High-Affinity Multivalent DNA Binding by Using Low-Molecular-Weight Dendrons

High-Affinity Multivalent DNA Binding by Using Low-Molecular-Weight Dendrons[†]

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This work was supported through the Socrates–Erasmus European Exchange Scheme (M.A.K.) and by the Engineering and Physical Sciences Research Council and Syngenta through the CASE Scheme (J.G.H.). We acknowledge Ian Ashworth and Colin Brennan (Syngenta) for support and Meg Stark (Department of Biology, University of York) for assistance with TEM measurements.

Keywords:

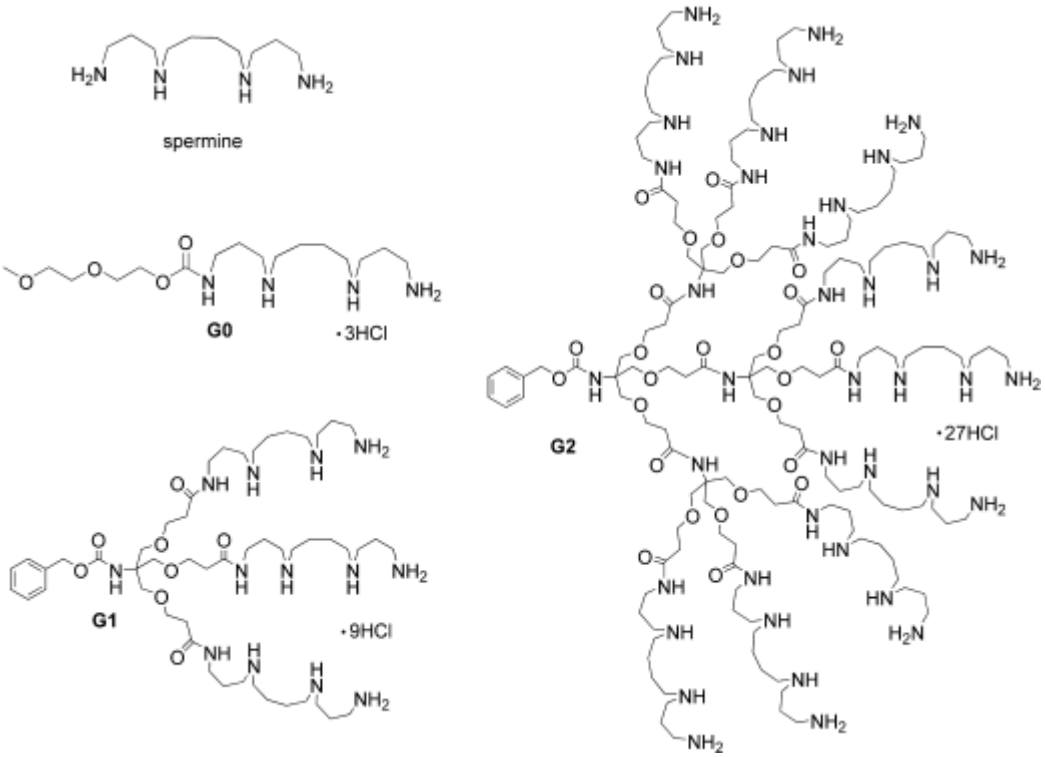
- dendrimers;
- DNA;
- multivalency;
- nanochemistry;
- self-assembly

High-affinity binding between nanoscale objects is an essential prerequisite for “bottom-up” fabrication.[1] In recent years, interest has focused on the use of dendritic macromolecules as supramolecular nanoscale building blocks.[2] The branched superstructure of dendrons and dendrimers offers specific advantages, for example, enhancement of weak binding by using multivalent arrays of recognition units on the dendritic surface. This multivalency principle, in which organized arrays amplify the strength of a weak binding process, such as the binding of saccharides to proteins on cell surfaces, is now well established.[3]

DNA constitutes a particularly interesting target for nanotechnological exploitation.[4] High-affinity binding of DNA is useful for protecting DNA and ultimately for delivering genetic information into cells.[5] Noncovalent interactions between dendritic macromolecules and DNA are, therefore, of considerable current interest.[6] Polyamidoamine (PAMAM) dendrimers were the first systems to be investigated,[7] while dendritic poly(L-lysine)[8] and poly(propylene imine)[9] have also been studied recently. In general, higher-generation, or structurally fractured, systems are usually more effective

for DNA binding and delivery. In an important recent study, however, Diederich and co-workers reported low-molecular-mass dendrons designed to self-assemble with DNA, which were capable of gene therapy.[10]

The interaction between a single protonated amine and the phosphate backbone of DNA is relatively weak and must compete with salt binding under biological conditions. Biology therefore uses tetraamines, such as spermine (Scheme 1), to achieve DNA binding.[11] Synthetic spermine derivatives are also widely used for applications in DNA binding and delivery.[12] However, although spermine is better than an isolated amine for binding DNA, the interaction is still relatively weak and the complex is mobile. Consequently, spermine struggles to compete with DNA-bound inorganic cations.[13]



Scheme 1. Spermine and target spermine derivatives **G0**, **G1**, and **G2**.

We became interested in optimizing DNA binding and developing low-molecular-mass dendrons with very high affinities for DNA—such systems would be particularly useful for DNA encapsulation and protection.[14] We therefore decided to develop dendrons that express multivalent spermine arrays on their surfaces. Some previously reported multivalent spermine arrays comprised multiple spermine groups grafted onto dextran polymers;[15] however, we wanted to develop multivalent systems with well-defined molecular structures. Such monodisperse systems enable an understanding of structure–activity relationships and, in addition, have a greater chance of being licensed for therapeutic applications in the longer term. Herein, we report on multivalent dendritic spermine constructs with extremely high, salt-independent binding affinities for DNA.

We used a divergent synthetic approach to construct target compounds **G1** and **G2** (Scheme 1, details of the synthesis can be found in the Supporting Information). Newkome-type branching[16] was used as the dendritic scaffold, as such structures are readily synthesized[17] and should be biocompatible. After dendron synthesis, the surface was functionalized with spermine groups which had been appropriately protected by using the methodology of Blagbrough and Geall.[18] Deprotection of the spermine groups with HCl gas then yielded target compounds **G1** and **G2**. Model compound **G0** was constructed by using a similar approach (Scheme 1). All synthetic steps were high yielding and all target compounds and intermediates were fully characterized with standard methods (see the Supporting Information).

Initially, the binding of the spermine derivatives to DNA was studied by using an ethidium bromide displacement assay. This method is commonly used to study the binding of polyammonium compounds to DNA.[19] The displacement of ethidium bromide from its complex with DNA can be monitored because it has enhanced fluorescence when intercalated. This is a powerful comparative method, although the resulting data reflect a competition assay, rather than an absolute binding strength, and furthermore give no information about binding stoichiometry.

Fluorescence titrations were performed in buffered water at pH 7.2 using **G0**, **G1**, **G2**, and spermine itself. The resultant titration profiles are shown in Figure 1. At this physiologically relevant pH value, the spermine groups are largely protonated whilst the DNA is largely deprotonated, so electrostatic interactions are maximized.

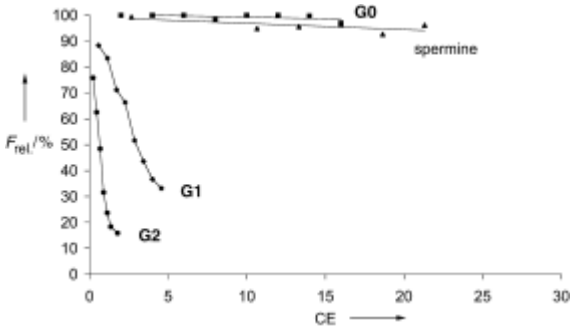


Figure 1. Fluorescence titration profiles for the addition of spermine, **G0**, **G1**, or **G2** to a solution of calf thymus DNA and ethidium bromide in buffered water (pH 7.2) in the presence of 150 mM NaCl.

The data can be presented in terms of C_{50} and CE_{50} values (Table 1). C_{50} values report the concentration of polyamine causing a 50 % decrease in fluorescence intensity. CE_{50} values represent the "charge excess"[20] required to achieve the same 50 % reduction in fluorescence—the very best DNA binders should have CE_{50} values below 1.0. Spermine binds to DNA with moderate strength under low-salt (9.4 mM NaCl) conditions (C_{50}=1.33 µM, CE_{50}=5.3), but a very large charge excess would have been required at physiological salt concentrations (150 mM NaCl) in order to achieve

50 % quenching of ethidium bromide fluorescence (C_{50}=390 µM, CE_{50}=1560). These results were in good agreement with literature data.[19a] Compound **G0** showed similar, if slightly weaker, DNA binding. This was expected, as one of the primary amines of spermine has been converted into an amide, which is incapable of protonation, and **G0** should therefore exhibit weaker electrostatic interactions with polyanionic DNA.

Table 1. Results for spermine, G0, G1, and G2 from an ethidium bromide displacement assay.[a]

Compound	[NaCl] [mM]	Nominal charge	C_{50} [µM]	CE_{50}
1. [a] Conditions: Measurements performed in buffered water at pH 7.2 (with 2 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) and 0.05 mM ethylenediaminetetraacetic tetraacetate (EDTA)). The calf thymus DNA (1 µM) and ethidium bromide (1.26 µM) concentrations were kept constant. The total added polyamine solution did not exceed 5 % of the total volume; therefore, corrections were not made for sample dilution. Results are an average of at least three titrations. Experimental errors ≤10 %.				
spermine	9.4	4+	1.33	5.3
G0	9.4	3+	20	60
G1	9.4	9+	0.076	0.68
G2	9.4	27+	0.030	0.81
spermine	150	4+	390	1560
G0	150	3+	220	660
G1	150	9+	0.300	2.70
G2	150	27+	0.028	0.76

The dendritic systems **G1** and **G2** showed significantly enhanced DNA binding. Under low-salt conditions, **G1** effectively displaced ethidium bromide from DNA (C_{50}=76 nM, CE_{50}=0.68). Notably, the affinity for DNA is considerably more than three times higher than that of **G0**. This indicates that the organization of three spermine units on the dendritic framework enables DNA-binding activity that is more than the simple sum of its individual parts—the multivalency principle[3] in operation. Compound **G1** is somewhat affected by the increase in salt concentration but still shows reasonable binding under these conditions (C_{50}=300 nM, CE_{50}=2.70).

Compound **G2** shows a similar binding affinity to **G1** under low-salt conditions (C_{50}=30 nM, CE_{50}=0.81) but demonstrates its power at physiological salt concentrations, where the binding remains just as strong (C_{50}=28 nM, CE_{50}=0.76). The binding is therefore *salt independent*—a proactive dendritic effect. The multivalent system can therefore compete with Na^+ ions for binding sites on the surface of the DNA helix. Indeed, compound **G2** exhibits one of the best binding profiles reported by using this method, thereby proving that the strategy of organizing spermine units into a well-defined multivalent array has considerable power.

Gel electrophoresis was used to confirm the affinities of the dendrons for DNA in a direct binding assay. The dendritic constructs **G1** and **G2** retarded the electrophoretic mobility of DNA as a consequence of charge neutralization, whilst the spermine and **G0** analogues were ineffective (Figure 2). The CE values at which mobility was retarded were in agreement with the results of the ethidium bromide displacement assays.

Figure 2. Gel electrophoresis of plasmid DNA (250 ng per lane). Lane 1: Plasmid DNA. Lane 2: Plasmid DNA + spermine (250 ng). Lane 3: Plasmid DNA + **G0** (250 ng). Lane 4: Plasmid DNA + **G1** (250 ng). Lane 5: Plasmid DNA + **G2** (250 ng).

Finally, transmission electron microscopy (TEM) was used to image the complexes (Figure 3). The addition of spermine at CE=1.8 to plasmid DNA in buffered water (pH 7.1, [NaCl]=9.4 mM) prior to deposition on a carbon-coated copper grid gave rise to large unsymmetrical aggregates approximately 250 nm in diameter (Figure 3 A). Compound **G0**, however, led to little or no compaction of DNA under the same conditions. On the other hand, use of **G1** or **G2** (CE=2.7) gave rise to well-defined, approximately spherical nanoscale complexes (**G1**: approximately 100 nm in diameter; **G2**: approximately 400 nm in diameter) with no free plasmid being detected (Figures 3 B, C3). The size range of the aggregates formed was relatively large. Nonetheless, these observations indicate that compounds **G1** and **G2** efficiently bind DNA and condense it into approximately spherical complexes.

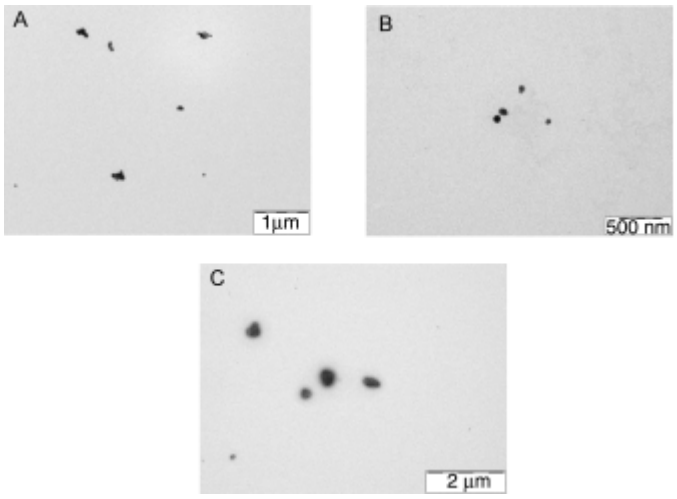


Figure 3. TEM images of DNA in the presence of A) spermine (CE=1.8), B) **G1** (CE=2.7), or C) **G2** (CE=2.7). Samples were deposited from buffered water (pH 7.1).

In summary, we have reported novel dendrons that bind DNA with remarkably high affinities. Notably, **G2** showed salt-independent DNA binding and it was a factor of ten more effective than the **G1** analogue under high-salt conditions, whilst **G1** was, in turn, significantly more effective than the **G0** analogue. It can be concluded that the expression of multiple spermine units, nature's own DNA binder, on the surface of a dendritic scaffold offers a powerful approach for achieving high-affinity DNA binding under physiological conditions. These molecules have potential for further synthetic variation, and in current and future work, we will be investigating the effect of structural modifications on DNA binding and nanoscale assembly, as well as looking at applications of the novel dendritic constructs in gene protection and delivery.

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Charge excess is defined as the nominal "number of positive charges" of the polyamine divided by the "number of negative charges" present on the DNA. A molecular weight of 660 g mol^{-1} per base pair and one negative charge per nucleotide were assumed.