TECHNICAL NOTES

Synthesis and Characterization of Covalently Linked Single-Stranded DNA Oligonucleotide−Dendron Conjugates

Stephen A. Bell,[†] Megan E. McLean,[†] Sang-Keun Oh,[†] Shane E. Tichy,[†] Wen Zhang,[†] Robert M. Corn,[‡] Richard M. Crooks,*[,†,§] and Eric E. Simanek*[,†,||]

Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, Texas 77842-3012 and Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706. Received September 25, 2002; Revised Manuscript Received November 14, 2002

A solution-phase synthesis and characterization of covalent DNA−dendron conjugates is presented. Thiol-terminated 12-base oligonucleotides were added to second- and third-generation triazine-based dendrons via thiol/disulfide exchange chemistry. Single-stranded DNA oligonucleotides were successfully attached to dendrons at the core, the periphery, and both. Proof of structure for these architectures is derived primarily from mass spectrometry and polyacrylamide gel electrophoresis and complemented by labeling analysis using Ellman's reagent and degradation analysis using a reducing agent.

INTRODUCTION

We report the solution-phase synthesis and characterization of covalent DNA−dendron conjugates. Using thiol/disulfide exchange chemistry, thiol-terminated 12-base oligonucleotide sequences were added to second- and third-generation triazine-based dendrons. The synthesis permits either one or two single-stranded DNA (ssDNA) oligonucleotides to be covalently attached to the dendron at the core, the periphery, or both (Scheme 1). Proof of structure for these architectures is derived primarily from mass spectrometry, polyacrylamide gel electrophoresis, labeling analysis using Ellman's reagent (*1*), and degradation analysis with a reducing agent.

The use of DNA constructs in DNA-machines (*2*−*5*), DNA-computing (*6*), antisense therapy (*7*−*9*), and biosensors (*4*, *10*, *11*) has fueled intense research into suitable methods for immobilizing DNA on surfaces (*12*, *13*) and preparing soluble DNA−nanoparticle assemblies (*7*, *14*−*17*). Most of the latter applications rely on nanoparticle modification with multiple copies of ssDNA oligonucleotides of a particular sequence. For example, multiple ssDNA oligonucleotides have been attached to soluble organic polymers (*18*) and gold nanoparticles (*19*). However, methods for covalent attachment of individual ssDNA oligonucleotides to soluble nanoparticles are surprisingly rare (*7*). Synthetic methods that provide a means for conjugating precise numbers of identical or different ssDNA oligonucleotides to particular locations on nanoparticles could allow for simultaneous probing of multiple target oligonucleotides or for constructing interesting, new recursive materials. If such materials also provide a means for attaching additional active components, such as fluorescent or electrochemical tags, their usefulness would be further increased.

The scaffolds used for these experiments were derived from melamine-based dendrimers, which were synthesized using methods similar to those we previously reported (*20*). These dendrimers offer opportunities for appending precise numbers of reactive groups to the periphery and the core. For the present work, dendrons incorporate reactive thiopyridyl groups (Scheme 2), which could subsequently undergo exchange with appropriately functionalized DNA oligonucleotides. Disulfide-terminated oligonucleotides were reduced to yield thiol-terminated ssDNA.

EXPERIMENTAL SECTION

Chemicals. Reagent grade chloroform, dichloromethane, methanol, and ethyl acetate were used without further purification during dendrimer synthesis. Tetrahydrofuran (THF) was dried over 4 Å molecular sieves prior to use. Deuterated solvents were used as supplied by Cambridge Isotope Laboratories (Cambridge, MA). Cyanuric chloride (98%), diisopropylethylamine (DIPEA, 99%), and piperazine (99+%) were used as received from Acros (Pittsburgh, PA). Immobilized tris(carboxyethyl)-phosphine (TCEP) and succinimidyl 3-(2-pyridylthio)-propionate (SPDP) were used as purchased from Pierce Chemical (Rockford, IL). Electrophoresis grade acrylamide:bisacrylamide (29:1), ethidium bromide (1% solution), and coomassie blue R250 were used as received from FisherBiotech (Pittsburgh, PA). Trisborate EDTA (0.45 M) and 10× DNA gel loading buffer were purchased from Eppendorf (Hamburg, Germany). Molecule **7** (*20*) and cysteamine hydrochloride (*21*) were synthesized according to literature procedures.

Synthesis of 1. BOC-$NHCH_2(CH_2OCH_2)_3CH_2NH_2$ (1.77 g, 6.05 mmol) in THF (15 mL) was added dropwise to a cooled (−6 °C), stirred THF solution (30 mL) of cyanuric

* To whom correspondence should be addressed.
[†] Texas A&M University.
[‡] University of Wisconsin.
[§] Phone 979-845-5629; fax 979-845-1399; e-mail crooks@tamu.edu.
[||] Phone 979-845-4242; fax 979-845-9452; e-mail simanek@tamu.edu.

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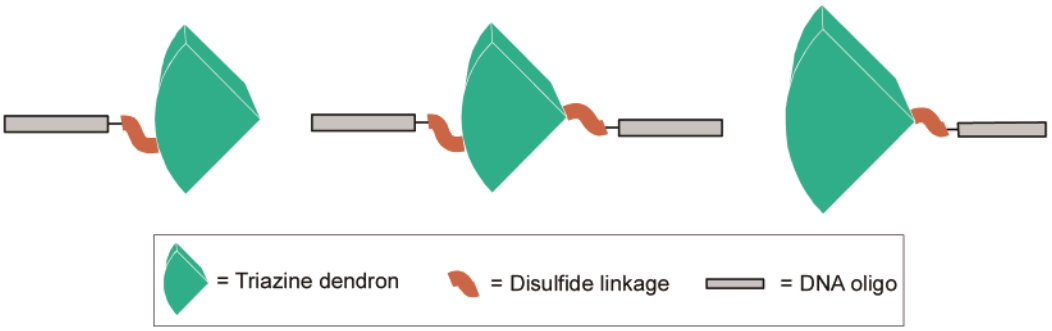
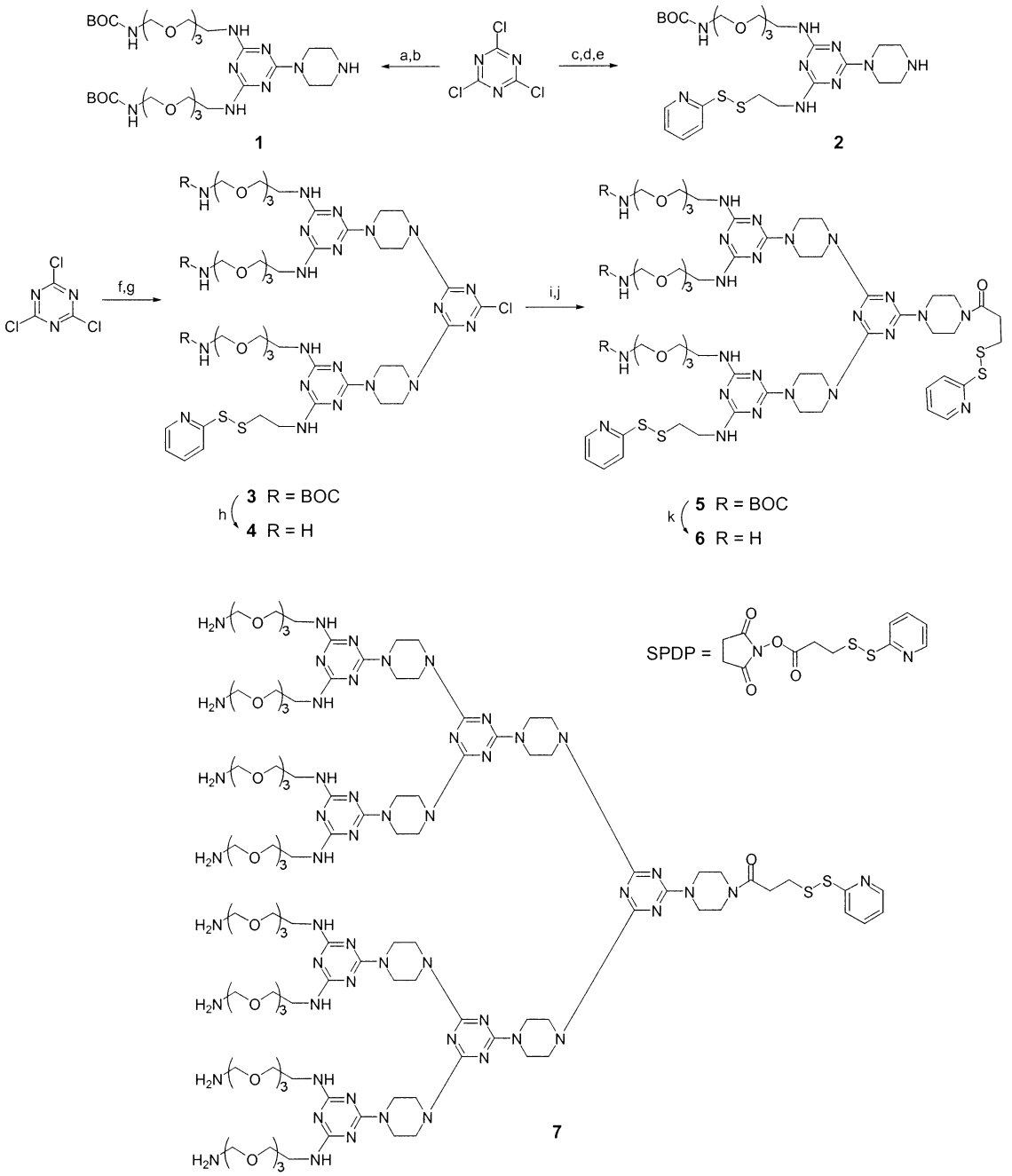
Scheme 1

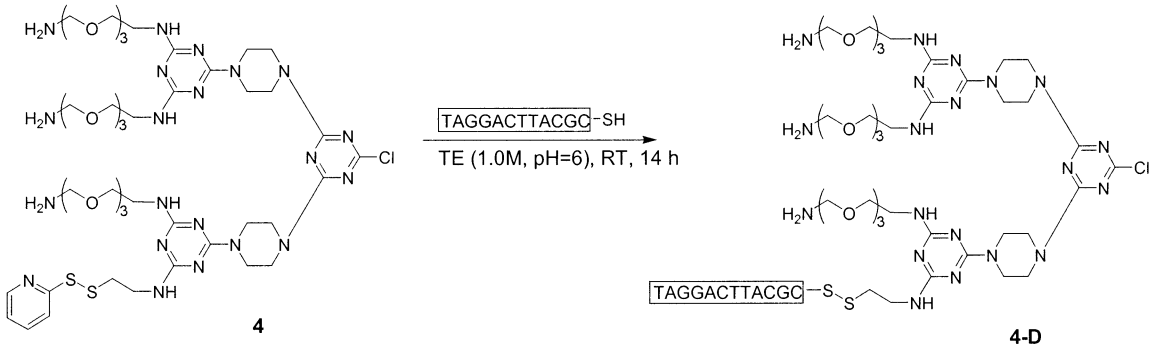


Scheme 2[a]



[a] (a) 2 equiv of $BocNHCH_2(CH_2OCH_2)_3CH_2NH_2$, DIPEA, THF, RT, 14 h; (b) piperazine, DIPEA, RT, 14 h; (c) $BocNHCH_2(CH_2OCH_2)_3$-CH_2NH_2, DIPEA, THF, −6 °C, 2 h; (d) pyridyl-S−S-CH_2CH_2-NH_2, DIPEA, THF, RT, 14 h; (e) piperazine, DIPEA, RT, 14 h; (f) **1**, DIPEA, THF, −6 °C; (g) **2**, DIPEA, THF, RT, 14 h; (h) trifluoroacetic acid (TFA), CH_2Cl_2 (1:1), 10 h, RT; (i) piperazine, DIPEA, RT, 14 h; (j) SPDP, DIPEA, THF, RT, 24 h; (k) TFA:CH_2Cl_2 (1:1), 10 h, RT.

Scheme 3



4 **4-D**

chloride (0.581 g, 3.17 mmol) and DIPEA (1.5 mL, 4.84 mmol). After 2 h the reaction mixture was warmed to room temperature, and more DIPEA (1.5 mL, 4.84 mmol) was added. Stirring for 14 h at room-temperature resulted in a pale yellow solution with white precipitate, yielding a single TLC spot (R_f = 0.65.) Piperazine (1.64 g, 19.02 mmol) and DIPEA (1.5 mL, 4.84 mmol) were added, and the mixture was stirred for 14 h at room temperature. After removal of solvent, the residue was partitioned between CH_2Cl_2 and water. The organic phases were concentrated to yield a pale yellow oil which was purified via silica gel column chromatography (100:3, CH_2Cl_2:MeOH eluent). A pale yellow oil was obtained (0.123 g, 82.3%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.72 (tr, 4H, NCH_2), 3.62 (m, 24H, CH_2OCH_2), 3.49 (tr, 4H,-C(O)NHCH_2CH_2), 3.20 (tr, 4H, NHCH_2CH_2), 2.79 (br, 4H, HNCH_2), 1.42 (s, 18H, OC(CH_3)$_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 166.2, 165.0, 157.1, 78.9, 70.4, 53.9, 43.7, 43.0, 40.4, 27.8; MALDI-TOF (*m*/*z*): [M + H]$^+$ calcd. for $C_{33}H_{63}N_9O_{10}$, 746.5; found, 746.8.

Synthesis of 2. BOC-NHCH_2(CH_2OCH_2)$_3$CH_2NH_2 (1.39 g, 4.75 mmol) in THF (10 mL) was added dropwise to a cooled (−6 °C), stirred solution of cyanuric chloride (0.908 g, 4.95 mmol) and DIPEA (3.3 mL, 9.82 mmol) in THF (30 mL). After 2 h the reaction mixture was warmed to room temperature, and more DIPEA (1.5 mL, 4.84 mmol) was added, followed by a THF solution (15 mL) of neutralized pyridyl cysteamine hydrochloride (1.10 g, 4.95 mmol). Stirring for 14 h at room-temperature resulted in a pale yellow solution with white precipitate, yielding a single TLC spot (R_f = 0.58.) A 6-fold excess of piperazine (2.55 g, 29.7 mmol) and DIPEA (1.5 mL, 4.84 mmol) was added, and the mixture was stirred for 14 h at room temperature. After removal of solvent, the residue was partitioned between CH_2Cl_2 and water. The organic phases were concentrated to yield a pale yellow oil which was purified via silica gel column chromatography (100:3, CH_2Cl_2:MeOH eluent). A pale yellow oil was obtained (1.56 g, 49%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.2 (tr, 1H, *o*-CH), 7.75 (m, 2H, *m*-C*H*), 7.2 (tr, 1H, *p*-C*H*), 3.75 (br, 4H, NCH_2) 3.63 (m, 12H, CH_2OCH_2), 3.50 (tr, 4H, C(O)NHCH_2CH_2 and NCH_2CH_2S), 3.21 (tr, 2H, NHCH_2CH_2), 3.02 (tr, 2H, NCH_2CH_2S), 2.82 (br, 4H, NHCH_2), 1.43 (s, 9H, OC(CH_3)$_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 167.6, 166.1, 161.2, 150.1, 139.2, 122.6, 121.2, 80.2, 71.4, 49.9, 46.3, 44.5, 41.4, 40.8, 39.6, 28.9; MALDI-TOF (*m*/*z*): [M + H]$^+$ calcd for $C_{27}H_{45}N_9O_5S_2$, 640.3; found, 640.4.

Synthesis of 3. Dendron **1** (0.601 g, 0.807 mmol) was dissolved in 15 mL of THF and added dropwise to a cooled (−6 °C), stirred solution THF solution (30 mL) of cyanuric chloride (0.148 g, 0.807 mmol) and DIPEA (1.5 mL, 4.84 mmol). After 2 h the reaction mixture was warmed to room temperature, and more DIPEA (1.5 mL, 4.84 mmol) was added, followed by a THF solution (15 mL) of **2** (0.505 g, 0.807 mmol). Stirring for 14 h at room-temperature resulted in a pale yellow solution with white precipitate. After removal of solvent, the residue was partitioned between CH_2Cl_2 and water. The organic phases were concentrated to yield a pale yellow oil which was purified via silica gel column chromatography (100:3, CH_2Cl_2: MeOH eluent). A white, sticky solid was obtained (0.86 g, 71.2% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 8.2 (m, 1H, *o*-C*H*), 7.78 (m, 2H, *m*-C*H*), 7.19 (tr, 1H, *p*-C*H*), 3.75 (br, 16H, NCH_2), 3.62 (m, 36H, CH_2OCH_2), 3.50 (br, 8H, C(O)NHCH_2CH_2 and NCH_2CH_2S), 3.22 (m, 6H, NCH_2) 3.04 (tr, 2H, NCH_2CH_2S), 1.43 (s, 27H, OC(CH_3)$_3$); ^{13}C NMR (75 MHz, CD_3OD) δ 169.5, 166.1, 165.0, 164.4, 160.0 157.0, 150.0, 137.8, 121.1, 119.8, 78.9, 71.2, 70.2, 43.5, 42.9, 40.3, 27.9. MALDI-TOF (*m*/*z*): [M + H]$^+$ calcd for $C_{63}H_{10}N_{21}O_9S_2$, 1495.5; found, 1495.8.

Synthesis of 4. Dendron **3** (0.86 g, 0.57 mmol) was reacted with 85% trifluoroacetic acid (TFA, 3 mL) for 14 h (1:1 TFA:CH_2Cl_2). A pale yellow oil was obtained after solvent evaporation (0.81 g, 94.2%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.12 (m, 1H, *o*-C*H*), 7.82 (m, 2H, *m*-C*H*), 7.45 (tr, 1H, *p*-C*H*), 3.84 (br, 14H, NCH_2), 3.78 (br, 6H, NH_2CH_2CH_2), 3.65 (m, 36H, CH_2OCH_2), 3.22 (m, 6H, N−CH_2), 3.04 (tr, 2H, NHCH_2CH_2S); ^{13}C NMR (75 MHz, CD_3OD) δ 166.1, 165.0, 164.4, 157.0, 150.0, 137.8, 121.1, 119.8, 71.2, 70.2, 43.5, 42.9, 40.3. NMR Spectra are in Supporting Information. MALDI-TOF (*m*/*z*): [M + H]$^+$ calcd for $C_{48}H_{82}ClN_{21}O_9S_2$, 1196.6; found, 1196.6.

Synthesis of 5. Dendron **3** (0.100 g, 0.17 mmol), piperazine (0.072 g, 1.40 mmol), DIPEA (0.160 mL, 1.4 mmol), and THF (15 mL) were heated at 70 °C for 14 h in a sealed, thick-walled reaction vessel. After removal of solvent, the residue was partitioned between CH_2Cl_2 and water. The organic phases were concentrated to yield a pale yellow oil which was purified via silica gel column chromatography (20:1, CH_2Cl_2:MeOH eluent). A pale yellow oil was obtained (42 mg, 35.3%). The oil was stirred at room temperature with THF (10 mL), DIPEA (0.25 mL, 0.807 mmol), and SPDP (9.0 mg, 0.029 mmol). After removal of solvent, the residue was partitioned between CH_2Cl_2 and water. The organic phases were concentrated to yield a pale, yellow oil which was purified via column chromatography (100:5, CH_2Cl_2:MeOH eluent) yielded an off-white viscous oil. ^{1}H NMR (300 MHz, CD_3OD) δ 8.37 (m, 2H, *o*-C*H*), 7.77 (m, 4H, *meta*-C*H*), 7.16 (tr, 2H, *p*-C*H*), 3.68 (br, 24H, NCH_2), 3.59 (m, 48H, CH_2OCH_2), 3.48 (tr, 2H, C(O)NHCH_2CH_2S), 3.21 (tr, 10H, NHCH_2), 3.04 (tr, 2H, C(O)NHCH_2CH_2S), 1.41 (s, 27H, OC(CH_3)$_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 171.2, 166.2, 165.9, 161.6, 158.0, 149.8, 122.1, 120.9, 119.2, 80.1, 71.5, 71.2, 71.1 44.2, 41.3, 36.2, 33.8, 31.9, 28.9. MALDI-TOF (*m*/*z*): [M + H]$^+$ calcd for $C_{75}H_{122}N_{24}O_{16}S_4$, 1743.7; found, 1743.7.

Synthesis of 6. Dendron **5** (100 mg, 0.069 mmol) was deprotected with TFA (1:1 TFA:CH_2Cl_2), yielding a sticky yellow oil (32 mg, 75.6%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.48 (d, 1H, *o*-CH_a), 8.40 (d, 1H, *o*-CH_b), 7.98 (m, 2H, *m*-CH_a), 7.88 (m, 2H, *m*-CH_b), 7.39 (tr, 1H, *p*-CH_a), 7.25 (tr, 1H, *p*-CH_b), 3.85 (br, 24H, N−CH_2), 3.67 (m, 48H, CH_2OCH_2), 3.56 (tr, 2H, C(O)NHCH_2CH_2), 3.13 (tr, 10H, NHCH_2), 2.92 (tr, 2H, C(O)CH_2CH_2S); ^{13}C NMR (75 MHz, CD_3OD) δ 170.5, 165.5, 162.4, 158.0, 149.4, 138.0, 121.1, 120.0, 119.9, 115.1, 70.3, 70.2, 70.0, 68.7, 66.7, 43.9, 42.8, 40.4, 39.4, 34.1, 32.2. NMR spectra are in Supporting Information. MALDI-TOF (*m*/*z*): $[M + H]^+$ calcd for $C_{60}H_{98}N_{24}O_{10}S_4$, 1443.5; found, 1443.7.

DNA Conjugation. All solvents and reagents used in DNA reactions were spectroscopic grade. Water, methanol, and acetonitrile were further purified by filtration through a 0.22 μm filter. Disulfide-modified oligonucleotides were purchased from Trilink Biotechnologies (San Diego, CA). The lyophilized powders were dissolved in UV/UF purified (Barnstead) water [0.1 OD/μL]. The fluorescently labeled oligo 5′-(Oregon Green 514)-TAGGACTTACGC-(C3-thiol), and nonlabeled 12mer 5′-TAGGACTTACGC-(C3-thiol) (referred to as DNA_a), were deprotected on immobilized TCEP (2-carboxyethylphosphine) for 15 min at room temperature and eluted with two volumes of TBE (45 mM Tris-borate, 1 mM EDTA) pH 8.3 buffer. The use of other buffers, including tris(hydroxymethyl) aminomethane buffer at pH 6.0 and 7.4, did not elute the DNA from the resin. In a typical conjugation, deprotected Oregon-Green labeled 12-mer [2.5 OD, 25 μL H_2O] was incubated with thiopyridyl-containing dendron **7** (41.8 nmol, 0.05 mg). After 12 h at room temperature the reaction was stopped, yielding the corresponding disulfide conjugated DNA−dendrimer. MALDI-TOF (*m*/*z*): $[M + H]^+$ calcd for DNA−dendron, **7-D**, 7227.4; found, 7227.6.

MALDI-TOF. An overlayer preparation was used with a 2,4,6-trihydroxyacetophenone (THAP) matrix (*22*). A 1:1:1 mixture of 1 μM aqueous reaction mixture, 10 mg/mL THAP matrix in methanol, and 15 mg/mL aqueous ammonium citrate was spotted in 1 μL aliquots on a bed of THAP matrix. The analyte-doped matrix crystals were washed repeatedly (~5−15 times) with 5 μL of cold water to remove alkali metals. MALDI-TOF mass spectra were acquired in positive- and negative-ion mode on a Voyager-DE STR mass spectrometer (Applied Biosystems, Framingham, MA) equipped with a pulsed nitrogen laser emitting at 337 nm. Samples were analyzed in linear mode using a delayed extraction time of 550 ns and an accelerating voltage of 20 kV. The laser light intensity was adjusted to provide the optimal signal-to-noise ratio. All spectra were the result of signal averaging 50−100 laser shots. Positive-ion mass calibrations were performed internally with the $[M + 2H]^{2+}$, $[M + H]^+$, and $[2M + H]^+$ ions of insulin (bovine). Negative-ion calibrations were performed externally with the $[M - H]^-$ and the $[2M - H]^-$ ions of DNA_a. Mass spectra for **4**, **6**, **7**, **6-D**, **6-D$_2$**, and **7-D** can be found in the Supporting Information.

Gel Electrophoresis. Polyacrylamide gels (20%) were prepared according to a standard protocol (*23*). Gels were run between 70 and 80 V for 2−3 h. The sample buffer was 0.25% bromophenol blue and 40% (w/v) sucrose in water. Samples were prepared by adding 0.1 volume of running buffer to each dendron and conjugate solution. The running buffer was TBE (90 mM Tris-Borate, 2 mM EDTA). Ethidium bromide and Coomassie Brilliant Blue R250 were used for staining and visualization.

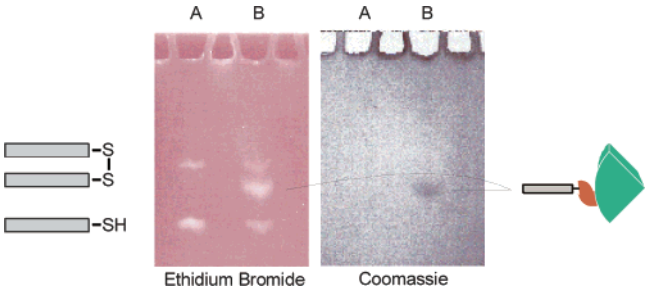


Figure 1. PAGE gels visualized by differential staining with ethidium bromide and Coomassie Brilliant Blue R-250. Lanes A contain DNA-thiol only, and lanes B covalently linked **4-D**. Removal of ethidium bromide, followed by staining with Coomassie Brilliant Blue R250 resulted in the second gel.

RESULTS AND DISCUSSION

The objectives of this paper are to present a synthetic approach for synthesizing DNA−dendrimer constructs and then to prove unambiguously that the DNA linkage to the dendrimer is covalent rather than electrostatic. Electrostatic complexes between DNA and dendrimers have been reported previously (*16*). The motivation for using cationic dendrimers stems from both the high water solubility that these groups afford, and the opportunity for electrostatic binding between the DNA and dendrimeric reactants, which may be useful for enhancing the rate of covalent bond formation between the two species.

Dendron synthesis reactions proceed cleanly and in high yields to give products that are readily purified by chromatography. All intermediates and products give satisfactory ^{1}H and ^{13}C NMR spectra and the appropriate parent ion by mass spectrometry. The two disulfides of **6** are not spectroscopically equivalent, as evidenced by the five relevant pyridyl peaks in the ^{1}H NMR (Supporting Information), indicating a subtle difference in the chemical environment of these groups.

To conjugate DNA to the dendrons, the disulfide-terminated oligonucleotides were first reduced using tris(2-carboxyethyl)phosphine (TCEP) in a trisborate EDTA (TBE) buffer (pH 8.3) to yield thiol-terminated ssDNA. Addition of this DNA to aqueous solutions of dendrons yielded the DNA−dendron conjugates upon thiol-disulfide exchange (Scheme 3).

To distinguish between covalent attachment and electrostatic association (*16*), the products were characterized using polyacrylamide gel electrophoresis (PAGE) and matrix-assisted laser desorption ionization − time-of-flight (MALDI-TOF) mass spectrometry (*24*). These DNA−dendron architectures (and the associated ssDNA starting materials) proved to be too small for agarose gel analysis. Instead, 20% polyacrylamide gels allowed successful separation of the initial G2-dendron, the ssDNA, and the DNA−dendron products (Figure 1). Column B of the gel shown in Figure 1 corresponds to the reaction of **4** with a deprotected 12-base oligonucleotide, in which we expect to make **4-D**. This gel was stained with ethidium bromide, which stains DNA but not the dendrons used in this study. Starting from the bottom of the gel in column B, the three bands correspond to unreacted DNA-thiol, DNA−dendron conjugate (**4-D**, Scheme 1), and a disulfide-coupled DNA dimer. To confirm these assignments, we ran a gel using a solution nominally containing only DNA-thiol (Column A). The two bands present in this control experiment correspond to DNA-thiol and the anticipated DNA dimer, which is produced by oxidation of two DNA-thiol oligonucleotides to a DNA-S−S-DNA disulfide.

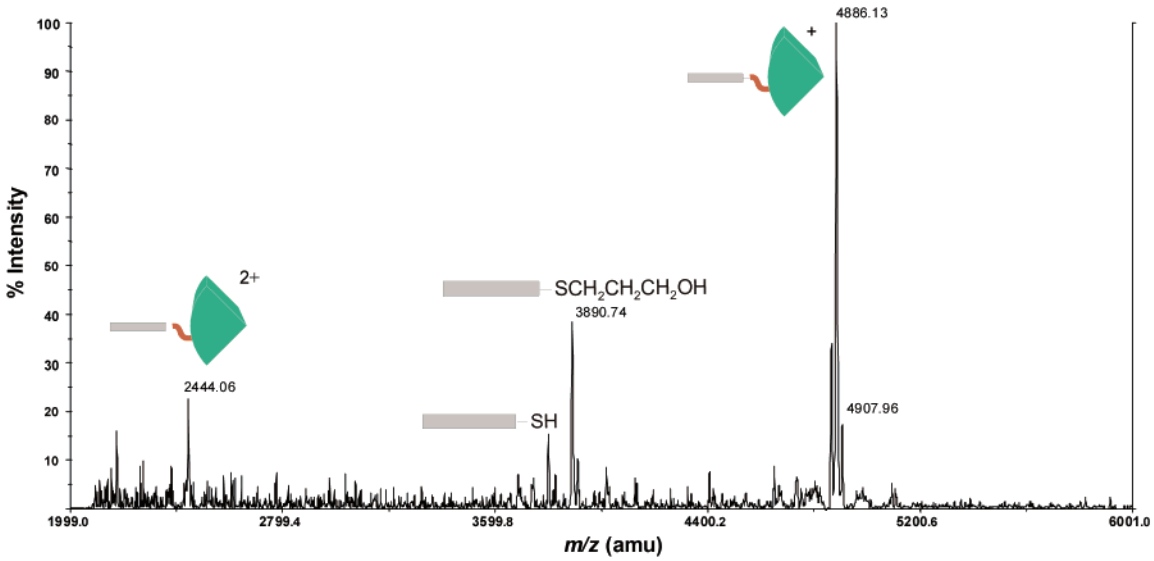
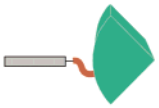
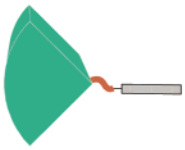


Figure 2. MALDI-TOF mass spectrum of **4-D**. Peaks at 4886.13 and 2444.06 correspond to the $[M + H]^+$ and $[M + 2H]^{2+}$ ions of **4-D**, and 3890.7 is the $[M + H]^+$ for DNA not deprotected by TCEP but with subsequent loss of the dimethoxytrityl ether.

Table 1

Reagents		Mass[a]	Products	Mass
 **4**	X = Pyr	1196.6 (1196.6)	 **4-D**	5561.76 (5561.97)[b] 4886.1 (4886.1)[c]
 **6**	X_1, X_2=Pyr	1443.7 (1443.7)	 **6-D_2**	10172.8 (10171.1)
 **7**	X = Pyr	2862.7 (2862.6)	 **7-D**	7227.4 (7227.6)

[a] Expected parent ion masses (*m*/*z*) shown (observed molecular weight in parentheses); [b] Internal calibration shows only a 36 ppm error in mass accuracy. [c] Same oligonucleotide sequence, but not functionalized with Oregon-Green dye.

The band assignments were further confirmed by staining with Coomassie Brilliant Blue R250, which stains the dendrons used in this study but not DNA (*25*). As shown in Column B, only the middle band, corresponding to the DNA-dendron, is visible. In contrast, the bands in Column A, corresponding to the DNA-thiol control experiment, are not stained. The data in Figure 2 shows the presence of the DNA-dendron conjugate, $[M + H]^+ = 4886.1$ and $[M + H]^{2+} = 2444.1$, along with a small amount of $[M + H]^+ = 3890.7$, which corresponds to DNA not deprotected by the original TCEP reduction with subsequent loss of dimethoxytrityl ether. We attribute the absence of a band corresponding to the cationic dendron to the inability of these charged species to enter the polyacrylamide gel (they would migrate toward the cathode).

Table 1 provides MALDI-TOF mass spectrometry data for the key reagents and products prepared for this study. Importantly, the molecular weights for **4-D**, **6-D**, **6-D_2**, and **7-D** correspond with the calculated masses for these DNA−dendron conjugates. As indicated previously, there are two potential linking motifs for the DNA−dendrons: electrostatic and covalent. The mass difference between these two adducts is only 2 Da. Nevertheless, it was

possible to distinguish between these two possible products using accurate-mass mass analysis. With bovine insulin as an internal calibrant ($[M + H]^+ = 5734.39$), the measured molecular weight of **4-D** was 5561.97. This compared with the expected $[M + H]^+$ of 5561.76, corresponding to an error of only 36 ppm. An electrostatic adduct ($[M + H]^+ = 5563.85$) would have a much larger mass error of 200 ppm. The same mass spectrometry technique was used to confirm the covalent addition of one and two ssDNA oligonucleotides to **6** to yield **6-D** and **6-D$_2$**, respectively.

In addition to mass spectrometry, Ellman's test (for free thiol) was used to distinguish between covalent and electrostatic attachment of DNA to the dendrons. Ellman's reagent oxidizes free thiols to mixed nitrobenzyl disulfides, which are easily identifiable by mass spectrometry. No Ellman's adduct was observed for **4-D**. Addition of a reducing agent, TCEP, to **4-D** led to disappearance of the mass spectral signal for the DNA-dendron conjugate, confirming covalent addition of DNA to the dendron.

SUMMARY AND CONCLUSIONS

In summary, we have outlined a general synthesis for covalent DNA−dendron conjugates. These materials were subjected to rigorous characterization. We are currently investigating hybridization and enzymatic manipulation of these DNA−dendron conjugates.

ACKNOWLEDGMENT

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Supporting Information Available: NMR spectra and MALDI-TOF mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Kuwata, K., Uebori, M., Yamada, K., and Yamazaki, K. (1982) Liquid chromatographic determination of alkylthiols via derivatization with Ellman's reagent. *Anal. Chem. 54*, 1082−1087.

(2) Shchepinov, M. S., Mir, K. U., Elder, J. K., Frank-Kamenetskii, M. D., and Southern, E. M. (1999) Oligonucleotide dendrimers: stable nano-structures. *Nucl. Acid. Res. 27*, 3035−3041.

(3) Nilsen, T. W., Grayzel, J., and Prensky, W. (1997) Dendritic nucleic acid structures. *J. Theor. Biol. 187*, 273−284.

(4) Capaldi, S., Getts, R. C., and Jayasena, S. D. (2000) Signal amplification through nucleotide extension and excision on a dendritic DNA platform. *Nucl. Acid. Res. 28*, e21.

(5) Yan, H., Zhang, X. P., Shen, X. Y., and Seeman, N. C. (2002) A robust DNA mechanical device controlled by hybridization topology. *Nature 415*, 62−65.

(6) Frutos, A. G., Smith, L. M., and Corn, R. M. (1998) Enzymatic ligation reactions of DNA "words" on surfaces for DNA computing. *J. Am. Chem. Soc. 120*, 10277−10282.

(7) Francis, M. B., Goh, S. L., Prasad, D., Padilla De Jesus, O. L. P., and Fréchet, J. M. J. (2001) DNA-dendrimer conjugates for drug delivery. *Polym. Mater. Sci. Eng. 84*, 12−13.

(8) Bielinska, A. U., Chen, C., Johnson, J., and Baker, J. R., Jr. (1999) DNA complexing with polyamidoamine dendrimers: Implication for transfection. *Bioconjugate Chem. 10*, 843−850.

(9) Gebhart, C. L., and Kabanov, A. V. (2001) Evaluation of polyplexes as gene transfer agents. *J. Controlled Release 73*, 401−416.

(10) Taton, T. A., Mirkin, C. A., and Letsinger, R. L. (2000) Scanometric DNA array detection with nanoparticle probes. *Science 289*, 1757−1760.

(11) Stears, R. L., Getts, R. C., and Gullans, S. R. (2000) A novel, sensitive detection system for high-density microarrays using dendrimer technology. *Physiol. Genom. 3*, 93−99.

(12) Smith, E. A., Wanat, M. J., Cheng, Y. F., Barreira, S. V. P., Frutos, A. G., and Corn, R. M. (2001) Formation, spectroscopic characterization, and application of sulfhydryl-terminated alkanethiol monolayers for the chemical attachment of DNA onto gold surfaces. *Langmuir 17*, 2502−2507.

(13) Frutos, A. G., Brockman, J. M., and Corn, R. M. (2000) Reversible protection and reactive patterning of amine- and hydroxyl- terminated self-assembled monolayers on gold surfaces for the fabrication of biopolymer arrays. *Langmuir 16*, 2192−2197.

(14) Kabanov, V. A., Sergeyev, V. G., Pyshkina, O. A., Zinchenko, A. A., Zezin, A. B., Joosten, J. G. H., Brackman, J., and Yoshikawa, K. (2000) Interpolyelectrolyte complexes formed by DNA and Astramol poly(propylene imine) dendrimers. *Macromolecules 33*, 9587−9593.

(15) Choi, J. S., Joo, D. K., Kim, C. H., Kim, K., and Park, J. S. (2000) Synthesis of a barbell-like triblock copolymer, poly(l-lysine) dendrimer-*block*-poly(ethylene glycol)-*block*-poly(l-lysine) dendrimer, and its self-assembly with plasmid DNA. *J. Am. Chem. Soc. 122*, 474−480.

(16) Ottaviani, M. F., Furini, F., Casini, A., Turro, N. J., Jockuch, S., Tomalia, D. A., and Messori, L. (2000) Formation of supramolecular structures between DNA and starburst dendrimers studied by EPR, CD, UV, and melting profiles. *Macromolecules 33*, 7842−7851.

(17) Reynolds, R. A., III., Mirkin, C. A., and Letsinger, R. L. (2000) Homogeneous, nanoparticle-based quantitative colorimetric detection of oligonucleotides. *J. Am. Chem. Soc. 122*, 3795−3796.

(18) Watson, K. J., Park, S.-J., Im, J.-H., Nguyen, S. T., and Mirkin, C. A. (2001) DNA-block copolymer conjugates. *J. Am. Chem. Soc. 123*, 5592−5593.

(19) Niemeyer, C. M. (2001) Nanoparticles, proteins, and nucleic acids: biotechnology meets material science. *Angew. Chem., Int. Ed. 40*, 4128−4158.

(20) Zhang, W., Nowlan, D. T., III., Thomson, L. M., Lackowski, W. M., and Simanek, E. E. (2001) Orthogonal, convergent syntheses of dendrimers based on melamine with one or two unique surface sites for manipulation. *J. Am. Chem. Soc. 123*, 8914−8922.

(21) Ebright, Y. W., Chen, Y., Pendergast, P. S., and Ebright, R. H. (1992) Incorporation of an EDTA-metal complex at a rationally selected site within a protein − application to EDTA-iron DNA affinity cleaving with catabolite gene activator protein (CAP) and CRO. *Biochemistry 31*, 10664−10670.

(22) Pieles, U., Zuercher, W., Schaer, M., and Moser, H. E. (1993) Matrix-assisted laser desorption ionization time-of-flight mass-spectrometry. A powerful tool for the mass and sequence-analysis of natural and modified oligonucleotides. *Nucl. Acid. Res., 21*, 3191−3196.

(23) Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning*, Cold Spring Harbor Press, 2nd ed.; New York, Vol. 1.

(24) Karas, M., and Hillencamp, F. (1988) Laser desorption ionization of proteins with molecular masses exceeding 10000 Daltons. *Anal. Chem., 60*, 2299−2301.

(25) Brothers, H. M., Piehler, L. T., and Tomalia, D. A. (1998) Slab-gel and capillary electrophoretic characterization of polyamidoamine dendrimers. *J. Chromatogr. A 814*, 233−246.

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