DNA Condensation With Spermine Dendrimers: Interactions in Solution, Charge Inversion, and Morphology Control

Supporting Information

Dennis Kurzbach,[a] Caroline Velte,[a] Philipp Arnold [b], Gönül Kizilsavas [c] and Dariush Hinderberger [a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

TEM Figures

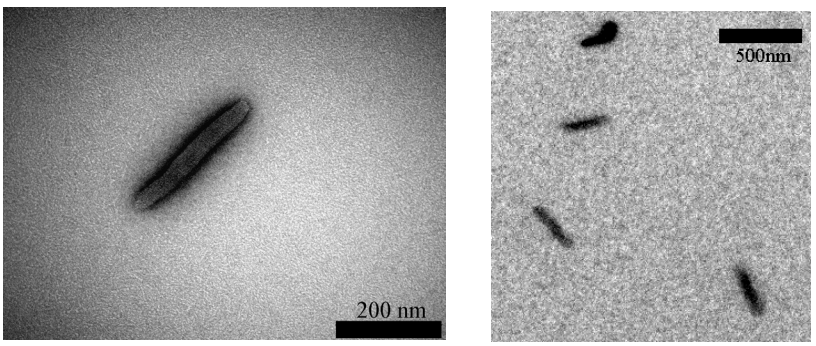


Figure S1: More TEM micrographs of DNA/SL-G2 thick rod-like condensates at cr = 2.3 and a nucleotide concentration of 0.23 mM in solution.

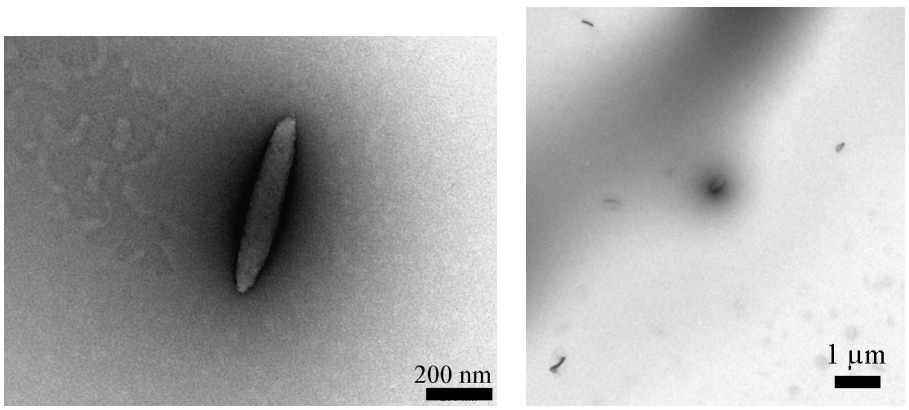


Figure S2: TEM of DNA/SL-G2 thick rod-like condensates at cr = 2.3 and a nucleotide concentration of 0.23 mM in solution.

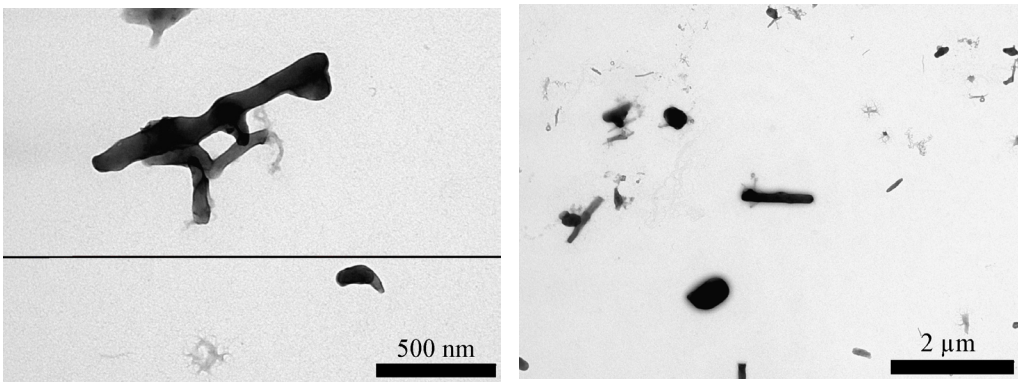


Figure S3: More TEM micrographs of aggregates of DNA/SL-G2 condensates at cr = 2.3 and 35 mM NaCl

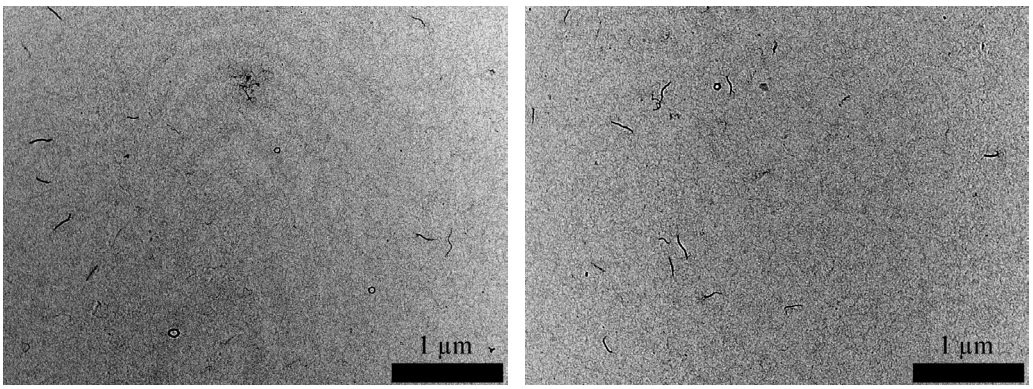


Figure S4: More TEM micrographs of thin rods formed from DNA/SL-G2 at cr = 2.3 and 35 mM NaCl after 10/1 dillution.

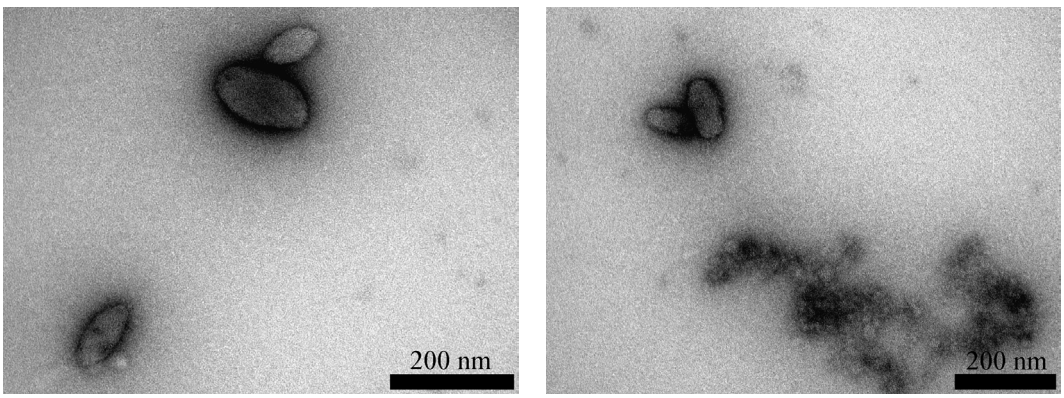


Figure S5: TEM of DNA/SL-G1 condensates (left) and condensates together with free DNA strands (right) at cr = 0.7.

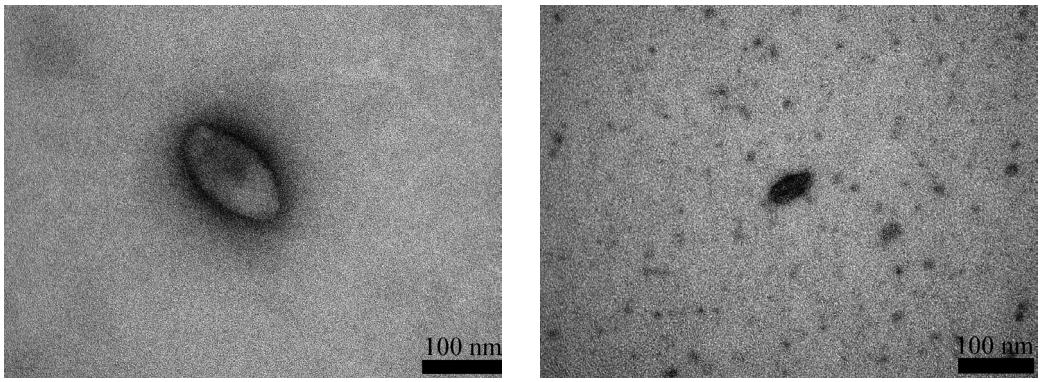


Figure S6: TEM of DNA/SL-G1 at cr = 1.0.

CW-EPR Spectra

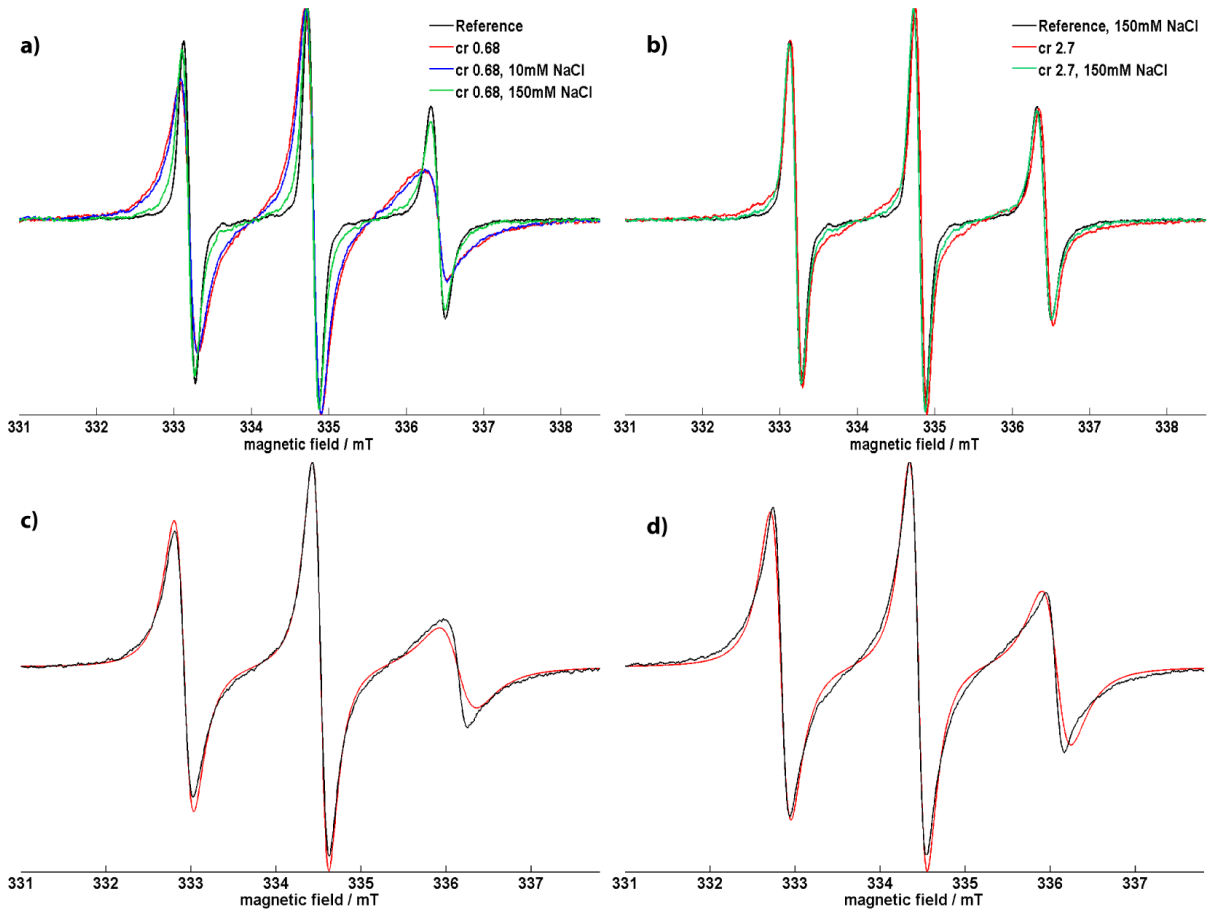


Figure S7: CW EPR spectra of G1-spermine in Tris-HCl buffer with different salt concentrations, measured at 20 °C. The reference is 0.01 mM G1-spermine without DNA, in 150 mM NaCl buffer solution. a) CR=0.68; b) CR=2.7; lower row: CW EPR spectral simulation of G1-spermine with DNA, CR=0.68, with 10 mM (c) and 150 mM NaCl (d), measured at 20° C. The measured spectra are plotted in black and the simulation is overlayed in red. For the simulation shown in c) a g-Tensor of [2.008 2.0043 2.003] was assumed and hyperfine-splitting constants of [4.5 4.5 39.5] G. An isotropic rotational correlation time τ_c of 1 ns was assumed. For the simulation in d), τ_c = 0.8 ns.

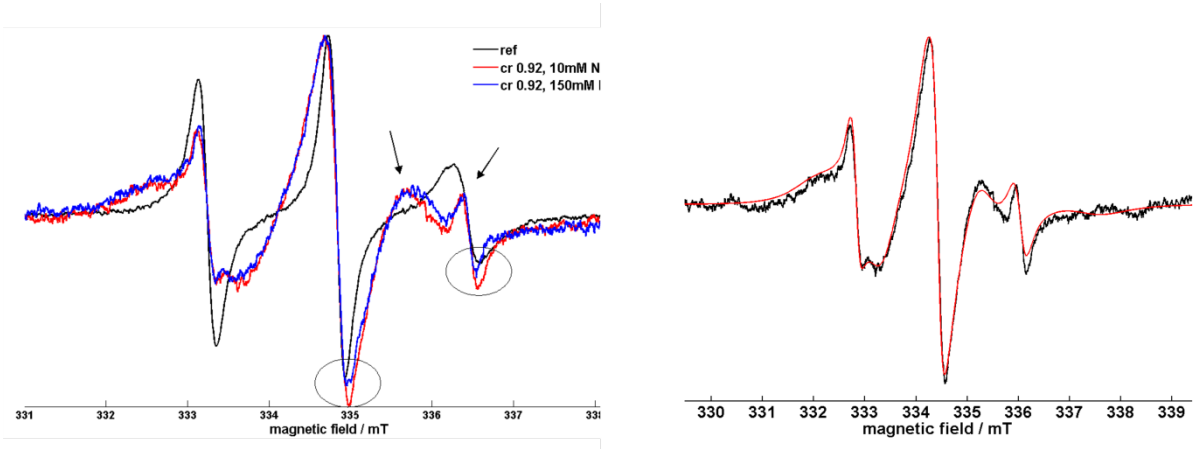


Figure S8: (a) CW-EPR spectra of G2-spermine. The reference (0.1 mM G2-spermine with 150 mM NaCl, black) and CR=0.92 exposed to 10 mM NaCl (red) and with 150 mM NaCl (blue), measured at 20 °C. (b) Simulation of G2-spermine with DNA, CR=0.92 with 10 mM NaCl (red), measured spectrum (black). g = [2.008 2.0043 2.003] and A = [4.5 4.5 39.5] G. Diffusion tensor D = [$7\cdot10^8$,$3\cdot10^8$,$1\cdot10^8$] 1/s for the fast and [$9\cdot10^7$,$9\cdot10^6$,$1\cdot10^8$] 1/s for the slow species. $x_{condensed} \approx 0.7$.

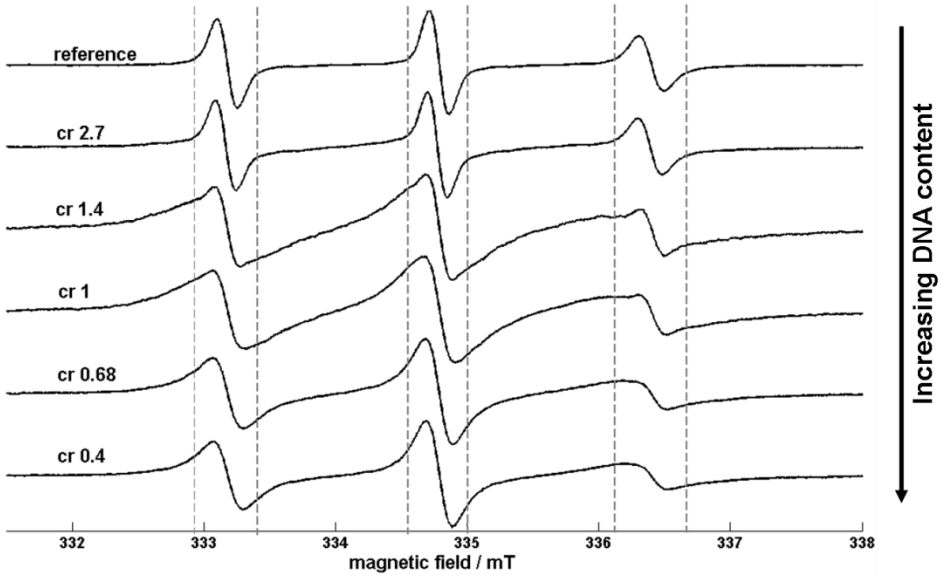


Figure S9: CW-EPR spectra of G1-spermine in Tris-HCl buffer with a salt concentration of 10 mM NaCl, measured at 20 °C. The CR is varied from 0.4 (bottom) to 2.7 (second to top). The reference is 0.1 mM pure G1-spermine without DNA. The dashed vertical lines are meant to guide the eye to the broadening of the linewidths with decreasing charge ratio.

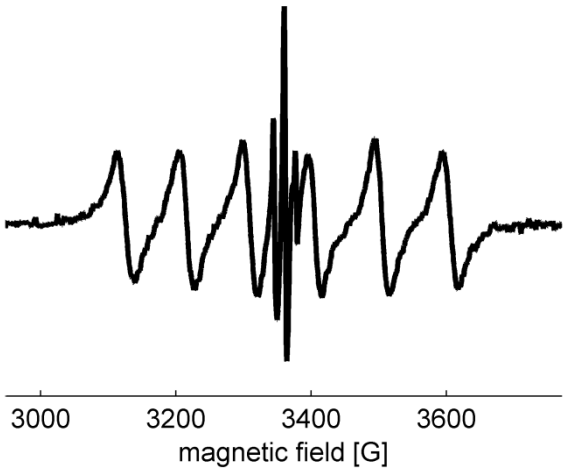


Figure S10: CW-EPR spectra of SL-G2 at cr =2.3 and 10 / 1 dilution (nucleotide concentration: 0.23 mM). Please note that the spectrum looks exactly as the one at a nucleotide concentration of 2.3. Therefore, one can infer that no structural variation takes place upon dilution, as confirmed by TEM. (Figures S1 and S2.)

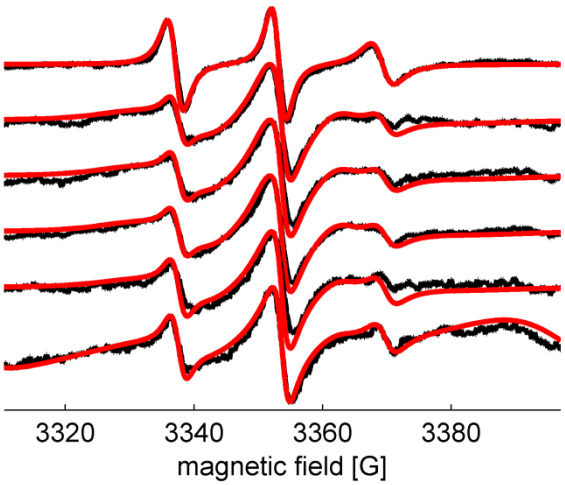
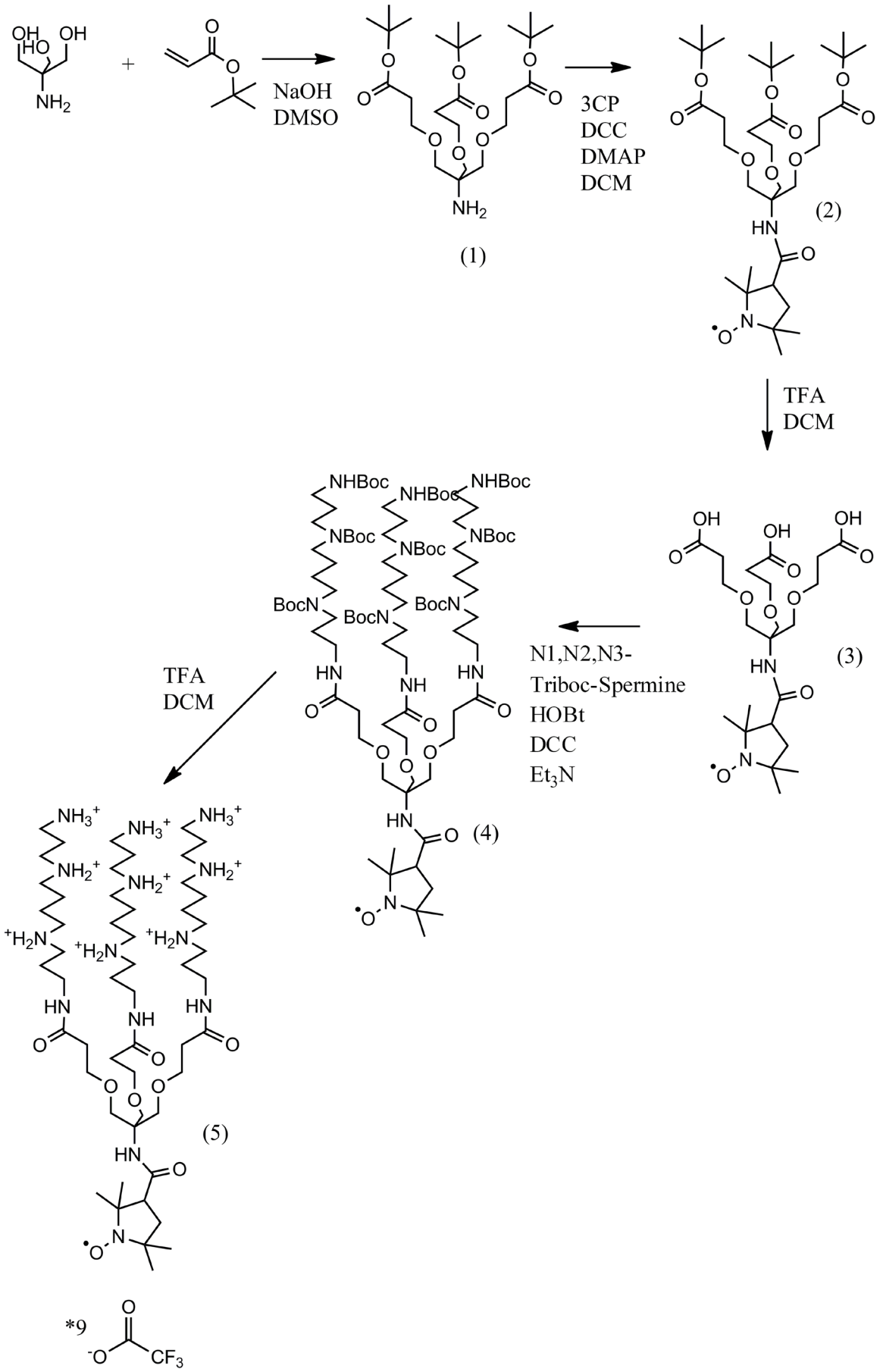


Figure S11: CW-EPR spectra of SL-G2 at different cr (top to bottom: 0.00, 0.35, 0.68, 1.20, 1.76, 2.30) and a nucleotide concentration of 2.3 mM. The spectra are shown in black, the simulations in red.

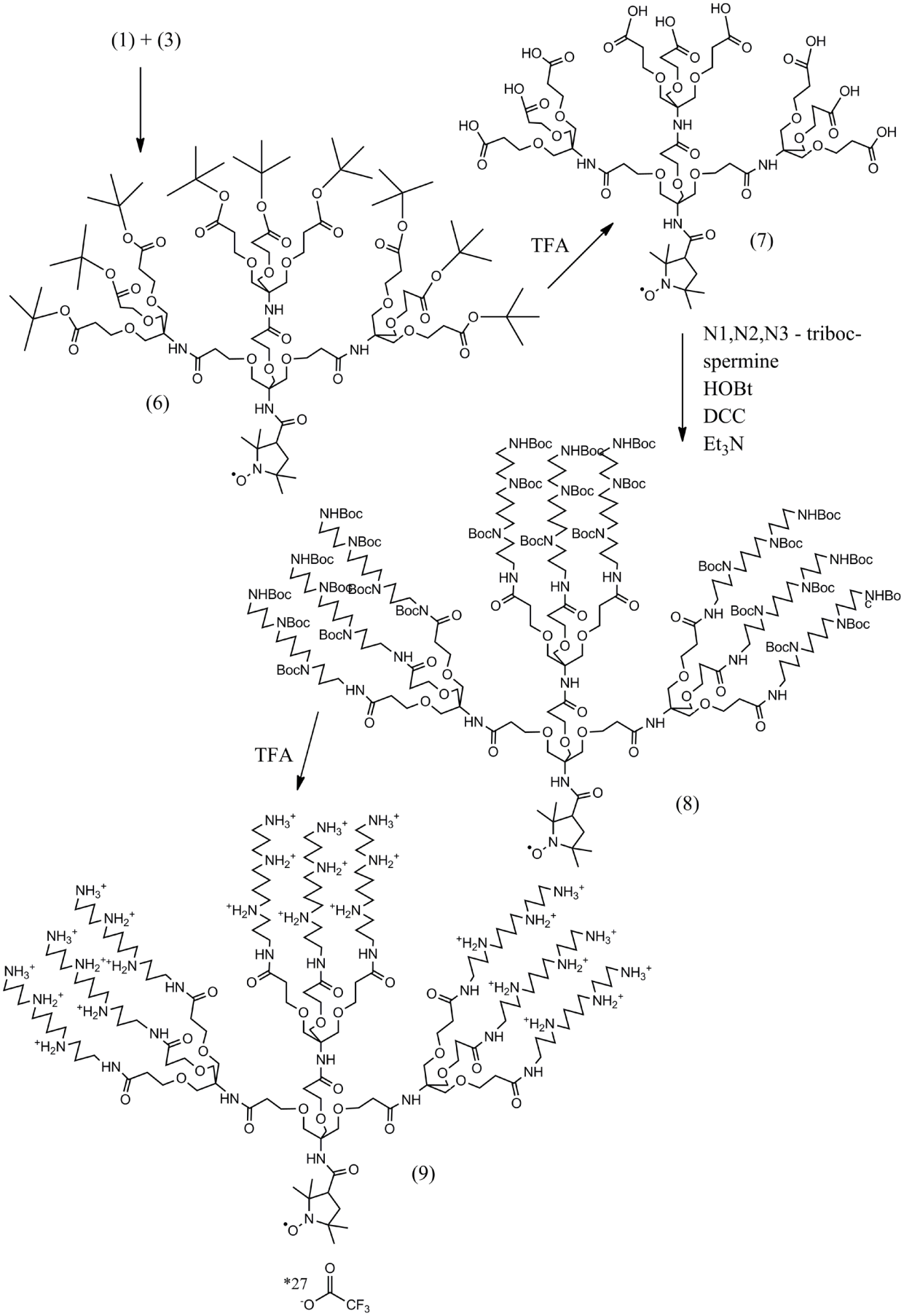
Simulations of SL-G2 at different charge ratios.

For spectral simulations EasySpin[1] implemented in MATLAB 8 was used. A model based on slow, anisotropic rotaion, developed by Schneider and Freed[2] was applied. In every case, the spectral components of the slow species were approximated by an anisotropic g-tensor of [2.0080 2.0043 2.0030]. Hyperfine coupling matrix elements were [4.5 4.5 39.5] G. An asymmetric diffusion tensor of [$9\cdot10^7$,$9\cdot10^6$,$1\cdot10^8$] 1/s with orientation of [0 70 70]° of its coordinate system relative to the molecular (g-) coordinate system was assumed.

The fast spectral componentes of the spectra were approximated applying a g-tensor of [2.009 2.0043 2.003] and HF values of [4.5 4.5 39.5] G. The diffusion tensor was [$7\cdot10^8$,$3\cdot10^8$,$1\cdot10^8$] 1/s, with orientations of [0 60 60]° relative to the molecular framework, indicating a faster rotation about the diffusional x-axis.



Scheme S1: Synthetic route towards SL-G1.



Scheme S2: Synthetic route towards SL-G2.

Synthesis of Spin-Labeled Spermine and its Derivatives

Mass spectra were done using FD techniques on a VG-Instruments TRIO-200 and ZAB 2-SE-FPD. NMR spectra were done with a Bruker Spectrospin 250 at room temperature. Chemical shifts are given relative to Tetramethylsilane in the δ-scale (ppm) and were calibrated through the deuterium-locking-signal. Analytical thin-layer-chromatography (TLC) was performed with Macherey-Nagel Alugram SIL G/ UV254 silica-gel plates. Detection of the Rf-values was performed through fluorescence quenching at 254 nm UV or staining with $KMnO_4$ solution.

Synthesis of SL-G1

N1,N2,N3-tri-Boc-Spermine was synthesized according to literature.[3]
Tris{[2-(tert-Butoxycarbonyl)ethoxy]methyl}methylamine (1) was synthesized according to literature.[4]

Proxyl-N-Tris{[2-(tert-Butoxycarbonyl)ethoxy]methyl}methylamide (2)

186.23 mg (1 mmol) 3-Carboxyl-Proxyl, 122.17 mg DMAP (1 mmol) and 533.71 mg (1 mmol) of (1) where solved in 10 ml dichloromethane and 1 ml dimethylformamide (DMF) at 0°C. The mixture was stirred 2 h at this temperature and additional 24 h at room temperature. After removing precipitated dicylcohexylurea (DCU) by filtration the crude product was concentrated to approximately 2 ml and purified by column chromatography (SiO2; DCM/MeOH 98:2 ' DCM/MeOH 95:5 ' DCM/MeOH 90:10 ' DCM/MeOH/NH4OH 70:10:1) and dried in vacuum. Yield: 528.5 mg (0.83 mmol).
TLC: R_f (SiO_2; hexane:EA 2:1.25): 0.36, visible through UV
FD-MS: 673.6 m/z

Proxyl-N-Tris{[2-carboxyethoxy]methyl}methylamide (3)

528.5 mg (0.83 mmol) of (2) were solved in 10 ml of CH_2Cl_2 and 10 ml TFA were added dropwise while stirring. After three hours CH_2Cl_2 and TFA were removed by evaporation. Yield: 390.35 mg (0.82 mmol).
TLC: R_f (SiO_2; hexane:EA 2:1.25): 0.04, visible through UV.
FD-MS: 505.8 m/z

SL-G1-tri-Boc-Spermine (4)

186.19 mg (0.37 mmol) (3), 681.82 mg (3.31 mmol) DCC, 334.39 mg (3.31 mmol) Et_3N and 446.51 mg HOBt (3.31 mmol) were solved in 12.5 ml of THF. 590.4 mg (1.176 mmol) N1,N2,N3-Tri-BOC-Spermine in 15 ml THF was added to the mixture dropwise while stirring. After three days precipitated DCU was removed by filtration. After concentrating the crude product to approx. 5-10 ml it was purified by column chromatography (SiO_2; DCM/MeOH 98:2 ' DCM/MeOH 95:5 ' DCM/MeOH 90:10 ' DCM/MeOH/NH_4OH 70:10:1) and dried in vacuum. Yield: 155.9 mg (0.08 mmol).
TLC: R_f (SiO_2; DCM/MeOH/NH_4OH 70:10:1): 0.64, visible through UV
FD-MS: 1956.2 m/z

SL-G1 (5)

155.9 mg (0.08 mmol) of (4) were solved in 10 ml of CH_2Cl_2 and 10 ml TFA were added dropwise while stirring. After three hours CH_2Cl_2 and TFA were removed by evaporation. Yield: 164.1 mg (0.08 mmol).
TLC: R_f (SiO_2; DCM/MeOH/NH_4OH 70:10:1): 0.01, visible through UV.

Synthesis of SL-G2

Proxyl-N-Tris[(2-{[(tris{[2-(tert-butoxycarbonyl)ethoxy]methyl}methyl)amino]\\carbonyl}ethoxy)methyl]methylamide (6)

462.79 mg (0.91 mmol) of acid (3), 566.99 mg (2.75 mmol) DCC, 371.31 mg (2,75 mmol) hydroxybenzotriazole (HOBt) and 278.07 mg (2.75 mmol) triethylamine were solved in 40 ml THF. Afterwards 1.39 g (2.75 mmol) of amine (1) in 10 ml THF were added dropwise while stirring. After 24 h precipitated DCU was extracted from the solution by filtration and after additional 48 h the suspension was filtered again. Afterwards the crude product was purified by column chromatography [SiO_2 (0.2-0.5mm); DCM/MeOH 20:1]. After removing the solvent and drying in vacuum orange oil remained. Yield: 381.46 mg (0.19 mmol)
TLC: R_f(DCM/MeOH 20:1): 0.27, visible through UV; FD-MS: 1970.8 m/z.

Proxyl-N-Tris[(2-{[(tris{[2-carboxyethoxy]methyl}methyl)amino]carbonyl}ethoxy)\\methyl]methylamide (7)

381.46 mg (0.19 mmol) of ester (6) were solved in 10 ml DCM. While stirring under nitrogen 10 ml TFA were added dropwise. After five minutes the solution started to darken. After 45 min. stirring TFA and DCM were removed in vacuum and brown viscous oil remained. Yield: 273.75 mg (0.19 mmol).
TLC: R_f (SiO_2; DCM/MeOH/NH_4OH 70:10:1): 0.01, visible through UV.

SL-G2-tri-Boc-Spermine (8)

273.75 mg (0.19 mmol) of acid (7), 652.00 mg (3.15 mmol) DCC, 426.98 mg (3.15 mmol) HOBt and 319.16 mg (3.15 mmol) triethylamine were solved in 80 ml ethylacetate. While stirring 1053.97 mg (2.09 mmol) of N1,N2,N3-tri-Boc-Spermine in 30 ml ethylacetate were added dropwise. In 24 h intervals 50 mg (0.10 mmol) N1,N2,N3-tri-boc-Spermine, 50 mg HOBt (0.37 mmol) and 50 mg (0.24 mmol) DCC were added to the solution after three days the precipitated DCU was removed by filtration. The crude product was purified by column chromatography [SiO_2 (0.2-0.5mm); DCM/MeOH/konz. NH_4OHaq 70:10:1]. Orange oil remained. Yield: 912.37 mg (0.16 mmol).

TLC: R_f(DCM/MeOH/konz. NH_4OHaq 70:10:1): 0.68, visible through UV; MALDI-TOF: 5352 m/z; 5855 m/z.

[MALDI-TOF measurements showed that next to the product also a smal fraction of a dendronwith only eight arms.Either because of fractionalization during ionization or because of incomplete chemical substitution.]

SL-G2 (9)

609.8 mg (0.10 mmol) of dendron (8) were solved in 25 ml DCM and washed with 25 ml of a saturated NaCl solution. Afterwards 25 ml TFA were added to the organic layer and the mixture was stirred for 45 min. After removing DCM and TFA by vaporisation the substance was washed two times with water and DCM. Brown oil remained that solidified slowly into a yellow solid. Yield: 649.81 mg (0.10 mmol).

TLC: R_f(DCM/MeOH/konz. NH_4OHaq 70:10:1): 0.01, visible through UV.

1. S. Stoll and A. Schweiger, *J. Magn. Reson.*, 2006, **178**, 42-55.
2. D. J. Schneider and J. H. Freed, *Biological Magnetic Resonance Vol. 8 - Theory and Applications*, Plenum Press, New York, 1989.
3. I. S. Blagbrough and A. J. Geall, *Tetrahedron Letters*, 1998, **39**, 439-442.
4. C. M. Cordona and R. E. Gawley, *J. Org. Chem.*, 2002, **67**, 1411-1413.

FD-MS and MALDI-TOF Data

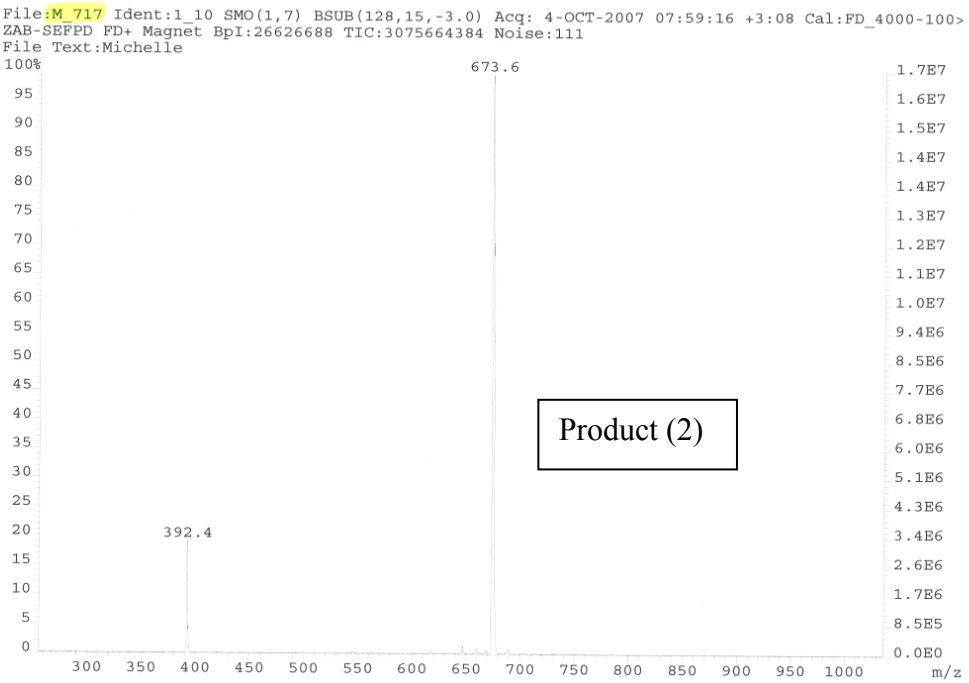


Figure S12: FD-MS of (2)

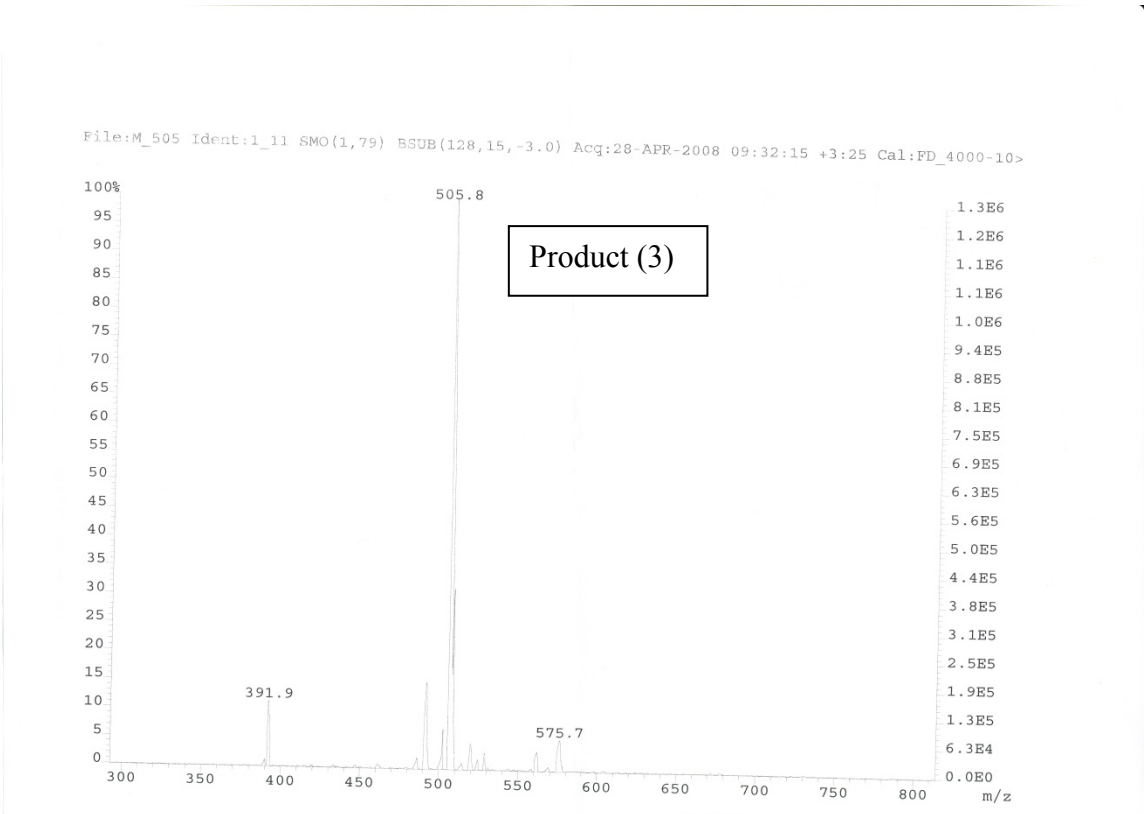


Figure S13: FD-MS of (3)

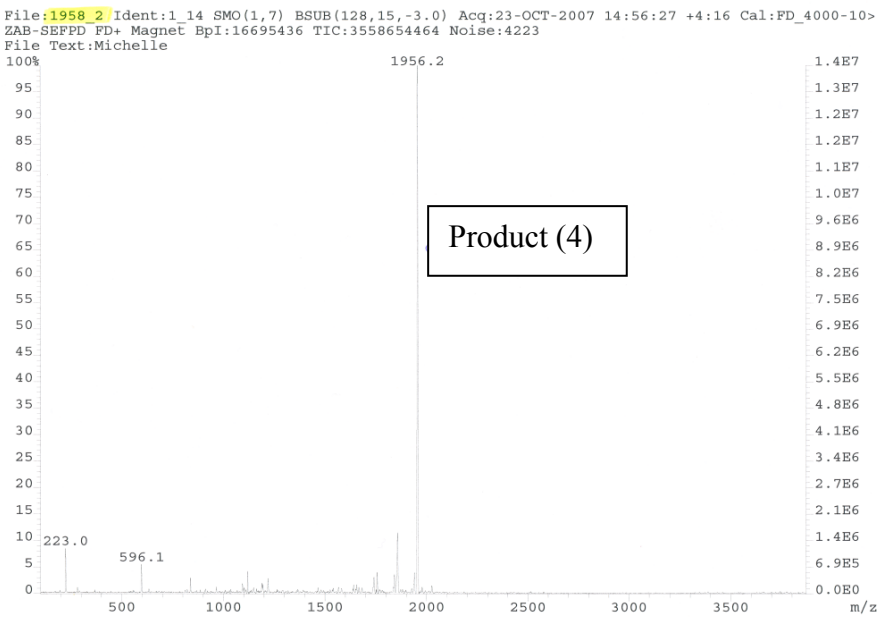


Figure S14: FD-MS of (4)

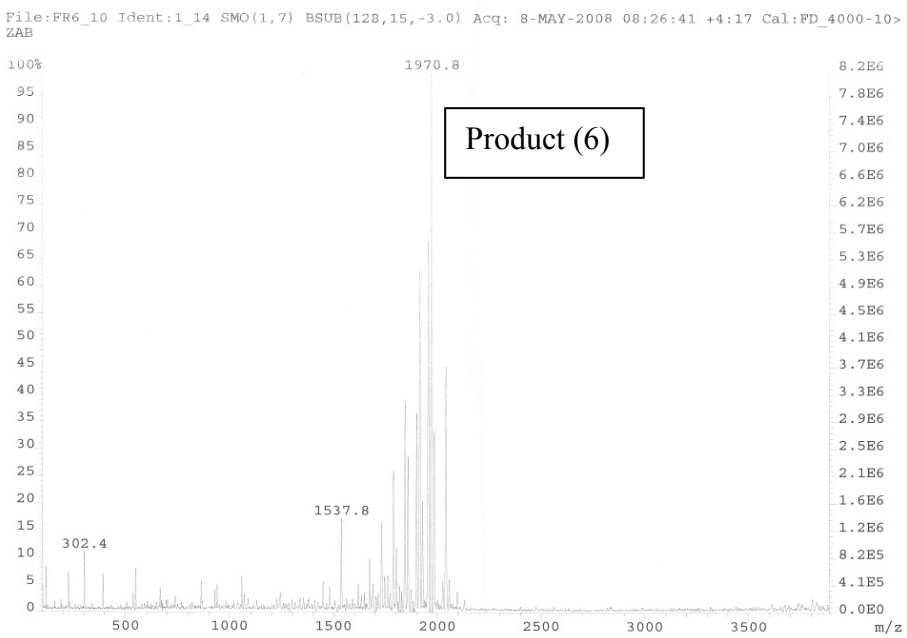


Figure S15: FD-MS of (6)

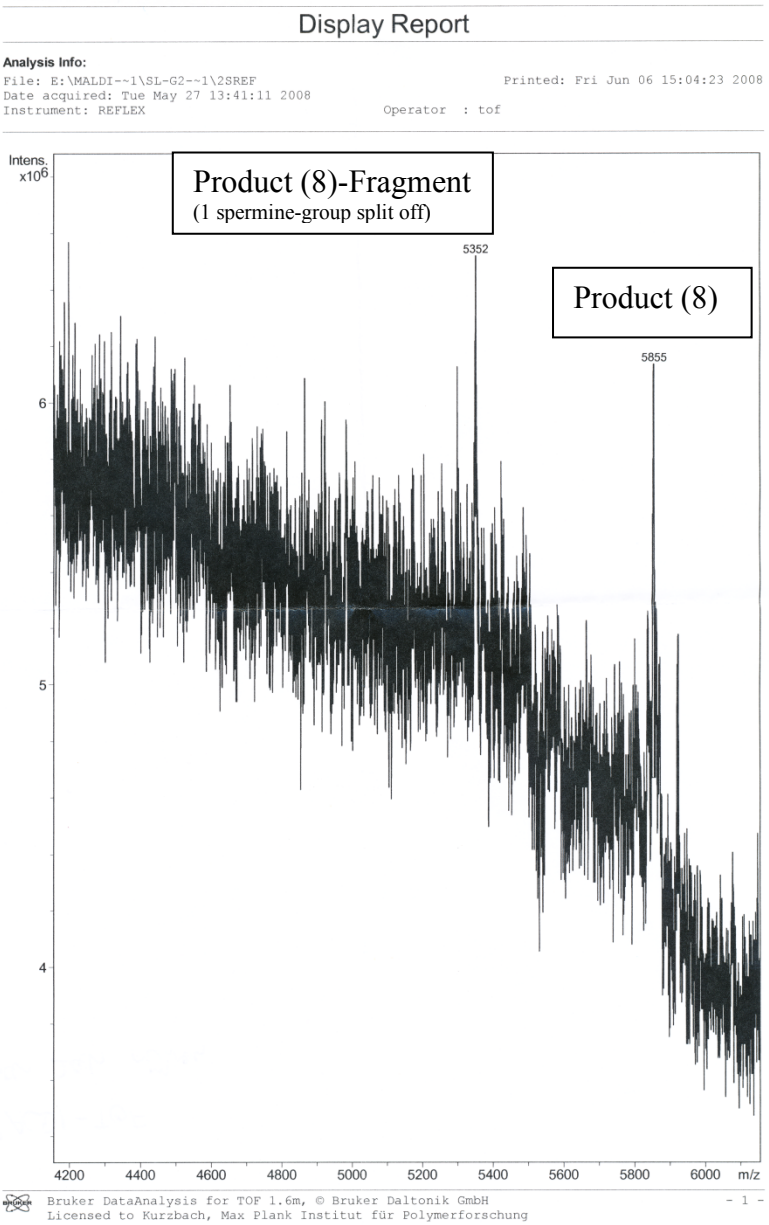


Figure S16: MALDI-TOF of (8)