DNA Nanostructures

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# Self-Assembly of a DNA Dodecahedron from 20 Trisoligonucleotides with $C_{3h}$ Linkers**

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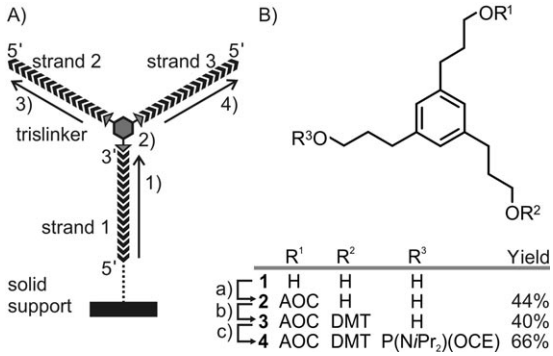
Since Seeman's pioneering work,[1a–c] DNA has been recognized as a building material for programmable hollow 3D nanoobjects. DNA nanoconstruction benefits from the structural rigidity of short DNA double strands, scalability, good accessibility of synthetic and chemically modified DNA, and the option for enzymatic amplification and processing. Four strategies for the construction of nanoobjects such as polyhedra exist so far. Strategy I is vertex-centered and goes from noncovalent junctions to covalent objects: Noncovalent three-way junctions are assembled from three linear oligonucleotides. Each arm contains a sticky end sequence which is hybridized to its complement in another junction and then covalently connected by DNA ligases.[1] Strategy II is the reverse of strategy I and makes use of trisoligonucleotides, in other words covalent junctions are noncovalently assembled to give the target nanoobjects.[2d,e] Non-natural modes of copying[2a] and amplifying[2b] were proposed to enable the replication of junctions and nanoconstructs from the latter.[2c] Strategy III is a face-centered approach, employing as many oligonucleotides as there are faces on the object while each oligonucleotide is composed of as many segments as there are edges surrounding the faces.[3a–d] Strategy IV first defines the longest path through the object by connecting all vertices using a very long DNA single strand; a set of shorter oligonucleotides generates suitable rigid motifs such as double crossovers, while additional connectivities are expressed by means of paranemic crossover motifs.[4] Recently the assembly of triangular prisms, cubes, pentameric and hexameric prisms, heteroprisms, and biprisms was reported. A set of single-stranded linear and cyclic DNA building blocks was used, and in the latter case rigid organic linker molecules were used as vertices.[5]

Herein we report on a new generation of trisoligonucleotides and their employment in benchmark experiments to evaluate strategy II. We selected a dodecahedron, as polyhedra with a smaller number of vertices have been described already.[1–5] The feasibility of constructing a dodecahedron that reflects the basic symmetry of a virus was forseen for strategy III,[1d] but so far this has not been achieved by any strategy.

Previously prepared trisoligonucleotides with three different arms were based on asymmetric linker constructs,[2a,e,f,6] so that in principle a set of three different sequences could be connected in three different ways. Linker scaffolds with $C_{3h}$ symmetry are thought to be advantageous because all vertices are expected to be subject to the same conformational constraints. Moreover, diastereomeric mixtures obtained by the utilization of commercially available racemic linker amidites[6b,7] are avoided here.

Until now trisoligonucleotides with $C_{3h}$ linkers were synthesized by chemical copying of connectivity, that is the usage of a 3′-connected trisoligonucleotide template for the trislinking of suitable 5′-functionalized linear oligonucleotides.[2a] $C_{3h}$-symmetric linker molecules have also been used to synthesize branched oligonucleotides having mixed sequence directions[8a] or equal sequence direction but two[8b] or three[8c] identical sequences. Very recently, a synthesis of branched oligonucleotides with a $C_{3h}$-symmetric linker has been described, which is similar to our work presented here.[8d]

Scheme 1 shows the principle of our approach. Target trisoligonucleotides with three different sequences are assembled on a DNA synthesizer by employing a trislinker amidite orthogonally protected with 4,4′-dimethoxytrityl (DMT) and




***Scheme 1.*** A) Trisoligonucleotide synthesis: 1) The first strand is constructed in the 5′→3′ direction using "reverse" 5′-nucleoside amidites. 2) Trislinker amidite **4** is coupled and subsequently detritylated. 3) The second strand is synthesized in the 3′→5′ direction using standard 3′-nucleoside amidites. 4) After Pd-catalyzed removal of the AOC protecting group, the third strand is assembled, again in the 3′→5′ direction. B) Synthesis of the trislinker amidite: a) 0.85 equiv allyloxycarbonyl chloride (AOC-Cl), 0.85 equiv pyridine, THF; b) 0.85 equiv 4,4′-dimethoxytrityl chloride (DMT-Cl), pyridine; c) 1.3 equiv 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylaminophosphorodiamidite, 1.3 equiv diisopropylammonium tetrazolide, dichloromethane. Yields calculated after chromatographic purification.

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

allyloxycarbonyl groups (AOC). The first strand is synthesized using reverse amidites (in the 5′–3′ direction).[9a,b] Following the coupling of trislinker **4**, which is derived from the $C_{3h}$ alcohol **1**, the second and third strands are subsequently synthesized after detritylation and Pd-catalyzed removal of the AOC protecting group, respectively (Scheme 1 A).

Trislinker amidite **4** is obtained from the trisalcohol 1,3,5-tris(hydroxypropyl)benzene (**1**) by successive treatment with a) allyloxycarbonyl chloride in the presence of pyridine to yield bisalcohol **2**, then with b) 4,4′-dimethoxytrityl chloride in pyridine to give alcohol **3,** and finally with c) Bannwarth's reagent in the presence of diisopropylammonium tetrazolide to give phosphoramidite **4** (Scheme 1 B).

The set of trisoligonucleotides was synthesized on a 1.3-μmol scale on a commercially available polystyrene support to which the 5′-starter nucleoside had been attached by means of a succinic ester linkage.[9c,12] After cleavage from the support, the trisoligonucleotides were purified by polyacrylamide gel electrophoresis (PAGE), extracted, desalted by size exclusion chromatography (NAP-25 columns), quantified by UV spectroscopy, and characterized by MALDI-MS. Yields were between 30 and 100 nmol after purification.

A regular dodecahedron consists of twelve regular pentagonal faces, three meeting at each vertex to give 30 edges and 20 vertices. To create a "stick model" of a dodecahedron, a set of 20 trisoligonucleotides is required: The centers of the trisoligonucleotides represent the vertices, the edges are formed by hybridization of complementary strands of proximate trisoligonucleotides. A pool of 30 independent 15mer double-stranded DNA sequences with narrow melting temperatures was designed using the DNA sequence generator developed by Feldkamp et al.[10] (conditions: $T_m$ between 52.3 and 56.2 °C, neutral pH, 50 mM NaCl). Double-stranded sequences were then designated as the edges of the dodecahedron, leading to a Schlegel representation for vertex enumeration (Figure 1) and a table of trisoligonucleotides defining the respective vertex connectivities (Table 1).

For the self-assembly of the dodecahedron all 20 trisoligonucleotides were combined in equimolar quantities (typical experiment: 0.5 μM per trisoligonucleotide, 10 mM HEPES buffer (pH 7.4), 100 mM NaCl). Best results were achieved when submicromolar concentrations of each trisoligonucleotide were used along with a temperature program that provides a melting step (90 °C, 5 min), an annealing step at 45 °C (approximately 10 °C below the melting point of the double strands) for at least 30 min, and a final cooling step down to 4 °C. Native agarose gel electrophoresis of these annealing mixtures showed one single discrete band with an electrophoretic mobility comparable to that of linear double-stranded DNA of 400 (3 % gel) to 450 base pairs (2 % gel); this is in agreement with the actual number of 450 base pairs for the completely assembled dodecahedron (Figure 2, see also the Supporting Information).

Treatment of the noncovalent product with mung bean nuclease did not lead to any change of electrophoretic mobility, while the digestion of assembly subsets resulted in such changes (Figure 2). This is to be expected because the fully assembled dodecahedron contains only double-stranded interconnections, while subsets necessarily contain digestible single-stranded arms. As observed in earlier studies on a trisoligonucleotide tetrahedron, the fully assembled product

***Table 1:*** Trisoligonucleotide sequences assigned to each of the 20 vertices of the dodecahedron; connectivities as in Figure 1.

| Sequence 5′–3′ | Vertex | Sequence 5′–3′ | Vertex |
|---|---|---|---|
| CGTACTGCCTCTTTC | v1 | CTCGAACCTGAACTG | v11 |
| GCATGACGGAGAAAG | | GAGACACGGAAGATC | |
| CACTAGCAGCTAGAC | | GGCGAAACGTTATAC | |
| GTCTAGCTGCTAGTG | v2 | GTAGCGGATATGCTG | v12 |
| CCTCACGAATCTAGC | | GTATAACGTTTCGCC | |
| CAGCATATCCGCTAC | | GAACGAAGGATTCGG | |
| GCTAGATTCGTGAGG | v3 | CCGAATCCTTCGTTC | v13 |
| CGCATATGGAAAGCC | | GTTCAGTCTTAGCCG | |
| CGGATCTTGCTAGAC | | GTCGCGTATCTATGG | |
| GGCTTTCCATATGCG | v4 | CTTTCTCCGTCATGC | v14 |
| CTGAGTCGCTTACAC | | CGGCTAAGACTGAAC | |
| CTGAGCTGTCGATAC | | GAGGTCATAAGCGAG | |
| GAAAGAGGCAGTACG | v5 | CTCTAGGAGATGCAG | v15 |
| GTATCGACAGCTCAG | | CTCGCTTATGACCTC | |
| CTACTCTGATCGCAC | | GAACAGATCGCTTGG | |
| GTGCGATCAGAGTAG | v6 | CCAAGCGATCTGTTC | v16 |
| CTGCATCTCCTAGAG | | GCATTCCGCAAAAAG | |
| CATTGCTGTATTGCG | | GTCTAAGGTCTTGCG | |
| CGCAATACAGCAATG | v7 | CCATAGATACGCGAC | v17 |
| CTCGTTCAAGACAGG | | CTTTTTGCGGAATGC | |
| CGTCTGATGTGTACG | | GATCACTCTTGCGTG | |
| GTGTAAGCGACTCAG | v8 | GATCTTCCGTGTCTC | v18 |
| CCTGTCTTGAACGAG | | CACGCAAGAGTGATC | |
| GAACGTTAATACGGC | | GATGACTCTGTAGCG | |
| GCCGTATTAACGTTC | v9 | CGCTAGTAAGGTGTG | v19 |
| CTTACGAATCCGGTG | | CGCTACAGAGTCATC | |
| CACACCTTACTAGCG | | GTTGTAGAGGAAGCG | |
| GTCTAGCAAGATCCG | v10 | CGTACACATCAGACG | v20 |
| CACCGGATTCGTAAG | | CGCAAGACCTTAGAC | |
| CAGTTCAGGTTCGAG | | CGCTTCCTCTACAAC | |

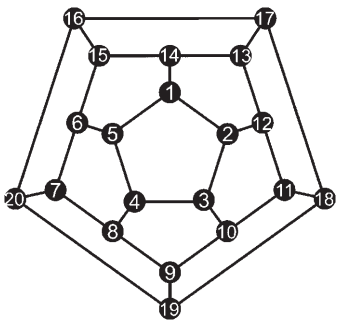


***Figure 1.*** Schlegel representation with numbers of trisoligonucleotides assigned to the vertices (see Table 1). Each 3′ end is attached to trislinker **1** by means of a phosphodiester linkage.

  

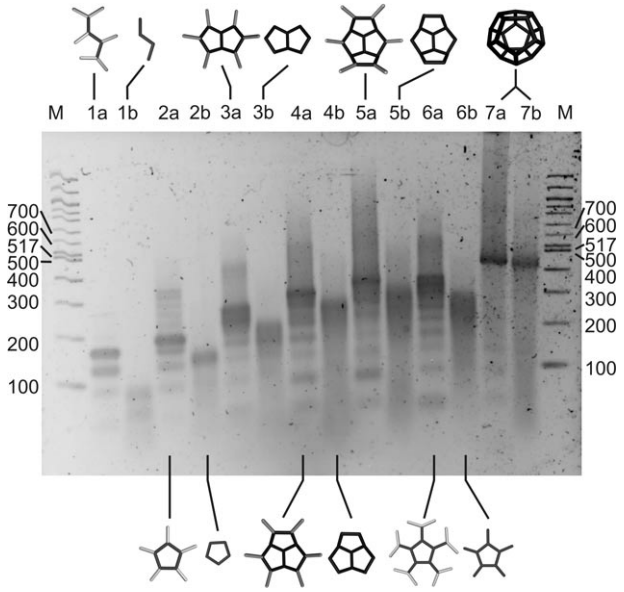


***Figure 2.*** Native agarose gel (2%) of the dodecahedron and assembly subsets a) before and b) after treatment with mung bean nuclease (digestion of single-stranded DNA). Lane M: 100-bp DNA ladder. Lanes 1a,b: v14–v17; lanes 2a,b: v13–v17; lanes 3a,b: v1, v2, v12–v17; lanes 4a,b: v1, v2, v11–v18; lanes 5a,b: v1, v2, v5, v6, v11–v18; lanes 6a,b: v1, v6, v12–v18, v20; lanes 7a,b: v1–v20.

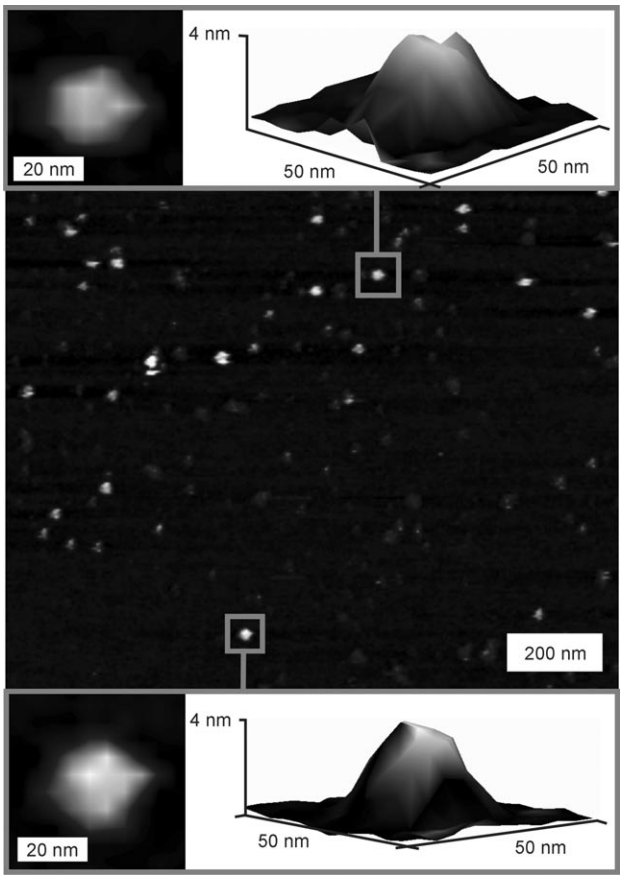


***Figure 3.*** AFM image of v1–v20 showing discrete objects having a size of about 20 nm when adsorbed on a mica surface (tapping mode, liquid phase, 10 mM HEPES buffer, pH 7, 100 mM NaCl, 1 mM $NiCl_2$).

appears as a single band while fragments often show two or more bands in the gel.[2e] We are tempted to explain the latter as an indication of cooperative structural integrity of the fully assembled object.

Atomic force microscopy of the self-assembled product in the liquid phase on mica showed quite uniform particles with a diameter of approximately 20 nm, which is in agreement with our expectations (Figure 3). The limited height of about 4 nm indicates that the dodecahedron has sufficient flexibility towards compression and adsorption. Future applications may benefit from the induced deformability of such "soft balls".

To demonstrate the usability of the dodecahedron as a scaffolding device, we assembled dodecahedra by employing one to six trisoligonucleotides in which one arm was extended to bear an overhang sequence. These objects were then exposed to solutions containing the corresponding complementary 5′-fluorescein-labeled oligonucleotides. Figure 4 shows the agarose gel of the assembled objects. As expected, the fluorescence intensity increases with the number of labels

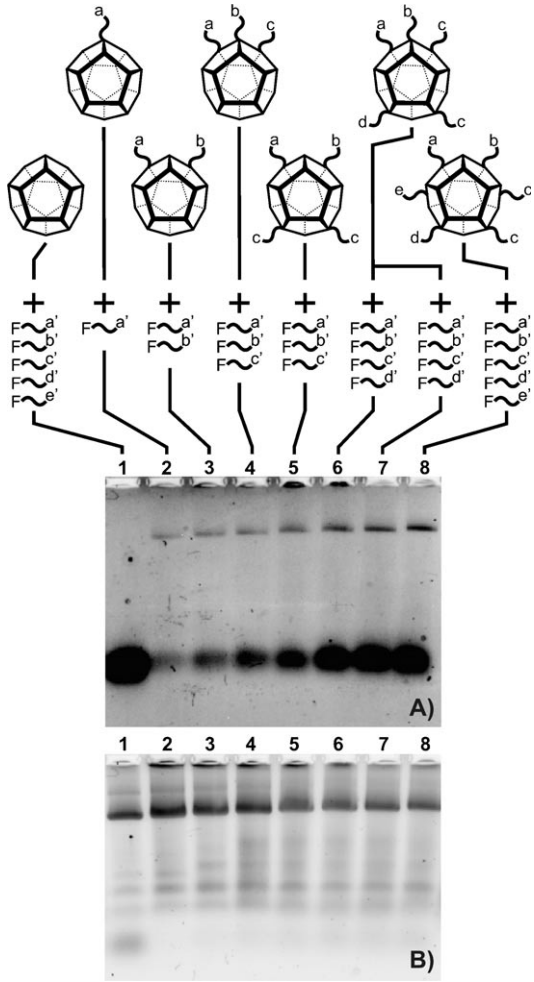


***Figure 4.*** Native agarose gel (3% agarose, 1×TBE buffer, 6.7 $V cm^{-1}$, 60 min at 5 °C) of dodecahedral assemblies containing different numbers of overhang sequences hybridized with fluorescein-labeled complementary oligonucleotides (see Table S8 in the Supporting Information), imaged A) before and B) after treatment with SYBR gold nucleic acid stain.

  

bound to the scaffold (Figure 4 A), while the concomitant decrease of electrophoretic mobility is barely detectable. Similar experiments were carried out with recently developed[11] gold clusters as labels (see the Supporting Information).

We have shown that the self-assembly of 3′-trisoligonucleotides having a $C_{3h}$-linker core generates the target dodecahedra as the main products. Sequence overhangs hybridize with hybrid molecules composed of a complementary sequence tag and modular function such as a dye or a nanoscaled cluster. Up to six positions have been successfully addressed so far, giving reason to believe that scaffolded multimodularity of highly complex assemblies is within the scope of this approach. Conceivable applications for such constructs are widespread and range from the trapping and functionalization of size-matched nanoscale objects to the construction of multimodular machines.

## Experimental Section

Compound **1** was prepared according to Ref. [12].

**2**: A solution of **1** (8.0 g, 31.7 mmol) and pyridine (2.2 mL, 2.1 g, 26.5 mmol) in 40 mL of anhydrous tetrahydrofuran under an argon atmosphere was stirred and cooled to 0 °C. A solution of allyloxycarbonyl chloride (2.85 mL, 3.24 g, 26.9 mmol) in 20 mL of dry tetrahydrofuran was added dropwise. The reaction mixture was allowed to warm up to room temperature while it was stirred for 3 h. The mixture was filtered and concentrated to dryness. The crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 3:1) to give 3.97 g (11.8 mmol, 44 %) of compound **2**. $^1$H NMR: (400 MHz, [D$_6$]DMSO): $\delta = 1.66$–$1.73$ (m, 4H, C$H_2$CH$_2$OH), 1.85–1.92 (m, 2H, C$H_2$CH$_2$OAOC), 2.52–2.59 (m, 6H, C$H_2$CH$_2$CH$_2$OH and C$H_2$CH$_2$CH$_2$OAOC), 3.41 (m$_c$, 4H, C$H_2$OH), 4.08 (t, $^3J = 6.48$ Hz, 2H, C$H_2$OAOC), 4.41 (t, $^3J = 5.08$ Hz, 2H, CH$_2$O$H$), 4.59 (dt, $^3J = 5.56$ Hz, $^5J = 1.5$ Hz, 2H, C$H_2$CH=CH$_2$), 5.23–5.35 (2 dm, $^3J_{trans} = 17.2$ Hz, $^3J_{cis} = 10.41$ Hz, 2H, CH=C$H_2$), 5.93 (m$_c$, 1H, C$H$=CH$_2$), 6.83 (s, 2H, ArH), 6.84 ppm (s, 1H, ArH). $^{13}$C NMR: (100.6 MHz, [D$_6$]DMSO): $\delta = 30.26$ (*C*H$_2$CH$_2$OAOC), 31.59 (*C*H$_2$CH$_2$CH$_2$OAOC), 32.04 (*C*H$_2$CH$_2$CH$_2$OH), 34.80 (*C*H$_2$CH$_2$OH), 60.63 (CH$_2$OH), 67.47 (*C*H$_2$OAOC), 68.14 (*C*H$_2$CH=CH$_2$), 118.69 (CH$_2$CH=*C*H$_2$), 126.08 126.48 (H-C$_{Ar}$), 132.72 (CH$_2$*C*H=CH$_2$), 141.15 142.55 (CH$_2$*C*$_{Ar}$), 154.83 ppm (O(CO)O); MALDI-TOF MS *m*/*z* calcd for $C_{19}H_{28}O_5Na$ ([*M*+Na]$^+$): 359.42; found: 359.43; ESI MS *m*/*z* calcd for [*M*+H]$^+$: 337.44; found: 337.1.

**3**: A solution of 4,4′-dimethoxytrityl chloride (3.38 g, 10 mmol) in 20 mL of anhydrous pyridine was added dropwise to a solution of **2** (3.97 g, 11.8 mmol) in 30 mL of anhydrous pyridine over a period of 30 min. The reaction mixture was stirred for 16 h at room temperature. The crude product was chromatographed on silica gel with cyclohexane/ethyl acetate (2:1, 0.5 % triethylamine) as eluent to give 2.57 g of compound **3** (4.0 mmol, 40 %). $^1$H NMR: (400 MHz, [D$_6$]DMSO): $\delta = 1.62$–$1.71$ (m, 2H, C$H_2$CH$_2$OH), 1.75–1.90 (m, 4H, C$H_2$CH$_2$O), 2.47–2.67 (m, 6H, C$H_2$CH$_2$CH$_2$), 2.97 (t, $^3J = 6.2$ Hz, 2H, C$H_2$ODMT), 3.40 (m, 2H, C$H_2$OH), 3.73 (s, 6H, OCH$_3$), 4.04 (t, $^3J = 7.0$ Hz, 2H, C$H_2$OAOC), 4.41 (t, $^3J = 5.0$ Hz, 1H, CH$_2$O$H$), 4.60 (dt, $^3J = 5.56$ Hz, $^5J = 1.52$ Hz, 2H, C$H_2$CH=CH$_2$), 5.21–5.36 (2 dm, $^3J_{trans} = 17.3$ Hz, $^3J_{cis} = 10.36$ Hz, 2H, CH=C$H_2$), 5.93 (m$_c$, 1H, C$H$=CH$_2$), 6.74–7.39 ppm (m, 16H, ArH). $^{13}$C NMR: (100.6 MHz, [D$_6$]DMSO): $\delta = 30.24$ (*C*H$_2$CH$_2$OAOC), 31.56 (*C*H$_2$CH$_2$ODMT), 31.59 (*C*H$_2$CH$_2$CH$_2$OAOC), 32.02 32.23 (*C*H$_2$CH$_2$CH$_2$O), 34.76 (*C*H$_2$CH$_2$OH), 55.43 and 55.40 (OCH$_3$), 60.63 (CH$_2$OH), 62.52 (*C*H$_2$ODMT), 67.44 (*C*H$_2$OAOC), 68.11 (*C*H$_2$CH=CH$_2$), 85.65 (C$_q$ DMT), 113.32 (C-3, C-3′, C-5, C-5′ DMT), 118.64 (CH$_2$CH=*C*H$_2$), 126.07 126.13 126.46 (H-C$_{Ar}$), 126.95 128.10 128.17 (C$_{Ar}$ DMT), 130.0 (C2, C2′, C6, C6′ DMT), 132.69 (CH$_2$*C*H=CH$_2$), 136.51 (C1, C1′ PhOCH$_3$ DMT), 141.09 142.03 142.52 (CH$_2$*C*$_{Ar}$), 145.68 (C1 Ph DMT), 154.83 (O(CO)O), 158.41 ppm (C4, C4′ PhOCH$_3$ DMT); MALDI-TOF MS *m*/*z* calcd for $C_{40}H_{46}O_7Na$ ([*M*+Na]$^+$): 661.80; found: 661.97; ESI MS *m*/*z* calcd for [*M*+Na]$^+$: 661.8; found: 661.3.

**4**: A solution of **3** (1.00 g, 1.57 mmol) **3**, diisopropylammonium tetrazolide (0.31 g, 1.86 mmol), and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylaminophosphorodiamidite (0.59 mL, 0.56 g, 1.86 mmol) in 20 mL of absolute dichloromethane was allowed to react at room temperature for 2 h in an argon atmosphere. The crude product was chromatographed on silica gel with cyclohexane/ethyl acetate (2:1, 0.5 % triethylamine) as eluent to give 0.87 g (1.03 mmol, 66 %) of compound **4**. $^1$H NMR: (400 MHz, CDCl$_3$): $\delta = 1.19$–$1.24$ (2 d, $^3J = 7.04$ Hz, 6.84 Hz, 12H, NCH(C$H_3$)$_2$), 1.88–2.02 (m, 6H, CH$_2$C$H_2$CH$_2$O), 2.63–2.70 (m, 8H, C$H_2$CH$_2$CH$_2$O and CH$_2$CN), 3.13 (t, 2H, $^3J = 6.32$ Hz, CH$_2$ODMT), 3.58–3.92 (m, 6H, OC$H_2$CH$_2$CN and C$H$(CH$_3$)$_2$ and CH$_2$CH$_2$C$H_2$OP), 3.82 (s, 6H, OCH$_3$), 4,18 (t, $^3J = 6.48$ Hz, 2H, CH$_2$OAOC), 4.66 (dt, $^3J = 5.8$ Hz, $^5J = 1.28$ Hz, 2H, C$H_2$CH=CH$_2$), 5.27–5.42 (2 dm, $^3J_{trans} = 17.2$ Hz, $^3J_{cis} = 10.4$ Hz, 2H, CH=C$H_2$), 5.92–6.04 (m, 1H, C$H$=CH$_2$), 6.81–6.87 (m, 7H, ArH), 7.20–7.48 ppm (m, 9H, ArH). $^{13}$C NMR: (100.6 MHz, CDCl$_3$): $\delta = 20.69$ 20.76 (*C*H$_2$CN), 24.90 24.97 25.04 (CH(*C*H$_3$)$_2$), 30.70 32.20 33.21 33.28 (*C*H$_2$CH$_2$O), 32.25 32.51 32.96 (*C*H$_2$CH$_2$CH$_2$O), 43.36 43.48 (*C*H(CH$_3$)$_2$), 55.54 (OCH$_3$), 58.61 58.80 (CH$_2$CH$_2$CH$_2$OP), 63.24 (*C*H$_2$ODMT), 63.33 63.50 (O*C*H$_2$CH$_2$CN), 67.87 (*C*H$_2$OAOC), 68.70 (*C*H$_2$CH=CH$_2$), 86.10 (C$_q$ DMT), 113.32 113.35 (C3, C3′, C5, C5′ DMT), 117.95 (CN), 119.20 (CH$_2$CH=*C*H$_2$), 126.31 126.41 126.71 (H-C$_{Ar}$), 126.93 128.13 128.59 (C$_{Ar}$ DMT), 130.38 (C2, C2′, C6, C6′ DMT), 132.04 (CH$_2$*C*H=CH$_2$), 137.07 (C1, C1′ PhOCH$_3$ DMT), 141.32 142.28 142.91 (CH$_2$*C*$_{Ar}$), 145.74 (C1 Ph DMT), 155.42 (O(CO)O), 158.71 ppm (C-4, C-4′ PhOCH$_3$ DMT). $^{31}$P NMR: (162 MHz, CDCl$_3$, phosphoric acid): $\delta =$ 148.71 ppm. ESI MS *m*/*z* calcd for [*M*+H]$^+$: 840.04; found: 839.4.

Polystryrene-based solid support for reverse DNA synthesis: Solid supports were synthesized by coupling 5′-*O*-succinate-3′-*O*-DMT nucleosides (synthesized according to Ref. [13]) to Custom Primer Support 200 Amino (GE Healthcare); 3′-*O*-succinate-5′-*O*-DMT nucleosides were synthesized according to Ref. [9b,c]. Coupling of the corresponding succinic acid esters to the solid support (200 μmol amino functions per gram) was achieved by combining equimolar quantities of 3′-*O*-succinate-5′-O-DMT nucleosides and amino-modified solid support with 1.3 equivalents of HBTU and 2 equivalents of triethylamine in anhydrous DMF (4 mL per gram of solid support) and gentle rotation of the reaction frit for 16 h at room temperature. Unreacted amino functions were capped by suspending 1.00 g of the modified solid support in a solution of 25 mg of DMAP and 0.5 mL of acetic acid anhydride in 7.5 mL of pyridine. The suspension was gently agitated by rotating the reaction frit for 16 h at room temperature. The resulting solid supports had loadings between 50 and 60 μmol per gram.

Trisoligonucleotide synthesis: Reverse amidites (ChemGene) were allowed to react for 5 min on a DNA synthesizer (Gene Assembler Plus) employing 5-benzylmercaptotetrazole (emp Biotec) as activator. Standard protocols for detritylation, capping, and oxidation were applied for all three strands. Coupling of linker **4** was performed in two consecutive injections (5 min each). Coupling times in the second strand were 1.5 min, while the third strand required $3 \times 15$ min for the first amidite and $3 \times 2$ min for the following. Allyl deprotection was carried out by circulating a solution of 17.1 mg of bis(diphenylphosphino)ethane, 24.7 mg of bis(dibenzylideneacetone)palladium (Acros organics), and 10.7 μL of pyrrolidine in 10.0 mL of acetonitrile for 15 min at 0.5 mL min$^{-1}$.

Trisoligonucleotide purification and characterization by MALDI-TOF MS: After cleavage from solid support (conc. ammonia, 55 °C, 16 h) products were purified by preparative denaturing PAGE, 12 % (450 V, 4 h, standard TBE buffer), extracted from the gel, and

  

desalted using NAP25 columns (Amersham Biosciences). MALDI MS analysis (Bruker Daltonics) was performed using 3-hydroxypicolinic acid (3-HPA) as the matrix.

Self-assembly studies: Trisoligonucleotides were combined stoichiometrically in HEPES buffer (10 mM, pH 7.4, 100 mM NaCl) to a final concentration of 0.5 μM. This solution was heated to 90 °C (5 min), cooled to 45 °C (0.1 °C $s^{-1}$, 40 min) and finally cooled to 4 °C (0.1 °C $s^{-1}$). Digestion of single-stranded DNA with mung bean nuclease (Promega) was performed for 30 min at 30 °C in acetate buffer (50 mM, pH 5, 1 mM $Zn^{2+}$) applying 10 units of mung bean nuclease per μg of DNA. AFM images (liquid phase, tapping mode) were recorded with silicon nitride tips (AU NM-10, tip radius 10 nm, Veeco) using a Solver Pro AFM (NT-MDT) equipped with an AU028NTF adjustment unit and processed using the Image Analysis 2.2.0 software. DNA nanostructures were fixed to mica in 10 mM HEPES, pH 7.4, 1 mM $NiCl_2$ and 100 mM NaCl.

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