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Organometallic Photonucleases: Synthesis and DNA-Cleavage Studies of Cyclopentadienyl Metal-Substituted Dendrimers Designed To Increase Double-Strand Scission

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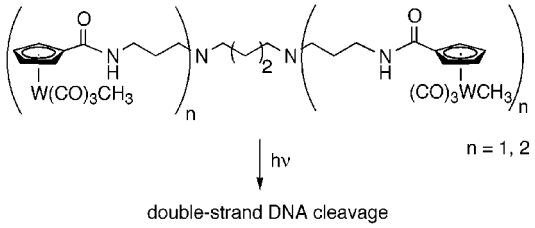
ABSTRACT



A series of metal complex-substituted polyamine dendrimers have been synthesized and examined for their ability to cleave plasmid DNA in a double-stranded manner. While photolysis of the dimetallic spermine derivative and the tetrametallic DAB-Am-4 complex led to double-strand scission, in the larger DAB-Am-8 and DAB-Am-16 systems, DNA aggregation/precipitation was the predominant competing process observed.

The cleavage of DNA in a double-stranded manner is a desirable goal in the development of new antibiotic and anticancer agents.[1] Thus, the initial structural elucidation of the neocarzinostatin chromophore in 1985[2] and subsequent identification and determination of mode of action of the calicheamicins and esperamicins in 1987[3] resulted in the rapid expansion of a new area of research,[4] including the isolation of other natural products with similarly elegant structures and mechanisms of action,[5] the preparation of synthetic analogues,[6] and the development of other simpler organic radical-based cleaving agents.[7] Common to all the naturally occurring enediynes are three important features: DNA-binding components, a triggering device, and an unsaturated core that can cycloaromatize to produce the diradical species that damages oligonucleotides, ultimately

(1) For lead references and examples, see: Melvin, M. S.; Tomlinson, J. T.; Saluta, G. R.; Kucera, G. L.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 6333−6334. Pamatong, F. V.; Detmer, C. A., III.; Bocarsly, J. R. *J. Am. Chem. Soc.* **1996**, *118*, 5339−5345. Branum, M. E.; Que, L., Jr. *J. Biol. Inorg. Chem.* **1999**, *4*, 593−600. Stubbe, J.; Kozarich, J. W.; Wu, W.; Vanderwall, D. E. *Acc. Chem. Res.* **1996**, *29*, 322−330. *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI Press: London 1992; Vol. 1, pp 217−243.

(2) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331−334.

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(4) For lead references, see: *Enediyne Antibiotics as Antitumor Agents*; Borders, D. B., Doyle, T. W., Eds.; Marcel Dekker: New York, 1995. Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103−2117. Armitage, B. *Chem. Rev.* **1998**, *98*, 1171−1200. Maier, M. E. *Synlett* **1995**, 13−26. For some recent examples, see: Myers, A. G.; Liang, J.; Hammond, M.; Harrington, P. M.; Wu, Y. S.; Kuo, E. Y. *J. Am. Chem. Soc.* **1998**, *120*, 5319−5320. Weinstein, D. S.; Nicolaou, K. C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 545−558. Churcher, I.; Hallett, D.; Magnus, P. *Tetrahedron* **1999**, *55*, 1597−1606. Shair, M. D.; Yoon T. Y.; Mosny, K. K.; Chou, T. C.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 9509−9525.

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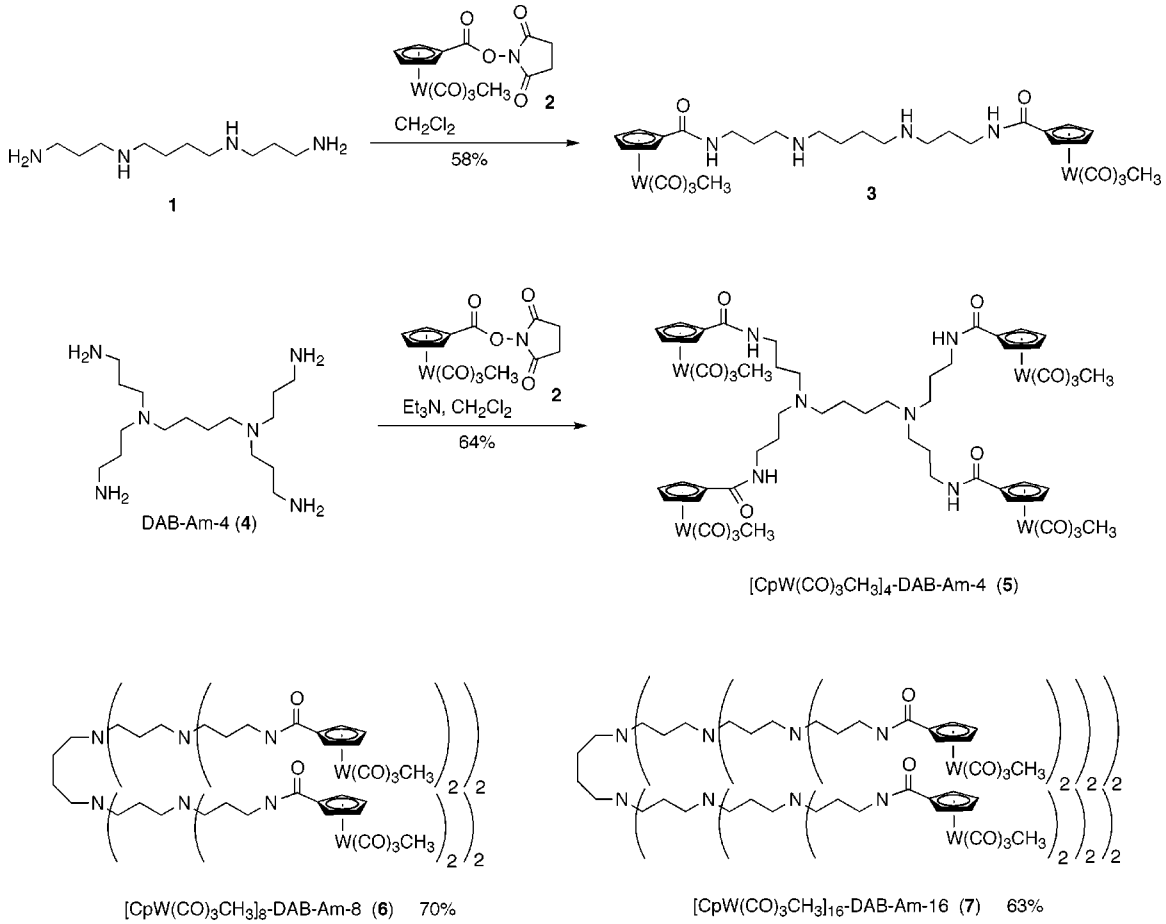
Scheme 1



leading to cell death. Although each of these systems produces two radical centers, not all result in the same amounts of the preferred double-strand cleavage,[8] which is more difficult than single-strand events for the cellular machinery to repair.[9]

In efforts to develop molecules that give rise to increased amounts of double-strand cleavage, several groups have designed enetetrayne-based systems with the potential of generating multiple radical centers; but these endeavors have met with varying degrees of success either in the synthetic approach or in cycloaromatization studies.[10] Unfortunately, none of the reported compounds undergo cycloaromatization under physiological conditions suitable for DNA cleavage studies.

One strategy to circumvent such difficulties is to connect multiple simple radical sources that have already proven effective for strand scission. As scaffolding for such systems, dendrimers are ideal because they are monodisperse and because the homogeneity of their terminal functionality allows for the rapid attachment of the cleaving agents. Polyamine (DAB-Am) dendrimers appear particularly well-suited for this application,[11] because they are protonated at physiological pH to give positively charged ammonium species that are electrostatically attracted to the negatively charged sugar−phosphate backbone of DNA[12] in the same manner as spermine and spermidine.[13] Additionally, this protonation increases their solubility in the aqueous conditions required for cleavage experiments; and various genera-

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(7) For selected recent examples: Wender, P. A.; Jeon, R. *Org. Lett.* **1999**, *1*, 2117−2120. Fernandez-Saiz, M.; Henderson, P. T.; Wilson, W. D.; Schuster, G. B. *Photochem. Photobiol.* **1999**, *70*, 847−852. Srinivasan, V.; Jebaratnam, D. J.; Budil, D. E. *J. Org. Chem.* **1999**, *64*, 5644−5649. Theodorakis, E.; Xiang, X.; Blom, P. *Chem. Commun.* **1997**, 1463−1464. Riordan, C. G.; Wei, P. *J. Am. Chem. Soc.* **1994**, *116*, 2189−2190.

(8) Epstein, J. L.; Zhang, X.; Doss, G. A.; Liesch, J. M.; Krishnan, B.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 6731−6738, and references therein.

tions of these dendrimers are commercially available. Therefore, we now describe the synthesis and DNA-cleaving behavior of such polyamine systems functionalized with either 2, 4, 8, or 16 equiv of tricarbonylcyclopentadienylmethyltungsten, a simple complex that has been shown previously to cleave DNA in a single-strand manner.[14]

The syntheses of the substituted polyamine dendrimers and functionalized spermine were accomplished in a straightforward manner (Scheme 1). Combining the activated succinimide ester **2**[15] with spermine (**1**) in dichloromethane gave a 58% yield of the dimetallic derivative **3**, and subjecting DAB-Am-4 (**4**) to similar conditions with the addition of triethylamine provided $[CpW(CO)_3CH_3]_4$-DAB-Am-4 (**5**) in 64% yield. The multimetallic complexes $[CpW(CO)_3CH_3]_8$-DAB-Am-8 (**6**) and $[CpW(CO)_3CH_3]_{16}$-DAB-Am-16 (**7**) were prepared in an analogous manner in 70% and 63% yields, respectively. For the substituted dendrimers, these overall conversions correspond to 89%, 96%, and 97% yields per amide bond formed, and the products were easily purified by recrystallization.

To assess the DNA-cleaving behavior of the new molecules, their photoinduced relaxation of plasmid DNA was investigated. Varying concentrations of the functionalized dendrimers were photolyzed in the presence of pBR322 DNA, and the results were analyzed by agarose gel electrophoresis (Figures 1 and 2) after precipitation of the DNA.

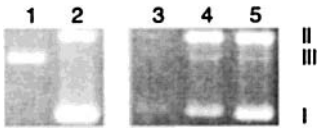

Figure 1. Cleavage of pBR322 DNA (60 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by **3**. Lane 1, form III marker, lanes 2 through 5, DNA + complex (30, 25, 20, and 15 μM, respectively). Reactions in lanes 3−5 were irradiated with Pyrex-filtered light from a 450 W Hanovia lamp for 20 min.

For the dimetallic species **3**, a band corresponding to form III DNA resulting from double-strand cleavage was observed at dendrimer concentrations from 0.042 to 0.33 molecules/bp (2.5 to 20 μM, Figure 1 and Supporting Information), a value better than that reported for light-induced cleavage of

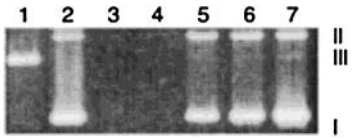

Figure 2. Cleavage of pBR322 DNA (60 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by **5**. Lanes 1, form III marker; lane 2, DNA alone; lanes 3 through 7, DNA + complex (80, 40, 20, 10, and 5 μM, respectively). Reactions in lanes 3−7 were irradiated with Pyrex-filtered light from a 450 W Hanovia lamp for 20 min.

DNA by the natural enediyne dynemicin (0.75 molecules/bp[16]). Form II DNA arising from single-strand scission was seen at ratios as low as 0.042 molecules/bp (see Supporting Information). This number corresponds to 0.084 equiv of the tungsten complex per base pair, representing an 18-fold improvement in single-strand cleaving efficiency over the simple complex, $CpW(CO)_3CH_3$, which gives single-strand cuts at 1.5 molecules/bp.[14] At higher concentrations (e.g., Figure 1, lane 3), the plasmid was cleaved almost completely to smaller linear fragments that migrate very fast through the gel. Control experiments demonstrated that both light (Figure 1, lane 2) and the organometallic species (Supporting Information) were required to effect cleavage.

To investigate whether the linear DNA arises from the accumulation of random single-strand breaks or from a true double-strand event, the bands from a number of gels (including Figure 1) were quantified.[17] The observation of all three forms of DNA in one reaction mixture indicates nonrandom double-strand cleavage,[1,18] and this is supported by ratios of single- to double-strand breaks. (n_1/n_2 in Table 1). For a variety of sets of reaction conditions, n_1/n_2 values

Table 1. Statistical Efficiency of Single-Strand (n_1) and Double-Strand (n_2) Cleavage by Spermine Derivative **3**[a]

[**3**] (μM)	precipitation method[b]	n_1[c]	n_2	n_1/n_2
20	A	0.656	0.068	9.7
10	A	0.383	0.036	10.6
15	B	0.707	0.033	21.4
12.5	B	0.331	0.017	19.5
10.0	B	0.288	0.024	12.0
7.5	B	0.248	0.034	7.3
2.5	B	0.173	0.025	6.9
20	B	0.592	0.056	10.6
15	B	0.436	0.022	19.8

[a] 60 μM pBR322 DNA, 10% DMSO in 10 mM Tris buffer, pH 8, 20 min irradiation). [b] See Supporting Information. [c] The number of single- and double-strand cleavage events were determined via the statistical test of Povirk et al.,[18] which assumes a Poisson distribution of strand cuts.

ranged from 6.9 to 21.4, which is significantly lower than the value expected from coincidental single-strand breaks (~750 for a 4361-bp plasmid).[19]

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(15) Gorfti, A.; Salmain, M.; Jaouen, G.; McGlinchey, M. J.; Bennouna, A.; Mousser, A. *Organometallics* **1996**, *15*, 142−151.

Photolysis of the substituted first generation dendrimer (**5**, Figure 2) also gave the appearance of form III DNA at dendrimer concentrations between 5 and 20 μM (0.083 to 0.33 molecules/bp). Quantitation of the amounts of the different forms of DNA further demonstrated that this was the result of double-strand breaks, with n_1/n_2 values of 20.9, 6.9, and 5.7 for lanes 5, 6, and 7, respectively.

Interestingly, when the substituted second- and third-generation dendrimers, **6** and **7** (containing 8 and 16 metal centers, respectively), were studied in similar experiments (data not shown), it was reproducibly noted at higher dendrimer concentrations that the DNA was absent, even in lanes for nonirradiated samples. Closer examination of the ethidium bromide-stained gels revealed that a significant amount of highly fluorescent material had remained in the loading wells. This substance is most likely composed of DNA/dendrimer aggregates that have precipitated from the reaction mixture, behavior that has been reported for numerous cationic oligomers and quantified for polyamidoamine dendrimers.[20] Despite numerous attempts to prevent the formation of these ensembles (by increasing the pH of the mixture) or to disassemble them after photolysis (by adding SDS[21]), no accurate data on double-strand cleavage by the second- or third-generation systems could be obtained.

In conclusion, it has been shown that connecting multiple radical sources together does increase the amount of true double-strand scission events observed in plasmid relaxation assays. The efficiency of single-strand cleavage is also improved by the attachment of these agents to scaffolds with an affinity for oligonucleotides; however, the DNA-recognition factors that lead to increased amounts of strand scission must be modulated to control DNA aggregation and precipitation.

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Supporting Information Available: Detailed descriptions of experimental procedures, spectral and analytical data for all new compounds, and photographs of the agarose gels of the products of the photolysis of **6** and **7** and pBR322 DNA and of the treatment of DNA with each of the new compounds without photolysis.

OL006030O

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(17) Quantification was accomplished via the NIH software program ImageJ. The amount of supercoiled DNA was multiplied by a factor of 1.22 to correct for reduced ethidium bromide intercalation into the form I plasmid DNA.

(18) Povirk, L. F.; Wübker, W.; Köhnlein, W.; Hutchinson, F. *Nucleic Acids Res.* **1977**, *4*, 3573−3580.

(19) The expected n_1/n_2 was determined with the Freifelder−Trumbo equation ($n_2 = n_1^2(2h + 1)/4L$, Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681), in which h is the maximum separation in base pairs between cuts on opposite strand that produces linear DNA ($h = 16$) and L is the number of phosphodiester bonds per strand of DNA (4361 for pBR322).

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