Dendronised block copolymers as potential vectors for gene transfection†

Tony J. Wigglesworth,[a] Francisco Teixeira Jr.,[b] Fabian Axthelm,[b] Sara Eisler,[a] Noemi S. Csaba,[c] Hans P. Merkle,[c] Wolfgang Meier[b] and François Diederich*[a]

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A series of block copolymers containing a dendronised cationic block for efficient DNA binding and a poly(ethylene glycol) block for encapsulation of the complex were synthesised in a modular fashion using a combination of click chemistry and ring-opening metathesis polymerisation. DNA binding experiments, investigated using gel electrophoresis, dynamic light scattering and transmission electron microscopy, showed that all polymers prepared in this study strongly complex DNA and self-assemble into polyion complex micelles with apparent hydrodynamic radii ranging from 20–120 nm at physiological pH (7.4). The *in vitro* transfection efficiency and toxicity of these potential non-viral vectors were also evaluated in HeLa‡ cells using plasmid DNA encoding for green fluorescent protein as the reporter gene.

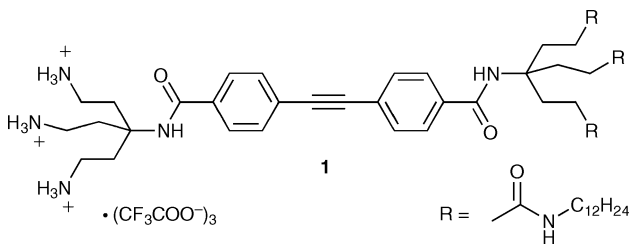
Introduction

The health concerns associated with viral gene delivery systems have spawned the development of non-viral vectors based on cationic lipids,[1] dendrimers[2] and block copolymers.[3] Despite significant advances, even the most successful platforms still have delivery efficiencies three orders of magnitude lower than viral DNA carriers, and the development of new, efficient vectors remains a challenge.[4]

Dendronised polymers are a subclass of comb polymers in which the linear combs have been replaced by dendrons. These unique macromolecules are interesting materials for potential use in therapeutic applications due to the large number of functionalisable peripheral groups, and their tunable size and shape.[5] The ability to modulate DNA complexation by synthetically varying the diameter of cationic dendronised polymers further suggests that these structures are attractive scaffolds for developing new non-viral vectors for gene delivery.[6]

The self-assembly of block copolymers is a powerful tool for forming ordered supramolecular structures in solution and is the basis of encapsulation/drug delivery approaches.[7] For gene therapy applications, block copolymers usually contain a cationic block for DNA binding and a hydrophilic segment for encapsulation of the complex.[3] Poly(ethylene glycol) (PEG) segments are commonly used in polymer therapeutics to sterically shield a drug or DNA core from metabolic/enzymatic cleavage and premature clearance by the mononuclear phagocyte system. This feature improves the solubility and increases the circulation lifetime *in vivo*.[3b]

Although the cationic blocks typically consist of linear poly(ethylene imine) (PEI) or poly-L-lysine,[3] the use of dendronised monomer units was envisaged. The high surface charge density provided by triply branching Newkome dendrons should favour strong DNA binding and endosomal escape once internalised. We have recently used this architecture in the development of small self-assembling amphiphilic dendrimers **1** for gene transfection and have shown that they constitute a suitable platform for developing new non-viral vectors.[8]



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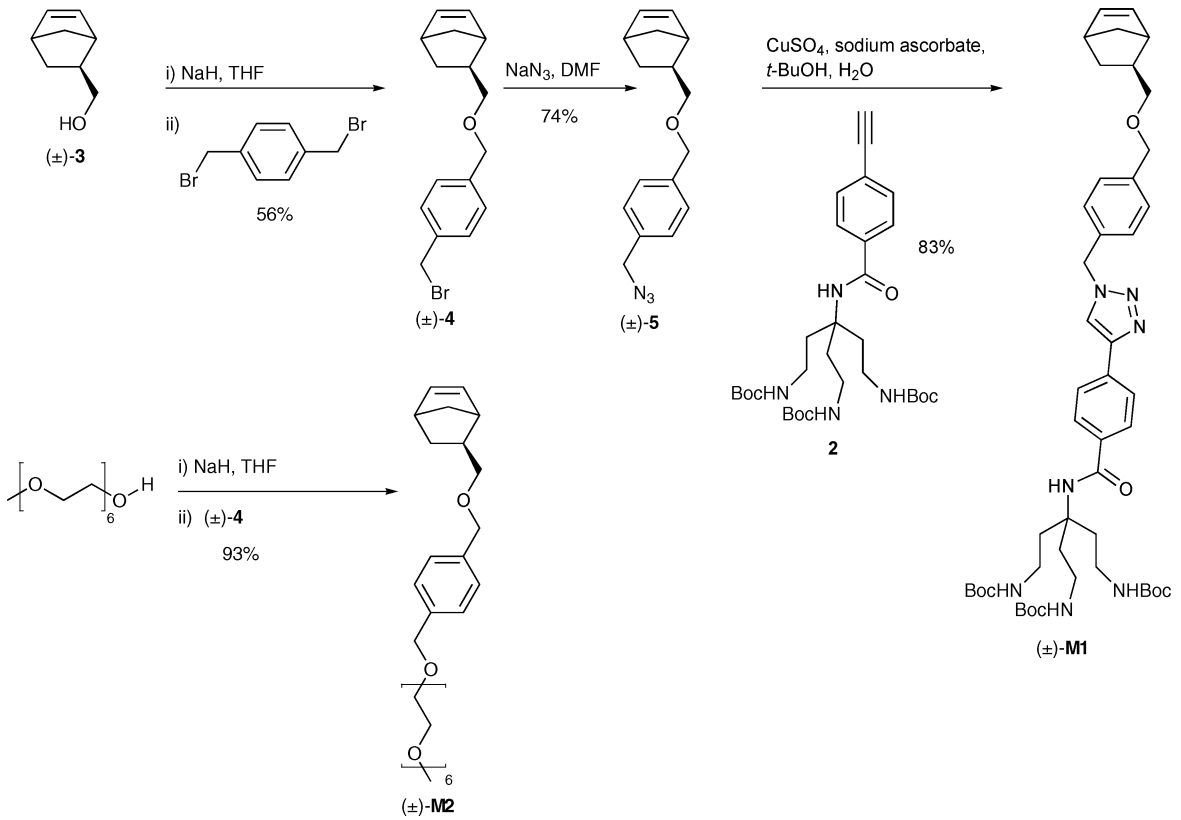
Block copolymers possessing a dendronised cationic block are expected to strongly complex DNA, and offer new opportunities for gene transfer applications because both the structure and generation of the dendronised block can be systematically varied to optimise their gene transfer properties. The construction of PEGylated dendronised block copolymers as effective gene transfection agents is a formidable task. We have shown that subtle changes in dendrimer structure can have a profound impact on transfection efficiency,[9] and wanted to develop a synthesis that is highly tolerant of functional groups and proceeds in virtually any solvent so that the polymer structure would not be hindered by the synthetic methodology. Combining click chemistry[10] and ring-opening metathesis polymerisation (ROMP)[11] is well suited for our purposes, as these reactions proceed in the presence of numerous functional groups, and are not limited by solvents, including water. In addition, the combination of click chemistry and ROMP has been recently used to prepare highly functionalised homopolymers[12] and block copolymers,[13] and was proven to be

[a]*Laboratorium für Organische Chemie, ETH-Zürich, 8093, Zürich, Switzerland. E-mail: diederich@org.chem.ethz.ch; Fax: +41 44 632 1109; Tel: +41 44 632 2992*
[b]*Department of Chemistry, University of Basel, 4056, Basel, Switzerland. E-mail: wolfgang.meier@unibas.ch*
[c]*Institut für Pharmazeutische Wissenschaften, ETH-Zürich, 8093, Zürich, Switzerland. E-mail: hmerkle@pharma.ethz.ch*

† Electronic supplementary information (ESI) available: Complete cell biological methods, plasmid DNA preparation, TEM images, light scattering data and ^{1}H and ^{19}F NMR spectral characterisation of polymers. See DOI: 10.1039/b802808g



Scheme 1 Modular synthesis of ROMP-reactive dendritic monomer (±)-**M1** and PEG monomer (±)-**M2**. Boc = *tert*-butyloxycarbonyl.

an effective technique for preparing structurally diverse polymers from common monomeric building blocks.

Results and discussion

We decided to use a "click-then-ROMP" strategy to prepare a series of block copolymers incorporating both a cationic dendronised block and a hydrophilic PEG block. This route was carefully chosen over a "ROMP-then-click" polymer postfunctionalisation route to maximize the purity of the copolymers and avoid any difficult purification steps.

Monomer synthesis

The synthesis of dendronised monomer (±)-**M1** was achieved by attaching a ROMP-reactive *exo*-norbornene scaffold to acetylenic dendron **2** using the modular click chemistry approach shown in Scheme 1. Monosubstitution of 1,4-bis(bromomethyl)benzene with the sodium anion of alcohol (±)-**3** provides convenient access to multi-gram quantities of benzylic bromide (±)-**4** with moderate yield. Conversion to benzylic azide (±)-**5** with sodium azide and a subsequent copper-catalysed click reaction completes the synthesis of monomer (±)-**M1**.

ROMP-based block copolymer nanoparticles, built from a hydrophilic block containing pendant PEG oligomers, have been recently developed for drug delivery applications.[14] Hexaethylene glycol chains were sufficient for surrounding a drug core and should also facilitate encapsulation of DNA. Accordingly, we prepared related ROMP-reactive PEGylated monomer (±)-**M2** from benzylic bromide (±)-**4** using a similar method (see Scheme 1).

Block copolymer synthesis

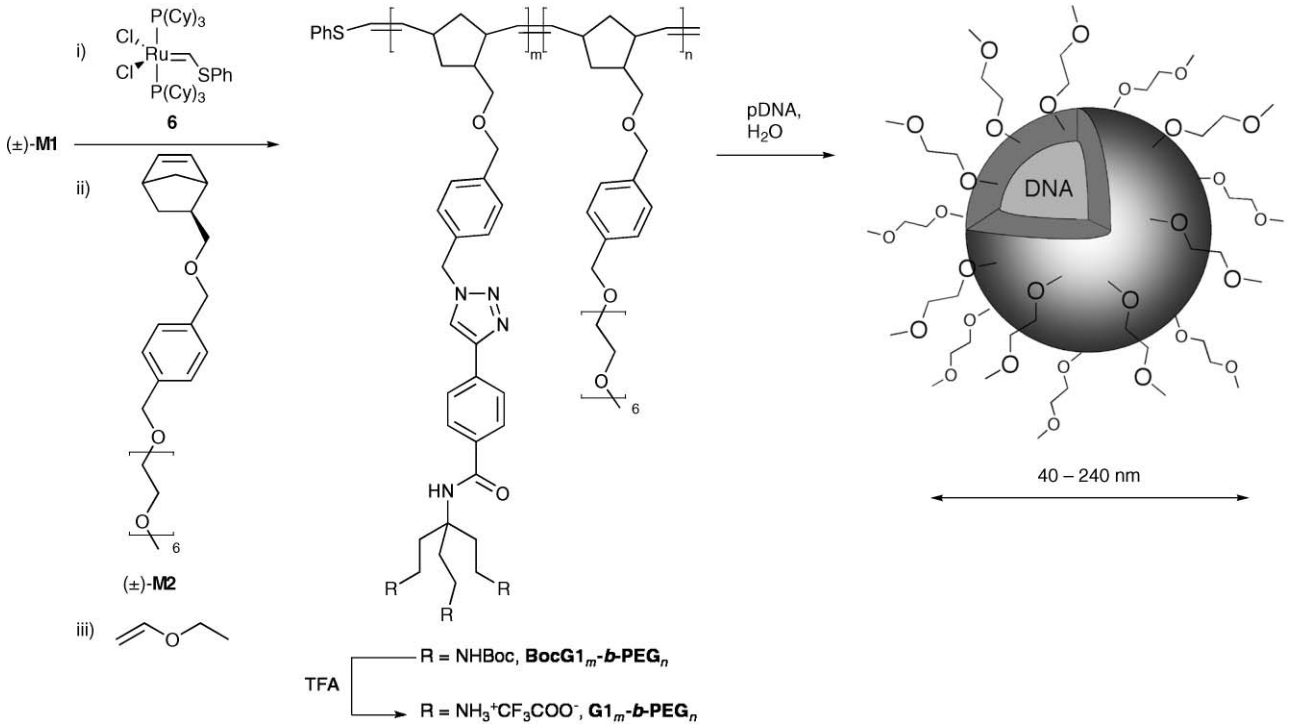
Preliminary ^{1}H NMR studies in CD_2Cl_2 indicated that dendronised monomer (±)-**M1** could be polymerised in an NMR tube using commercially available, air-stable metathesis catalyst bis(tricyclohexylphosphine)[(phenylthio)methylene]ruthenium(II) dichloride (**6**) (see Scheme 2).[15] The polymerisation reaction was complete after 90 minutes as indicated by the disappearance of the ^{1}H NMR signals corresponding to the strained bicyclic olefin and the subsequent appearance of new broad ^{1}H NMR signals of the polymer (see ESI†). A series of block copolymers was prepared under similar conditions by first polymerising dendronised monomer (±)-**M1** using catalyst **6** (see Scheme 2). After stirring for 90 minutes, monomer (±)-**M1** was completely polymerised as indicated by TLC. The reaction mixtures were then treated with different amounts of PEG monomer (±)-**M2**, stirred for an additional 90 minutes and quenched with ethyl vinyl ether.

All block copolymers **BocG1$_m$-*b*-PEG$_n$** were isolated in high purity and good yield, by pouring the crude reaction mixture into cold hexane, collecting the resulting precipitate by vacuum filtration and drying under high vacuum for 18 h. Control over the block length and molecular weight was affected by varying the monomer/catalyst ratios. All polymers were characterised by ^{1}H

Table 1 Properties of dendronised block copolymers **BocG1$_m$-*b*-PEG$_n$**

Entry	Copolymer	Yield (%)	m [a]	n [a]	m/n [b]	M_n ($\times 10^{-3}$)[c]	PDI[c]	Size/nm [d]	Polyplex size/nm [e]
1	**G1$_{100}$**	92	100	—	—	185	1.68	1	20
2	**G1$_{20}$-*b*-PEG$_{20}$**	89	20	20	1.2	66.6	2.72	6	50
3	**G1$_{40}$-*b*-PEG$_{40}$**	89	40	40	1.1	70.9	2.60	5	120
4	**G1$_{60}$-*b*-PEG$_{60}$**	92	60	60	0.8	110	2.52	5	120
5	**G1$_{20}$-*b*-PEG$_{10}$**	92	20	10	2.0	96.1	1.33	4	65
6	**G1$_{60}$-*b*-PEG$_{30}$**	92	60	30	2.0	201	2.70	5	105

[a] Calculated on the basis of the reaction stoichiometry. [b] Determined by ^{1}H NMR spectroscopy. [c] Estimated by GPC with poly(methyl methacrylate) standards using 1 mg cm^{-3} LiBr in DMF as eluent. [d] Apparent R_h of copolymers at physiological pH (7.4) as determined by DLS. [e] Apparent R_h of copolymer/pDNA complexes at CE = 2 as determined by DLS.



Scheme 2 Synthesis of dendronised block copolymers **G1$_m$-*b*-PEG$_n$** and their proposed self-assembly with pDNA. The figure of the proposed self-assembly is adapted from Nguyen and coworkers.[14b] Boc = *tert*-butyloxycarbonyl, TFA = trifluoroacetic acid.

NMR spectroscopy and gel permeation chromatography (GPC) (see Table 1). The GPC results shown in Table 1 indicate that higher than expected molecular weights (M_n) and broad molecular weight distributions (PDI) were obtained for all the polymers prepared in this study. This suggests that the propagation rate was much higher than the initiation rate with this catalyst.[16] These results are consistent with the results of a study by Hult and coworkers who polymerised first to fourth generation dendronised norbornene monomers using Grubb's 2nd generation catalyst and obtained broad molecular weight distributions and higher than expected molecular weights, although much higher yields were obtained in our case.[17]

To facilitate DNA binding all polymers were subsequently deprotected with TFA. ^{1}H and ^{19}F NMR analysis of the deprotected vectors **G1$_m$-*b*-PEG$_n$** indicated that the Boc protecting groups were removed quantitatively and that the polymer backbone and the PEG chains were not adversely affected by the deprotection conditions (see ESI†).

DNA binding and copolymer self-assembly

Polyion complex (PIC) micelles were subsequently prepared from the resulting cationic polymers by adding different amounts of block copolymers **G1$_m$-*b*-PEG$_n$** to 25 μg of plasmid DNA (pDNA) encoding for green fluorescent protein at physiological pH (7.4). The resulting PIC micelles were analysed by gel electrophoresis, dynamic light scattering (DLS) and transmission electron microscopy (TEM). Gel electrophoresis experiments, shown in Fig. 1a, revealed that block copolymers **G1$_m$-*b*-PEG$_n$** completely retard the migration of pDNA towards the cathode at a charge excess ratio of 2 (CE‡). This indicates that all polymers prepared

‡ The human cervix epithelial adenocarcinoma cell line (HeLa) was used and the transfection efficiency (TE) was evaluated using fluorescence-activated cell-sorting (FACS). The charge excess (CE) ratio is defined as the number of positive charges in the copolymer divided by the number of negative charges in pDNA and was estimated based on the ^{1}H NMR data shown in Table 1.

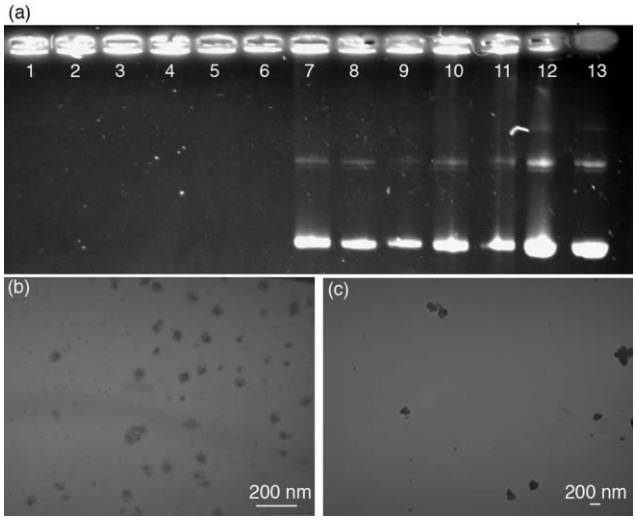


Fig. 1 (a) Gel electrophoresis of pDNA (250 ng per lane). Lane 1: $\mathbf{G1_{100}}$/pDNA (CE = 2). Lane 2: $\mathbf{G1_{20}}$-*b*-$\mathbf{PEG_{20}}$/pDNA (CE = 2). Lane 3: $\mathbf{G1_{40}}$-*b*-$\mathbf{PEG_{40}}$/pDNA (CE = 2). Lane 4: $\mathbf{G1_{60}}$-*b*-$\mathbf{PEG_{60}}$/pDNA (CE = 2). Lane 5: $\mathbf{G1_{60}}$-*b*-$\mathbf{PEG_{30}}$/pDNA (CE = 2). Lane 6: $\mathbf{G1_{20}}$-*b*-$\mathbf{PEG_{10}}$/pDNA (CE = 2). Lane 7: $\mathbf{G1_{100}}$/pDNA (CE = 1). Lane 8: $\mathbf{G1_{20}}$-*b*-$\mathbf{PEG_{20}}$/pDNA (CE = 1). Lane 9: $\mathbf{G1_{40}}$-*b*-$\mathbf{PEG_{40}}$/pDNA (CE = 1). Lane 10: $\mathbf{G1_{60}}$-*b*-$\mathbf{PEG_{60}}$/pDNA (CE = 1). Lane 11: $\mathbf{G1_{60}}$-*b*-$\mathbf{PEG_{30}}$/pDNA (CE = 1). Lane 12: $\mathbf{G1_{20}}$-*b*-$\mathbf{PEG_{10}}$/pDNA (CE = 1). Lane 13: pDNA. (b, c) Representative TEM images of copolymer/pDNA complexes. (b) $\mathbf{G1_{100}}$/pDNA (CE = 2), (c) $\mathbf{G1_{40}}$-*b*-$\mathbf{PEG_{40}}$/pDNA (CE = 2).

in this study strongly complex DNA, even at low charge excess ratios.

DLS measurements at a 90° scattering angle were used to estimate the apparent hydrodynamic radii (R_h) of the resulting polyplexes (see ESI†). At low CE ratios (2) the complexes formed stable PIC micelles and their sizes could be estimated by DLS (see Table 1). The light scattering measurements indicated that the apparent R_h of the PIC micelles ranged from 20–120 nm, dependent on both the polymer molecular weight and the block lengths. At higher CE ratios, DLS measurements were uninformative, due to the formation of complex mixtures of larger aggregates, with $R_h > 1$ μm, and free polymer chains. Control DLS measurements on aqueous solutions of the pure block copolymers (0.5 mg cm^{-3}) at pH 7.4 indicated that the polymers do not form large aggregates at this concentration (see Table 1). This illustrates that the observed aggregates are due to the formation of PIC micelles with a pDNA core surrounded by a hydrophilic PEG corona (for a model visualisation, see Scheme 2).[18]

The size and shape of the polyplexes was further investigated by TEM experiments. Copolymer/pDNA complexes (CE = 2) were drop-cast on carbon grids, stained with 1% aqueous uranyl acetate, air-dried and imaged by TEM (see ESI†). Representative examples are shown in Fig. 1b,c. The polyplexes, dispersed on carbon grids, are small spherical aggregates with diameters ranging from 50–200 nm. The polyplex sizes observed by TEM correlate with the DLS measurements and further suggest that the polymers self-assemble with pDNA into a core-shell structure, as illustrated in Scheme 2.

The transfection efficiency (TE) and toxicity of the polymers were evaluated in comparison with commercial vectors lipofectamine 2000™ and linear poly(ethylene imine) (PEI) using HeLa‡ cells as a model system (see ESI†). In general, the TEs were much lower than for the commercial vectors due to the high toxicity of the polymers, in particular at high CE values (see ESI†). It is interesting to note that polymer $\mathbf{G1_{100}}$ had the lowest TE of all the polymers prepared in this study, which suggests that gene transfer is partially mediated by the PEG block. Perhaps, pDNA binding by $\mathbf{G1_{100}}$ might be too strong to permit transfection and the effect of the PEG chains is disruptive enough to permit moderate TE at high CE. This effect has also been observed in PEI-*b*-PEG graft copolymers, particularly at high CE.[19]

In general, the block ratio (*m*/*n*, Table 1) was critical and polymers with higher PEG content had increased TE and decreased toxicity. The molecular weight also had some impact on the biological activity as lower molecular weight polymers had higher TE at low CE (see ESI†). The molecular weight and block lengths impact the polyplex size, toxicity and the overall TE of the polymers. All of these factors are now considered in the design of future delivery vectors based on this polymer backbone.

Conclusions

We have developed a series of dendronised block copolymers in a modular fashion and demonstrated their ability to complex pDNA and self-assemble into core–shell nanoparticles with apparent hydrodynamic radii from 20–120 nm. The biological activity of these potential gene carriers is low due their high toxicity to cell cultures. Taking the initial structure–activity relationships (SARs) into account we are currently preparing a diverse series of dendritic monomers for constructing new dendronised block copolymers in hopes of discovering less toxic and more efficient vectors for gene transfection. They should become readily available using the synthetic "click-then-ROMP" methodology introduced in this study.

Experimental

General details

Solvents and reagents were purchased as reagent-grade and used without further purification. Hexaethylene glycol monomethyl ether and air stable ROMP catalyst bis(tricyclohexylphosphine)-[(phenylthio)methylene]ruthenium(II) dichloride **6** were purchased from ABCR and Strem respectively. Acetylenic dendron **2**[8] and ROMP-reactive alcohol (±)-**3**[20] were synthesised according to a literature procedure. All reactions were carried out under an Ar or N_2 atmosphere unless otherwise stated. CH_2Cl_2 and THF were freshly distilled over CaH_2 and sodium/benzophenone, respectively. Solvents for polymerisation were freshly distilled and deoxygenated by bubbling Ar through the solvent for 30 min prior to use. All products were dried under high vacuum (10^{-2} Torr, room temperature) before analytical characterisation. TLC was performed on glass-backed plates coated with SiO_2-60 UV_{254} (Macherey-Nagel), visualisation by UV light at 254 nm or staining with a solution of $KMnO_4$ (2.5 g), K_2CO_3 (16 g), NaOH (0.25 g) in H_2O (250 cm^3). Column chromatography (CC) was performed on SiO_2-60 (230–400 mesh) (Silicylce). Melting points (M.p.) were obtained using a Büchi-510 apparatus and are uncorrected. IR spectra were obtained using a Perkin Elmer Spectrum BX FTIR System spectrometer (ATR-unit, Attenuated Total Reflection,

Golden Gate) and are reported in wavenumbers (ν_{max}/cm^{-1}). The peak intensities are described as s (strong), m (medium) or w (weak). NMR spectra (^{1}H, ^{13}C, ^{19}F) were acquired using a Varian Gemini-300 spectrometer (^{1}H, ^{13}C) or a Bruker DRX-400 spectrometer (^{19}F) and were recorded at 25 °C using the solvent peak as an internal reference. Coupling constants (J) are given in Hz. The resonance multiplicities are described as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br m (broad multiplet). High-resolution mass spectra (HR-MS) were obtained by MALDI using a IonSpec Ultima FT-ICR with 3-hydroxypicolinic acid (3-HPA) as matrix or by EI using a Micromass AutoSpec-Ultima. Elemental analyses (EA) were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich. Gel permeation chromatography (GPC) measurements were performed by Mr Martin Colussi at the Department of Materials, ETH Zürich. GPC measurements were carried out using a PL-GPC 220 instrument equipped with a 2 PL-Gel Mix-B LS column set (2 × 30 cm) and refractive index (RI), viscosity and light scattering (LS; 15° and 90° angles) detectors (Polymer Laboratories Ltd, UK). DMF containing LiBr (1 mg cm^{-3}) was used as eluent at 45 °C. Universal calibration was performed with poly(methyl methacrylate) standards in the range of Mp = 2680–1 520 000 (Polymer Laboratories Ltd, UK).

Complex formation

Block copolymers **G1$_m$-*b*-PEG$_n$** and pDNA encoding for green fluorescent protein were complexed by electrostatic interactions. A given quantity of the respective copolymer solution (1.0 mg cm^{-3}, 5% MeOH in deionised water) was added to pDNA solution (0.1 mg cm^{-3} in Tris-EDTA (TE) buffer, pH 7.4) to obtain a dispersion of the complex with the respective charge excess (CE) ratio. To ensure efficient mixing, the resulting dispersions containing copolymer/pDNA complexes were pipetted up and down, and incubated for 30 min at 37 °C to complete complex formation.

Gel electrophoresis

Pre-formed copolymer/pDNA complexes (CE = 1–2) were loaded onto 1 wt% agarose gels (250 ng of pGFP/lane) with Blue/Orange 6× load dye (0.001 cm^3 per lane) from Invitrogen. The copolymer/pDNA complexes were analysed by gel electrophoresis (110 V, BioRad) using 1× Tris-borate (TBE) buffer (pH 7.8) and stained with an aqueous ethidium bromide solution (0.5 μg cm^{-3}) for 30 min prior to analysis. Gels were visualised with a BioRad Gel Doc XR and analysed using QuantiyOne software (BioRad, Hercules, CA, USA).

Dynamic light scattering

Polyplex solutions containing 25 μg of pDNA and excess copolymer (CE = 2) were diluted to a final volume of 1 cm^3 with TE buffer (pH 7.4) and analysed by DLS using an ALV-500 Multiple Tau Correlator equipped with a 632 nm laser (ALV, Germany) at 20 °C with a scattering angle of 90° and a correlation time of 300 s. The DLS correlation data was fit to a regularised DLS-exponential (g2(t)) fit model using ALV-Correlator software (ALV, Germany). DLS measurements on aqueous solutions of the copolymers (0.5 mg cm^{-3}) at pH 7.4 were acquired and analysed under identical conditions.

Transmission electron microscopy

TEM grids were prepared by pre-coating 300 mesh copper grids with a layer of parlodion and a layer of carbon. Polyplex solutions containing 2.5 μg of pDNA and excess copolymer (CE = 2) were deposited onto TEM grids by letting 0.005 cm^3 of solution rest for 75 s on the grid and removing the excess of solution by dabbing with a Kim wipe, leaving the polyplexes deposited onto the film. After staining the polyplex samples with a 1 wt% aqueous uranyl acetate solution for 10 s, the staining solution was removed by dabbing, and the dry grid bearing the stained polyplex samples was imaged using a Philips Morgagni 268D TEM Microscope (FEI Company, Hillsboro, OR, USA) with an accelerating voltage of 80 kV (max. 100 kV).

Synthetic procedures

***exo*-5-({[4-(Bromomethyl)benzyl]oxy}methyl)bicyclo[2.2.1]hept-2-ene ((±)-4).** A suspension of NaH (1.45 g, 60 mmol) in anhydrous THF (150 cm^3) was treated dropwise with (±)-**3** (5.00 g, 40 mmol) and heated to 80 °C under N_2. After 1 h, the heating source was removed, the reaction was cooled to room temperature, treated with a solution of α,α′-dibromo-*p*-xylene (12.8 g, 49 mmol) in anhydrous THF (100 cm^3) in one portion *via* a cannula and heated to 80 °C under N_2. After 16 h, the heating bath was removed, the reaction was cooled to room temperature and quenched with a saturated aqueous NH_4Cl solution (100 cm^3). The layers were separated and the aqueous layer was extracted with toluene (4 × 200 cm^3). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated to dryness *in vacuo*. The crude product was purified by CC (SiO_2, 30% CH_2Cl_2 in hexane), yielding (±)-**4** (6.9 g, 56%) as a colourless oil; ν_{max}/cm^{-1} 2958 m, 2862 m, 1568 w, 1513 w, 1093 s, 705 s and 605 s; δ_H($CDCl_3$, 300 MHz) 7.38 (2H, d, J = 8 Hz), 7.33 (2H, d, J = 8 Hz), 6.11 (1H, dd, J = 6, 3 Hz), 6.06 (1H, dd, J = 6, 3 Hz), 4.52 (2H, s), 4.50 (2H, s), 3.55 (1H, dd, J = 9, 6 Hz), 3.38 (1H, dd, J = 9, 9 Hz), 2.79 (2H, s), 1.79–1.69 (1H, m), 1.35–1.22 (3H, m) and 0.91–0.89 (1H, m); δ_C ($CDCl_3$, 75 MHz) 139.1, 136.9, 136.6, 136.5, 129.1, 127.9, 75.1, 72.5, 45.0, 43.7, 41.5, 38.9, 33.4 and 29.7; HR-EI-MS calcd for $C_{16}H_{19}O^+$ ([M − Br]$^+$): 227.1430; found: 227.1431.

***exo*-5-({[4-(Azidomethyl)benzyl]oxy}methyl)bicyclo[2.2.1]hept-2-ene ((±)-5).** A solution of (±)-**4** (1.50 g, 4.88 mmol) in anhydrous DMF (5 cm^3) was treated with NaN_3 (0.33 g, 5.13 mmol) in one portion and stirred at room temperature under N_2. After 18 h, the reaction mixture was poured into H_2O (25 cm^3) and extracted with Et_2O (3 × 50 cm^3). The combined organic layers were washed with H_2O (3 × 25 cm^3), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified by CC (SiO_2, 4% EtOAc in hexane), yielding (±)-**5** (0.97 g, 74%) as a colourless oil; ν_{max}/cm^{-1} 2958 m, 2862 m, 2093 s (N_3), 1514 w, 1094 s and 706 s; δ_H($CDCl_3$, 300 MHz) 7.38 (2H, d, J = 8 Hz), 7.30 (2H, d, J = 8 Hz), 6.11 (1H, dd, J = 6, 3 Hz), 6.06 (1H, dd, J = 6, 3 Hz), 4.54 (2H, s), 4.34 (2H, s), 3.53 (1H, dd, J = 9, 6 Hz), 3.38 (1H, dd, J = 9, 9 Hz), 2.79 (2H, s), 1.78–1.70 (1H, m), 1.34–1.22 (3H, m) and 1.15–1.89 (1H, m); δ_C($CHCl_3$, 75 MHz)

138.8, 136.6, 136.5, 134.4, 128.2, 127.9, 75.0, 72.5, 54.5, 44.9, 43.6, 41.4, 38.8 and 29.6; EI-HR-MS calcd for $C_{16}H_{18}NO^+$ ([M − N_2H]$^+$): 240.1382; found: 240.1380.

***tert*-Butyl (3-({4-[1-(4-{[(2-*exo*)-bicyclo[2.2.1]hept-5-en-2yl-methoxy]methyl}benzyl)-1*H*-1,2,3-triazol-4-yl]benzoyl}amino)-5-[(*tert*-butoxycarbonyl)amino]3-{2-[(*tert*-butoxycarbonyl)amino]-ethyl}pentyl)carbamate ((±)-M1).** A solution of dendritic alkyne **2** (0.60 g, 1.0 mmol) in a mixture of *t*-BuOH (8 cm^3) and deionised H_2O (4 cm^3) was deoxygenated by bubbling Ar through the solution. After 30 min, the mixture was treated with benzylic azide (±)-**5** (0.41 g, 1.5 mmol), $CuSO_4$ (0.02 g, 0.1 mmol) and sodium ascorbate (0.04 g, 0.2 mmol), and stirred at room temperature under Ar. After 18 h, the mixture was poured into CH_2Cl_2 (200 cm^3), washed with H_2O (2 × 30 cm^3) and a saturated aqueous NaCl solution (30 cm^3), dried (Na_2SO_4), filtered and concentrated to dryness *in vacuo.* The crude product was purified by CC (SiO_2, 33% hexane in EtOAc), yielding monomer (±)-**M1** (0.70 g, 83%) as a white solid; M.p. 99.6–101.8 °C; ν_{max}/cm^{-1} = 2972 m, 1694 s, 1515 s, 1163 s, 858 m, 770 m and 707 m; δ_H($CDCl_3$, 300 MHz) 7.95 (d, J = 8 Hz, 2H), 7.82 (d, J = 8 Hz, 2H), 7.71 (s, 1H), 7.38 (d, J = 8 Hz, 2H), 7.30 (d, J = 8 Hz, 2H), 6.08 (dd, J = 5, 3 Hz, 1H), 6.04 (dd, J = 5, 3, 1H), 5.56 (s, 2H), 4.83 (br s, 3H), 4.52 (s, 2H), 3.52 (dd, J = 9, 6 Hz, 1H), 3.37 (dd, J = 9, 9 Hz, 1H), 3.17 (m, 6H), 2.78 (s, 1H), 2.76 (s, 1H), 2.07 (m, 6H), 1.76–1.67 (m, 1H), 1.40 (s, 27H), 1.32–1.20 (m, 3H), and 1.20–1.07 (m, 1H); δ_C($CDCl_3$, 75 MHz) 166.9, 156.2, 147.3, 139.6, 136.6, 136.5, 134.2, 133.6, 133.3, 128.2, 128.2, 127.8, 125.4, 120.1, 79.4, 75.3, 72.4, 56.9, 54.0, 45.0, 43.7, 41.5, 38.8, 36.3, 36.1, 29.7, and 28.4; MALDI-HR-MS (3-HPA) calcd for $C_{47}H_{67}N_7O_8Na^+$ ([M + Na]$^+$): 880.4949; found: 880.4946; EA calcd for $C_{47}H_{67}N_7O_8$: C, 65.8; H, 7.9; N, 11.4; found C, 65.6; H, 7.9; N, 11.2%.

1-(4-{[(2-*exo*)-Bicyclo[2.2.1]hept-5-en-2ylmethoxy]methyl}phenyl)-2,5,8,11,14,17,20-heptaoxahenicosane ((±)-M2). A suspension of NaH (0.09 g, 2.0 mmol) in anhydrous THF (10 cm^3) was treated with hexaethylene glycol monomethyl ether (0.50 g, 1.7 mmol) in one portion and heated to 80 °C under N_2. After 1 h, the heating bath was removed, the reaction was cooled to room temperature, treated with benzylic bromide (±)-**3** (0.54 g, 1.8 mmol) in one portion and heated to 80 °C under N_2. After 5 h, the heating bath was removed, the reaction was slowly cooled to room temperature and stirred under N_2. After 16 h, the THF was removed *in vacuo* and the residue was dissolved in CH_2Cl_2 (100 cm^3), filtered to remove NaBr and concentrated *in vacuo.* The crude product was purified by CC (SiO_2, 3% MeOH in CH_2Cl_2), yielding monomer (±)-**M2** (0.82 g, 93%) as a colourless oil; ν_{max}/cm^{-1} = 2862 m, 1515 w, 1094 s and 707 m; δ_H($CDCl_3$, 300 MHz) 7.32 (4H, s), 6.16 (1H, dd, J = 6, 3 Hz), 6.05 (1H, dd, J = 6, 3 Hz), 4.56 (2H, s), 4.52 (2H, s), 3.66–3.64 (24H, m), 3.51 (1H, dd, J = 9, 6 Hz), 3.38 (3H, s), 3.35 (1H, dd, J = 9, 9 Hz), 2.78 (2H, s), 1.77–1.68 (1H, m), 1.33–1.21 (3H, m) and 1.14–1.07 (1H, m); δ_C($CDCl_3$, 75 MHz) 138.0, 137.5, 136.6, 136.6, 127.8, 127.6, 74.9, 73.0, 72.7, 71.9, 70.6, 69.4, 59.0, 45.0, 43.7, 41.5, 38.9 and 29.7 (18 of 29 carbons found); MALDI-HR-MS (3-HPA) calcd for $C_{29}H_{46}O_8Na^+$ ([M + Na]$^+$): 545.3090, found: 545.3087; EA calcd for $C_{29}H_{46}O_8$: C, 66.6; H, 8.9; found: C, 66.4; H, 8.8%.

General procedure for block copolymer synthesis

A vigorously stirred solution of monomer (±)-**M1** (0.1–0.3 mmol) in deoxygenated, anhydrous CH_2Cl_2 (1 cm^3) was treated with a solution of catalyst **6** (1–12 μmol, 0.02–0.05 molar equiv.) in deoxygenated, anhydrous CH_2Cl_2 (1 cm^3) in one portion *via* a cannula. After rinsing with deoxygenated, anhydrous CH_2Cl_2 (1 cm^3), the mixture was stirred under Ar. After 1.5 h, monomer (±)-**M1** was completely polymerised as indicated by TLC. The solution was treated with a solution of glycol monomer (±)-**M2** (0.11–0.23 mmol) in anhydrous, deoxygenated CH_2Cl_2 (1 cm^3) in one portion *via* a cannula and was stirred under Ar. After 1.5 h, the reaction was quenched with excess ethyl vinyl ether (1 cm^3) and stirred while exposed to the atmosphere. After 5 min, the crude reaction mixture was poured into cold hexane (80 cm^3) and the resulting precipitate was collected by vacuum filtration and rinsed with hexane (10 cm^3). The pure block copolymers **BocG1$_m$-*b*-PEG$_n$** were isolated as off-white solids and dried under high vacuum for 24 h; typical δ_H($CDCl_3$, 300 MHz) 7.89 (br s), 7.76 (br s), 5.50 (br s), 5.29 (br s), 5.02 (br s), 4.54 (br s), 4.45 (br s), 3.65–3.64 (br m, PEG-OCH_2CH_2O), 3.41 (br s), 3.37 (s, PEG-OCH_3), 3.17 (br s), 2.79 (br, s), 2.47 (br s), 2.08 (br s), 1.89 (br, s), 1.73 (br s), 1.55 (br s) and 1.39 (br s, Boc-CH_3).

General procedure for block copolymer deprotection

A solution of (co)polymer **BocG1$_m$-*b*-PEG$_n$** (0.05–0.18 g) was dissolved in anhydrous CH_2Cl_2 (3 cm^3), cooled to 0 °C and treated with TFA (4 cm^3). After 5 min, the cooling bath was removed and the resulting solutions were warmed to room temperature. After 4 h, the reactions were quenched by the addition of methanol (5 cm^3), concentrated to a viscous oil *in vacuo* and precipitated with Et_2O (10 cm^3). All solvents were removed *in vacuo* and the deprotected polymers **G1$_m$-*b*-PEG$_n$** were dried under high vacuum for 24 h; typical ν_{max}/cm^{-1} = 2902 m, 1674 s, 1538 w, 1199 s, 1176 s, 1124 s, 1084 s, 835 m, 798 m and 721 m; δ_H((CD_3)$_2$SO, 300 MHz) 8.76 (s), 7.98 (s), 7.90 (s), 7.32 (s), 7.22 (s), 5.61 (br s), 5.27 (br s), 4.41 (br s), 3.46 (br s, PEG-OCH_2CH_2O), 3.20 (s, PEG-OCH_3), 2.83 (br s), 2.12 (br s) and 1.77–1.63 (br m); δ_F((CD_3)$_2$SO, 376 MHz) −73.9 (s, CF_3COO^-).

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