Electronic Supplementary Material (ESI) for Nanoscale.

SUPPORTING INFORMATION

Boron Clusters as a Platform for New Materials: Composites of Nucleic Acids and Oligofunctionalized Carboranes ($C_2B_{10}H_{12}$) and their Assembly into Functional Nanoparticles

Damian Kaniowski,[1] Katarzyna Ebenryter-Olbinska,[1] Katarzyna Kulik,[1] Slawomir Janczak,[2] Anna Maciaszek,[2] Katarzyna Bednarska-Szczepaniak,[2] Barbara Nawrot, [1]* Zbigniew Lesnikowski [2]*

[1]*Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland.*

[2]*Institute of Medical Biology, Polish Academy of Sciences, Laboratory of Molecular Virology and Biological Chemistry, 106 Lodowa St., 92-232 Lodz, Poland.*

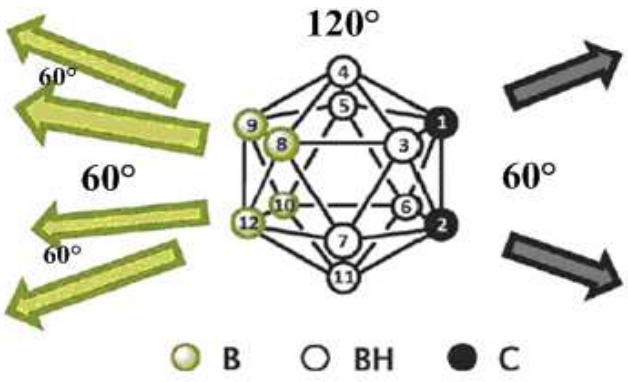
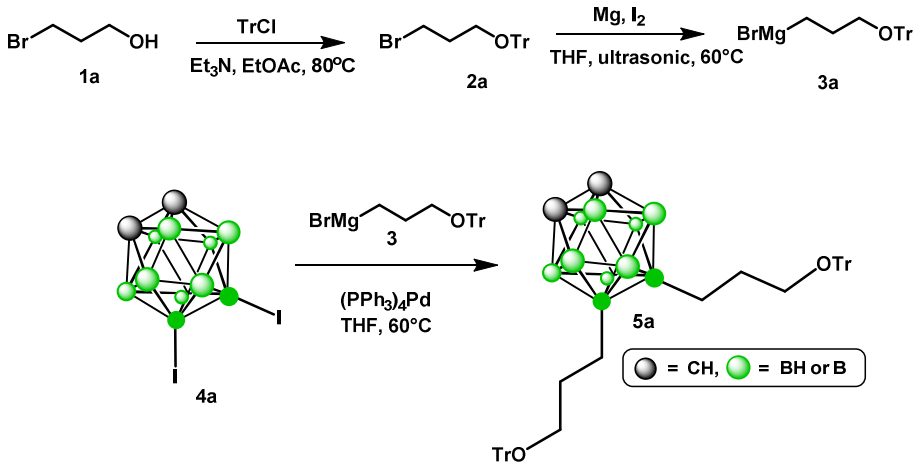


Figure S1. Schematic representation of the possible spatial orientation of the substituents attached to the boron or cluster atoms in 1,2-dicarba-*closo*-dodecaborane.



Scheme S1. Synthesis of 9,12-bis(3-O-tritylprop-1-yl)-1,2-dicarba-closo-dodecaborane (**5a**)

Materials

3-Bromo-1-propanol, 97%, was obtained from Acros Organics (Geel, Belgium), tetrakis (triphenylphosphine) palladium (0) and 1,2-dicarba-closo-dodecaborane was from Katchem (Rež n/Prague).

Chemical synthesis

Synthesis of 9,12-diiodo-1,2-dicarba-closo-dodecaborane (4a) was performed according to the literature.[1]

Synthesis of a long chain alkylamine controlled pore glass (LCA CPG) solid support 1 loaded with boron cluster triped, 9,12-bis(3-O-tritylprop-1-yl)-1,2-dicarba-closo-dodecaborane starting from 9,12-di(3-O-tritylprop-1-yl)-1,2-dicarba-closo-dodecaborane was performed as described previously.[1,2,3]

Procedure S1:

Synthesis of 3-bromo-1-trityloxypropane (2a). Compound **2a** was obtained following the procedure described for the synthesis of (2S,3S,4R)-2-N-Benzoylamino-1-O-trityl-1,3,4-octade-canetriol. Briefly, trityl chloride (9.75 g, 34.97 mmol) was added to a stirred solution of 3-bromo-1-propanol (97%, from Acros Organics (Geel, Belgium)) (**1**, 3.1 mL, 34.28 mmol) in EtOAc (95 mL) containing anhydrous Et_3N (5.8 mL, 41.62 mmol) and maintained at 80 °C. The suspension was stirred at 80 °C for 3 h. Next, the reaction mixture was cooled to room temperature and successively washed with aqueous HCl (1 M, 3 × 40 mL) and aqueous $NaHCO_3$ (10 %, 1 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4, and the organic solvent was evaporated to dryness. The crude product was purified with silica gel column chromatography using hexane/EtOAc (5:1, v/v) as the eluting solvent. **Yield:** 11.1 g, 85%, white crystals. **TLC** (hexane/EtOAc 4:1): R_f = 0.59; **UV-Vis** (Et_2O): λ_{max} = 239, 254, 260 nm; **ATR IR** (cm^{-1}): ν = 3086, 3055, 3032, 3020 (C-H_{arom}); 2959, 2933, 2902, 2878 (C-H_{alif}); 1595, 1489; 1474, 1446 (C-H_{alif}), 1060 (C-O); 760, 744, 706 (C-H_{arom}); **^{1}H NMR** (CD_3CN , 600 MHz): δ= 2.09–2.13 (m, 4H, CH_2-CH_2-CH_2), 3.17–3.20 (t, 2H, CH_2-OTr), 3.60–3.62 (t, 2H, CH_2-Br), 7.25–7.47 ppm (m, 15H, $H^{arom.}$ Tr). **HRMS**: m/z calculated for $C_{22}H_{21}OBr$ 380.0776, found: 403.0676 [M+Na] (Figures S6-S9).

Procedure S2:

In situ synthesis of 1-trityloxypropyl-magnesium bromide (3a). Suitable amounts of magnesium turnings were added to a flame-dried and argon-flushed 50 mL round-bottom flask. Magnesium was washed first with anhydrous toluene (2 x 3 mL) and next with anhydrous Et_2O, and then, it was maintained over P_2O_5 under reduced pressure overnight. 3-Bromo-1-trityloxypropane was re-crystallized from heptane and then kept overnight over P_2O_5 under reduced pressure. Anhydrous THF was stored over 3 Å molecular sieves and flushed with argon before use. Iodine (three small crystals) was added to the magnesium turnings (885.0 mg, 36.4 mmol) with anhydrous THF (3 mL), and then, the reaction mixture was placed in an ultrasonic bath at 60 °C, protected against moisture. After 15 minutes, when the reaction

solution changed color from dark brown to white, a solution of 3-bromo-1-trityloxypropane (**2**, 5.78 g, 15.15 mmol) in anhydrous THF (6 mL) was added. The flask containing 3-bromo-1-trityloxypropane was rinsed with anhydrous THF (3 mL), and the washings were added to the reaction mixture. The reaction was carried out in an ultrasonic bath at 60 °C for 4 h, and the obtained solution **3** was directly used in the synthesis of **5**.

Procedure S3:

Synthesis of 9,12-di(3-O-tritylprop-1-yl)-1,2-dicarba-closo-dodecaborane (5a). Anhydrous THF was stored over 3 Å molecular sieves and flushed with argon before use. 9,12-Diiodo-1,2-dicarba-*closo*-dodecaborane (**4**, 300 mg, 0.76 mmol) (1,2-Dicarba-closo-dodecaborane was obtained from Katchem (Rež n/Prague)) and tetrakis (triphenylphosphine) palladium (0) (obtained from Sigma Aldrich (St. Louis, MO, USA) (30 mg, 0.026 mmol) were placed in a flame-dried and argon-flushed 50 mL round-bottom flask equipped with a magnetic stirring bar, which was kept over P_2O_5 under reduced pressure overnight. Next, anhydrous THF (6 mL) was added, and the resultant solution was cooled to 0 °C, at which point the solution of Grignard reagent **3** prepared *in situ* from **2** was added. The reaction mixture was stirred at 60 °C for 48 h. Then, the reaction was quenched with aqueous HCl (5 mL, 1 M), followed by diethyl ether (5 mL). The organic layer was separated, and the aqueous layer was extracted with diethyl ether (3 x 5 mL). The organic layer and ether washings were combined and washed with water (2 x 3 mL) and then with brine (1.5 mL). The organic layer was then dried over anhydrous $MgSO_4$, and the solvent was evaporated to dryness. The resulting crude **5** was purified by silica gel column chromatography (230–400 mesh) using a gradient of hexane/Et_2O (4:1 – 1:2; v/v) as for elution. **Yield:** 423 mg (75%); **TLC** (hexane/Et_2O 1:4): R_f = 0.63; ; **UV-Vis** (Et_2O): λ_{max} = 239, 253, 259; **ATR IR** (cm^{-1}): ν = 3060, 3032 (C-H_{arom}); 2974, 2918, 2862, 2851 (C-H_{alif}); 2613, 2589, 2552 (B-H); 1596, 1489 (C-H_{alif}); 1071(C-O); 761, 745, 705 (C-H_{arom}); **^{1}H NMR** (acetone-d6, 600 MHz): δ= 0.60–0.68 (m, 4H, CH_2-$\underline{CH_2}$-CH_2), 1.56–1.63 (m, 4H, CH_2-$B^{carborane}$), 1.35–2.75 (8H, $BH^{carborane}$), 2.97–3.03 (m, 4H, CH_2-OTr), 4.31 (s, 2H, $CH^{carborane}$), 7.19–7.47 ppm (m, 30H, $H^{arom.}$ Tr); **^{11}B NMR** {^{1}H} (acetone-d6, 192.6 MHz): δ = -18.00– -11.70 (6B), -10.00– -7.55 (2B), 5.63 – 9.98 (2B); **MS** (FAB): calculated for $C_{46}H_{52}O_2B_{10}$ 745.015, m/z found: 744.4 $[M]^-$, (Figures S10-S13).

References

1 A.S. Batsanov, M.A. Fox, J.A.K. Howard, A.K. Hughes, A.L. Johnson and S.J. Martindale, 9,12-diiodo-1,2-dicarba-*closo*-dodecaborane(12), *Acta Cryst., C.*, 2003, **59**, 74.

2 S. Janczak, A. Olejniczak, S. Balabańska, M.K. Chmielewski, M. Lupu, C. Viñas, and Z.J. Lesnikowski, The boron clusters as a platform for new materials: synthesis of functionalized o-carborane ($C_2B_{10}H_{12}$) derivatives incorporating DNA fragments, *Chem. Eur. J.*, 2015, **21**, 15118.

3 R.J.B.H.N. van den Berg, C.G.N. Korevaar, H.S. Overkleeft, G.A. van der Marel and J.H. van Boom, Effective, High-Yielding, and Stereospecific Total Synthesis of d-erythro-(2R,3S)-Sphingosine from d-ribo-(2S,3S,4R)-Phytosphingosine, *J. Org. Chem.*, 2004, **69**, 5699.

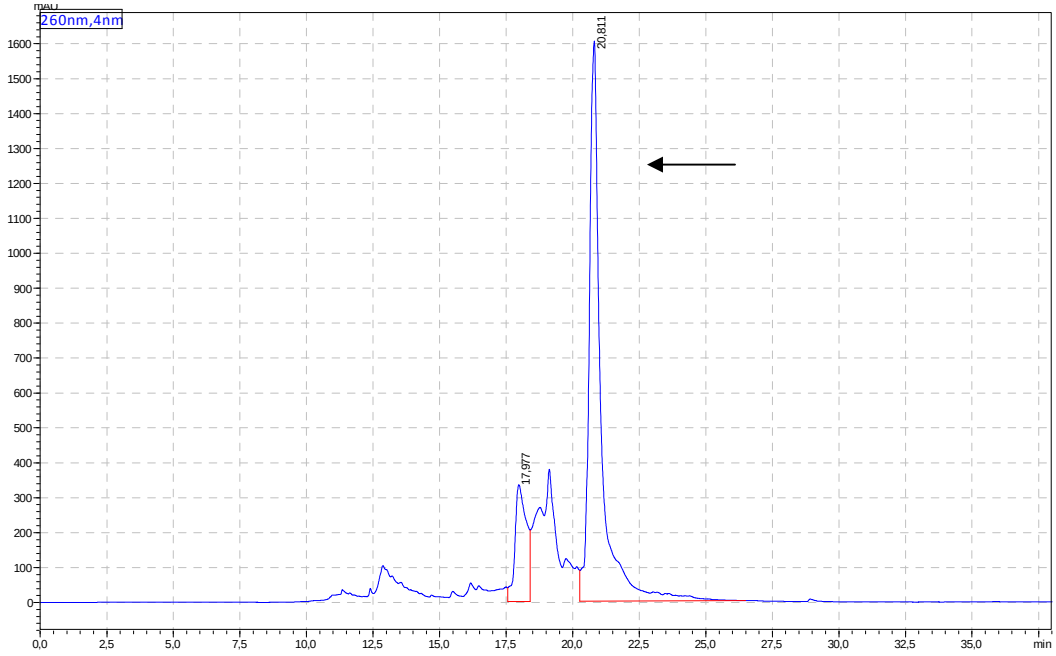


Figure S2A. RP HPLC profile of crude "trityl on" triped **4** (the peak of the collected material indicated by arrow). RP-HPLC conditions were as follow: the buffer A (0.1 M CH_3COONH_4) and buffer B (100% CH_3CN). The buffer B gradient: 0→2 min 0%; 2→21 min 0-40%; 21→24 min 40-45%; 24→28 min 45-0%; 28→32 min 0%.

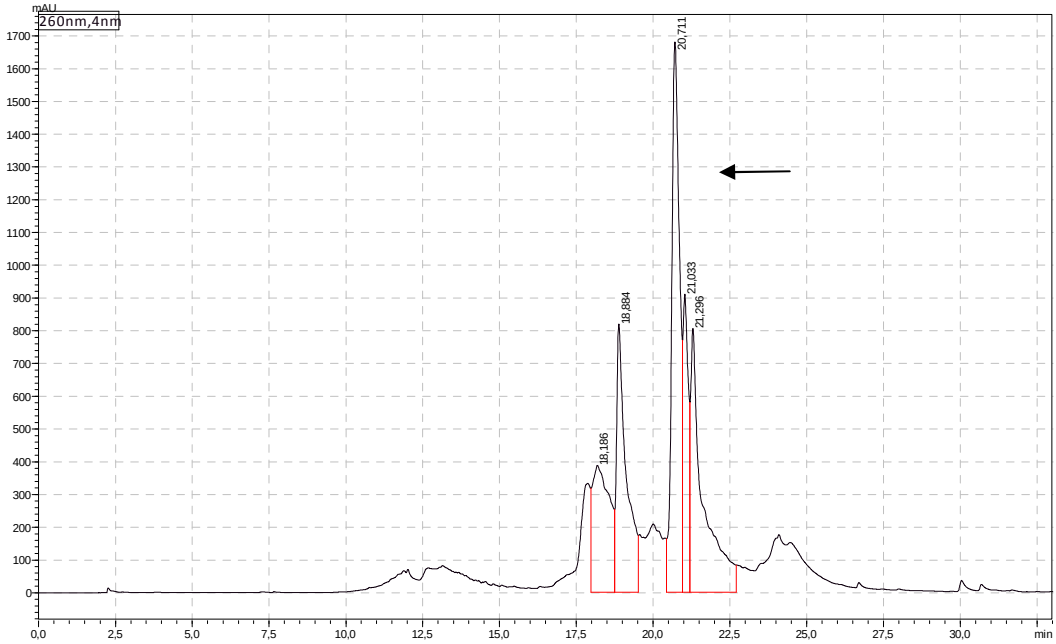


Figure S2B. RP HPLC profile of crude "trityl on" triped **5** (the peak of the collected material indicated by arrow). RP-HPLC conditions were as follow: the buffer A (0.1M CH_3COONH_4) and buffer B (100% CH_3CN). The buffer B gradient was as follows: 0→2 min 0%; 2→25 min 0-45%; 25→28 min 45-60%; 28→30 min 60-0%; 30→33 min 0%.

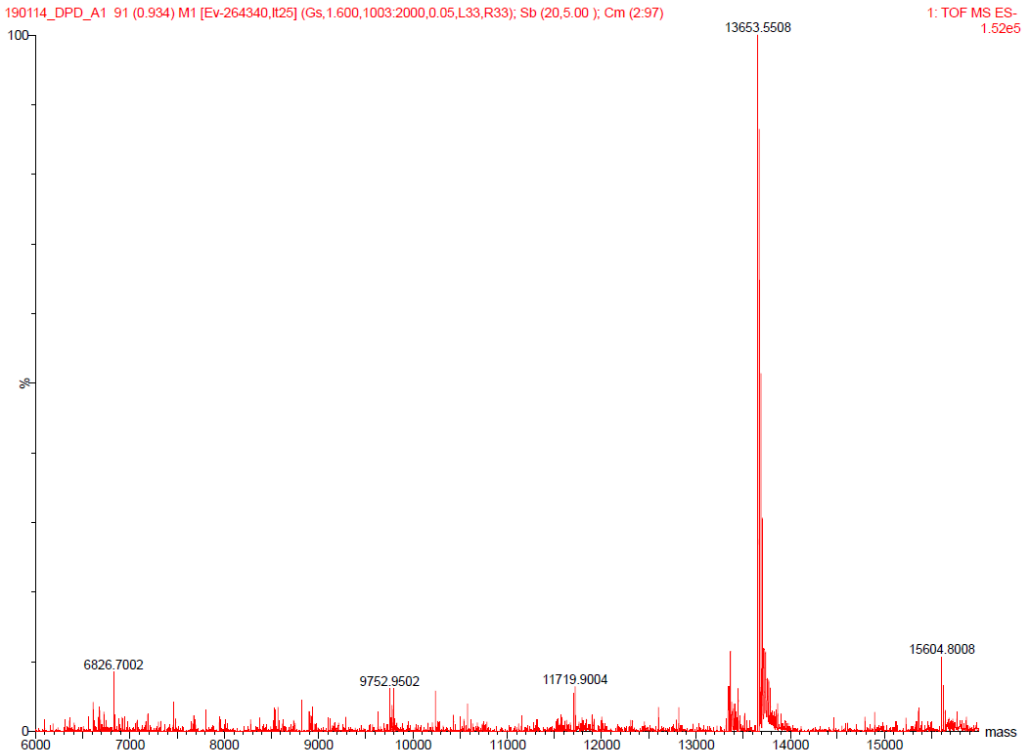


Figure S3A. ESI-Q-TOF mass spectrometry analysis of triped **4.** M.W. calc: 13652.35; *m/z*: 13653.5508.

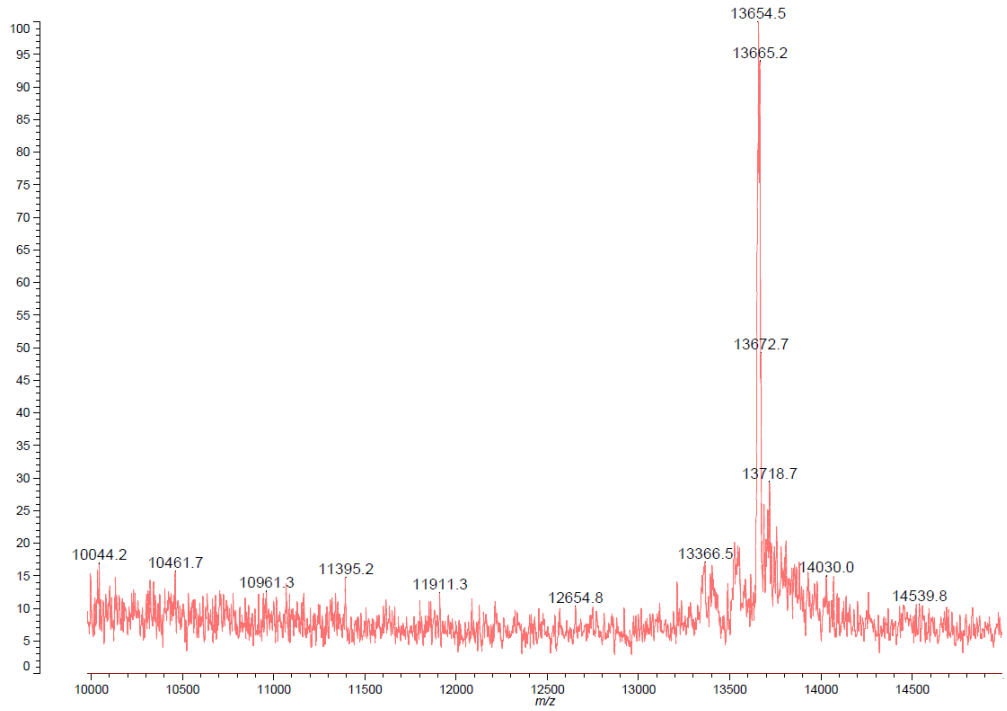


Figure S3B. MALDI - TOF mass spectrometry analysis of triped **4**. M.W. calc: 13652.35; *m/z*: 13654.5.

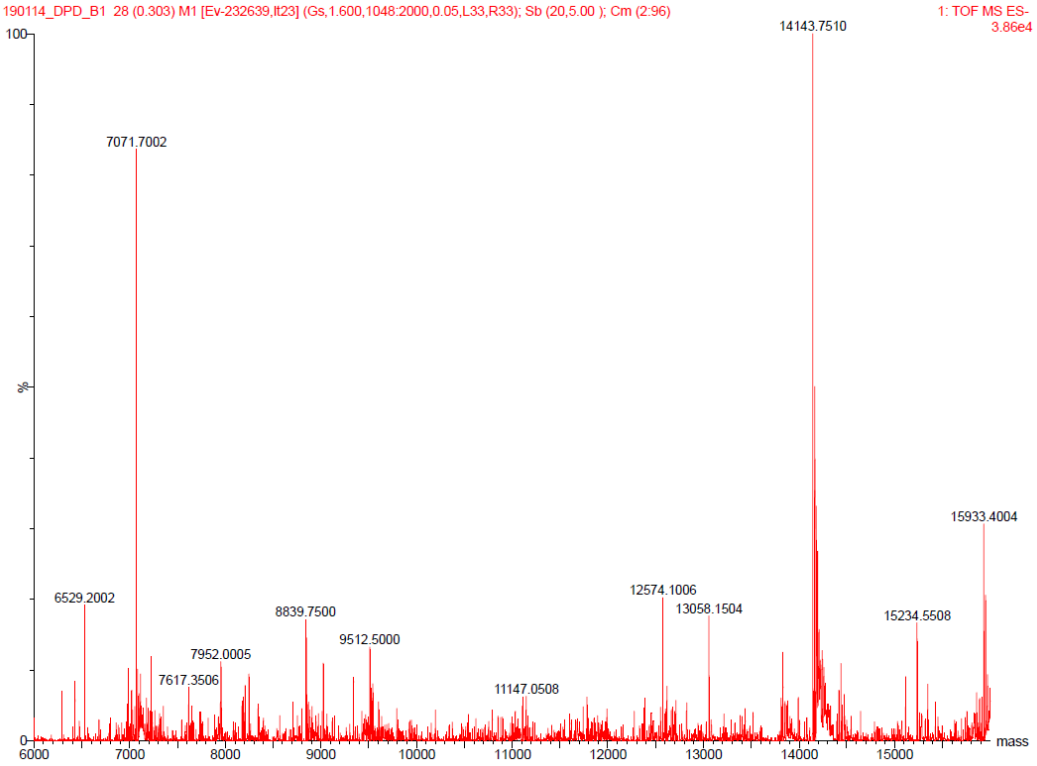


Figure S3C. ESI-Q-TOF mass spectrometry analysis of triped **5.** M.W. calc: 14143.31; *m/z:* 14143.7510.

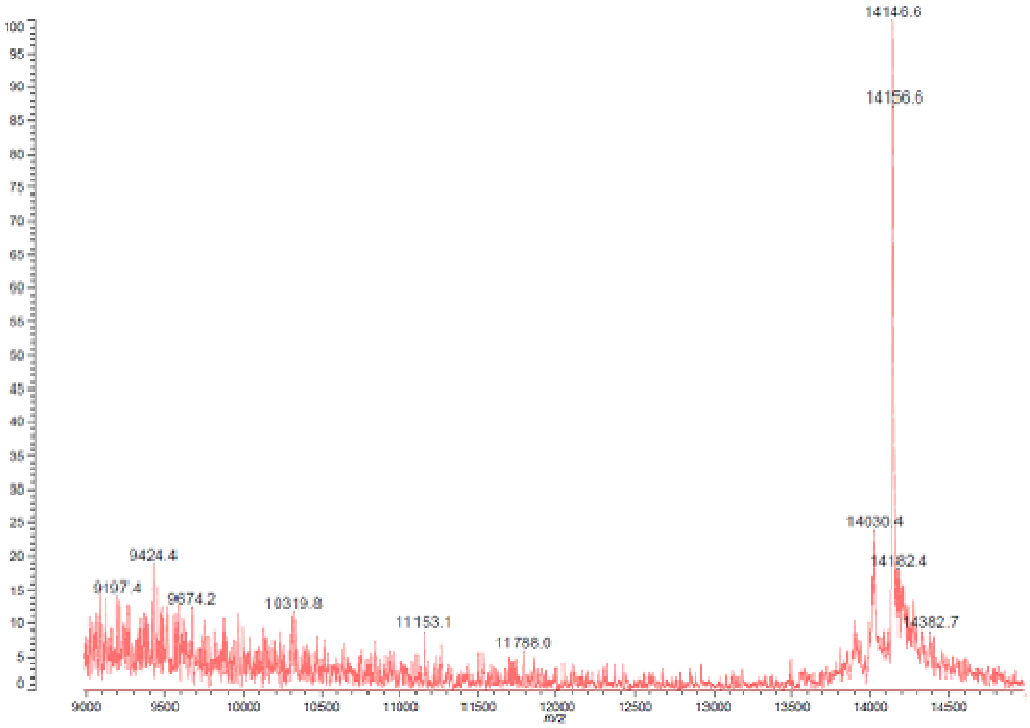


Figure S3D. MALDI-TOF mass spectrometry analysis of triped **5**. M.W. calc: 14143.31; *m/z*:14146.6.

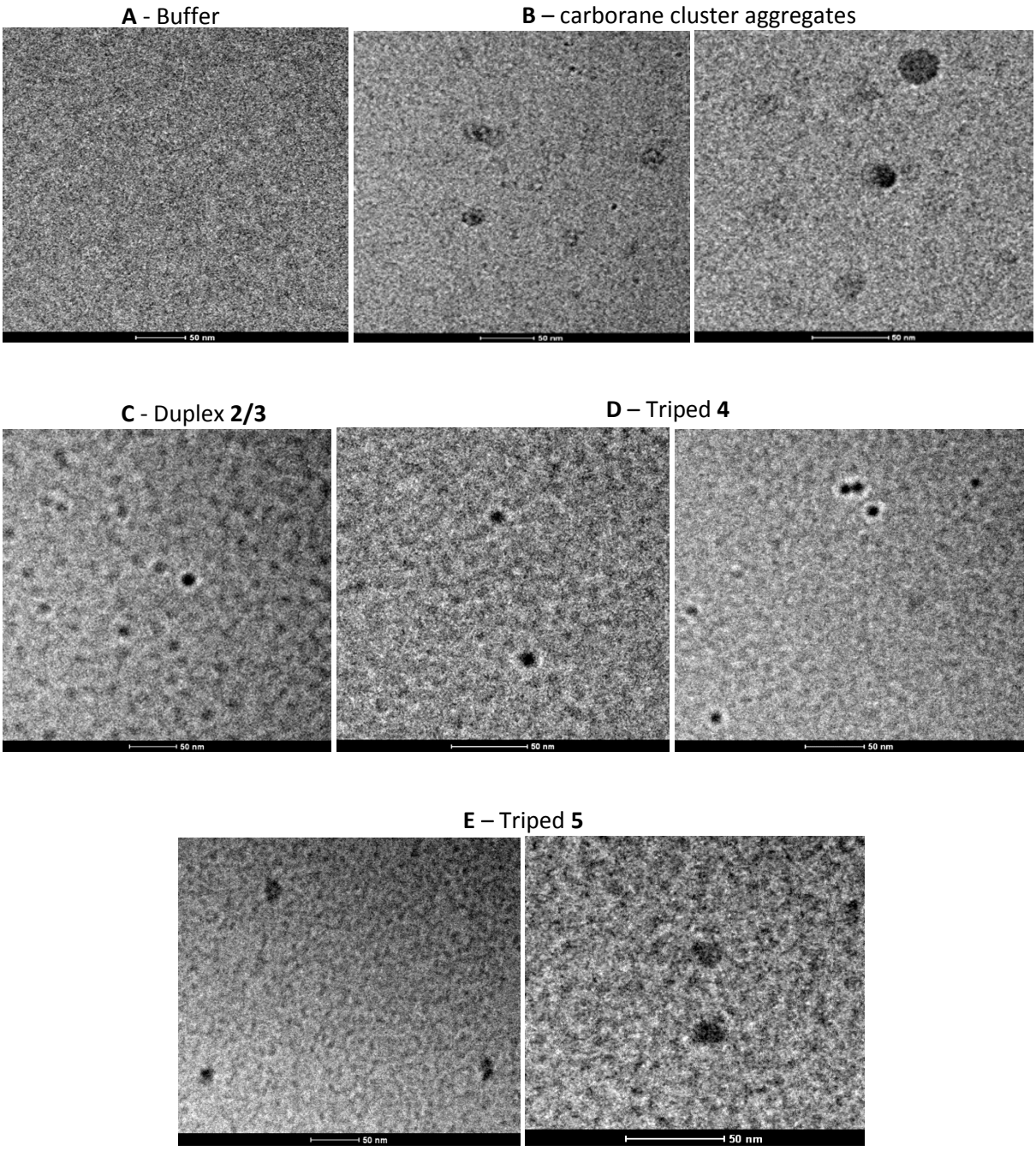


Figure S4. The cryo-TEM images: A) buffer (20 mM Tris-HCl pH 8, 10 mM $MgCl_2$, 50 mM NaCl), B) 1,2-dicarba-*closo*-dodecaborane cluster ($C_2B_{10}H_{12}$) aggregate, C) duplex **2/3**, D) single stranded triped **4** and E) single stranded triped **5.** Images are shown on a scale 50 nm.

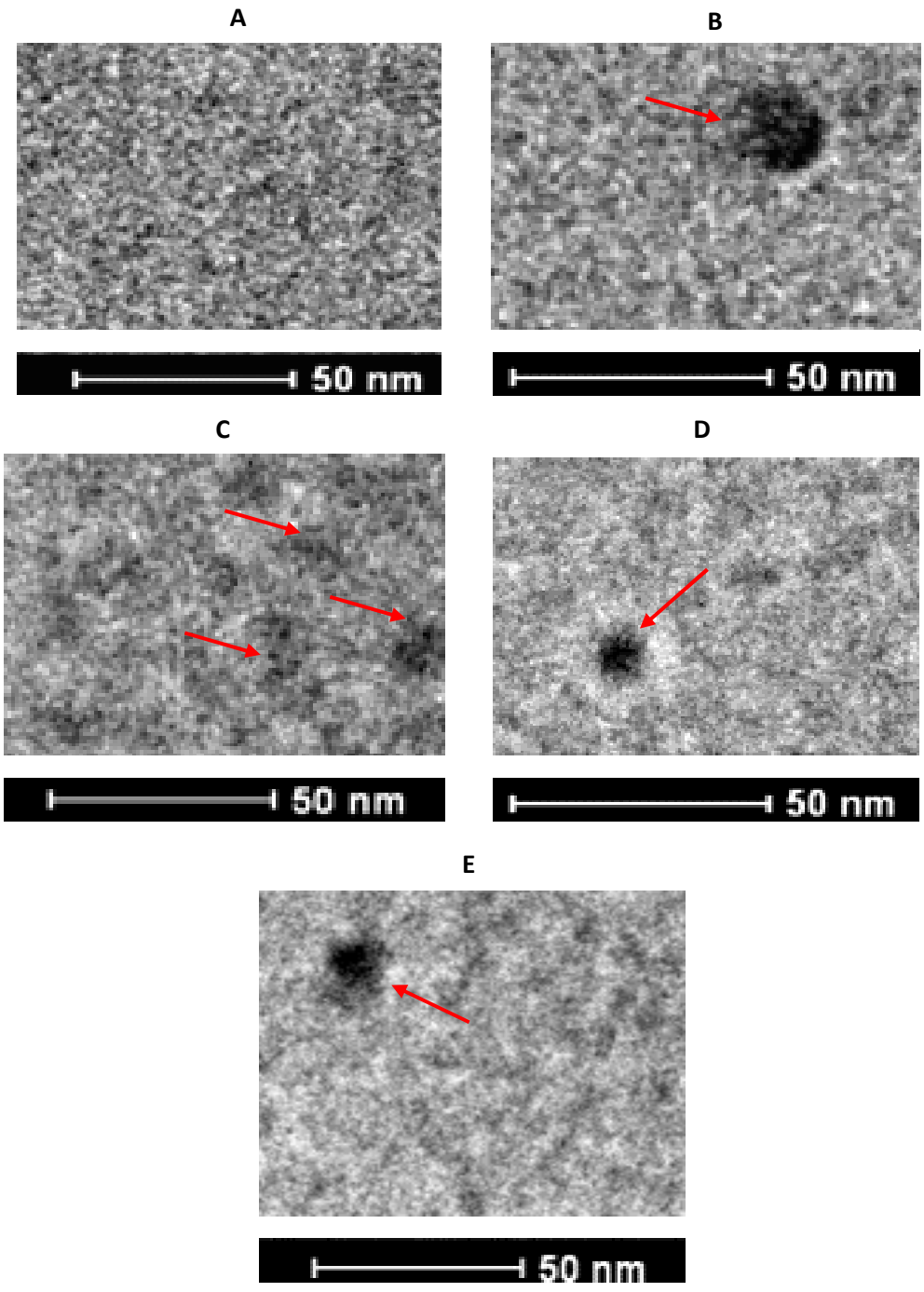


Figure S5. The cryo-TEM images: A) buffer (20 mM Tris-HCl pH 8, 10 mM $MgCl_2$, 50 mM NaCl), B) boron clusters ($C_2B_{10}H_{12}$) aggregate, C) duplex **2/3**, D)aggregates of single stranded tripeds **4** and E) **5**, (16 times enlarged images from Figures S4A-E, respectively, scale bar: 50 nm). Red arrows show the individual structures.

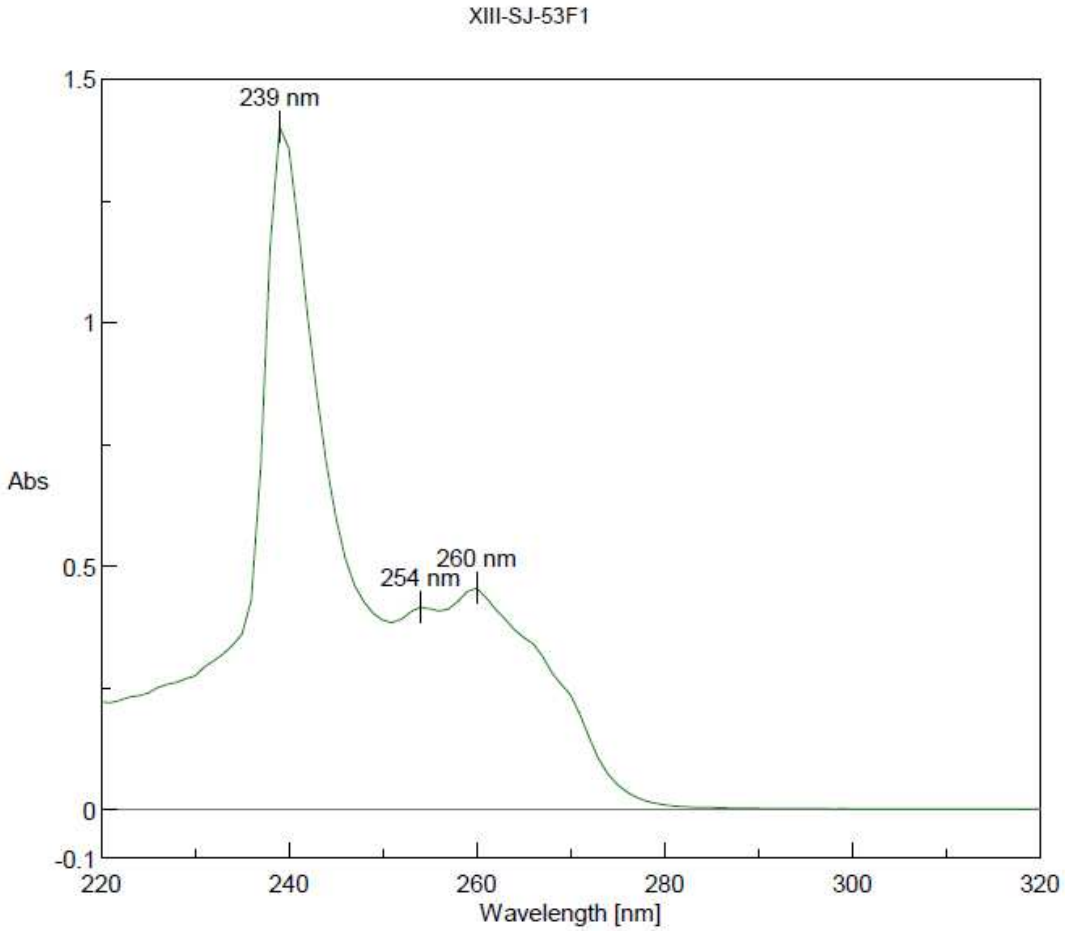


Figure S6. UV-Vis spectrum of 3-bromo-1-trityloxypropane (**2a**).

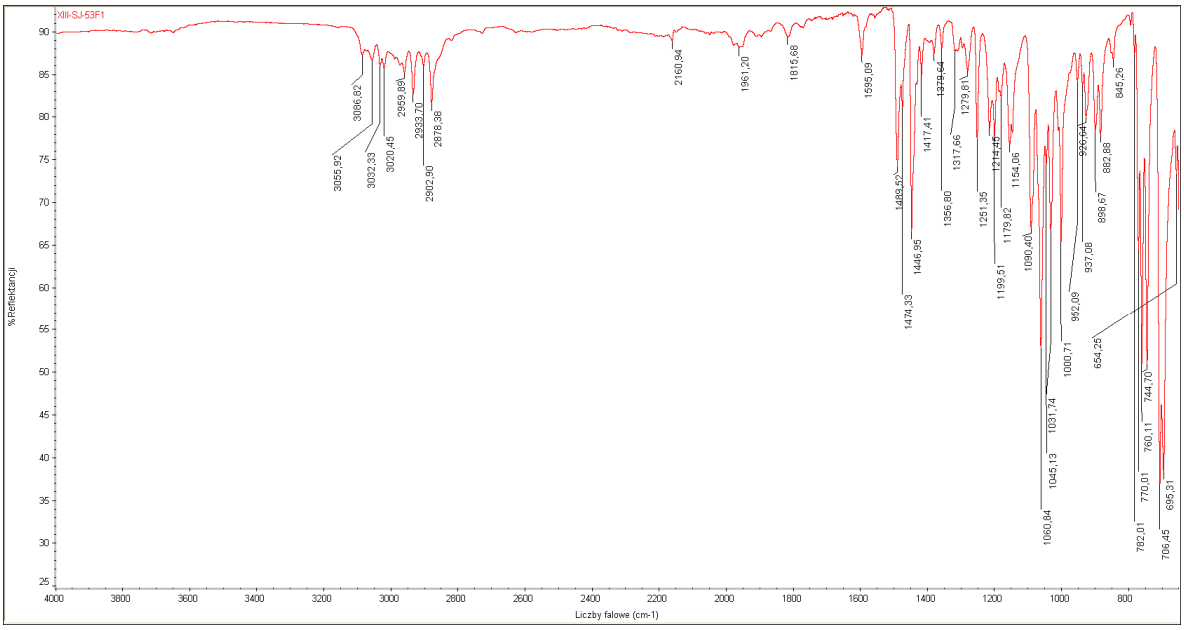


Figure S7. ATR IR (cm^{-1}): spectrum of 3-bromo-1-trityloxypropane (**2a**).

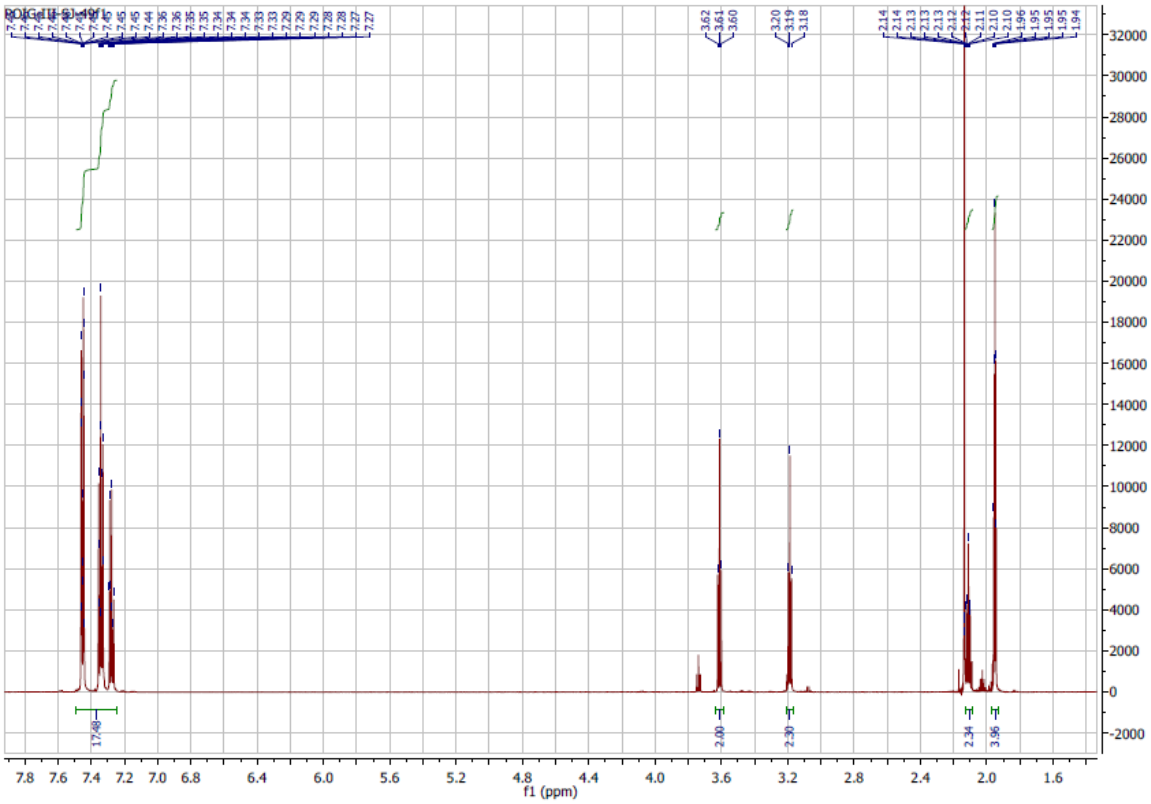


Figure S8. ^{1}H NMR (CD_3CN , 600 MHz, 25˚C, TMS) spectrum of 3-bromo-1-trityloxypropane (**2a**).

Figure S9. HRMS: spectrum of 3-bromo-1-trityloxypropane (**2a**).

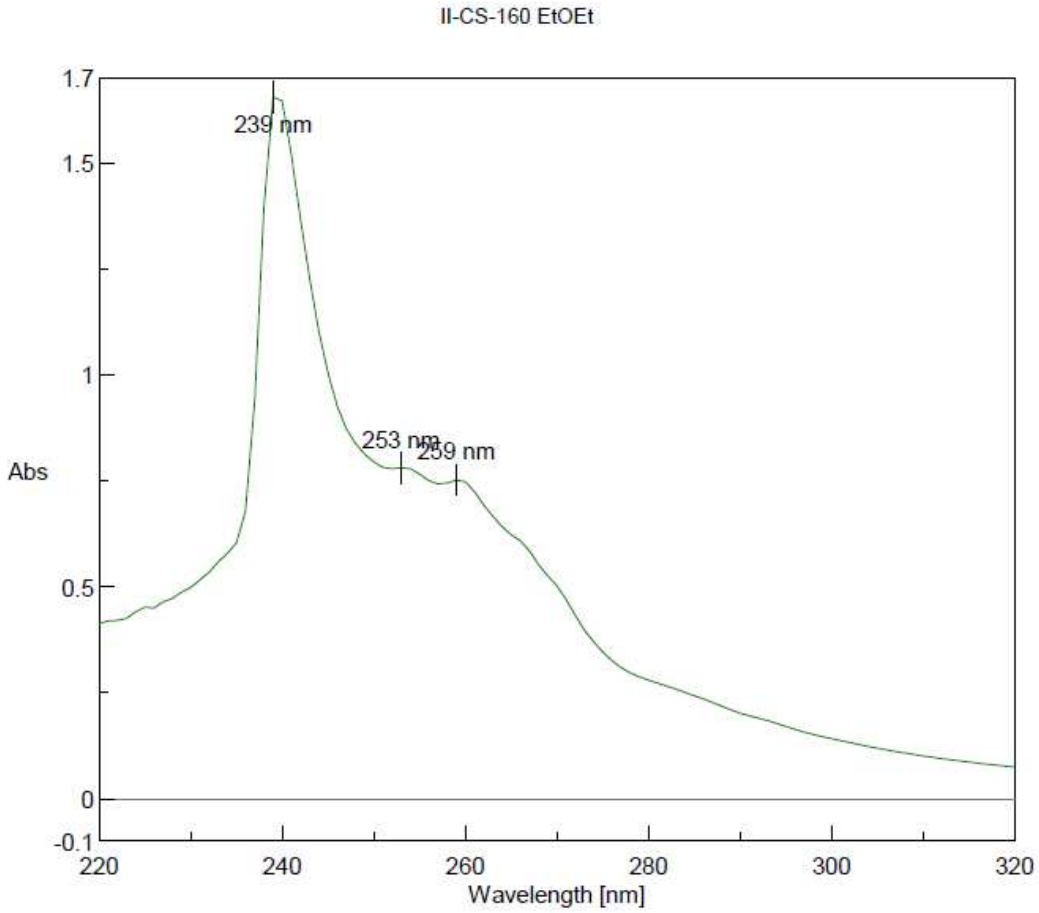


Figure S10. UV-Vis spectrum of 9,12-di(3-O-tritylprop-1-yl)-1,2-dicarba-*closo*-dodecaborane (**5a**).

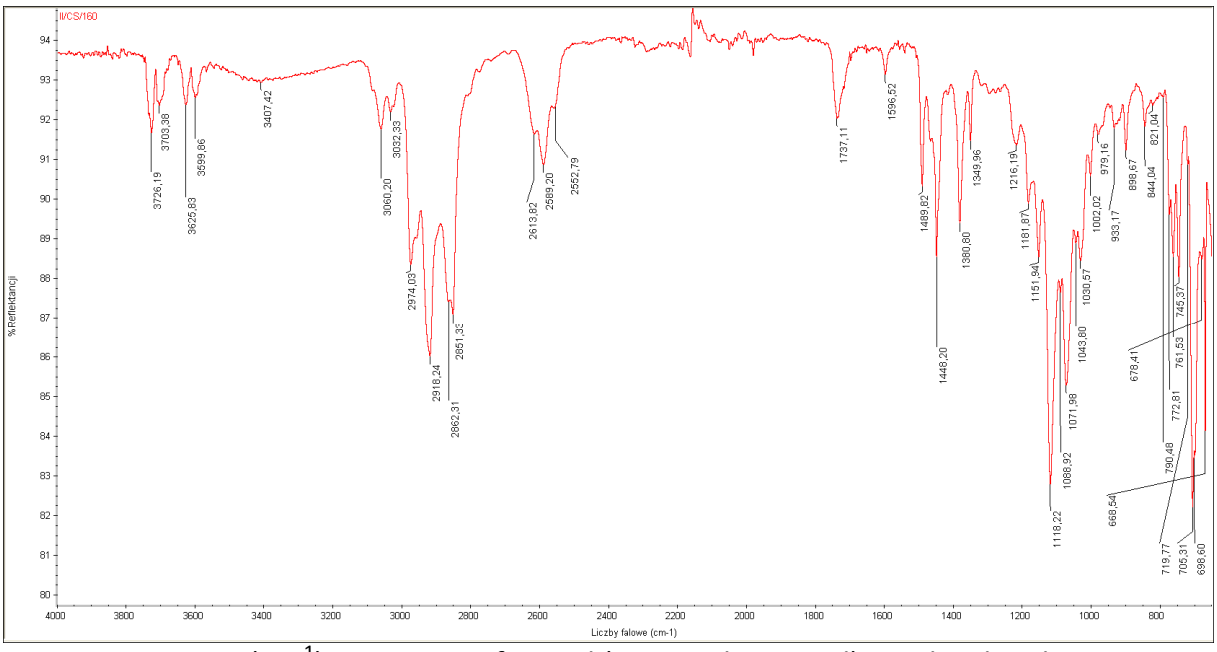


Figure S11. ATR IR (cm^{-1}): spectrum of 9,12-di(3-O-tritylprop-1-yl)-1,2-dicarba-*closo*-dodecaborane (**5a**).

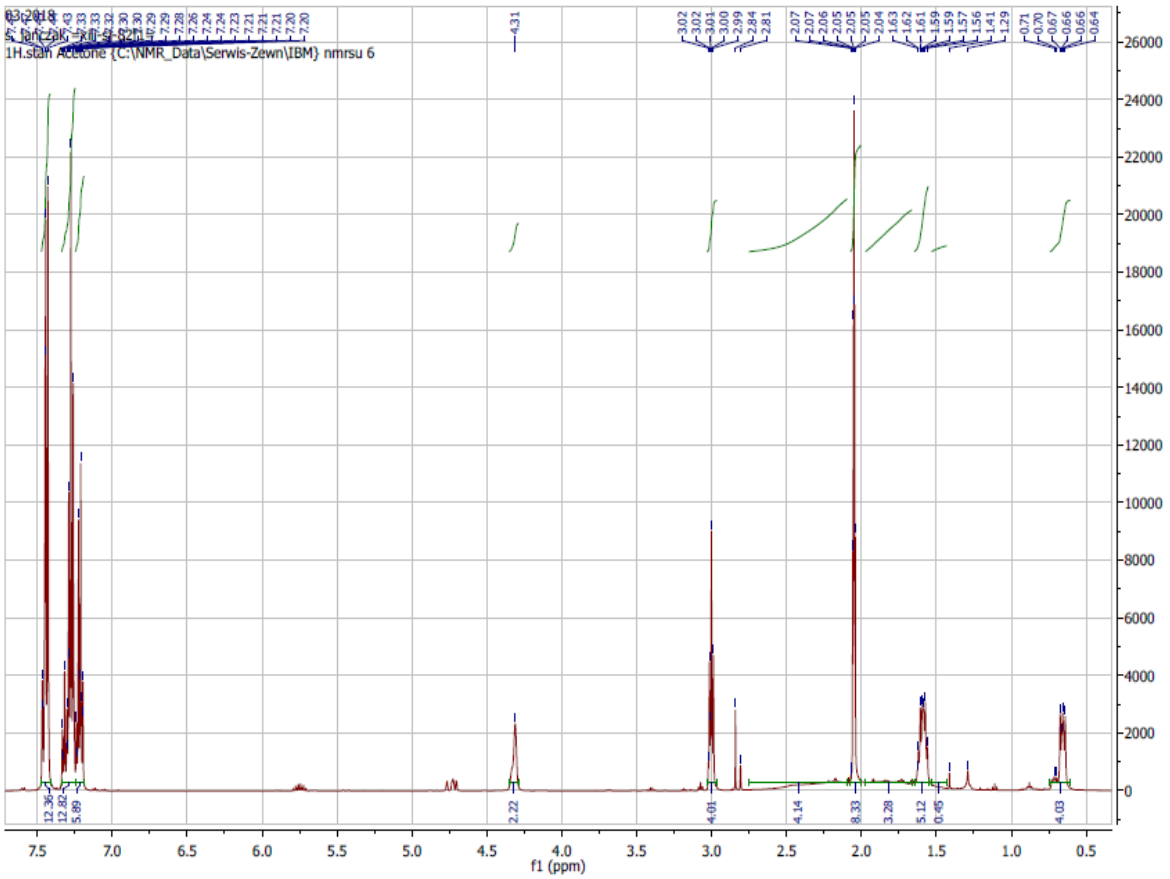


Figure S12. ^{1}H NMR (acetone-d6, 600 MHz, 25˚C, TMS) spectrum of 9,12-di(3-O-tritylprop-1-yl)-1,2-dicarba-*closo*-dodecaborane (**5a**).

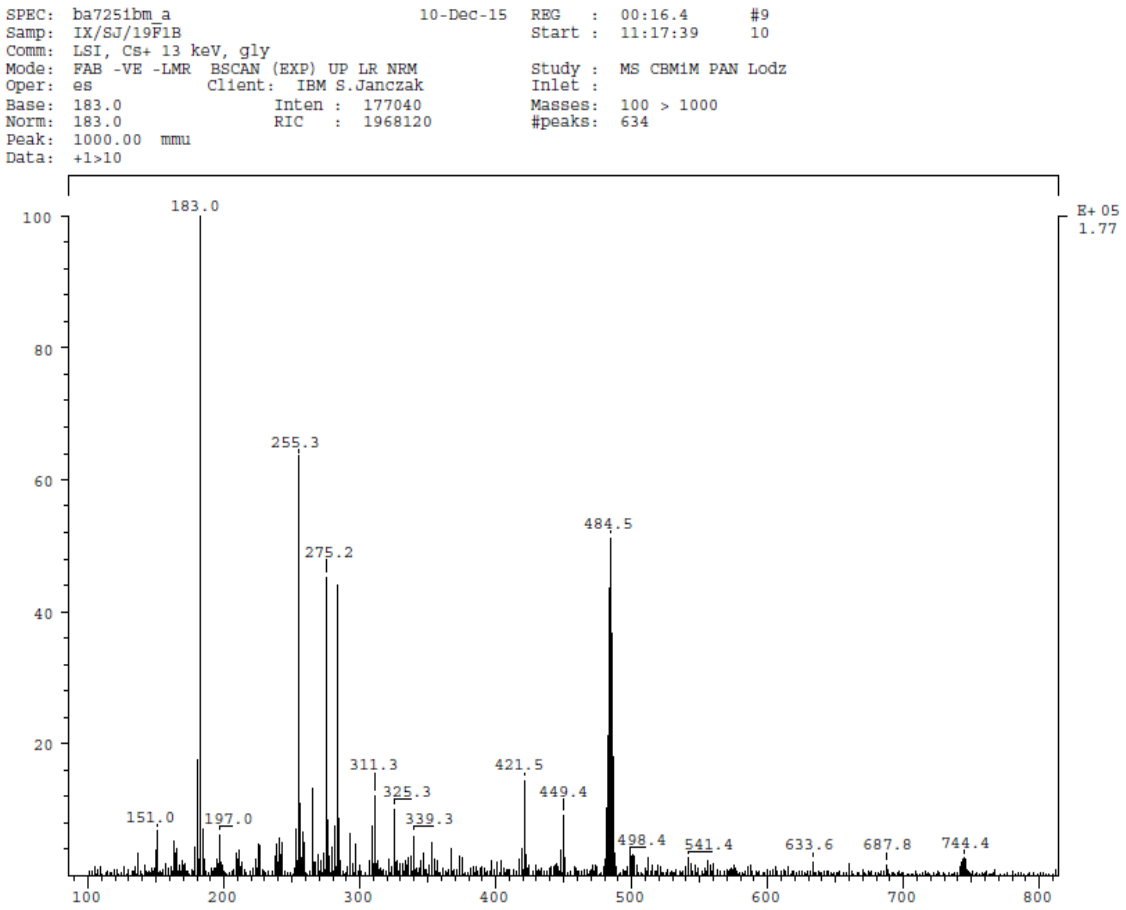


Figure S13. MS (FAB): spectrum of 9,12-di(3-O-tritylprop-1-yl)-1,2-dicarba-*closo*-dodecaborane (**5a**).

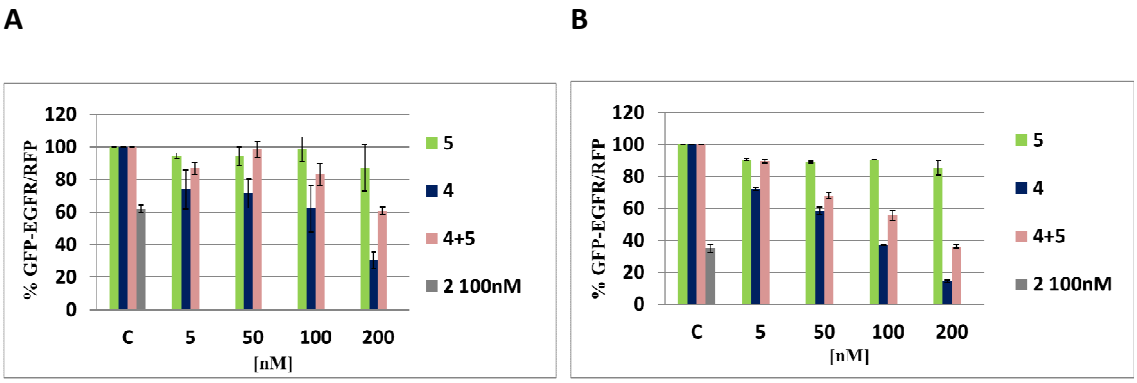


Figure S14. Concentration-dependent silencing activity of nanoconstructs **4/5** and their components - tripeds **4** and **5**, as determined by a pEGFP-EGFR/RFP dual fluorescence assay. HeLa cells transfected with increasing amounts of tripeds **4**, **5** and nanoconstruct **4/5** (5-200 nM), incubated for 48 h (A) and 72 h (B). The cells were transfected with the pEGFP-EGFR and pDsRED-N1 plasmids and then treated (in the presence of Lipofectamine 2000) with the oligonucleotide components. The relative EGFP/RFP fluorescence of the cells transfected with the plasmids only was taken as 100 %. The expression of the target protein in the presence of a reference non-modified oligonucleotide was used as a positive control (**2** 100 nM). The results are mean values from at least three independent experiments. Standard deviation is given (± SD).

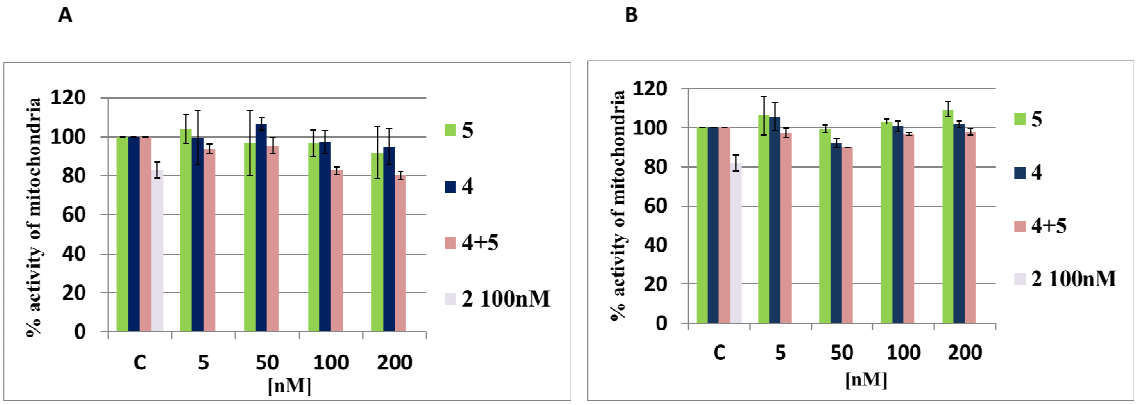


Figure S15. Mitochondrial activity of HeLa cells determined by the MTT assay. Cells were transfected with tripeds **4**, **5**, nanoconstructs **4/5** or with the non-modified oligonucleotide **2**, in the presence of Lipofectamine 2000, at the concentration of 5-200 nM (100 nM for **2**), for 48 h (A) and 72h (B). Standard deviation is given (± SD).

Table S1. Hybridization parameters and melting temperatures of the dissociation and association transitions for the duplex formed by tripeds **4** and **5** and the non-modified control DNA duplex formed by **2** and **3**. The results are the mean values from three independent experiments. Standard deviation is given (±SD).

Duplex	Tm [°C]	ΔH [kcal/mol]	ΔS [cal/Kmol]	ΔG [kcal/mol]
2/3 (dissociation)	69.1 ± 0.3	-128.0 ± 13.6	-345.6 ± 39.6	-20.8 ± 1.4
4/5 (dissociation)	69.5 ± 0.8	-138.6 ± 9.6	-377.0 ± 28.0	-21.6 ± 1.0
2/3 (association)	68.3 ± 0.1	-131.0 ± 9.9	-338.5 ± 0.7	-20.9 ± 0.9
4/5 (association)	68.7 ± 0.9	-149.6 ± 8.8	-380.7 ± 25.0	-21.5 ± 1.1