Supporting Information

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Supplementary information

Optically Triggered Release of DNA from Multivalent Dendrons by Degrading and Charge-Switching Multivalency


1. THF, DCC/HOBt, Et3N, 79%
2. DMAP/DCC/HOBt, pyridine, DCM, 65%
3. HCl (g)/EtOAc, 98%
4. DMAP/DCC/HOBt, pyridine, DCM, 65%
5. HCl (g)/EtOAc, 98%
6. HCl (g)/EtOAc, 100%

pll-G0  pll-G1  pll-G2

1  2  3  4  5  6
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1. Experimental procedures: synthesis and methods

1.1 General procedures and materials
All reagents were commercially available and used as supplied without further purification. Column chromatography was performed on silica gel provided by Merck Ltd. (60, 40-63 µm) while TLC was performed on Merck aluminium-backed plates coated with silica gel (F\textsubscript{254} 10-12 µm). Spots were visualised either by UV, or by use of an appropriate stain (ninhydrin stain: 1 g ninhydrin, 100 mL ethanol, 5 drops glacial acetic acid or cerium molybdate stain: 180 mL H\textsubscript{2}O, 20 mL conc. H\textsubscript{2}SO\textsubscript{4}, 5 g ammonium dimolybdate, 2 g cerium sulfate). Preparative gel permeation chromatography was carried out using Biobeads SX-1 supplied by Bio-Rad. NMR chemical shifts (\(\delta\)) are reported in ppm downfield of tetramethylsilane using residual solvent as internal reference. All spectra were recorded on a Bruker (\(^1\)H 400 MHz, \(^{13}\)C 100 MHz) spectrometer. Electrospray mass spectrometric data was obtained at the University of Oulu mass spectrometry laboratory.

1.2 Synthesis
BOCspermine (1), Z-G1-acid (2) and Z-G2-acid (3) were synthesised as reported previously (subject to minor modifications), and all data were in full agreement with those previously published.\textsuperscript{[1,2,3]}

Phtolabile linker-BOCspermine (pll-BOCsp) (4)
BOCspermine (1) (2.02 g, 4.01 mmol) and PLL (1 g, 3.34 mmol) were dissolved into 50 mL of THF. DCC (0.83 g, 4.01 mmol), HOBt (0.61 g, 4.01 mmol) and Et\textsubscript{3}N (0.41 mg, 4.01 mmol) were added and the mixture was left to stir at RT for 16 h. Dicyclohexylurea precipitate was removed by filtration. The crude product was purified by silica column (97:3 DCM/MeOH). Solvent evaporation yielded a light yellow solid (2.04 g, 78%).

R\textsubscript{f} 0.11 (97:3 DCM/MeOH, ninhydrin stain). \(^1\)H NMR (CDCl\textsubscript{3}, 400 MHz) \(\delta\) 1.42-1.70 (br m, (CH\textsubscript{3})\textsubscript{3}C, 27H: CH\textsubscript{2}CH\textsubscript{2}N, 8H); 1.52 (d, J = 6.4 Hz, CHCH\textsubscript{3}, 3H); 2.17 (quintet, J = 6.8 Hz, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CO, 2H); 2.40 (t, J = 7.2 Hz, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CO, 2H); 3.10-3.23 (br m,  CH\textsubscript{2}N, 12H); 3.95 (s,CH\textsubscript{3}, 3H); 4.09 (t, J = 6.4 Hz, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CO, 6H); 5.31 (br m, NH carbamate, 1H); 5.54 (m, J = 6.4 Hz, CHCH\textsubscript{3}, 1H); 6.83 (br m, NH amide, 1H); 6.82 (s, aromatic CH –m-NO\textsubscript{2}, 1H); 7.54 (s, aromatic CH –o-NO\textsubscript{2}, 1H). ESI-MS (m/z): Calc. value for C\textsubscript{38}H\textsubscript{65}N\textsubscript{5}O\textsubscript{12} 783.949; found: 806 (100%, [M+Na]\textsuperscript{+}).
Phtolabile linker-spermine ·3 HCl (pll-G0)

PLL-BCSpermine (4) (65 mg, 0.083 mmol) was first dissolved in small amount of EtOAc after which 5 mL of HCl gassed EtOAc was added. The reaction was allowed to stir for 1 h, after which time the solvent was removed under reduced pressure, and a light yellow solid was recovered (48 mg, 98%). Yield calculated for HCl salt, FW: 593.

\[ R_f \ 0 \ (9:1 \ DCM/MeOH, \ ninhydrin \ stain). \]

**\[ \text{H NMR (D}_2\text{O, 400 MHz) \delta 1.51 (d, J = 6 \ Hz, CHCH}_3, \ 3H); \ 1.76-1.78 \ (br \ m, \ NHCH}_2CH_2CH_2NH, \ 4H); \ 1.88 \ (quintet, J = 6.8 \ Hz, \ CONHCH}_2CH_2N, \ 2H); \ 2.10-2.15 \ (br \ m, \ CH_2NH, \ 2H: OCH}_2CH_2CO, \ 2H); \ 2.46 \ (t, J = 7.2 \ Hz, \ OCH}_2CH_2CO, \ 2H); \ 3.00-3.19 \ (br \ m, \ CH_2N, \ 10H); \ 3.28 \ (t, J = 6.8 \ Hz, \ CONHCH}_2CH_2N, \ 2H); \ 4.16 \ (t, J = 6.4 \ Hz, \ OCH}_2CH_2CONH, \ 2H); \ 5.50 \ (q, J = 6.4 \ Hz, \ CHCH}_3, \ 1H); \ 7.33 \ (s, \ aromatic \ C, 1H); \ 7.66 \ (s, \ aromatic \ CH –o-NO_2, 1H). \]**

ESI-MS (\textit{m/z}): Calc. value for C_{23}H_{41}N_{5}O_{6} 483.602; found: 484 (100%, [M+H]^+).

Z-G1-PLL-BCSpermine (5)

PLL-BOCSpermine (4) (200 mg, 0.26 mmol), Z-G1-acid (2) (30 mg, 0.064 mmol), DCC (39 mg, 0.19 mmol), HOBt (3 mg 0.02 mmol), DMAP (2 mg, 0.016 mmol) were dissolved into 4 mL of dry DCM under argon atmosphere and cooled on ice, after which pyridine (50 µL) was added. The mixture was stirred at 0°C for 10 min and allowed to reach room temperature. The mixture was left refluxing for 16 h. Dicyclohexylurea precipitate was removed by filtration. The crude product was purified with GPC (Biobeads, 90:10 DCM/MeOH) and silica column (97:3 DCM/MeOH). Solvent evaporation yielded a light yellow solid (113 mg, 65%).

\[ R_f \ 0.11 \ (97:3 \ DCM/MeOH, \ ninhydrin \ stain). \]

**\[ \text{H NMR (CDCl}_3, \ 400 MHz) \delta 1.42-1.70 \ (br \ m, \ (CH}_3)_3C, \ 81H; \ CH}_2CH_2N, \ 24H); \ 1.57 \ (d, J = 6.4 \ Hz, \ CHCH}_3, \ 9H); \ 2.17 \ (quintet, J = 6.4 \ Hz, \ OCH}_2CH_2CH_2CO, \ 6H); \ 2.39 \ (br \ t, J = 7.2 \ Hz, \ OCH}_2CH_2CH_2CO, \ 6H); \ 2.45-2.60 \ (br \ m, \ OCH}_2CH_2CO, \ 6H); \ 3.10-3.24 \ (br \ m, \ CH}_2N, \ 36H); \ 3.50-3.61 \ (br \ m, \ OCH}_2CH_2CO, \ 6H: \ CCH}_2O, \ 6H); \ 3.92 \ (s, \ OCH}_3, \ 9H); \ 4.08 \ (t, J = 6.4 \ Hz, \ OCH}_2CH_2CH_2CO, \ 6H); \ 4.99 \ (s, \ benzylic \ CH}_2, \ 2H); \ 5.10-5.31 \ (br \ m, \ NH \ carbamates, \ 4H); \ 6.45 \ (m, J = 6.4 \ Hz, \ CHCH}_3, \ 3H); \ 6.84 \ (br \ m, \ NH \ amides, \ 3H); \ 6.99 \ (s, \ aromatic \ CH –m-NO_2, 4H); \ 7.31 \ (br \ m, \ Z-aromatic \ CH, \ 5H); \ 7.54 \ (s, \ aromatic \ CH –o-NO_2, 3H). \]**

ESI-MS (\textit{m/z}): Calc. value for C_{135}H_{218}N_{16}O_{44} 2769.257; found: 1407 (100, [M+2Na]^2+); 946 (75, [M+3Na]^3+).
Z-G1-pll-spermine · 9 HCl (pll-G1)

Z-G1-pll-BOCspermine (4) (40 mg, 0.014 mmol) was first dissolved in small amount of EtOAc after which 5 mL of HCl gassed EtOAc was added. The reaction was allowed to stir for 1 h, after which time the solvent was removed under reduced pressure, and a light yellow solid was recovered (31 mg, 98%). Yield calculated for HCl salt, FW: 2196.4

Rf 0 (9:1 DCM/MeOH, ninhydrin stain). $^1$H NMR (D$_2$O, 400 MHz) $\delta$ 1.60 (br m, CHCH$_3$, 9H); 1.78-1.80 (br m, NHCH$_2$CH$_2$CH$_2$CH$_2$NH, 12H); 1.89 (quintet, $J = 7.2$ Hz, CONHCH$_2$CH$_2$CH$_2$N, 6H); 2.06-2.15 (br m, CH$_2$CH$_2$NH$_2$, 6H: OCH$_2$CH$_2$CH$_2$CO, 6H); 2.41 (t, $J = 7.2$ Hz, OCH$_2$CH$_2$CH$_2$CO, 6H); 2.56 (t, $J = 5.6$ Hz, OCH$_2$CH$_2$CO, 6H); 3.02-3.19 (br m, CH$_2$N, 30H); 3.27 (t, $J = 7.2$ Hz, CONHCH$_2$CH$_2$CH$_2$N, 6H) 3.31-3.71 (br m, OCH$_2$CH$_2$CO, 6H: CHCH$_2$O, 6H); 3.93 (s,OC$_3$H$_3$, 9H); 4.10 (br m, OCH$_2$CH$_2$CH$_2$CONH, 6H); 4.90 (s, benzylic CH$_2$, 2H); 6.32 (m , CHCH$_3$, 3H); 7.16 (br m, Z-aromatic CH, 5H); 7.30 (m, aromatic CH –m-NO$_2$, 3H); 7.54 (m, aromatic CH –o-NO$_2$, 3H). ESI-MS ($m/z$): Calc. value for C$_{90}$H$_{146}$N$_{16}$O$_{26}$ 1868.214.

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<tr>
<td>[M+3H]$^{3+}$</td>
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Z-G2-pll-BOCspermine (6)

pll-BOCsp (4) (200 mg, 0.26 mmol), Z-G2-acid (3) (30 mg, 0.021 mmol), DCC (52 mg, 0.26 mmol), HOBt (7 mg 0.043 mmol), DMAP (5 mg, 0.043 mmol) were dissolved into 4 mL of dry DCM under argon atmosphere and cooled on ice, after which pyridine (50 µL) was added. The mixture was stirred at 0°C for 10 min and allowed to reach room temperature. The mixture was left refluxing for 72 h. Dicyclohexylurea precipitate was removed by filtration. The crude product was purified with GPC (Biobeads, 90:10 DCM/MeOH) and silica column (97:3 DCM/MeOH). Solvent evaporation yielded a light yellow solid (120 mg, 65%).

Rf 0.1 (97:3 DCM/MeOH, ninhydrin stain). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 1.41-1.66 (br m, (CH$_3$)$_3$C, 243H: CHCH$_3$, 27H: CH$_2$CH$_2$N, 72H); 2.17 (quintet, $J = 6.8$ Hz, OCH$_2$CH$_2$CH$_2$CO, 18H); 2.41 (br t, $J = 6.8$ Hz, OCH$_2$CH$_2$CH$_2$CO, 18H); 2.47-2.63 (br m, OCH$_2$CH$_2$CO, 24H); 3.14-3.28 (br m, CH$_2$N, 108H); 3.55-3.69 (br m, OCH$_2$CH$_2$CO, 24H: CHCH$_2$O, 24H); 3.95 (s,OC$_3$H$_3$, 27H); 4.08 (br t, $J = 6.4$ Hz, OCH$_2$CH$_2$CH$_2$CO, 18H); 5.00 (s, benzylic CH$_2$, 2H); 5.10-5.31 (br m, NH carbamates, 10H); 6.45 (m, $J = 6.4$ Hz, CHCH$_3$, 9H); 6.89 (br m, NH amides,
12H); 7.02 (s, aromatic CH –m-NO2, 9H); 7.28 (br m, Z-aromatic CH, 5H); 7.54 (s, aromatic CH –o-NO2, 9H). ESI-MS (m/z): Calc. value for C402H659N49O134 8322.783.

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Z-G2-pll-spermine ·27 HCl (pll-G2)
Z-G1-pll-BOCspermine (4) (80 mg, 0.0093 mmol) was first dissolved in small amount of EtOAc after which 5 mL of HCl gassed EtOAc was added. The reaction was allowed to stir for 1 h, after which time the solvent was removed under reduced pressure, and a light yellow solid was recovered (65 mg, 100%). Yield calculated for HCl salt, FW: 7028.7

Rf 0 (9:1 DCM/MeOH, ninhydrin stain). 1H NMR (D2O, 400 MHz) δ 1.52 (CHCH3, 27H); 1.76-1.83 (br m, NHCH2CH2CH2CH2NH, 36H); 1.9 (quintet, J = 7.2 Hz, CONHCH2CH2CH2N, 18H); 2.04-2.13 (br m, CH2CH2NH2, 18H: OCH2CH2CH2CO, 18H); 2.39 (t, J = 7.2 Hz, OCH2CH2CH2CO, 18H); 2.57 (br m OCH2CH2CO, 18H); 3.02-3.19 (br m, CH2N, 90H); 3.27 (br t, J = 6.8 Hz, CONHCH2CH2CH2N, 6H) 3.39-3.68 (br m, CCH2O, 24H: OCH2CH2, 24H); 3.93 (s,CH3, 27H); 3.94-4.09 (br m, OCH2CH2CH2CONH, 18H); 4.89 (s, benzylc CH2, 2H); 6.25 (m, CHCH3, 9H); 7.10-7.19 (br m, Z-aromatic CH, 5H: aromatic CH –m-NO2, 9H); 7.47 (m, aromatic CH –o-NO2, 9H). ESI-MS (m/z): Calc. value for C267H443N49O80 5619.657

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<td>[M+9H]+</td>
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1.3 UV-Vis spectroscopy
Absorption properties of pll-G0, pll-G1 and pll-G2 were studied as a function of UV irradiation time. All samples were dissolved in Milli-Q water at a concentration of 40 µg/mL and irradiated for a given time in quartz cuvettes using Rayonet photochemical reactor. The UV-Vis spectra were measured using a Perkin-Elmer Lambda 950 spectrophotometer in the wavelength range 200-500 nm.
1.4 UV light irradiation

Samples were irradiated in quartz cuvettes for different time periods using a Rayonet photochemical reactor (Southern New England Ultraviolet Co., Middletown, CT, USA) equipped with 16 RPR-3500Å lamps (intensity approximately 9.2 mW/cm², λ=350 nm).

1.5 Ethidium bromide displacement assay

A Varian Cary Eclipse photofluorimeter was used to record the data. Excitation of the sample was done in 3 mL quartz cuvette with 546 nm excitation light and emission was measured at 595 nm.

The buffer designated 0.01 SHE was of ionic strength 0.01 and contained 2 mM of HEPES, 10 µM EDTA, and 9.4 mM of NaCl. The pH was adjusted to 7.2 with NaOH. Biological SHE contained 2 mM of HEPES, 10 µM EDTA, and 150 mM of NaCl. The pH was adjusted to 7.2 with NaOH. Ethidium bromide was dissolved in the buffer to provide 1.26 µM concentration. After mixing the fluorescence was measured. Type III DNA from salmon testes (1 mM of nucleotide concentration in 0.01 SHE) was added to provide a concentration of 1 µM and increasing the fluorescence to measurement maxima.

Ethidium bromide displacement (Figure 2 a,b): The test agent (spermine, pll-G0, pll-G1, pll-G2) in aqueous solution (30 µM – 0.04 M, depending on the compound) was added in small portions to reduce the fluorescence of DNA-ethidium complex to 50% (or plateau).

Complex relaxation by chondroitin sulfate B (Figure 2 c,d): DNA was first completely complexed with the test agent (see above, spermine CE=10, pll-G0 CE=60, pll-G1, CE=2, pll-G2 CE=2), resulting in EthBr displacement and fluorescence decrease. Chondroitin sulfate B was then added in small portions to relax the formed complexes, allowing EthBr re-intercalation and fluorescence increase.

Complex degradation with UV irradiation (Figure 2 e,f): DNA was first completely complexed with the test agent (see above, G1 CE=10, pll-G1, CE=2, pll-G2 CE=2), resulting in EthBr displacement and fluorescence decrease. Higher CE value for G1 was used because binding of G1 is weaker than pll-G1 and pll-G2. G1 complexes DNA in similar manner at CE=10 compared to pll-dendrons at CE=2. At CE=2 DNA complexation with G1 is ineffective. Complexes were then irradiated with UV light to degrade photolabile dendrons, allowing EthBr re-intercalation and fluorescence increase. EthBr fluorescence was recorded after different time periods.

Each fluorescence measurement was repeated two times and each titration series was repeated three times. Finally, the results were averaged.

DNA: a molecular weight of 330 g mol⁻¹ and one negative charge per nucleotide were assumed.
Chondroitin sulfate B: a molecular weight of 444 g mol$^{-1}$ and one negative charge per repeat unit were assumed.

### 1.6 Light scattering and $\zeta$-potential measurements

Scattering intensity and $\zeta$-potential were determined with Malvern Zetasizer 3000HS. DNA-polyamine complexes were prepared at room temperature, by the addition of $\text{pll-G2}$ solution into a solution of plasmid DNA (12.5 µg/mL) (6.7 kilobase pair expression vector, gWiz-Luc (Aldevron, Fargo, ND, USA)) in 150 mM SHE to achieve CE=2 ratio. The resultant complexes were incubated at RT for 5 min and then measured.

### 1.7 Gel retardation assay

Appropriate amounts of polyamine and 250 ng of DNA plasmid (6.7 kilobase pair expression vector, gWiz-Luc (Aldevron, Fargo, ND, USA)) solutions in 150 mM SHE were added to a further 10 µL of 150 mM SHE to achieve the desired polyamine/DNA ratio. The resultant complexes were incubated at RT for 5 min. 2 µL loading dye was added and the mixtures incubated at room temperature for a further 5 min, after which 15 µL was run on a 0.8% agarose gel (70 V, 45 min). DNA was visualized with Ethidium Bromide staining (Bio-Rad, Hercules, CA).

### 2. Supporting information figures

The photolytic degradation of $\text{pll-G0}$ and $\text{pll-G1}$ in aqueous solution was studied with UV-Vis spectroscopy. Irradiation of these compounds with UV light at 350 nm led to significant changes in the UV-Vis spectra. Decrease of absorbance at 245 nm was observed along with clear increase at 268 nm and 349 nm, which typically indicate the photolytic reaction proposed in scheme 1 b. These results are consistent with $\text{pll-G2}$ as shown in figure 1 (see article).
Figure S1. UV-Vis spectra of a) pll-G0 and b) pll-G1 (40 µg/mL) after different UV irradiation (λ=350 nm) times.

Longer irradiation (after approximately 4 minutes) leads to further changes in the absorption spectra, for example decrease of absorption at 330-400 nm (figure S2)

Figure S2. UV-Vis spectra of a) pll-G0 b) pll-G1 and c) pll-G2 (40 µg/mL) after different irradiation times (4-40 min) with UV light (λ=350 nm).

3. Supporting information references

4. Gradient selected –COSY NMR spectra for compounds:
4. pll-G0, 5. pll-G1, 6. and pll-G2
060801 Z-G1-photol. link-trieBOCsperrnine (Z-G1-ppi-BOCsp)

ppm (f2)

0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0

ppm (f1)
060803 Z-G1-photol. link- spermine 'deBOC' (Z-G1-phot-sp)

P11-67