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Flexibility of C_{3h}-Symmetrical Linkers in Tris-oligonucleotide-Based Tetrahedral Scaffolds

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Flexibility of tris-oligonucleotides is determined by the length of their connecting hydrocarbon chains. Tris-oligonucleotides are branched DNA building blocks with three oligonucleotide arms attached to a C_{3h}-symmetrical linker core at these chains. Four tris-oligonucleotides hybridise into a tetrahedral nanocage by sequence-determined self-assembly. The influence of methylene, ethylene and propylene chains was studied by synthesising sets of tris-oligonucleotides and analysing the relative stability of the hybridisation products against digestion by mung bean nuclease by using gel electrophoresis. Linkers with ethylene chains showed sufficient flexibility, whereas methylene-chain linkers were too rigid. Tris-oligonucleotides based on the latter still formed tetrahedral scaffolds in intermixing experiments with linkers of higher flexibility. Thus, a new generation of versatile isocyanurate-based linkers was established.

Introduction

The design strategy in DNA nanotechnology is based on sequence-controlled assembly into higher-order hybridisation motifs. It was influenced by significant contributions from Seeman[1,2] and Rothemund.[3] DNA nanostructure formation relies on the specificity of Watson–Crick base pairing as the major force for structural self-assembly[4] into nano-objects like hollow polyhedra.[5–7] These constructs are attractive as nanoscale containers for cargo transport.[8–10] The DNA backbone is unaltered, thus potentially leading to structural limitations, such as the fixed persistence length of double strands. Chemical linkers can change the chemical or structural behaviour of DNA nanostructures.[11–15] They can, for instance, connect several linear oligonucleotides together and modify the structure of a design by acting as flexible hinges.[16–20] The resulting DNA nanoconstructs can be more compact compared to DNA junction- and origami-based designs.

We previously established tris-oligonucleotides as branched DNA building blocks that use C_{3h}-symmetrical covalent linkers to connect three oligonucleotides with individual sequences.[21] The achirality of the linker, 1,3,5-trihydroxypropylbenzene, avoids diastereomeric mixtures in preparation,[22,23] and its propylene connectors enable unhindered folding of the oligonucleotide arms into polyhedral scaffolds. A set of 20 tris-oligonucleotides was successfully self-assembled into a dodecahedron with a diameter of 20 nm.[21]

However, the feasibility of shorter alkyl chains at the linker core in tris-oligonucleotide-based assembly was not explored. Here we report a systematic study on the flexibility of methylene, ethylene, and propylene chains in tris-oligonucleotide-based tetrahedral scaffolds (Scheme 1 B). Four tris-oligonucleotides are sufficient to self-assemble into a DNA tetrahedron (Scheme 1 B), the geometry of which is the simplest case of a Platonic body. It entails a stiffness unlike the case for the collapsible cube and higher-order geometries, as discussed previously.[24] In Buckminster Fuller's principle of tensegrity,[25,26] an intrinsic tension allows the tetrahedron to maintain structural stability even if an external force is applied. The highest constraint for the alkyl arms at the linker is found in this geometry and it is therefore the most interesting scaffold to study the effect of linker flexibility.

Results and Discussion

We prepared three phosphoramidites of orthogonally protected linkers for tris-oligonucleotide synthesis: the "T1 trislinker" bearing methylene chains, the "T2 trislinker" with ethylene chains and "T3 trislinker" with propylene chains (Scheme 1 A; see also Scheme S1 and S2 in the Supporting Information). The synthesis of the T3 trislinker[21] was highly improved by following a palladium(II)-catalysed Heck-type coupling reaction of *1,3,5*-tribromobenzene with methyl acrylate (following a procedure by Majchrzak et al.)[27] and allyl benzyl ether (Scheme 2). The new strategy saved up to six synthetic steps compared to previous efforts.[21,28]

All trislinkers were orthogonally protected with 4,4′-dimethoxytrityl chloride (DMT-Cl), allyl chloroformate (AOC-Cl) and (in this work) were phosphitylated in the presence of *N,N*-diiso-

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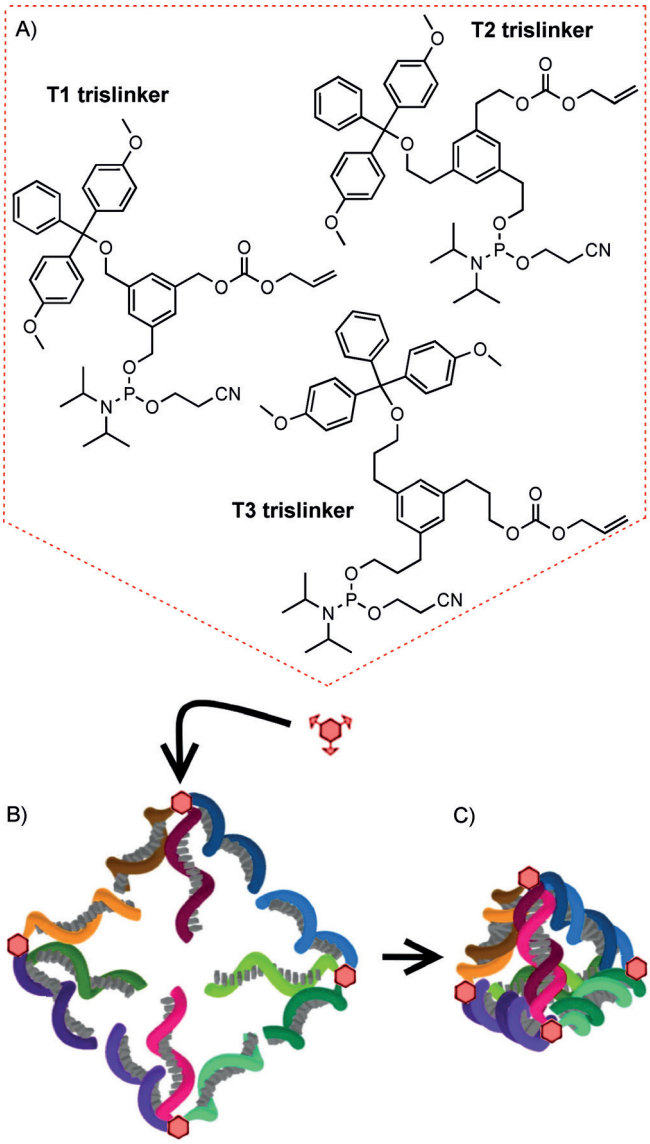
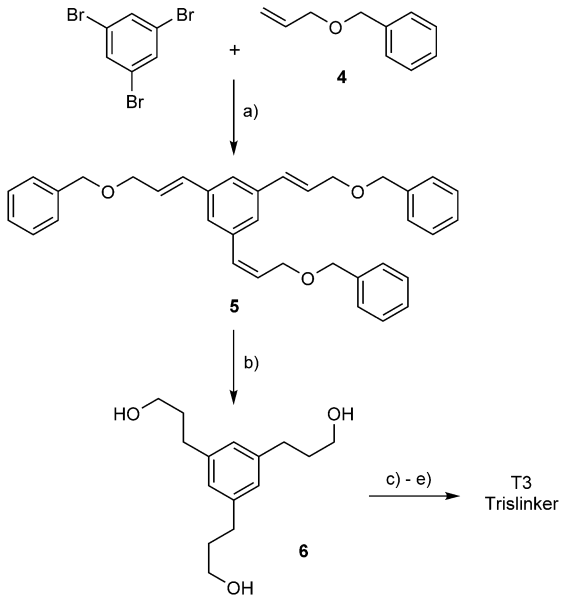


Scheme 1. A) Linker designs. B) Self-Assembly of tris-oligonucleotides into a C) tetrahedral scaffold.



Scheme 2. Synthesis of the T3 trislinker core by a Heck-coupling-type reaction of 1,3,5-tribromobenzene with allylbenzyl ether and one-step hydrogenation and hydrogenolysis. a) 3% $Pd(OAc)_2$, 6% Ph_3P, TEA, ACN, reflux, 1 d, 37%; b) 40 psi H_2,10% Pd/C, MeOH, RT, 16 h, 91%; c) AOC-Cl, Py_{abs}, THF_{abs}, 0 °C–RT, 3 h, 47%; d) DMT-Cl, Py_{abs}, RT, 16 h, 38%; e) 1.3 equiv 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, 1.3 equiv DIPEA, DCM_{abs}, RT, 2 h.

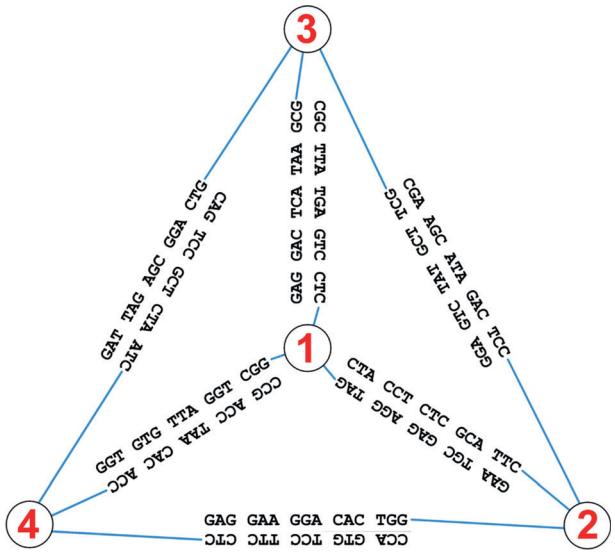


Figure 1. Schlegel representation of a tris-oligonucleotide-based tetrahedron and assigned sequences. Each tris-oligonucleotide is numbered at the trislinker, with the 3′-ends at the linker. Sequence design and numbering are identical for all sets.

propylethylamine (DIPEA) with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite.[29,30]

The sequences were designed with the DNA sequence generator program of Feldkamp et al.[31] The output consisted of a pool of 15-mer sequences with melting points of 58.3–58.4 °C at a neutral pH for a 1.25 μM solution in 110 mM sodium chloride (Table S1). The program used SantaLucia parameters[32] and the Nearest Neighbour model for duplex energy calculations. The first and last nucleotides in the sequences were G and C, in order to reduce fraying at the blunt ends. Six unique sequences were picked and assigned together with the six complementary sequences onto a Schlegel representation of a tris-oligonucleotide-based tetrahedral scaffold (Figure 1).

Three sets of four tris-oligonucleotides were prepared on a DNA synthesiser on a 1.3 μmol scale by using a previously established protocol[21] with commercial polystyrene supports, with the starter nucleoside attached by a succinic ester linkage.[34] The first strand was synthesised in the 5′→3′ direction by using reverse amidites, and followed by coupling of one trislinker per set (Figure S1). Subsequently, the second arm was synthesised after linker detritylation, and then the third arm was synthesised after palladium-catalysed removal of the AOC protecting group (synthesis direction changed to 3′→5′ for the second and third arm).

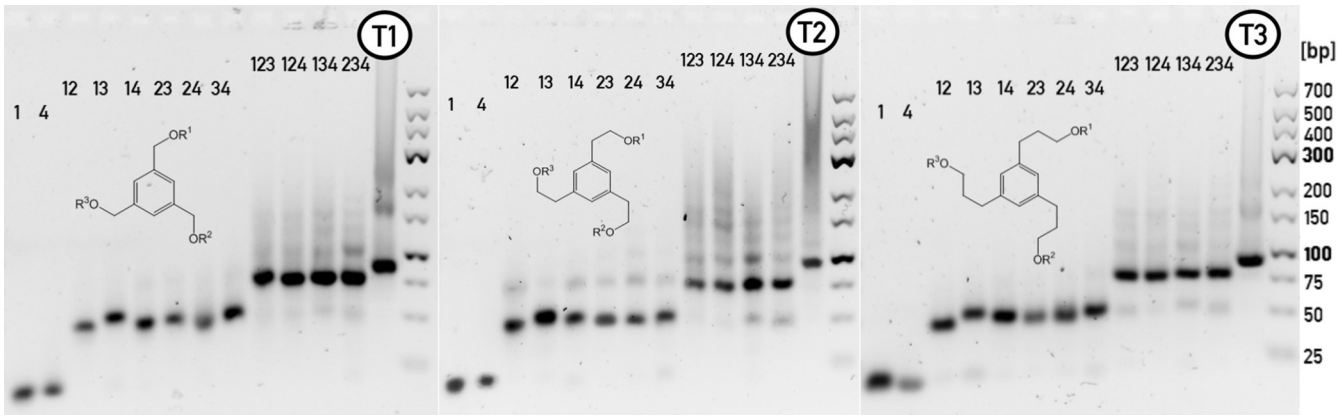


Figure 2. Gels for fully assembled A) T1, B) T2 and C) T3 showing all dimer and trimer permutations (e.g., "12" = monomers 1 and 2; "123" = monomers 1, 2 and 3). Single monomers "1" and "4" added for reference; the last band is the final tetramer; 1.25 μM in hybridisation buffer; native 3% Agarose 1000, 1× TBE, 100 V, 75 min; GelStar nucleic acid gel stain (in-gel).

Following automated synthesis and cleavage from the solid support, each tris-oligonucleotide was purified by preparative denaturing PAGE. The products were manually extracted, desalted by size-exclusion chromatography with NAP-25 columns and quantified by UV spectroscopy (yield: 26 to 208 nmol (2–16%) after purification). Aqueous stock solutions were prepared at 125 μM, and the masses were confirmed by MALDI-TOF-MS (Table S2). The purity of each product was confirmed by agarose gel electrophoresis (Figure S3).

The stock solutions were diluted to 1.25 μM for assembly experiments with a hybridisation buffer (10 mM HEPES, pH 7.5, with 100 mM NaCl). Tris-oligonucleotides were combined in equimolar amounts for each set by denaturing (5 min at 95 °C), annealing (0.3 °C s^{-1}; 5 min at 40 °C; ~20 °C below the theoretical melting point of single strands) and a final low-temperature step (0.1 °C s^{-1}; 15 min at 0 °C; Table S3).

Self-assembly (Figure 2) was carried out for every possible combination of building blocks. The assembly was analysed by native agarose gel electrophoresis for each set to test for errors in the sequences leading to the assembly of all four tris-oligonucleotides. Six distinct binary combinations lead to dimers connected at one arm (Figure 2, "12" to "34"; numbers according to Figure 1), four trimer combinations containing three double stranded arms ("123" to "234") and one unique tetramer combination with six double strands. Two of the four monomers ("1" and "4") were added as references. The bands showed full addressability of each arm and no sequence errors. The faint higher-order bands and smearing can be explained by mismatching side reactions and the formation of higher aggregates (most noticeable in the T2 set). A noticeable band (~180 bp) above the tetramers was assigned to a cube-shaped side product (Scheme S3). All mixtures assembled into tetramers ("T1", "T2", "T3") with the expected electrophoretic mobility below the 100 bp marker.

Treatment with mung bean nuclease (Figure 3, "+E") showed distinct digestion patterns for trimer "123" (of the T3 set) and the T1 tetramer, whereas no change in electrophoretic mobility was observed for the T2 and T3 tetramers. Mung bean nuclease preferably digests single-strands, which were

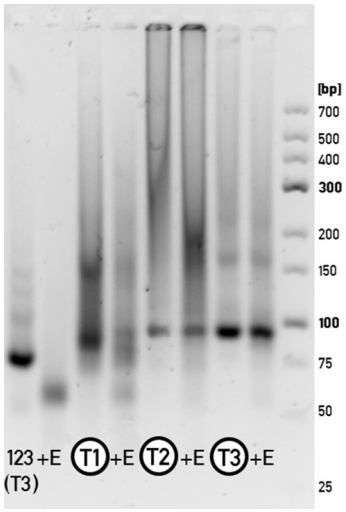
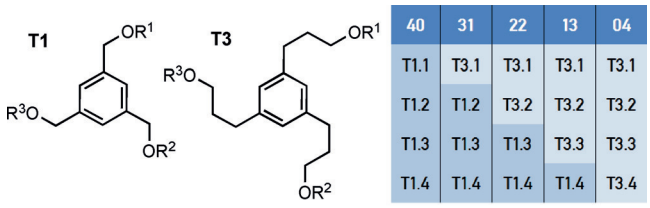


Figure 3. Digestion of trimer 123 of the T3 set and tetramers T1, T2, T3; "+E": incubation with 5 U mung bean nuclease for 10 min at 30 °C; 1.25 μM; native 3% Agarose 1000, 1× TBE, 100 V, 2 h; GelStar nucleic acid gel stain (in-gel).

present in the test trimer subset "123". The T3 tetramer band (Figure 3) showed no digestion pattern, as previously observed for fully paired tris-oligonucleotide nanostructures such as dodecahedra. Thus, T3 contained only non-digestible double strands needed for a fully assembled 3D object. T1, in contrast, produced single strands upon digestion. It can be concluded that the T1 trislinker alone is too rigid to enable strain-free folding into a tetrahedral scaffold.

It was, however, possible to successfully intermix T1 monomer tris-oligonucleotides with the more flexible T3 monomers to result in more-stable constructs (Figure 4). The setup started with the assembly of the four T1 monomers ("40": four units of set T1 and zero units of T3) and replacement stepwise with the analogous tris-oligonucleotide of the T3 set. Stability was again analysed with mung bean nuclease. The substitution of just one tris-oligonucleotide (Figure 4, "31+E") resulted in a major increase in stability (minimal digestion). Apparently,



40	31	22	13	04
T1.1	T3.1	T3.1	T3.1	T3.1
T1.2	T1.2	T3.2	T3.2	T3.2
T1.3	T1.3	T1.3	T3.3	T3.3
T1.4	T1.4	T1.4	T1.4	T3.4

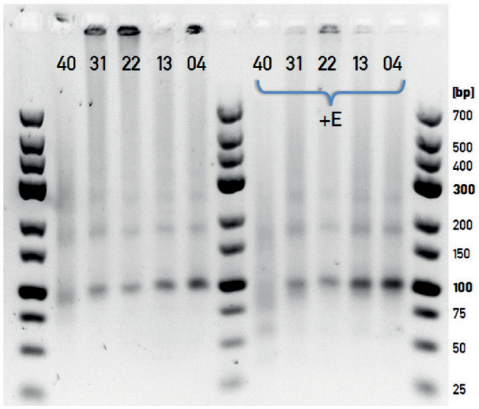


Figure 4. Mixed assemblies between the T1 and the T3 set; 1.25 μM; "+E": 5 U mung bean nuclease for 10 min at 30 °C; native 3% Agarose 1000, 1× TBE, 100 V, 60 min; GelStar nucleic acid gel stain (in-gel).

the more flexible T3 propylene chains were able to decrease the strain caused by the rigidity of the T1 methylene chains and allowed the formation of a stable tetrahedron. Stability further increased for a 50:50 mix (Figure 4, "22+E"). The tetramer of the T2 set (Figure 3) behaved similarly to the T3 set with respect to enzymatic digestion. Thus, a fully folded tetrahedral scaffold is assumed.

Because the ethylene chains proved sufficiently flexible, a new generation of trislinkers ("TN") was established by using commercial 1,3,5-tris(2-hydroxyethyl)-*N*,*N*′,*N*′′-isocyanurate (THEIC), which has a geometry similar to that of the T2 trislinker core. Synthesis of the linker core is omitted, and the TN trislinker thus only requires the introduction of the protection groups and the phosphoramidite (Scheme 3). The linker can thus be prepared faster and at lower cost, thus alleviating a bottle-neck in the bulk preparation of tris-oligonucleotides.

Flexibility and stability of the TN trislinker in tris-oligonucleotides was tested as for the T1–T3 sets. A set of four tris-oligonucleotides was synthesised and studied in self-assembly experiments (Figure S4). The assembled product of all four tris-oligonucleotides (Figure 5, "TN") showed no digestion for at least 20 min (Figure 5, "TN+E"), unlike the test trimer (Figure 5, "123+E"). All single strands were therefore hybridised, thus also indicating a fully closed tetrahedral scaffold and confirming the findings for the T2 set. The TN set was fully intermixable with the T3 set and had a similar stabilising effect in T1 intermixes (Figure S5). Isocyanurate-based tris-oligonucleotides and T1 decompose much faster than T2 and T3, when stored in aqueous solution at >0 °C, but are sufficiently stable for at least one year when stored at −20 °C.

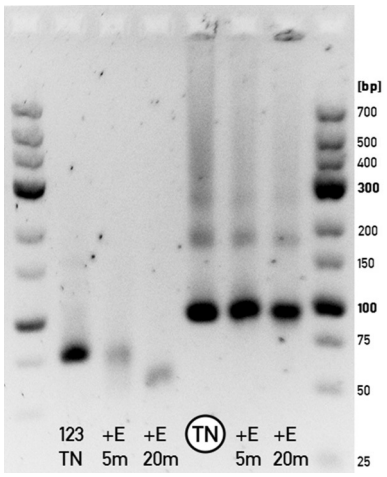
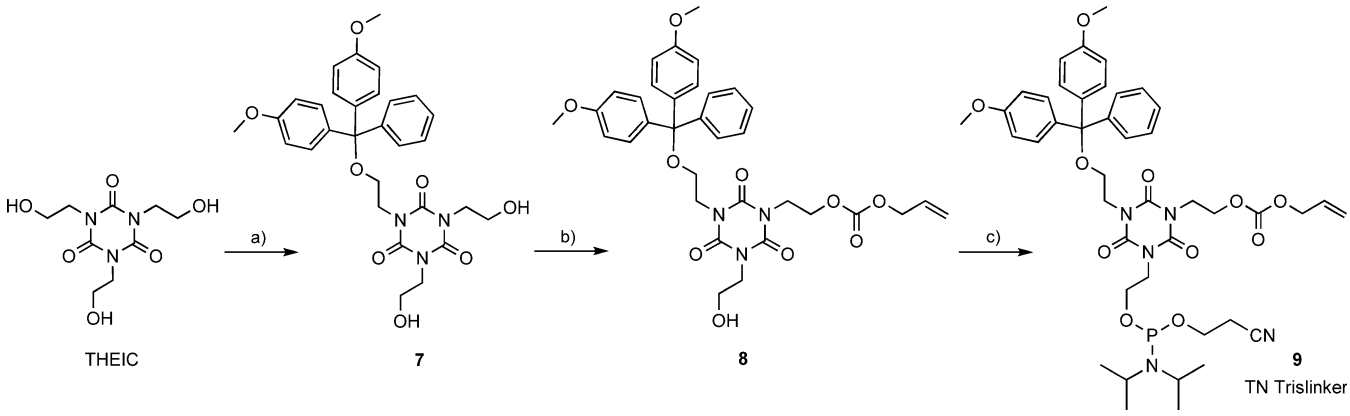


Figure 5. Digestion of trimer 123 of the TN set and tetramer TN; "+E": 5 U mung bean nuclease for 5 or 20 min at 30 °C; 1.25 μM; native 3% Agarose 1000, 1× TBE, 100 V, 65 min; GelStar nucleic acid gel stain (in-gel).



Scheme 3. Complete synthesis of the TN trislinker including the orthogonal protection group chemistry and the phosphoramidite reaction. a) 0.8 equiv DMT-Cl, 1:1 DMF_{abs}/Py_{abs}, RT–60 °C, 16 h, 30%; b) 0.8 equiv AOC-Cl, 1.2 equiv Py, THF_{abs}, 0 °C–RT, 4 h, 30%; c) 2 equiv 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, 2 equiv DIPEA, DCM_{abs}, RT, 2 h, 45%.

Conclusion

We have studied the effect of short alkylene chains of linkers in tris-oligonucleotide-based tetrahedral scaffolds by hybridisation and digestion experiments. Unlike methylene, ethylene chains at the linker are sufficiently flexible to allow folding into tetrahedral nano-objects. THEIC provides a new generation of cheap and versatile C_{3h}-symmetrial trislinkers, compatible and sufficiently flexible for use in tris-oligonucleotide-based nanostructures.

Experimental Section

General: See the Supporting Information for organic preparations and experimental procedures. All solvents and chemicals were of analytical grade unless otherwise noted. NMR spectra were recorded with DPX-200, DPX-250 and DRX-400 spectrometers (Bruker). MS analysis was performed on an Autoflex spectrometer (Bruker) equipped with an LTB MNL106 nitrogen-laser. Tris-oligonucleotides were synthesised on a Gene Assembler Plus machine (Pharmacia Biotech). The custom 3′ solid support was based on Primer Support 200 Amino (GE Healthcare) with 3′-end dC- and dG-starter nucleosides (~50 μmol g^{-1}) on a 1.3 μmol scale (previously synthesised according to ref. [34]). Synthesis, purification and characterisation of tris-oligonucleotides were according to ref. [21]. Mung bean endonuclease (40 units μL^{-1}) was purchased from Roboklon (Berlin, Germany).

MALDI mass analysis: A sample (1.5 μL, 1–10 pmol μL^{-1}) was mixed with a few beads of an ion-exchange resin (NH_4^+) on top of a plastic paraffin film (Parafilm; Pechiney Plastic Packaging). After ~15 s, the supernatant (1 μL) was mixed with 3-HPA (3 μL, Fluka) on top of the parafilm (matrix: 3-HPA (80 mg) was diluted in ddH_2O/ACN (1:1; 2 mL) and stored at −20 °C). An aliquot of the mixture (2–4 μL) was placed on the steel target (MTP 384, Bruker Daltonics) and allowed to dry at room temperature for at least 30 min.

Tris-oligonucleotide extraction process: After automated synthesis and separation by preparative PAGE, the gel band was extracted with a scalpel. In order to exclude mutants only $^3/_4$ of the upper part of band was extracted. The gel extract was placed in a 50 mL centrifuge tube and crushed with a spatula. ddH_2O (50 mL) was added and vortexed for 2 min. The gel/water suspension was centrifuged (5500 rpm, GS15R, S4180 rotor, 15 min; Beckmann), and the supernatant was collected in a 250 mL round-bottom flask. Occasionally the supernatant contained traces of gel, which were also collected. The process was repeated: water (50 mL) was added and shaken into a gel/water suspension, but the mixture was then placed on a shaker for 16 h prior to centrifugation. The collected aqueous sample (~150 mL) was evaporated into a white residue and then dissolved again in ddH_2O (5 mL; final ~8 mL). The extracts were split into three aliquots and desalted with three Illustra NAP-25 columns (Sephadex G-25 DNA grade; GE Healthcare) by following the manufacturer's standard protocols. Desalting was repeated with NAP-10 columns if necessary. The combined solutions were evaporated, and aqueous stock solutions (125 μM) were prepared.

Analytical native agarose gel electrophoresis: Agarose gel electrophoresis was executed on a Mini-Sub Cell GT system (Bio-Rad). TBE-buffer (20 mL) was added to 3% Agarose 1000 (0.6 g; Invitrogen) and stirred until the suspension homogenised (~10 min). It was then heated in a microwave to boiling, and then heated in short intervals (~10 s) until the agarose was fully dissolved. GelStar Nucleic Acid Stain (1 μL; Lonza) was added for in-gel staining, and the mixture was stirred until it cooled to 40–50 °C. The warm solution was then cast in a 7×7 cm tray placed in a gel caster (Sub-Cell GT UV-Transparent Mini-Gel Trays, Bio-Rad) and either an 8- or a 15-well comb was then added into the gel. The gel was covered in aluminium foil and allowed to cool to room temperature. The gels had a thickness of 4–7 mm. The gels had a pre-run for 15 min at 100 V and at ~8 °C in a cooling chamber. Gel loading buffer (1 μL; Sigma–Aldrich) was added to each sample. GeneRuler Low Range DNA Ladder (25–700 bp; 50 μg; Life Technologies) served as an internal reference for DNA and as an external reference with the added dyes. Electrophoresis was executed at 100 V and ~8 °C in a cooling chamber for 30–120 min.

Enzymatic digestion: DNA (4 μL, picomol) was diluted in ddH_2O (5 μL) with reaction buffer (1 μL, 30 mM sodium acetate, pH 5.0, 50 mM NaCl, 1 mM $ZnCl_2$, Roboklon) and 0.125 μL mung bean nuclease (In storage buffer containing 10 mM Tris·HCl, pH 7.5, at 22 °C, 0.1 mM zinc acetate, 50% (*v*/*v*) glycerol, Roboklon) was added. Samples were incubated at 30 °C on a thermocycler for between 5 and 30 min. The samples were transferred to a cooling chamber (~8 °C), mixed with gel loading buffer (1 μL; Sigma–Aldrich) and placed as soon as possible onto the gel.

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Keywords: DNA structures · isocyanurate · nanotechnology · self-assembly · tris-oligonucleotide

[1] N. C. Seeman, *Nature* **2003**, *421*, 427–431.
[2] N. C. Seeman, *Biochemistry* **2003**, *42*, 7259–7269.
[3] P. W. K. Rothemund, *Nature* **2006**, *440*, 297–302.
[4] K. M. M. Carneiro, P. K. Lo, H. F. Sleiman in *Supramolecular Chemistry: From Molecules to Nanomaterials, Vol. 5: Self-Assembly and Supramolecular Structures* (Eds.: J. W. Steed, P. A. Gale), Wiley, Hoboken, **2012**, pp. 2263–2274.
[5] F. Zhang, J. Nangreave, Y. Liu, H. Yan, *J. Am. Chem. Soc.* **2014**, *136*, 11198–11211.
[6] S. S. Simmel, P. C. Nickels, T. Liedl, *Acc. Chem. Res.* **2014**, *47*, 1691–1699.
[7] C. J. Serpell, T. G. W. Edwardson, P. Chidchob, K. M. M. Carneiro, H. F. Sleiman, *J. Am. Chem. Soc.* **2014**, *136*, 15767–15774.
[8] X. Liu, Y. Xu, T. Yu, C. Cliffford, Y. Liu, H. Yan, Y. Chang, *Nano Lett.* **2012**, *12*, 4254–4259.
[9] H. Lee, A. K. R. Lytton-Jean, Y. Chen, K. T. Love, A. I. Park, E. D. Karagiannis, A. Sehgal, W. Querbes, C. S. Zurenko, M. Jayaraman, C. G. Peng, K. Charisse, A. Borodovsky, M. Manoharan, J. S. Donahoe, J. Truelove, M. Nahrendorf, R. Langer, D. G. Anderson, *Nat. Nanotechnol.* **2012**, *7*, 389–393.
[10] C. M. Erben, R. P. Goodman, A. J. Turberfield, *Angew. Chem. Int. Ed.* **2006**, *45*, 7414–7417; *Angew. Chem.* **2006**, *118*, 7574–7577.
[11] Y. P. Ohayon, R. Sha, O. Flint, W. Liu, B. Chakraborty, H. K. K. Subramanian, J. Zheng, H. O. Abdallah, X. Wang, X. Zhang, N. C. Seeman, *ACS Nano* **2015**, *9*, 10304–10312.
[12] M. R. Hartman, D. Yang, T. N. N. Tran, K. Lee, J. S. Kahn, P. Kiatwuthinon, K. G. Yancey, O. Trotsenko, S. Minko, D. Luo, *Angew. Chem. Int. Ed.* **2013**, *52*, 8699–8702; *Angew. Chem.* **2013**, *125*, 8861–8864.

[13] M. Tintoré, R. Eritja, C. Fábrega, *ChemBioChem* **2014**, *15*, 1374 – 1390.
[14] A. Schwenger, C. Gerlach, H. Griesser, C. Richert, *J. Org. Chem.* **2014**, *79*, 11558 – 11566.
[15] E. Paredes, X. Zhang, H. Ghodke, V. K. Yadavalli, S. R. Das, *ACS Nano* **2013**, *7*, 3953 – 3961.
[16] O. A. Vinogradova, D. V. Pyshnyi, *Russian Chem. Rev.* **2012**, *81*, 130 – 157.
[17] C. K. McLaughlin, G. D. Hamblin, H. F. Sleiman, *Chem. Soc. Rev.* **2011**, *40*, 5647 – 5656.
[18] J. Tumpane, P. Sandin, R. Kumar, V. E. C. Powers, E. P. Lundberg, N. Gale, P. Baglioni, J.-M. Lehn, B. Albinsson, P. Lincoln, L. M. Wilhelmsson, T. Brown, B. Nordén, *Chem. Phys. Lett.* **2007**, *440*, 125 – 129.
[19] M. Mattarella, L. Berstis, K. K. Baldridge, J. S. Siegel, *Bioconjugate Chem.* **2014**, *25*, 115 – 128.
[20] B. M. Laing, R. L. Juliano, *ChemBioChem* **2015**, *16*, 1284 – 1287.
[21] J. Zimmermann, M. P. J. Cebulla, S. Mönninghoff, G. von Kiedrowski, *Angew. Chem. Int. Ed.* **2008**, *47*, 3626 – 3630; *Angew. Chem.* **2008**, *120*, 3682 – 3686.
[22] M. Chandra, S. Keller, C. Gloeckner, B. Bornemann, A. Marx, *Chem. Eur. J.* **2007**, *13*, 3558 – 3564.
[23] M. S. Shchepinov, E. M. Southern, *Russ. J. Bioorg. Chem.* **1998**, *24*, 794 – 797.
[24] G. von Kiedrowski, L.-H. Eckardt, K. Naumann, W. M. Pankau, M. Reimold, M. Rein, *Pure Appl. Chem.* **2003**, *75*, 609 – 619.
[25] R. B. Fuller in *Portfolio & Art News Annual No. 4* (Ed. A. Frankfurter), The Art Foundation Press, **1961**, pp. 112 – 127.
[26] D. E. Ingber, *Annu. Rev. Physiol.* **1997**, *59*, 575 – 599.
[27] M. W. Majchrzak, J. N. Zobel, D. J. Obradovich, G. A. Peterson, *New J. Org. Synth.* **1997**, *29*, 361 – 364.
[28] Y. Yamagiwa, Y. Koreishi, S. Kiyozumi, M. Kobayashi, T. Kamikawa, M. Tsukino, H. Goi, M. Yamamoto, M. Munakata, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3317 – 3323.
[29] M. Vaman Rao, C. B. Reese, V. Schehimann, P. S. Yu, *J. Chem. Soc. Perkin Trans. 1* **1993**, 43 – 55.
[30] N. D. Sinha, J. Biernat, H. Köster, *Tetrahedron Lett.* **1983**, *24*, 5843 – 5846.
[31] U. Feldkamp, S. Saghafi, W. Banzhaf, H. Rauhe in *DNA Computing, 7th International Workshop on DNA-Based Computers* (Eds.: N. Jonoska, N. C. Seeman), Springer, **2001**, pp. 23 – 32.
[32] J. SantaLucia, H. T. Allawi, A. Seneviratne, *Biochemistry* **1996**, *35*, 3555 – 3562.
[33] M. D. Matteucci, M. H. Caruthers, *Tetrahedron Lett.* **1980**, *21*, 3243 – 3246.
[34] H. Roesch, A. Froehlich, J. F. Ramalho-Ortigao, M. Montenarh, H. Seliger, *Eur. Pat. EP0464638*, **1992**.

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