroxide (25%, 10 mL) for 5 h at 55 °C. Evaporation yielded crude **1** (216 mg). Cartridge purification on Sep-Pak C_{18} with a gradient of CH_3CN (0-25%) in 10 mM NH_4Ac buffer, led to elution of **1** at 8% CH_3CN. The title compound was obtained in a yield of 29.1 mg (9.8 µmol, 38%). A small sample was purified by HPLC, using a gradient of CH_3CN (1-30% in 35 min) in 10 mM TEAA buffer, with elution of **1** at t_R = 16 min. MALDI-TOF-MS *m/z* calcd for $C_{110}H_{128}N_{32}O_{52}P_8$ $[M-H]^-$ 2977, obsd 2978.

Extension Cycle (General Protocol A). The following is the chain extension cycle leading to $(A^{Bz})_4$TOA (**13a**). This protocol is representative. The specifics of each individual coupling are given below for each intermediate or target compound. A sample of tetraol **9** (40 mg, 0.2 mmol) was mixed with the phosphoramidite (**12a**, 1.37 g, 1.5 mmol, 8 equiv), and molecular sieves (3 Å, 10 beads) were added. The mixture was dried for 2 h at 45 °C and 0.001 mbar. The flask was flushed with argon and sealed with a septum. Then, DMF (5 mL) and tetrazole solution (5.3 mL of a commercial 0.45 M solution in CH_3CN, known as "activator solution" for DNA synthesis, 12 equiv) were added. The flask was placed in an ultrasonic bath for two minutes until the components were fully dissolved. The flask was placed on an orbital shaker for 30 min and then stored at 5 °C for 24 h. Then, *tert*-butyl hydroperoxide (216 µL, 5.5 M in decane, 0.24 mmol, 12 equiv) was added. The reaction mixture was allowed to react for 15 min at 5 °C. After addition of CH_2Cl_2 (20 mL), the mixture was washed with phosphate buffer (20 mL, 0.2 M, pH 7). The aqueous phase was removed and back-extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried over Na_2SO_4 filtered and concentrated *in vacuo*. The residue was coevaporated twice from MeOH, dissolved in a minimal amount of CH_2Cl_2 (approx. 10 mL), followed by precipitation with MTBE (45 mL) and separation by centrifugation. The sequence of dissolving and precipitation was repeated three times. The solid thus obtained was treated with MeOH (5 mL) for 5 min in an ultrasonic bath, followed by centrifugation (5 min). The supernatant was discarded, and the residue was dissolved in CH_2Cl_2 (4 mL), followed by precipitation with MTBE (45 mL) and centrifugation. The residue was dissolved in CH_2Cl_2 (5 mL), separated from any remaining particles and the solution concentrated *in vacuo* to yield the fully protected hybrid (720 mg). Then, the DMT groups were removed by dissolving in CH_2Cl_2 (4 mL) and addition of H_2O (47 µL, 2.6 mmol) and dichloroacetic acid (6 mL, 6% in CH_2Cl_2). After 10 min, the reaction was quenched by addition of methanol (3 mL). The solution was then concentrated by rotary evaporation to remove the dichloromethane, and the detritylated hybrid was precipitated by addition of MTBE (15 mL). The precipitate was isolated by centrifugation, redissolved in a minimal volume of CH_2Cl_2/CH_3OH (1 mL, 4:1, *v/v*) and precipitated again by addition of MTBE (15 mL). This process was repeated two more times to give **13a** (418 mg, 0.2 mmol, quant). The hybrid thus obtained was used for the next coupling cycle without further purification.

Full Deprotection with Aqueous Ammonia (General Protocol B). Fully deprotected hybrids were obtained by treating the DMT-free hybrid

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with ammonium hydroxide (30-25%, 1 mL per 10 mg) for 5 h at 55 °C. Then, excess ammonia was removed by passing a stream of N_2 onto the solution until the sample was odorless. The remaining solution was lyophilized to yield the crude hybrid. The crude was then purified on a Sep-Pak C_{18} cartridge, using a gradient of 0-25% CH_3CN in 10 mM NH_4Ac buffer or by reversed-phase HPLC (C_{18} column, 250 × 20 mm), using a gradient of 1-15% CH_3CN in 10 mM TEAA buffer at 55°C.

$(G^{Bz})_4$TOA (**13g**). General Protocol A was employed, starting from **9** (300 mg, 1.5 mmol), **12g** (10 g, 12 mmol) in DMF (19 mL) and tetrazole solution (0.45 M, 31.9 mL, 14.3 mmol) with 48 h coupling time at 5°C, followed by oxidation with *t*-BuOOH (2.8 mL, 15.6 mmol), DMT deprotection with CH_2Cl_2 (25 mL), water (336 µL) and DCA solution (6% in CH_2Cl_2, 50 mL), and precipitations from CH_2Cl_2/MeOH (4 × 5 mL, 3/2, *v/v*) with MTBE (45 mL). Yield 2.85 g (1.42 mmol, 95%). ^{31}P NMR (121.5 MHz, DMSO-d_6): δ = -7.58 (broad peak: mixture of diastereomers); MALDI-TOF-MS *m/z* calcd for $C_{78}H_{100}N_{24}O_{32}P_4$ $[M-H]^-$ 2008, obsd 2006 $[M-H]^-$ and 1951 $[M-H-CE]^-$. **$(G)_4$TOA** (**14g**). Compound **14g** was obtained from **13g** using General Protocol B. Hybrid **14g** was purified by HPLC, using a gradient of CH_3CN (1-15% in 45min), t_R (**14g**) = 21 min, MALDI-TOF-MS *m/z* calcd for $C_{50}H_{64}N_{20}O_{28}P_4$ $[M-H]^-$ 1516, obsd 1515.

$(C^{Bz})_4$TOA (**13c**). The synthesis followed General Protocol A, starting from **9** (40 mg, 0.2 mmol) and **12c** (1.33 g, 1.6 mmol, 8 equiv) in DMF (3.5 mL) and tetrazole solution (5.3 mL, 2.4 mmol, 1.5 equiv) for 44 h at 5°C, followed by oxidation with *t*-BuOOH (0.4 mL, 2.4 mmol, 1.5 equiv), DMT deprotection with CH_2Cl_2 (5 mL), water (44 µL) and DCA solution (6% in CH_2Cl_2, 7 mL), and precipitations from CH_2Cl_2/MeOH (4 mL, 3:1, *v/v*) with MTBE (a 45 mL). Detritylated hybrid **13c** was obtained in a yield of 241 mg (121 µmol, 61%). MALDI-TOF-MS *m/z* calcd for $C_{86}H_{92}N_{16}O_{32}P_4$ $[M-H]^-$ 1984, obsd 1984 $[M-H]^-$, 1931 $[M-H-CE]^-$. **$(C)_4$TOA** (**14c**). Compound **14c** was obtained from **13c** using General Protocol B. MALDI-TOF-MS *m/z* calcd for $C_{46}H_{64}N_{12}O_{28}P_4$ $[M-H]^-$ 1355, obsd 1354.

$(T^*)_4$TOA (**13t**). The synthesis followed General Protocol A, starting from **9** (20 mg, 0.1 mmol) and **1t** (483 mg, 0.65 mmol, 6.5 equiv) in DMF (1.5 mL) and tetrazole solution (2.16 mL, 0.97 mmol, 1.5 equiv) for 21 h at 5 °C, followed by oxidation with *t*-BuOOH (177 µL, 0.97 mmol, 1.5 equiv), DMT deprotection with CH_2Cl_2 (3 mL), water (27 µL) and DCA solution (6% in CH_2Cl_2, 3.5 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 3:1, *v/v*) with MTBE (45 mL). Hybrid **13t** was isolated in a yield of 110 mg (67.5 µmol, 68%). ^{31}P NMR (121.5 MHz, DMSO-d_6): δ = -7.48 (broad peak: mixture of diastereomers); MALDI-TOF-MS *m/z* calcd for $C_{62}H_{80}N_{12}O_{32}P_4$ $[M-H]^-$ 1628, obsd 1628 $[M-H]^-$, 1574 $[M-H-CE]^-$.

$(A^{Bz})_4$TOA (**13a**). General Protocol A was followed verbatim (*vide supra*), and **13a** was isolated in a yield of 418 mg (0.2 mmol, quant). MALDI-TOF-MS *m/z* calcd for $C_{90}H_{92}N_{24}O_{28}P_4$ $[M-H]^-$ 2080, obsd 2080 $[M-H]^-$, 2026 $[M-H-CE]^-$. $(A)_4$TOA (**14a**). General Protocol B was employed to obtained **14a**, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min) in 100 mM TEAA buffer, t_R = 37.5 min, MALDI-TOF-MS *m/z* calcd for $C_{50}H_{64}N_{20}O_{24}P_4$ $[M-H]^-$ 1452, obsd 1452.

$(C^{Bz}G^{iBu})_4$TOA (**5p**). The hybrid **5p**, where "p" denotes the protected form of **5**, was prepared following General Protocol A via coupling of **13g** (2.8 g, 1.4 mmol) and **12c** (10.6 g, 12.8 mmol, 9 equiv) in DMF (22 mL) and tetrazole solution (36.9 mL, 16.6 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (3.5 mL, 19.2 mmol, 1.5 equiv), DMT deprotection with CH_2Cl_2 (26 mL), water (229 µL) and DCA solution (6% in CH_2Cl_2, 70 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 3:2, *v/v*) with MTBE (45 mL). The detritylated hybrid was isolated in a yield of 4 g (1.14 mmol, 81%). ^{31}P NMR (121.5 MHz, DMSO-d_6): δ = 2.71, -7.3, -7.9 (broad peaks, mixture of diastereomers); MALDI-TOF-MS *m/z* 3741 $[M-H-CE]^-$. **$(CG)_4$TOA** (**5**). The synthesis of **5** was performed according to General Protocol B, starting from **5p** (40 mg, 10.5 µmol). One fraction was purified by HPLC, using a gradient of CH_3CN (1-15% in 30 min), t_R of **5** = 16.6 min. Alternatively, cartridge purification used a gradient of CH_3CN in 10 mM NH_4Ac buffer, 0-20%. Hybrid **5** eluted at 1.5-5%, and was obtained in an overall yield of 25 mg (9.4 µmol, 67%). MALDI-TOF-MS *m/z* calcd for $C_{86}H_{112}N_{32}O_{52}P_8$ $[M-H]^-$ 2672, obsd 2673.

$(G^{iBu}C^{Bz})_4$TOA (**25**). Hybrid **25** was prepared following General Protocol A, starting with coupling of **13c** (377 mg, 0.19 mmol) and **12g** (1.2 g, 1.4 mmol, 7.5 equiv) in DMF (3.2 mL) with tetrazole solution (3.8 mL, 1.7 mmol, 1.35 equiv) for 26 h at 5 °C, followed by oxidation with *t*-BuOOH (389 µL, 2.2 mmol, 1.5 equiv), DMT removal with CH_2Cl_2 (5 mL), water (55 µL) and DCA solution (6% in CH_2Cl_2, 11 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 3:2, *v/v*) with MTBE (45 mL). The detritylated hybrid **25** was isolated in a yield of 715 mg (188 µmol, 99%). MALDI-TOF-MS *m/z* 3737 $[M-H-CE]^-$. **$(GC)_4$TOA** (**25d**). Hybrid **25d** was prepared following General Protocol B, starting from **25** (180 mg, 47.5 µmol). An analytical sample was purified by HPLC, using a gradient of CH_3CN (1-15% in 50 min), t_R = 21.5 min. Alterantively, cartridge purification used a gradient of CH_3CN in 10 mM NH_4Ac buffer, 0-20%, **25d** eluted from 1.6% to 5% CH_3CN; yield 82 mg (30.6 µmol, 64%). MALDI-TOF-MS *m/z* calcd for $C_{86}H_{112}N_{32}O_{52}P_8$ $[M-H]^-$ 2672, obsd 2672.

$(T^*T^*)_4$TOA (**15**). The extension cycle leading to **15** was performed according to General Protocol A, starting with cupling with **13t** (476 mg, 292 µmol) and **12t** (1.7 g, 2.3 mmol, 8 equiv) in DMF (6 mL) and tetrazole solution (7.8 mL, 3.5 mmol, 1.5 equiv) for 23 h at 5 °C, followed by oxidation with *t*-BuOOH (637 µL, 3.5 mmol, 1.5 equiv), DMT removal with CH_2Cl_2 (15 mL), water (53 µL) and DCA solution (6% in CH_2Cl_2, 16 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 1:1, *v/v*) with MTBE (45 mL) The detritylated hybrid **15** was obtained in a yield of 654 mg (214 µmol, 73%). ^{31}P NMR (121.5 MHz, DMSO-d_6): δ = 2.5, -7.2, -7.8 (broad peaks; mixture of diastereomers); MALDI-TOF-MS *m/z* 3004 $[M-H-CE]^-$. **$(TT)_4$TOA** (**15d**). General Protocol B was employed to obtaind **15d**, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 26 min, MALDI-TOF-MS *m/z* calcd for $C_{90}H_{120}N_{16}O_{60}P_8$ $[M-H]^-$ 2632, obsd 2633.

$(A^{Bz}A^{Bz})_4$TOA (**18**). General Protocol A was used, starting from **13a** (102 mg, 49 µmol) and **12a** (378 mg, 441 µmol, 9 equiv) in DMF (1 mL) and tetrazole solution (1.27 mL, 0.57 mmol, 1.3 equiv) for 47 h at 5 °C, followed by oxidation with *t*-BuOOH (120 µL, 0.66 mmol, 1.5 equiv), detritylation with CH_2Cl_2 (2.5 mL), water (11 µL) and DCA solution (6% in CH_2Cl_2, 4 mL), and precipitations from CH_2Cl_2/MeOH (1 mL, 4:1, *v/v*) with MTBE (15 mL). Hybrid **18** was isolated in a yield of 169 mg (43 µmol, 87%). **$(AA)_4$TOA** (**18d**). The deprotected hybrid was obtained from **18** using General Protocol B. Crude hybrid was purified by HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 24.4 min, MALDI-TOF-MS *m/z* calcd for $C_{90}H_{112}N_{40}O_{44}P_8$ $[M-H]^-$ 2704, obsd 2706.

$(C^{Bz}A^{Bz})_4$TOA (**21**). Hybrid **21** was prepared following General Protocol A, starting from **13a** (1 g, 485 µmol) and **12c** (3.2 g, 3.9 mmol) in DMF (7.3 mL) and tetrazole (10.4 mL, 4.68 mmol, 1.2 equiv.) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (922 µL, 5.2 mmol, 1.3 equiv.), DMT removal with CH_2Cl_2 (4 mL), water (104 µL) and DCA solution (6% in CH_2Cl_2, 8 mL), and precipitations. The detritylated hybrid **21** was isolated in a yield of 1.8 g (480 µmol, 98 %). **$(CA)_4$TOA** (**21d**). Compound **21d** was obtained from **21** using General Protocol B, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 20.8 min, MALDI-TOF-MS *m/z* calcd for $C_{86}H_{112}N_{32}O_{48}P_8$ $[M-H]^-$ 2608, obsd 2606.

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($C^{Bz}G^{iBu}C^{Bz}$)$_4$TOA (**26**). General Protocol A was used, starting from **25** (715 mg, 188 µmol) and **12c** (1.25 g, 1.5 mmol, 8 equiv) in DMF (3.7 mL) and tetrazole (4.35 mL, 1.96 mmol, 1.3 equiv) for 44 h at 5 °C, followed by oxidation with *t*-BuOOH (356 µL, 1.96 mmol, 1.5 equiv), detritylation with CH_2Cl_2 (3.5 mL), water (38 µL) and DCA solution (6% in CH_2Cl_2, 13 mL), and precipitations from CH_2Cl_2/MeOH (4 mL, 3:1, *v/v*) with MTBE (45 mL). Hybrid **26** could be isolated with a yield of 744 mg (137 µmol, 75%). **(CGC)$_4$TOA** (**26d**). General Protocol B was employed to obtained **26d** followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 24 min, MALDI-TOF-MS *m/z* calcd for $C_{126}H_{168}N_{44}O_{76}P_{12}$ $[M-H]^-$ 3831, obsd 3832.

(T*T*T*)$_4$TOA (**16**). The synthesis followed General Protocol A, starting from **15** (309 mg, 101 µmol) and **12t** (678 mg, 0.9 mmol, 9 equiv.), in DMF (2.1 mL) and tetrazole (2.6 µL, 1.18 mmol, 1.3 equiv) for 47 h at 5 °C, followed by oxidation with *t*-BuOOH (248 µL, 1.36 mmol, 1.5 equiv), washing with MeOH (4 mL), DMT removal with CH_2Cl_2 (3 mL), water (26 µL) and DCA solution (6% in CH_2Cl_2, 6 mL), and precipitations from CH_2Cl_2/MeOH (4 mL, 2:1, *v/v*) with MTBE (10 mL). The detritylated hybrid **16** was isolated in a yield of 414 mg (92 µmol, 91%). **(TTT)$_4$TOA** (**16d**). General Protocol B was employed to obtained **16d**, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R (**16d**) = 27 min, MALDI-TOF-MS *m/z* calcd for $C_{130}H_{172}N_{24}O_{88}P_{12}$ $[M-H]^-$ 3849, obsd 3849.

($A^{Bz}A^{Bz}A^{Bz}$)$_4$TOA (**19**). General Protocol A was employed, starting from **18** (129 mg, 32.5 µmol) and **12a** (279 mg, 320 µmol, 10 equiv) in DMF (0.8 mL) and tetrazole solution (0.94 mL, 0.42 mmol, 1.3 equiv) for 47 h at 5 °C, followed by oxidation with *t*-BuOOH (89 µL, 0.48 mmol, 1.5 equiv), washing with MeOH (5 mL), DMT deprotection with CH_2Cl_2 (2 mL), water (7 µL) and DCA solution (6% in CH_2Cl_2, 3 mL), and precipitations from CH_2Cl_2/MeOH (1 mL, 4:1, *v/v*) with MTBE (10 mL). Hybrid **19** could be isolated with a yield of 174 mg (29 µmol, 92%). **(AAA)$_4$TOA** (**19d**). General Protocol B was employed to obtained **19d** followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 29 min, MALDI-TOF-MS *m/z* calcd for $C_{130}H_{160}N_{60}O_{64}P_{12}$ $[M-H]^-$ 3957, obsd 3958.

(T*$C^{Bz}A^{Bz}$)$_4$TOA (**22**). The extension cycle leading to **22** was performed according to General Protocol A, starting with coupling with **21** (1.4 g, 349 µmol) and **12t** (2.3 g, 3.1 mmol, 9 equiv.) in DMF (5.9 mL) and tetrazole (8.4 mL, 3.7 mmol, 1.2 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (743 µL, 4.1 mmol, 1.3 equiv.), DMT removal with CH_2Cl_2 (5 mL), water (66 µL) and DCA solution (6% in CH_2Cl_2, 20 mL), and precipitations. Hybrid **21** was obtained in a yield of 1.6 g (310 µmol, 87%). **(TCA)$_4$TOA** (**22d**). General Protocol B was employed to obtained **22d**, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 26.4 min, MALDI-TOF-MS *m/z* calcd for $C_{126}H_{164}N_{40}O_{76}P_{12}$ $[M-H]^-$ 3825, obsd 3822.

(T*T*T*T*)$_4$TOA (**17**). The synthesis followed General Protocol A, starting from **16** (314 mg, 70 µmol) and **12t** (512 mg, 0.7 mmol, 10 equiv), in DMF (1.6 mL) and tetrazole (2 mL, 0.9 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (191 µL, 1 mmol, 1.5 equiv), washing with MeOH (4 mL), DMT deprotection with CH_2Cl_2 (2 mL), water (10 µL) and DCA solution (6% in CH_2Cl_2, 3 mL), and precipitations from CH_2Cl_2/MeOH (3 mL, 3:1, *v/v*) with MTBE (20 mL). Hybrid **17** was isolated in a yield of 253 mg (40 µmol, 57%). **(TTTT)$_4$TOA** (**17d**). The synthesis of **17d** was performed according to General Protocol B, starting from **17** (30 mg, 5 µmol). An analytical sample was purified by HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R (**17d**) = 35 min, MALDI-TOF-MS *m/z* calcd for $C_{170}H_{224}N_{32}O_{116}P_{16}$ $[M-H]^-$ 5066, obsd 5066.

($A^{Bz}A^{Bz}A^{Bz}A^{Bz}$)$_4$TOA (**20**). The extension cycle leading to **20** was performed according to General Protocol A, starting with coupling with **19** (133 mg, 23 µmol) and **12a** (215 mg, 250 µmol, 11 equiv) in DMF (579 µL) and tetrazole (724 µL, 0.32 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (68 µL, 0.37 mmol, 1.5 equiv), washing with MeOH (5 mL), DMT removal with CH_2Cl_2 (2 mL), water (5 µL) and DCA solution (6% in CH_2Cl_2, 10 mL), and precipitations from CH_2Cl_2/MeOH (2 mL, 2:1, *v/v*) with MTBE (10 mL). Hybrid **20** was obtained in a yield of 130 mg (16 µmol, 73%). **(AAAA)$_4$TOA** (**20d**). General Protocol B was employed to obtained **20d**, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 30 min, MALDI-TOF-MS *m/z* calcd for $C_{170}H_{208}N_{80}O_{84}P_{16}$ $[M-H]^-$ 5210, obsd 5211.

($G^{iBu}C^{Bz}G^{iBu}C^{Bz}$)$_4$TOA (**27**). Hybrid **27** was prepared following General Protocol A, starting from **26** (745 mg, 137 µmol) and **12g** (1.03 g, 1.2 mmol, 9 equiv) in DMF (3 mL) and tetrazole (3.6 mL, 1.6 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (336 µL, 1.85 mmol, 1.5 equiv), DMT removal with CH_2Cl_2 (2 mL), water (22 µL) and DCA solution (6% in CH_2Cl_2, 8 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 8:1, *v/v*) with MTBE (45 mL). The detritylated hybrid **27** was isolated in a yield of 807 mg (109 µmol, 82%). **(GCGC)$_4$TOA** (**6**). Hybrid **6** was prepared following General Protocol B, starting from **27** (90 mg, 12 µmol). The product **6** was purified by HPLC, using a gradient of CH_3CN (1-25% in 60 min), with elution of hybrid **6** at t_R = 25 min, yield 34 mg (6.6 µmol, 55%). MALDI-TOF-MS *m/z* calcd for $C_{162}H_{208}N_{64}O_{100}P_{16}$ $[M-H]^-$ 5146, obsd 5145.

($C^{Bz}T^*C^{Bz}A^{Bz}$)$_4$TOA (**23**). The synthesis followed General Protocol A, starting from **22** (1.5 g, 279 µmol) and **12c** (2.3 g, 2.79 mmol, 10 equiv.) in DMF (4.9 mL) and tetrazole (6.95 mL, 3.1 mmol, 1.2 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (650 µL, 3.6 mmol, 1.3 equiv.), DMT deprotection with CH_2Cl_2 (5 mL) and H_2O (60 µL) and DCA solution (6% in CH_2Cl_2, 20 mL), and precipitations. Detritylated hybrid **23** was obtained in a yield of 1.8 g (248 µmol, 89%). **(CTCA)$_4$TOA** (**23d**). Compound **23d** was obtained from **23** using General Protocol B, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 27.8 min, MALDI-TOF-MS *m/z* calcd for $C_{162}H_{212}N_{52}O_{112}P_{16}$ $[M-H]^-$ 4982 obsd 4983.

(T*T*T*T*T*)$_4$TOA (**2p**). The hybrid **2p**, where "p" denotes the protected form of **2**, was prepared following General Protocol A involving coupling of **17** (205 mg, 35 µmol) and **12t** (284 mg, 0.38 mmol, 11 equiv.), in DMF (0.8 mL) and tetrazole (1.1 mL, 0.5 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (104 µL, 0.57 mmol, 1.5 equiv), washing with MeOH (4 mL), DMT removal with CH_2Cl_2 (2 mL), water (3 µL) and DCA solution (6% in CH_2Cl_2, 3 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 3:1, *v/v*) with MTBE (45 mL). The detritylated was isolated in a yield of 119 mg (19 µmol, 55%). **(TTTTT)$_4$TOA** (**2**). The synthesis of **2** was performed according to General Protocol B, starting from **2p** (119 mg, 19 µmol), followed by purification via HPLC, using a gradient of CH_3CN (1-20% in 65 min), t_R (**2**) = 37 min, yield 83 mg (13.2 µmol, 38%). MALDI-TOF-MS *m/z* calcd for $C_{210}H_{276}N_{40}O_{144}P_{20}$ $[M-H]^-$ 6283, obsd 6280.

($A^{Bz}A^{Bz}A^{Bz}A^{Bz}A^{Bz}$)$_4$TOA (**3p**). The extension cycle leading to **3p** performed according to General Protocol A, starting with coupling with **20** (100 mg, 13 µmol) and **12a** (133 mg, 0.15 mmol, 12 equiv.), in DMF (0.36 mL) and tetrazole (0.5 mL, 0.25 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (42 µL, 0.23 mmol, 1.5 equiv), washing with MeOH (5 mL), DMT deprotection with CH_2Cl_2 (2 mL), water (2 µL) and DCA solution (6% in CH_2Cl_2, 4 mL), and precipitations from CH_2Cl_2/MeOH (1 mL, 4:1, *v/v*) with MTBE (5 mL). Hybrid **3p** was isolated in a yield of 83 mg (8.6 µmol, 67 %). **(AAAAA)$_4$TOA** (**3**). General

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Protocol B was employed to obtained **3**, starting from **3p** (83 mg, 8.6 µmol), followed by purification via HPLC, using a gradient of CH_3CN (1-20% in 65 min), with elution of hybrid **3** at t_R = 31 min., yield 38 mg (5.8 µmol, 45%). MALDI-TOF-MS *m/z* calcd for $C_{210}H_{256}N_{100}O_{104}P_{20}$ $[M-H]^-$ 6463, obsd 6463.

$(C^{Bz}G^{iBu}C^{Bz}G^{iBu}C^{Bz})_4$TOA (**28**). The extension cycle leading to **28** was performed according to General Protocol A, starting with coupling with **27** (706 mg, 92 µmol) and **12c** (736 mg, 915 µmol, 10 equiv) in DMF (2.1 mL) and tetrazole (2.6 mL, 1.19 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (250 µL, 1.4 mmol, 1.5 equiv), washing with MeOH (5 mL), DMT removal with CH_2Cl_2 (4 mL), water (10 µL) and DCA solution (6% in CH_2Cl_2, 6 mL), and precipitations from CH_2Cl_2/MeOH (1 mL, 5:3, *v/v*) with MTBE (10 mL). Hybrid **28** was isolated in a yield of 608 mg (64 µmol, 70%). **$(CGCGC)_4$TOA** (**28d**). The hybrid **28d** was prepared following General Protocol B, starting from **28** (90 mg, 9.5 µmol). An analytical sample was purified by HPLC, using a gradient of CH_3CN (1-20% in 65 min), t_R = 30 min, MALDI-TOF-MS *m/z* calcd for $C_{198}H_{256}N_{76}O_{124}P_{20}$ $[M-H]^-$ 6303, obsd 6308.

$(C^{Bz}C^{Bz}T^*C^{Bz}A^{Bz})_4$TOA (**24**). General Protocol A was employed, starting from **23** (1.6 g, 229 µmol, 1 equiv.) and **12c** (2.1 g, 2.5 mmol, 11 equiv.) in DMF (4.7 mL) and tetrazole (6.8 mL, 2.8 mmol, 1.2 equiv.) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (598 µL, 3.3 mmol, 1.3 equiv.), detritylation with CH_2Cl_2 (5 mL), H_2O (45 µL) and DCA solution (6 % in CH_2Cl_2, 23 mL), and precipitations. Hybrid **24** was isolated in a yield of 1.7 g (188 µmol, 82%). **$(CCTCA)_4$TOA** (**24d**). Compound **24d** was obtained from **24** using General Protocol B, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R = 30.8 min, MALDI-TOF-MS *m/z* calcd for $C_{198}H_{260}N_{64}O_{124}P_{20}$ $[M-H]^-$ 6139, obsd 6139.

$(G^{iBu}C^{Bz}G^{iBu}C^{Bz}G^{iBu}C^{Bz})_4$TOA (**29**). Hybrid **29** was prepared following General Protocol A, starting with coupling of **28** (508 mg, 55.4 µmol) and **12g** (511 mg, 609 µmol, 11 equiv) in DMF (1.5 mL) and tetrazole (1.7 mL, 0.79 mmol, 1.3 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (166 µL, 0.9 mmol, 1.5 equiv), washing with MeOH (5 mL), DMT removal with CH_2Cl_2 (4 mL), water (11 µL) and DCA solution (6% in CH_2Cl_2, 7 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 5:1, *v/v*) with MTBE (15 mL). Detritylated hybrid **29** was isolated in a yield of 516 mg (47 µmol, 85%). **$(GCGCGC)_4$TOA** (**7**). Hybrid **7** was prepared following General Protocol B, starting from **29** (68 mg, 6.2 µmol). Product **7** was purified by HPLC, using a gradient of CH_3CN (1-20% in 60 min), with elution of hybrid **7** at t_R = 28 min, yield 43 mg (5.6 µmol, 77%). MALDI-TOF-MS *m/z* calcd for $C_{238}H_{304}N_{96}O_{148}P_{24}$ $[M-H]^-$ 7619, obsd 7623.

$(T^*C^{Bz}C^{Bz}T^*C^{Bz}A^{Bz})_4$TOA (**4p**). The synthesis followed General Protocol A, starting from **24** (1.5 g, 173 µmol,) and **12t** (1.6 g, 2.1 mmol, 12 equiv.) in DMF (3.9 mL) and tetrazole (6.6 mL, 2.5 mmol, 1.2 equiv.) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (492 µL, 2.7 mmol, 1.3 equiv.), DMT deprotection with CH_2Cl_2 (6 mL), H_2O (22 µL), and DCA (16 mL, 6 % in CH_2Cl_2), and precipitations. Hybrid **4p** was isolated in a yield of 1.68 g (146 µmol, 84%). **$(TCCTCA)_4$TOA** (**4**). General Protocol B was employed to obtained **4**, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R (**4**) = 34 min, yield 40 µmol, 23%. MALDI-TOF-MS *m/z* calcd for $C_{238}H_{312}N_{72}O_{152}P_{24}$ $[M-H]^-$ 7353, obsd 7357.

$(C^{Bz}G^{iBu}C^{Bz}G^{iBu}C^{Bz}G^{iBu}C^{Bz})_4$TOA (**30**). The extension cycle leading to **30** was performed according to General Protocol A, starting with coupling with **29** (429 mg, 39 µmol) and **12c** (390 mg, 468 µmol, 12 equiv) in DMF (1 mL) and tetrazole (1.4 mL, 0.56 mmol, 1.2 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (128 µL, 0.7 mmol, 1.5 equiv), washing with MeOH (5 mL), DMT removal with CH_2Cl_2 (3 mL), water (7 µL) and DCA solution (6% in CH_2Cl_2, 5 mL), and precipitations from CH_2Cl_2/MeOH (1 mL, 5:3, *v/v*) with MTBE (15 mL). Detritylated hybrid **30** was isolated in a yield of 449 mg (35 µmol, 90%). **$(CGCGCGC)_4$TOA** (**30d**). Hybrid **30d** was prepared following General Protocol B, starting from **30** (64 mg, 5 µmol). An analytical sample was purified by HPLC, using a gradient of CH_3CN (1-15% in 45 min), t_R (**30d**) = 29 min, MALDI-TOF-MS *m/z* calcd for $C_{274}H_{352}N_{108}O_{172}P_{28}$ $[M-H]^-$ 8776, obsd 8777.

$(G^{iBu}C^{Bz}G^{iBu}C^{Bz}G^{iBu}C^{Bz}G^{iBu}C^{Bz})_4$TOA (**8p**). The hybrid **8p**, where "p" denotes the protected form of **8**, was prepared following General Protocol A involving coupling of **30** (377 mg, 30 µmol) and **12g** (322 mg, 383 µmol, 13 equiv) in DMF (1.5 mL) and tetrazole (1.5 mL, 0.56 mmol, 1.2 equiv) for 48 h at 5 °C, followed by oxidation with *t*-BuOOH (104 µL, 0.7 mmol, 1.5 equiv), washing with MeOH (5 mL), DMT deprotection with CH_2Cl_2 (3 mL), water (4 µL) and DCA solution (6% in CH_2Cl_2, 4 mL), and precipitations from CH_2Cl_2/MeOH (5 mL, 5:1, *v/v*) with MTBE (15 mL). Hybrid **8p** was isolated in a yield of 350 mg (24 µmol, 80%). **$(GCGCGCGC)_4$TOA** (**8**). General Protocol B was employed to obtained **8**, followed by purification via HPLC, using a gradient of CH_3CN (1-15% in 45 min), **8** t_R (**8**) = 34 min., yield 17.1 µmol, 57%. MALDI-TOF-MS *m/z* calcd for $C_{314}H_{400}N_{128}O_{196}P_{32}$ $[M-H]^-$ 10093, obsd 10095.

Acknowledgements

The authors thank Shiliang He for discussions, Tanja Walter for measuring melting curves, Peter Tremmel for the acquisition of NMR spectra, and Helmut Griesser for a review of parts of the manuscript. This work was supported by DFG (grant No. RI 1063/15-1 to C.R.).

Keywords: Oligonucleotides • DNA • solution-phase synthesis • phosphoramidites • adamantane

[1] N. C. Seeman, *Nature* **2003**, 421, 427-431.
[2] B. Saccà, C. M. Niemeyer, *Angew. Chem. Int. Ed.* **2012**, *51*, 58-66.
[3] F. Zhang, J. Nangreave, Y. Liu, H. Yan, *J. Am. Chem. Soc.* **2014**, *136*, 11198–11211.
[4] F. A. Aldaye, A. L. Palmer, H. F. Sleiman, *Science* **2008**, *321*, 1795-1799.
[5] L. H. Tan, H. Xing, Y. Lu, *Acc. Chem. Res.* **2014**, *47*, 1881−1890.
[6] M. R. Jones, N. C. Seeman, C. A. Mirkin, *Science* **2015**, *347*, 840.
[7] C. B. Reese, *Org. Biomol. Chem.,* **2005**, *3*, 3851-3868.
[8] A. M. Michelson, A. Todd, *J. Chem. Soc.,* **1955**, 2632-2638.
[9] H. G. Khorana, W. E. Razzell, P. T. Gilham, G. M. Tener, E. H. Pol, *J. Am. Chem. Soc.*, **1957**, *79*, 1002-1003.
[10] R. L. Letsinger, K. K. Ogilvie, *J. Am. Chem. Soc.* **1969**, *91*, 3350-3355.
[11] C. B. Reese, *Tetrahedron* **1978**, *34*, 3143-3179.
[12] K. L. Agarwal, H. Büchi, M. H. Caruthers, N. Gupta, H. G. Khorana, K. Kleppe, A. Kumar, E. Ohtsuka, U. L. Rajbhandary, J. H. Van de Sande, V. Sgaramella, H. Weber, T. Yamada, *Nature* **1970**, *227*, 27-34.
[13] R. B. Merrifield, *Angew. Chem. Int. Ed.* **1985**, *24*, 799-810.
[14] H. Köster, W. Heidmann, *Angew. Chem. Int. Ed.* **1973**, *12*, 859-860.
[15] M. H. Caruthers, A. D. Barone, S. L. Beaucage, D. R. Dodds, E. F: Fisher, L. J. McBride, M. Matteucci, Z. Stabinsky, J.-Y. Tang, *Meth. Enzymol.* **1987**, *154*, 287-326.
[16] M. J. Gait, H. W. D. Matthes, M. Singh, B. S. Sproat, R. C. Titmas, *Nucleic Acid Res.* **1982**, *10*, 6243-6254.
[17] B. C. Froehler, P. G. Ng, M. D. Matteucci, *Nucleic Acids Res*, **1986**, *14*, 5399-5407.
[18] M. D. Matteucci, M. H. Caruthers, *J. Am. Chem. Soc.* **1981**, *103*, 3185-3191.
[19] M. H. Caruthers, *Science* **1985**, *230*, 281-285.

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[20] E. M. LeProust, B. J. Peck, K. Spirin, H. Brummel McCuen, B. Moore, E. Namsaraev, M. H. Caruthers, *Nucleic Acids Res.* **2010**, *38*, 2522-2540.
[21] M. H. Caruthers, *J. Biol. Chem.* **2013**, *288*, 1420-1427.
[22] T. Wada, Y. Sato, F. Honda, S. Kawahara, M. Sekine, *J. Am. Chem. Soc.* **1997**, *119*, 12710-12721.
[23] M. Sekine, A. Ohkubo, K. Seio, *J. Org. Chem.*, **2003**, *68*, 5478-5492.
[24] V. Kungurtsev, P. Virta, H. Lönnberg, *Eur. J. Org. Chem.* **2013**, 7886-7890.
[25] J. F. Kim, P. R. J. Gaffney, I. B. Valtcheva, G. Williams, A. M. Buswell, M. S. Anson, A. G. Livingston, *Org. Process Res. Dev.* **2016**, *20*, 1439-1452.
[26] M. C. De Koning, A. B. T. Ghisaidoobe, H. I. Duynstee, P. B. W. Ten Kortenaar, D. V. Filippov, G. A. van der Marel, *Org. Process Res. Dev.* **2006**, 10, 1238-1245
[27] T. Abramova, *Molecules* **2013**, *18*, 1063-1075.
[28] R. Raetz, B. Appel, S. Müller, *Chem. Today* **2016**, *34*, 14-17.
[29] B. Virnekäs, L. Ge, A. Plückthun, K. C. Schneider, G. Wellnhofer, S. E. Moroney, *Nucleic Acids Res.* **1994**, *22*, 5600-5607.
[30] J. Yáñez, M. Argüello, J. Osuna, X. Soberón, P. Gaytán, *Nucleic Acids Res.* **2004**, *32*, e158.
[31] B. J. Hong, I. Eryazici, R. Bleher, R. V. Thaner, C. A. Mirkin, S. T. Nguyen, *J. Am. Chem. Soc.* **2015**, 137, 8184−8191.
[32] R. Pathak, A. Marx, *Chem. Asian J.* **2011**, *6*, 1450-1455.
[33] H. Bußkamp, S. Keller, M.Robotta, M. Drescher, A. Marx, *Beilstein J. Org. Chem.* **2014**, *10*, 1037-1046.
[34] M. Meng, C. Ahlborn, M. Bauer, O. Plietzsch, S. A. Soomro, A. Singh, T. Muller, W. Wenzel, S. Bräse, C. Richert, *ChemBioChem* **2009**, *10*, 1335-1339.
[35] A. Singh, M. Tolev, M. Meng, K. Klenin, O. Plietzsch, C. I. Schilling, T. Muller, M. Nieger, S. Bräse, W. Wenzel, C. Richert, *Angew. Chem., Int. Ed.* **2011**, *50*, 3227-3231.
[36] A. Schwenger, C. Gerlach, H. Griesser, C. Richert, *J. Org. Chem.* **2014**, *79*, 11558-11566.
[37] G. R: Newkome, Z. Yao, G. R. Baker, V. K. Gupta, *J. Org. Chem.* **1985**, *50*, 2003-2004.
[38] D. A. Tomalia, A. M. Naylor, W. A. Goddard, *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138-175.
[39] A. Singh, M. Tolev, C. I. Schilling, S. Bräse, H. Griesser, C. Richert, *J. Org. Chem.* **2012**, *77*, 2718-2728.
[40] A. Schwenger, W. Frey, C. Richert, *Chem. Eur. J.* **2015**, *21*, 8781-8789.
[41] H. Stetter, C. Wulff, *Chem. Ber.* **1960**, *93*, 1366-1371.
[42] H. Stetter, M. Krause, *Liebigs Ann. Chem.* **1968**, *717*, 60-63.
[43] H. Griesser, M. Tolev, A. Singh, T. Sabirov, C. Gerlach, C. Richert, *J. Org. Chem.* **2012**, *77*, 2703-2717.
[44] B. J. Hong, V. Y. Cho, R. Bleher, G. C. Schatz, S. T. Nguyen, *J. Am. Chem. Soc.* **2015**, *137*, 13381-13388.

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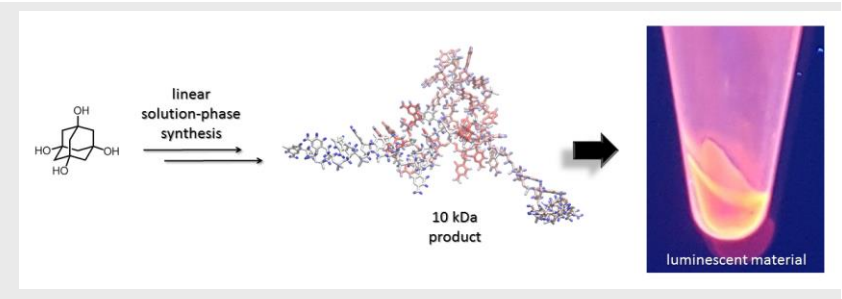

Oligonucleotide Synthesis

*A. Schwenger, N. Birchall, C. Richert**

Page No. – Page No.

Solution-Phase Synthesis of Branched Oligonucleotides with up to 32 Nucleotides and the Reversible Formation of Materials

Table Contents Text

A protocol for the solution-phase synthesis of branched oligodeoxynucleotides was developed that can be scaled up readily and that produces products with the ability to form materials through base pairing.

Key Topic

DNA Synthesis

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