a 25-m Carbowax 20 M column, which has quite different retention properties.

The alkane $R'_2R'_2$ had also been isolated previously,[12,13a] and we confirmed it by spiking on the two GLC columns using an authentic sample.[16] The alkane R_2R_2 was isolated by LC, and its ^1H NMR at 270 MHZ compared with that of a synthetic sample;[17] the isolated sample had the same peaks but appeared to be contaminated with a small amount of $R'_2R'_2$. The structure of R_2R_2 was confirmed by spiking on two columns with an authentic sample. The peak assigned to R'_2R_2 remained next to that of R_2R_2 on both GLC columns.

The alkenes **4** and **5** had also been isolated previously,[12] but were also isolated from our reaction products by GLC and LC. The ^1H NMR spectra were compared with those of authentic samples and the GLC peaks confirmed by spiking on two columns.

A mixture of the phenylbutenes **6**, **7**, and **8** was isolated from the decomposition products from **2** by GLC and the 270-MHZ ^1H NMR spectrum compared to that of a mixture made by the dehydration of a synthetic sample of the alcohol R''_2OH. The GLC peaks of this mixture were assigned to the isomers on the basis of the intensities in the integrated ^1H NMR spectrum. The synthetic mixture was used to identify these alkenes in various product mixtures by spiking on the two GLC columns.

The esters R_2OOCR_2 and R'_2OOCR_2 have been found in the products from the decomposition of **2** in hexachloroacetone and characterized by Shevlin and Hansen.[13c] The esters were isolated from our product mixtures by GLC and LC and their IR and NMR spectra compared with those reported in the literature and with those of our synthetic samples. The peaks were identified in various product mixtures by spiking with authentic samples on both GLC columns.

The lactone L_2 was isolated by GLC and LC and its ^1H NMR spectrum compared with that of a synthetic sample.[13d] This sample was used to confirm the lactone in various product mixtures by spiking on both columns.

The alcohols R_2OH and R'_2OH were isolated by GLC and LC and the NMR spectra compared with those of synthetic samples.[13b] They were accompanied by traces of the alcohol R″OH identified by peaks corresponding to those of a synthetic sample. Synthetic samples were used to identify GLC peaks by spiking on both columns.

The lactone L_1, R_1OOCR, R_1OH, R'_1OH, styrene, R_1H, $R_1C_6H_5$, and R_1R_1 were identified by spiking with authentic samples.

1-Phenyl-1-methylcyclopropane was synthesized from α-methylstyrene and methylene iodide[14] and characterized by its ^1H NMR (δ) spectrum: aromatic multiplet, 5 H, 7.01; CH_3, 3 H, 1.35; CH_2CH_2, 2 H, m, 0.65; 2 H, m, 0.78. This compound did not correspond to any GLC peak in our product mixtures, although it has been reported as a product from **2** and related peroxides decomposing in solution.[12,13c]

Product Analysis Runs. The peroxide was put on the silica from a hexane solution using either of the two methods of ref 3; the solvent was then removed by tumbling in a rotary evaporation apparatus until the silica was free flowing, and then the silica was transferred to an amplitude for degassing. Degassing was usually continued until the pressure reached 10^{-5}mmHg. For photolysis runs, the ampule was a quartz tube cooled by a stream of water, that was rotated continuously so as to tumble the particles during the photolysis and to ensure uniform illumination.

Products were removed from the silica in two stages. In the first stage, the silica was simply washed with several portions of ether. The ether solution was extracted with bicarbonate to remove the acid, then dried, and concentrated for the subsequent analysis of the neutral products.

In the second stage, the extracted silica was stirred with 5% H_2SO_4 at 50 °C for 1 h, and then this mixture was extracted with ether. This second ether extract was the source of the alcohols identified in the tables as ROSi.

In a few experiments the first and second ether extracts were hydrolyzed with dilute H_2SO_4 while connected to a gas adsorption train to detect evolution of CO_2 during the hydrolysis.

The yields of acid were determined by weighing the isolated acids.

Yields of neutral products were determined by gas chromatography using a Hewlett-Packard computer-controlled GC with a flame ionization detector and a 25-m Supelco SP 2100 quartz capillary column and a glass inlet splitter. The more volatile components, alkanes, alkenes, and alcohols, were determined by using *p*-bromotoluene as the internal standard, an initial temperature of 60 °C, and a temperature program that eluted the last alcohol at about 17 min and a temperature of about 125 °C. The less volatile components, lactone, esters, and dimeric hydrocarbons, were determined by using phenyl benzoate as the internal standard and a temperature program beginning at 160 °C. The less volatile ester emerged at about 16 min at which point the temperature was about 240 °C.

Kinetic Runs. Samples for kinetic runs were prepared as described for product studies except that the silica, after rotary evaporation to the free flowing stage, was partitioned approximately among a number of separate ampules. After the thermolysis or photolysis the ampule was opened and the bulk of the contents quickly transferred to a weighing bottle; this avoids increases in weight due to adsorption of atmospheric moisture. The sample, consisting of a 1–2-g aliquot, was then transferred to a flask, and 15 mL of acetic acid saturated with CO_2 and 1.5 mL of saturated aqueous KI and a pellet of Dry Ice were added. The flask was then heated on the stream bath for 7 min, diluted with 50 mL of H_2O, and titrated to a starch end point, with thorough stirring, by using 0.05 M sodium thiosulfate. A CO_2 atmosphere was maintained by adding small pellets of Dry Ice.

Acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for the support of this research.

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Sequence-Specific Osmium Reagents for Polynucleotides. 2. A Method for Thymine–Cytosine Pairs

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Abstract: We have modified the dinucleoside monophosphate, deoxythymidylyldeoxycytidine (d-TpC), by replacement of the exocyclic amino group of cytosine with a series of ligands, $H_2N(CH_2)_nN(CH_3)CH_2CH_2N(CH_3)_2$, where n = 2, 4, and 6. The kinetics of the reactions of these modified dinucleoside monophosphates with osmium tetroxide were measured. The modified nucleotides react with osmium tetroxide 4200, 7900, and 1200 times faster than the unmodified species for n = 6, 4, and 2, respectively. The products of the reactions are macrocyclic oxoosmium(VI) esters for which 360-MHz proton NMR spectra are reported.

Osmium(VIII) reagents add to the 5,6-double bond of pyrimidines to form osmium(VI) esters.[1] The reactivity order for the common pyrimidine residues is thymine > uracil > cytosine. The common purines are unreactive. This kinetic specificity for

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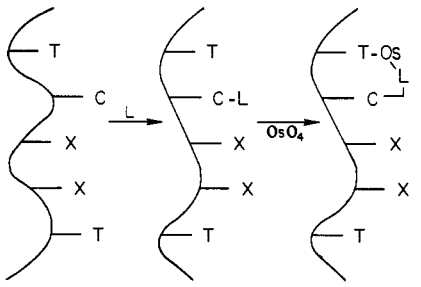


Figure 1. General scheme for pair-specific osmium reagents.

Table I. Overall Yields (%) of the Alkyl Triamines from Their Corresponding Bromoalkylphthalimides

	synthetic method		
$H_2N(CH_2)_nN(CH_2)_2N(CH_3)_2$	trimethylethylenediamine route	tetramethylethylenediamine route	
n		$LiAlH_4$	PhS^-Na^+
2	10	2	33
4	21	11	43
6	31	17	48

thymine or uracil residues has been useful in a number of applications including DNA cleavage[2] and single-atom visualization.[3] The utility and potential for crystallographic work is clear as osmium atoms have been selectively placed at specific sites in yeast t-RNA Phe,[4] t-RNA fMet,[5] and t-RNA Tyr.[6] The selectivity of the osmium(VIII) reagent is a function of the ligand used in conjunction with the osmium reagent. For example, pyridine gives a more selective but less reactive reagent than does 2,2'-bipyridyl.[7] The (2,2'-bipyridyl)osmium(VI) esters are more stable to both hydrolysis and transesterification than are the corresponding bis(pyridine) esters.[8] Reactions in the absence of ligands are slow, and the esters formed are hydrolytically unstable.[9] We describe here the chemistry of a system designed for application to polynucleotides which gives kinetic selectivity to osmium(VI) esters formed at thymine residues which are in the vicinity of modified cytosine residues. This is accomplished by attaching an appropriate ligand to cytosine residues and using that ligand to accelerate the reaction of osmium(VIII) with neighboring thymine residues. The osmium(VI) esters so formed are also hydrolytically stable as compared with nonliganded esters.[9] The general scheme is illustrated in Figure 1. Because there will exist two types of thymine residues in a generalized polynucleotide, namely, those in the vicinity of modified cytosine residues and those not so placed, this scheme represents a method of selectively labeling certain thymine–cytosine pairs in a polynucleotide. A preliminary account of this work has appeared.[10]

Results and Discussion

1. Synthesis of Ligands. We carried out the syntheses of the required ligands by the two routes based on the Gabriel synthesis[11] shown in Scheme I. The route through the quaternary salt **5** followed by demethylation using sodium thiophenoxide gave the best overall yields (Table I). Physical properties of the ligands are given in the experimental section and in ref 13.

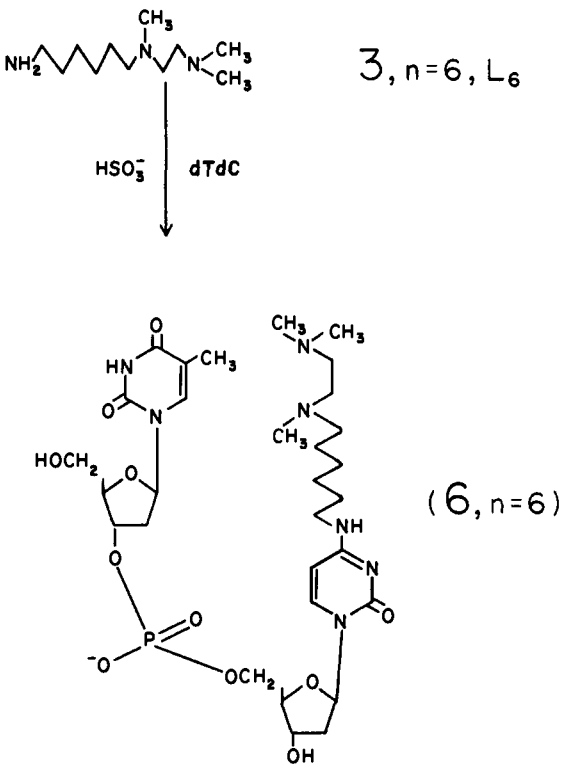
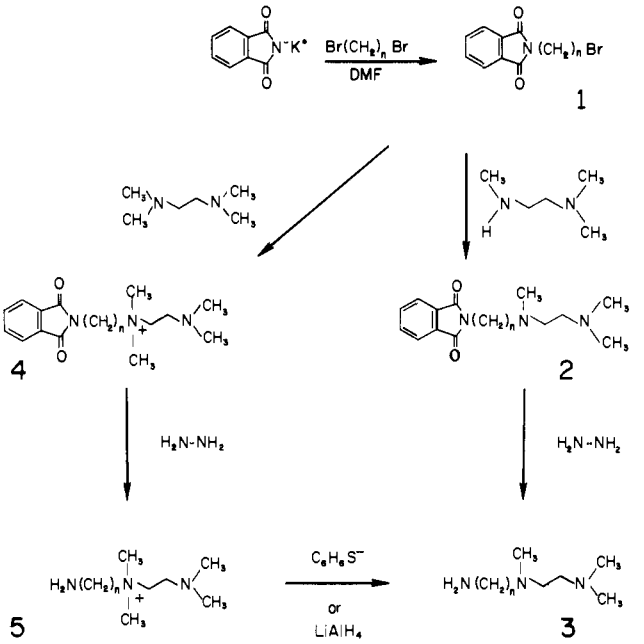


Figure 2. Synthesis of d-TpC–L_6.

Scheme I. Syntheses of Ligands



2. Transamination of d-TpC. Transamination reactions between d-TpC and various ligands were carried out by using the general procedure described by Shapiro and Weisgras.[12] Figure 2 illustrates the reaction for the ligand **3**, $n = 6$. We will refer to this ligand as L_6. The progress of the transamination reactions was monitored by gel chromatography on Sephadex G-10. This is illustrated in Figure 3. Note that the transaminated product moves more slowly than d-TpC, presumably due to chemical

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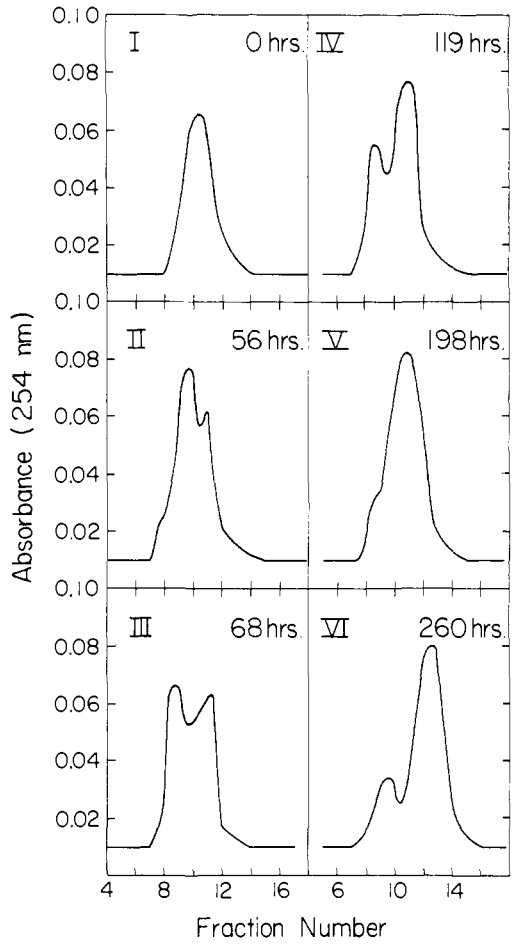

Figure 3. Progress of the transamination reaction between d-TpC and L_6 as monitored by Sephadex G-10 chromatography. The new peak in panel 6 is d-TpU.

interaction with the gel network. The disappearance of d-TpC as measured by integration of the peak area shows a half-time of about 75 h at 38 °C in the presence of 1.4 M L_6 and 0.86 M sodium bisulfite, pH 7.1. The progress of the reaction could also be followed by electrophoresis on paper at pH 2.7. The nucleotides were detected by their UV absorption and the free ligands by their reaction with ninhydrin. The transaminated products were isolated by preparative tlc on cellulose following removal of excess ligand, bisulfite, and other salts as described in Experimental Section. The bands were eluted with water; the solution was concentrated and then rechromatographed on a Sephadex G-10 column to yield material which was electrophoretically and chromatographically pure. Table II summarizes the electrophoretic mobilities, R_f values, and UV data. Yields were estimated by assuming that the extinction coefficients for the modified nucleotides are the same as those for the parent, d-TpC (21 500 M^{-1} cm^{-1}, pH 7, 272 nm). On this basis, the yields were in the range 65–70%. All of these modified nucleotides showed maxima at 272 nm, pH 7. Further, the 270/280 nm ratio was close to that measured for d-TpC itself (Table II).

3. Proton NMR Spectra of the Nucleotides Modified at the Cytosine Residue, d-TpC–L_n. The 360-MHz NMR data shown in Table III and Figure 4 are consistent with the assigned structures for the modified nucleotides. Ford has concluded[13] that d-TpC–L_6 is present in solution (pD 7.4, 21 °C) with both pyrimidines in the unstacked anticonformation, and further that the 0.15-ppm upfield shift of cytosine H-6 in the modified nucleotide (as compared with d-TpC itself) is due to elimination of the phosphate ion induced deshielding of this proton because of ion pairing between the ligand and the phosphate.

Table II. R_f Values, Electrophoretic Mobilities, and UV Data for Some Modified Nucleotides

compd	R_f Values: solvent B[a]	solvent C[a]	solvent D[b]	electrophoretic mobilities[c] R_m, pH 2.7	A(270 nm)/A(280 nm), pH 7
d-TpC–L_6	0.80	0.33	0.29	0.87	1.21
d-TpC–L_4	0.80	0.27	0.22	0.85	1.17
d-TpC–L_2	0.61	0.26	0.31	0.59	1.21
dC–L_6	0.93	0.35			
5′-dCMP–L_6	0.44	0.39			
dC	0.70	0.60		1.0	
d-TpC	0.30			0.05	1.26

[a] R_f values were determined by using cellulose TLC (Eastman). [b] Chromatography was carried out on silica gel TLC (250 μm). [c] R_m values were calculated by using deoxycytidine (dC) as reference and 0.1 M ammonium formate (pH 2.7) as buffer.

Table III. ^{1}H NMR Resonace Assignments (δ) for Some Modified Dinucleoside Monophosphates, d-TpC–L_n

assignt[a]	d-TpC–L_2 (pD 8.06)	d-TpC–L_4 (pD 4.70)	d-TpC–L_6 (pD 8.24)
	Thymidine		
C_5–CH_3	1.88 (s)	1.88 (s)	1.88 (s)
C_6–H	7.63 (s)	7.63 (s)	7.60 (s)
C_1'–H	6.21 (t)	6.21 (t)	6.21 (t)
C_2'–H	2.34	2.43	2.34
C_3'–H	4.8 (m)	4.8 (m)	4.8 (m)
C_4'–H	4.14 (m)	4.14 (m)	4.14 (m)
C_5'–H	3.79 (m)	3.79 (m)	3.77 (m)
	Cytidine		
C_5–H	5.98 (d)	5.97 (d)	5.94 (d)
C_6–H	7.78 (d)	7.77 (d)	7.72 (d)
C_1'–H	6.31 (t)	6.31 (t)	6.31 (t)
C_2'–H	2.34	2.34 (m)	2.34
C_3'–H	4.57 (m)	4.57	4.57 (m)
C_4'–H	4.14 (m)	4.14 (m)	4.14 (m)
C_5'–H	4.08, 4.14	4.07, 4.14	4.07, 4.14
	Ligand HN		
CH_2	3.18 (t)	3.28 (t)	3.30 (t)
CH_2		1.66 (m)	1.55 (m)
$(CH_2)_2$			1.33 (m)
CH_2		1.77 (m)	1.55 (m)
CH_2	2.80	3.37	2.69 (t)
N–CH_3	2.34 (s)	2.92 (s)	2.47 (s)
$(CH_2)_2$	2.80	3.62 (s)	2.78
			2.87
N–$(CH_3)_2$	2.78 (s)	2.97 (s)	2.41 (s)
			2.45 (s)

[a] See ref 14 for the spectrum of d-TpC.

4. Analysis of the Proton NMR Spectrum of the Osmate Ester Formed from d-TpC–L_6. Figure 4 compares the NMR spectra of dTpC–L_6 and that of its reaction products with osmium tetroxide. Figure 5 illustrates the chemistry of the reaction. The most important changes are those of the H-6 singlet of the thymine residue at δ 7.62 which appears in the products as a set of three singlets at δ 5.47, 5.16, and 5.05, the thymine methyl singlet at δ 1.88 which appears as three upfield singlets of δ 1.66, 1.64, and 1.61, the *N*-methyl resonances of the ligand which shift downfield to a complex group of 9–12 resonances centered at δ 2.95, and the cytosine H-5 and H-6 doublets which each form a pair of doublets.

With the exception of the cytosine resonances, the observed shifts are all in the expected directions in accord with previous findings on analogous systems.[8,14] The multiplicities can be accounted for by considering the possible diastereomers that should

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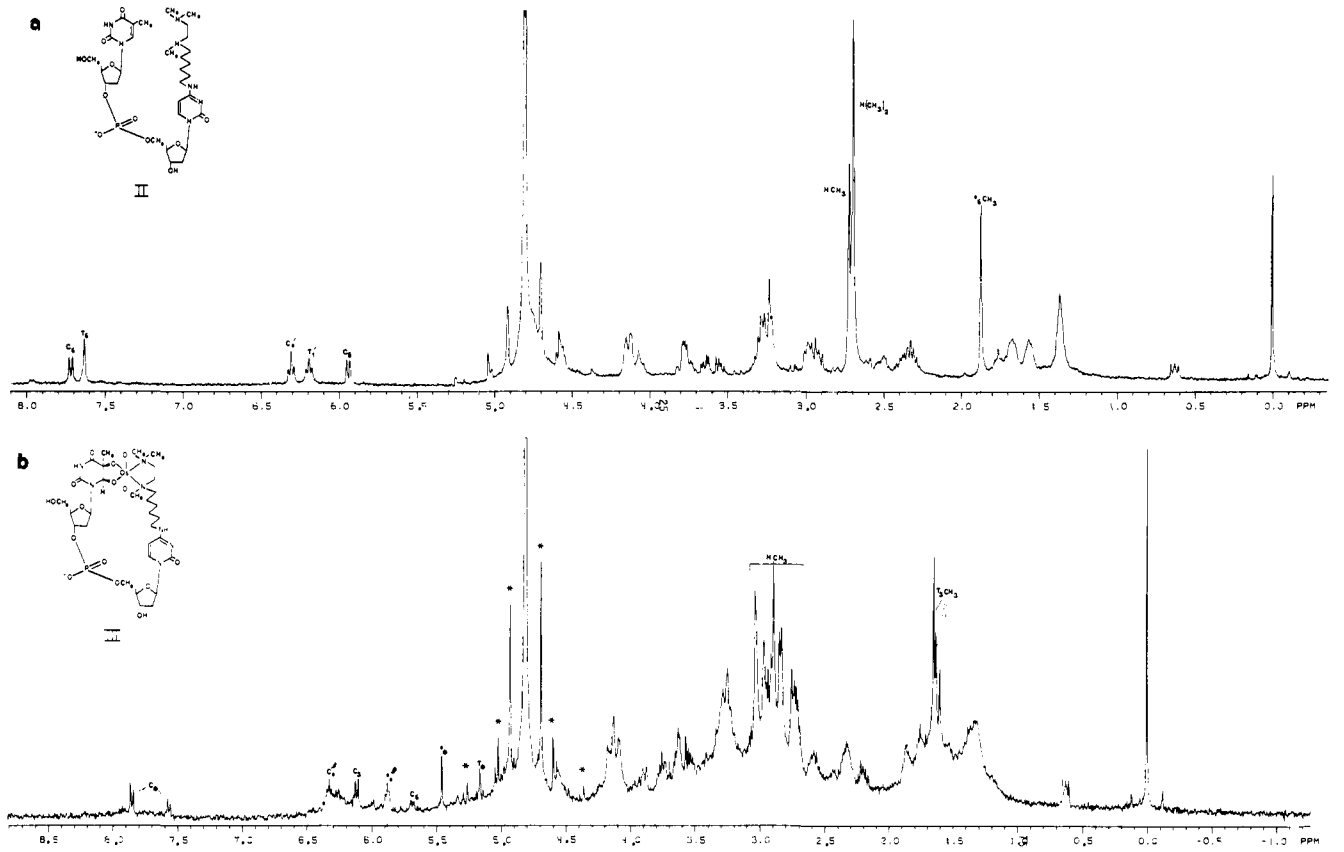


Figure 4. (a) 360-MHz proton NMR spectrum of d-TpC–L_6. (b) 360-MHz proton NMR spectrum of the osmate esters formed from d-TpC–L_6 and OsO_4.

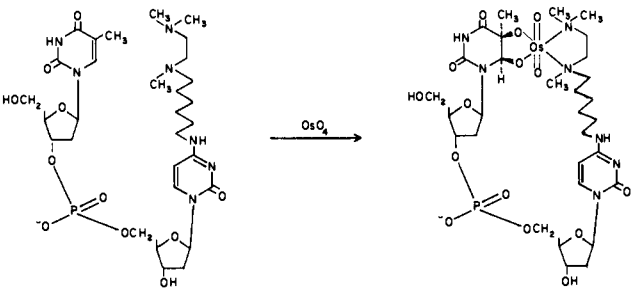


Figure 5. The reaction between d-TpC–L_6 and OsO_4. Only one diastereomer is shown.

be formed by cis addition of the ligand–osmate system to the thymine ring (Figure 6). Formation of at least three of these would account for the three thymine methyl and H-6 singlets as well as the multiplicity observed for the *N*-methyl resonances of the ligand.

CPK models of the macrocycle suggest the probable origin of the doubled cytosine resonances.[13] Two conformations are evident: in one of these the cytosine protons at the 5- and 6-positions are directed toward the interior of the macrocyclic ring (shielded); in the other, they are directd outward (deshielded). For further discussion, see ref 13.

5. Changes in the NMR Spectra of the Macrocycles with Time. (a) d-TpC–L_6 Osmate Ester. After 48 h at 4 °C, there are no substantial changes in the NMR spectrum of the mixture of diastereomeric osmate esters. After 1 month, however, a new resonance appears at δ 1.38. We assign this singlet to a thymine glycol residue by analogy with the similar shifts observed for the simpler glycols.[15] The extent of this hydrolytic process after 1 month was about 15% as estimated by the intensity of the resonance at δ 1.38. After 6 months, hydrolysis had proceeded only to the extent of 20%. This suggests that the diastereomers are undergoing hydrolysis at very different rates from each other. This idea is confirmed by the changes in relative intensities in the thymine ester methyl and H-6 resonances with time. Among the set of three H-6 resonances, the one at δ 5.16 decreases relative to the other two and is absent after 1 month. Likewise, among the three methyl resonances, that one at δ 1.66 decreases relative to the other two with time. We also note that no OsO_2 is formed during storage up to 10 months as shown by the absence of a black precipitate. The hydrolysis can thus probably be represented by eq 1.

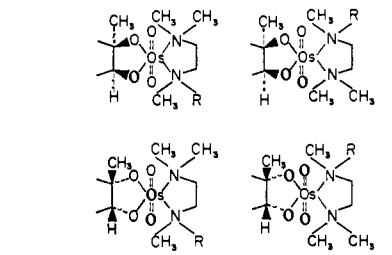
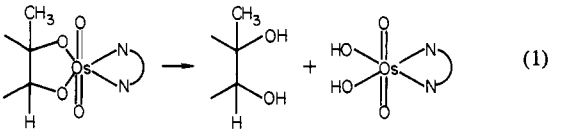


Figure 6. The diastereomers which could result from reaction of d-TpC–L_n with OsO_4.



(1)

The cytosine H-6 and H-5 doublets also undergo complex changes during the hydrolysis.[13]

(b) d-TpC–L_4 Osmate Ester. This ester behaved similarly to d-TpC–L_6 osmate ester except that it was hydrolyzed more rapidly.

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Table IV. Rate Data for the Reaction of the Modified Dinucleoside Monophosphates d-TpC–L_n with OsO_4 and Some Comparative Data[a]

substrate	ligand	k_0, M^{-1} min^{-1}	k, M^{-2} min^{-1}	10^{10} rate, M min^{-1}	rel rate
thymine		0.25[b]		22.5	1
thymine	TMEN[c]	0.25[b]	6400 ± 700[d]	40	1.8
thymine	L_6	0.25[b]	10000 ± 800[e]	49.5	2.2
d-TpC	L_6	0.25[b]	10700 ± 800[f]	53	2.4
d-TpC–L_6		1042 ± 96[g]		94000	4200
d-TpC–L_4		1986 ± 300[g]		179000	7900
d-TpC–L_2		309 ± 8[g]		28000	1200

[a] General conditions: 25 °C, 0.1 M sodium phosphate buffer, pH 7. Rates were calculated for [L] = [S] = 3×10^{-5} M; $[OsO_4] = 3 \times 10^{-4}$ M using the rate laws given in text. [b] Extrapolated from the value at 8 °C (Ragazzo and Behrman[7]) using E_a = 9.5 kcal mol^{-1} (Subbaraman et al.[9]). [c] *N,N,N′,N′*-tetramethylethylenediamine. [d] Measured by the increase in absorbance at 360 nm under pseudo-first-order conditions: $OsO_4 = 4 \times 10^{-4}$; thymine = 5×10^{-3}; TMEN = $(0.5–1.0) \times 10^{-2}$ M. [e] Measured as in footnote *d* but with $L_6 = 1.1 \times 10^{-2}$ M. [f] Measured by the decrease in absorption at 270 nm under pseudo-first-order conditions: d-TpC = 3×10^{-5}; $OsO_4 = 3.2 \times 10^{-4}$; $L_6 = 4.85 \times 10^{-2}$ M. [g] Measured as in footnote *f* under second-order conditions: d-TpC–$L_n = 1 \times 10^{-4}$; $OsO_4 = 1 \times 10^{-4}$ M. Corresponding second-order rate constants for these reactions run under pseudo-first-order conditions with substrate limiting and with OsO_4 limiting, respectively, are as follows: d-TpC–L_6. 386 ± 24, 435 ± 24; d-TpC–L_4. 1040 ± 18, 1266 ± 122; d-TpC–L_2. not determined, 270 ± 20 M^{-1} min^{-1}.

About 5–10% hydrolysis was deduced from appearance of the thymine glycol resonance after 48 h at 4 °C and about 25% after 1 month.

(c) d-TpC–L_2 Osmate Ester. Although the NMR spectra of solutions of this ester showed the characteristic upfield shifts for the H-6 and methyl resonances of the thymine residue, these, as well as the other resonances, were broad. The solutions darkened rapidly. These observations and the TLC results to be discussed suggest rapid decomposition.

6. Thin-Layer Chromatography. TLC on glass-supported silica gel using Solvent D gave resolution of the diastereomers. It is worth noting that a number of other solvent systems and solid phases were tried, but these showed only one mobile osmium-containing spot in all cases. Figure 7 summarizes the TLC results. Not only are several osmium-containing products formed in each case (in agreement with the NMR results), but different patterns are obtained depending on the ratio of the reactants. A black spot, mobility zero, is formed only when there is an excess of osmium tetroxide. Figure 7 also shows changes in the pattern of osmium-containing materials with time in agreement with the NMR results. The L_2 derivative, in particular, shows the most extensive changes. We note that the initial pattern of multiple spots is probably not due to instability of the products in the TLC system since the *N,N,N′N′*-tetramethylethylenediamine osmate ester of thymine shows only one spot (R_f 0.58).

7. Kinetics. The Reaction of Osmium Tetroxide with d-TpC–L_n. The rate law for the reaction of osmium tetroxide with olefins in the presence of a free bidentate ligand contains two terms, one for the reaction without and one for the reaction with ligand.[7,16]

$$v = (k_0 + k[L])[OsO_4][S] \quad (2)$$

When the ligand, L, is attached to the substrate (as in d-TpC–L_n) the rate law will consist of only one term if we make the reasonable assumption (see below) that no osmate ester is formed without participation of the ligand.

$$v = k[OsO_4][SL] \quad (3)$$

The kinetics were measured either by the decrease in absorbance at 270 nm due to saturation of the olefinic bond of thymine or by measuring the formation of the product by an increase in absorbance at 360 nm. Reactions were run under second-order conditions with both substrate and osmium tetroxide at equal concentrations, under pseudo-first-order conditions with osmium tetroxide limiting and under pseudo-first-order conditions with substrate limiting. We have also compared these rates with those for the unmodified substrate in the presence of free ligand. These data are given in Table IV. The data (Table IV, footnote *g*) show approximately a twofold variation in k_0 depending on whether the measurement was made under second-order or under pseudo-first-order conditions; the measured rates under equimolar second-order conditions are larger. Thin-layer chromatographic analysis of the reaction mixtures shows that this variation is probably due to the formation of different products under different conditions. Comparison of the rate constants for reaction of d-TpC–L_n with osmium tetroxide and for reaction of thymine in the absence of external ligand can be made directly since the rate laws are of the same form. But the interesting comparison for the reaction of d-TpC with osmium tetroxide in the presence of added external ligand cannot be made directly since in this case the rate law contains two terms (eq 2). We therefore compared rates under specified conditions. These comparisons are also shown in Table IV.

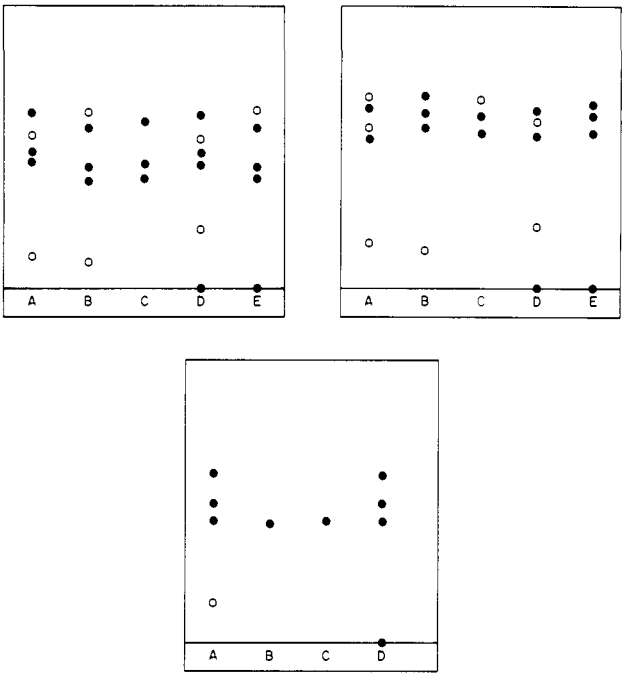

Figure 7. TLC (silica gel, solvent D) of the osmium-containing products of the reactions between d-TpC–L_n and OsO_4. Spots were revealed both by UV absorption and by reaction with thiourea. Panel 1, n = 4: A–C, $[OsO_4]$: [d-TpC–L_4] = 1; reaction time = 15 min, 6 days, 6 months; D and E, $[OsO_4]$:[d-TpC–L_4] = 10; reaction time = 15 min, 6 days. Panel 2, n = 6: A–C, $[OsO_4]$:[d-TpC–L_6] = 1; reaction time = 15 min, 6 days, 12 months. D and E, $[OsO_4]$:[d-TpC–L_6] = 10; reaction time = 15 min, 7 days. Panel 3, n = 2: A–C, $[OsO_4]$:[d-TpC–L_2] = 1; reaction time = 15 min, 18 hours, 6 months; D, $[OsO_4]$:[D-TpC–L_2] = 10; reaction time = 15 min.

The data show that under equimolar second-order conditions, the dinucleoside monophosphate modified with L_6, L_4, and L_2 will react with OsO_4 4200, 7900, and 1200 times more rapidly than does the unmodified dinucleoside monophosphate. For work with a polynucleotide, the L_4 derivative would be chosen for kinetic advantage and the L_6 derivative for the advantage of greater stability.

(16) R. L. Clark and E. J. Behrman, *Inorg. Chem.*, 14, 1425–1426 (1975).

Experimental Section

Materials. d-TpC was a product of the Sigma Chemical Co.; the Aldrich Chemical Co. supplied most of the other reagents.

Methods. Routine measurements were carried out as previously described.[8] High-resolution proton NMR spectra were measured at the Purdue University Magnetic Resonance Facility at 360 MHz, 21 ± 1 °C. Paper electrophoresis was carried out at about 40 V/cm. The following solvents were used for TLC: solvent A, 2-propanol–water (80:20 v/v); solvent B, 2-propanol–NH_4OH (concentrated)–water (7:2:1); solvent C, 2-propanol–water–HCl (concentrated) (7:2:1); solvent D, 2-propanol–NH_4OH (concentrated)–acetic acid–water (4:1:2:2); solvent E, 2-propanol–water–NH_4OH (7:2:1).

Kinetics were followed by measuring the decrease in absorptivity at 270 nm due to saturation of the 5,6-double bond of thymine or the increase in absorptivity at 360 nm due to osmate ester formation. Infinity values were taken after at least 10 half-lives. All values are the average of at least three determinations. Log plots were linear for at least 3 half-lives. General conditions were 25 °C, pH 7, and 0.1 M sodium phosphate buffer. The UV spectra of the osmate esters of d-TpC–L_n showed quantitatively the expected decrease in absorbance at 270 nm due to saturation of the olefinic bond of the thymine residue. The absorbance ratios at 270 nm before and after reaction with osmium tetroxide were 1.37, 1.40, and 1.34 for n = 6, 4, and 2 respectively. The calculated ratio based on cytosine and the osmate ester of thymine with TMEN as ligand is 1.38.

NMR Studies and Reactions of d-TpC–L_n with OsO_4. High-resolution ^1H NMR samples were prepared by lyophilizing the required amount of sample in the NMR tube (Wilmad 528 PP) and adding 0.30 mL of 0.1 M phosphate buffer to the tube followed by lyophilization. The samples were then lyophilized three times from 99.8% D_2O and once from 100.0% D_2O. The final solutions were made up to 0.30 mL in 100.0% D_2O. One molar equivalent of OsO_4 in 100% D_2O was added and the spectrum recorded immediately. Samples were also prepared by adding 2–3 equiv of OsO_4 in water to the buffered sample in the NMR tube, reacting 30–45 minutes, and then removing both water and excess osmium tetraoxide by lyophilization. Repeated lyophilization from D_2O was carried out as described above with final dilution to 0.30 mL with 100.0% D_2O. The spectra were recorded several hours to several days after the final dilution. Concentration of osmate esters in the NMR tubes was 2.7 × 10^{-3} M. The samples were stored at 4 °C. DSS was used as internal standard.

Synthetic Procedures. *N*-(6-Bromohexyl)phthalimide (1, n = 6). Potassium phthalimide (77.0 g, 0.416 mol) was added to a solution of 253 g (1.04 mol) of 1,6-dibromohexane (Aldrich) in 250 mL of dimethylformamide (DMF). The temperature of the mixture was raised to 90 °C, and the reaction was allowed to continue for 75 min with stirring. DMF and the excess dibromohexane (135 g) were removed by distillation (6 mmHg) using an oil bath (90–130 °C). The solid residue was dissolved in 50 mL of chloroform and the mixture filtered if necessary, extracted with water (2 × 75 mL), washed with 40 mL of 0.1 M NaOH (to remove unreacted phthalimide), and washed with water (50 mL) again. The chloroform was removed under reduced pressure and the residue triturated with ether (300 mL), and the solution filtered. The solid diphthalimidohexane (17 g, mp 172–175 °C) was discarded. The ether was removed from the filtrate. The solid residue was recrystallized from 250 mL of 75% ethyl alcohol and dried in vacuo to yield 85 g (66%) of *N*-(6-bromohexyl)phthalimide: mp 55.5–57.2 °C; NMR (CCl_4, Me_4Si) δ 7.72 (m, 4, C_6H_4), 3.64 (t, 2, $(CO)_2NCH_2$), 3.37 (t, 2, CH_2Br), 1.64 (m, 8, $(CH_2)_4CH_2Br$).

***N,N,N'*-Trimethyl-*N'*-(6-(phthalimido)hexyl)ethylenediamine (2, n = 6).** *N*-(6-Bromohexyl)phthalimide (64 g, 0.206 mol) and *N,N,N'*-trimethylethylenediamine (Aldrich), (24 g, 0.235 mol) were heated at 100 °C with stirring for 3 h. The mixture was cooled, and 100 mL of water and 65 mL of 6 N hydrochloric acid were added. The mixture was shaken vigorously until the brown gum dissolved. The aqueous mixture (pH <2) was extracted with chloroform (3 × 200 mL), the pH adjusted to 8.5, and the mixture extracted with diethyl ether (4 × 100 mL) keeping the pH constant between extractions. The combined ether extracts were dried (Na_2SO_4) and evaporated to give a yellow oil, 23 g (34%) of **2**, n = 6. The product was used without further purification: NMR ($CDCl_3$, $CHCl_3$) δ 7.83 (m, 4, C_6H_4), 3.73 (t, 2, $(CO)_2NCH_2$), 2.45 (s, 3, NCH_3), 2.4 (m, 6, $CH_2N(CH_3)(CH_2)_2N(CH_3)_2$), 1.47 (m, 8, $NCH_2(CH_2)_4CH_2N$).

***N,N,N'*-Trimethyl-*N'*-(4-(*N*-phthalimido)butyl)ethylenediamine and *N,N,N'*-Trimethyl-*N'*-(2-(*N*-phthalimido)ethyl)ethylenediamine (2, n = 4, 2).** These compounds were prepared by methods analogous to those used to prepare **2**, n = 6. The yields of these compounds were 11 and 23%, respectively. These compounds were brown and yellow oils, respectively: NMR data (**2**, n = 4) ($CDCl_3$, Me_4Si) δ 7.82 (m, 4, C_6H_4), 3.73 (t, 2, $(CO)_2NCH_2$), 2.43 (s, 3, $N(CH_3)_2$, 2.35 m, 6, $CH_2N(CH_3)(CH_2)_2N(CH_3)_2$), 2.23 (s, 6, $N(CH_3)_2$), 1.63 (m, 4, $NCH_2(CH_2)_2CH_2N$); (**2**, n = 2) ($CDCl_3$, Me_4Si) δ 7.92 (m, 4, C_6H_4), 3.82 (t, 2, $(CO)_2NCH_2$), 2.6 (m, 6, $CH_2N(CH_3)(CH_2)_2N(CH_3)_2$), 2.40 (s, 3, $N(CH_3)$), 2.23 (s, 6, $N(CH_3)_2$).

***N,N,N'*-Trimethyl-*N'*-(6-aminohexyl)ethylenediamine. (3, n = 6, L_6).** Hydrazinolysis was accomplished by refluxing a mixture of the *N,N,N'*-trimethyl-*N'*-(6-*N*-phthalimido)hexyl)ethylenediamine (15.0 g, 0.045 mol) and 2.67 mL of an 85% aqueous hydrazine hydrate solution (0.045 mol) in 100 mL of methanol for 1 h. After the solution was cooled, 50 mL of water and the methanol was removed under reduced pressure. To the aqueous mixture was added 50 mL of hydrochloric acid (concentrated). The mixture was refluxed for 1 h, cooled, and filtered. The filtrate was concentrated to remove most of the hydrochloric acid. The residue was dissolved in 50 mL of water and the solution filtered again to remove any insoluble material. The filtrate was made basic (pH >11) with 10% sodium hydroxide and extracted with chloroform (3 × 30 mL). The chloroform was dried (Na_2SO_4) and evaporated to yield L_6 (8.5 g, 94%) as a yellow oil. The product was distilled to give 7.4 g (82%) of a colorless, hygroscopic oil: bp 76–78 °C (0.2 mmHg); NMR ($CDCl_3$, Me_4Si) δ 2.67 (m, 2, H_2NCH_2), 2.42 (s, 3, $N(CH_3)$, 2.33 (m, 6, $CH_2N(CH_3)(CH_2)_2N(CH_3)_2$), 2.23 (s, 6, $N(CH_3)_2$), 1.38 (m, 10, $H_2NCH_2(CH_2)_4$), H_2N exchanges readily with D_2O; IR (neat) 3360 cm^{-1} and 3295 (N–H stretch), 1600 (N–H bend), 1040 (C–N stretch), 840 (N–H wag); chemical ionization mass spectrum, protonated molecular ion m/e (relative intensity) 202 (100); calculated mass of MH^+ 202. Anal. Calcd for $C_{11}H_{27}N_3 \cdot 0.5H_2O$: C, 62.80; H, 13.42; N, 19.97. Found: C, 62.51; H, 13.68; N, 19.74. The picrate salt: mp 199–199.5 °C. Anal. Calcd for $C_{11}H_{27}N_3 \cdot 3C_6H_3N_3O_7$: C, 39.20; H, 4.08, N, 18.90. Found: C, 38.93; H, 4.32; N, 18.60. Molecular weight: calcd, 888.7; found, 903 ± 1%, based on $\epsilon_{380nm}^{ethanol}$ = 13 440 for picrate.[17]

***N,N,N'*-Trimethyl-*N'*-(4-aminobutyl)ethylenediamine and *N,N,N'*-Trimethyl-*N'*-(2-aminoethyl)ethylenediamine (3, n = 4, 2, L_4 and L_2).** These compounds were prepared by methods analogous to those described for the hexyl derivative. The products were obtained as colorless oils in yields of >90%. The physical and spectral properties are given below. **3**, n = 4, L_4: b.p. 61–63 °C (0.2 mmHg): NMR ($CDCl_3$, Me_4Si) δ 2.7 (m, 2, H_2NCH_2), 2.42 (s, 3, $N(CH_3)$), 2.23 (s, 6, $N(CH_3)_2$), 2.37 (m, 6, $CH_2N(CH_3)(CH_2)_2N(CH_2)_2$), 1.47 (m, 6, $H_2NCH_2(CH_2)_2$), H_2N exchanges readily with D_2O; IR (neat) 3375 cm^{-1} and 3260 (N–H stretch), 1605 (N–H bend), 1038 (C–N stretch), 842 (N–H wagging). Anal. Calcd for $C_9H_{23}N_3 \cdot 0.65H_2O$: C, 58.63; H, 12.93; N, 22.80. Found: C, 58.36; H, 12.80; N, 23.23. The picrate salt: mp 232.5–233.8 °C. Anal. Calcd for $C_9H_{23}N_3 \cdot 3C_6H_3N_3O_7$: C, 36.07; H, 3.39; N, 20.19. Found: C, 36.30; H, 3.50, N, 20.00. Molecular weight: calcd, 860; found, 886 ± 1.9%.[17] **3**, n = 2, L_2: bp 38–43 °C (0.2mmHg); NMR ($CDCl_3$, Me_4Si) δ 2.73 (m, 2, H_2NCH_2), 2.4 (s, 3, $N(CH_3)$), 2.38 (m, 6, $CH_2N(CH_3)(CH_2)_2N(CH_3)_2$), 2.22 (s, 6, $N(CH_3)_2$), 1.37 (s, 2, H_2N), H_2N exchanges readily with D_2O; IR (neat 3360 cm^{-1} and 3280 (N–H stretch), 1580 (N–H bend), 1038 (C–N stretch), 845 (N–H wagging). Anal. Calcd for $C_7H_{19}N_3 \cdot 0.5H_2O$: C, 54.50; H, 13.07; N, 27.24. Found: C, 55.00; H, 13.32; N, 26.18. The picrate salt: mp 193–194.5 °C. Anal. Calcd for $C_7H_{19}N_3 \cdot 3C_6H_3N_3O_7$: C, 37.68; H, 3.75; N, 19.53. Found: C, 37.75; H, 3.85; N, 19.32. Molecular weight: calcd, 832; found, 852 ± 0.8%.[17]

(2-(*N*-Phthalimido)ethyl)(2-(dimethylamino)ethyl)dimethylammonium Bromide (4, n = 2). *N*-(2-(Bromoethyl)phthalimide (25 g, 0.098 mol) and 28.5 g (0.24 mol) of *N,N,N',N'*-tetramethylethylenediamine were heated at 100 °C for 3 h with stirring. After the mixture was cooled, 200 mL of chloroform was added and the mixture again refluxed for 1 h, cooled, and filtered. The solid product was washed with cold chloroform and ether and then dried in vacuo to yield 27 g (76%) of a white granular solid: mp 192 °C; NMR (D_2O, DSS) δ 7.93 (s, 4, C_6H_4), 4.22 (m, 2, $(CO)_2NCH_2$), 3.72 (m, 4, $CH_2N^+(CH_3)_2CH_2$), 3.33 (s, 6, $N(CH_3)_2$), 2.93 (m, 2, $CH_2N(CH_3)_2$), 2.4 (s, 6, $N(CH_3)_2$). The preparation of the butyl and hexyl derivatives (**4**, n = 4, 6) was carried out by methods analogous to those given for the ethyl derivative. These compounds were isolated in similar yields. **4**, n = 4, was a white granular solid; mp 168 °C. **4**, n = 6, was a gummy white solid. **4**, n = 4: NMR (D_2O, DSS) δ 7.73 (m, 4, C_6H_4), 3.77 (m, 6, $(CO)_2NCH_2$, $CH_2N^+(CH_3)_2CH_2$), 3.45 (s, 6, $N^+(CH_3)_2$), 2.73 (m, 2, $CH_2N(CH_3)_2$), 2.28 (s, 6, $N(CH_3)_2$), 1.85 (m, 4, $(CO)_2NCH_2(CH_2)_2$). **4**, n = 6: NMR (D_2O, DSS) δ 7.43 (m, 4, C_6H_4), 3.1 (m, 8, $(CO)_2NCH_2(CH_2)_4CH_2N^+(CH_3)_2(CH_2)_2N(CH_3)_2$), 3.06 (s, 6, $N^+(CH_3)_2$), 2.28 (s, 6, $N(CH_3)_2$), 1.5 (m, 8, $(CO)_2NCH_2(CH_2)_4$).

(2-(Dimethylamino)ethyl)(2-aminoethyl)dimethylammonium Chloride (5, n = 2). A mixture of (2-(*N*-phthalimido)ethyl(2-(dimethylamino)-ethyl)dimethylammonium bromide (**4**, n = 2) (20.2 g, 0.054 mol), 3.2

(17) K. G. Cunningham, W. Dawson, and F. S. Spring, *J. Chem. Soc.*, 2305–2306 (1951).

mL of 85% aqueous hydrazine hydrate solution (0.054 mol), and 50 mL of methanol was refluxed for 1 h and cooled and 50 mL of water added. The methanol was removed under reduced pressure, and 50 mL of hydrochloric acid (concentrated) was added to the remaining aqueous solution. The mixture was refluxed for 1 h, cooled, and filtered. The filtrate was evaporated to dryness. Water was added, and the solution was extracted with chloroform (3 × 30 mL). The pH was raised to ~7 (10% NaOH), and the solution was extracted with chloroform. The aqueous solution was evaporated, 100 mL of ethanol added, and the solution again evaporated. This process was repeated three times. Finally, ethanol was added and the solution refluxed for 15 min and filtered to remove NaCl. The filtrate was dried ($MgSO_4$), evaporated, and dried in vacuo to yield a glassy yellow hygroscopic solid (9.6 g, 91%): NMR (D_2O, DSS) δ 3.3 (m, 8, $H_2N(CH_2)_2N^+(CH_3)_2(CH_2)_2N(CH_3)_2$), 3.20 (s, 6, $N^+(CH_3)_2$), 2.35 (s, 6, $N(CH_3)_2$). The butyl and hexyl derivatives were prepared in a similar manner, with similar yields. **5**, $n = 4$: NMR (D_2O, DSS) δ 3.4 (m, 8, $H_2NCH_2(CH_2)_2CH_2N^+(CH_3)_2CH_2CH_2N(CH_3)_2$), 3.15 (s, 6, $N^+(CH_3)_2$), 2.37 (s, 6, $N(CH_3)_2$), 1.83 (m, 4, $H_2NCH_2(CH_2)_2$). **5**, $n = 6$: NMR (D_2O) δ 3.2 (m, 8, $H_2NCH_2(CH_2)_4CH_2N^+(CH_3)_2CH_2CH_2N(CH_3)_2$), 3.06 (s, 6, $N^+(CH_3)_2$), 2.32 (s, 6, $N(CH_3)_2$), 1.5 (m, 8, $H_2NCH_2(CH_2)_4$).

***N,N,N'*-Trimethyl-*N'*-(2-aminoethyl)ethylenediamine, L_2, by Demethylation of 5, $n = 2$.** To (2-(dimethylamino)ethyl)(2-aminoethyl)dimethylammonium chloride (**5**, $n = 2$) (7.0 g, 0.036 mol) in 150 mL of ethanol (100%) was added a solution of 9.5 g (0.072 mol) of sodium thiophenoxide in 20 mL of ethanol and the mixture was stirred for 20 min, filtered, and washed with ethanol. The filtrate was evaporated in vacuo, and 400 mL of 2-butanone (freshly distilled from zinc dust) was added to the residue. The mixture was refluxed under nitrogen for 72 h. Following removal of the solvent, 40 mL of water and 50 mL of chloroform were added, the chloroform layer was removed, and the aqueous layer was extracted three times with chloroform (3 × 30 mL). The chloroform fractions were combined and evaporated. After 40 mL of 10% hydrochloric acid was added to the residue, the solution was repeatedly extracted with ether. The aqueous layer was made basic (pH >11, 10% NaOH), extracted with ether (3 × 30 mL), and then extracted with chloroform. The chloroform extracts were dried (Na_2SO_4) and evaporated to yield a brown oil which was distilled to give 2.4 g (46%) of L_2 as a colorless oil. The properties of this compound were identical with the triamine isolated from the *N,N,N'*-trimethylethylenediamine route. In some cases the oil contained contaminants which could not be removed upon distillation. The oil can be purified by using a silicagel column. The butyl (L_4) and hexyl (L_6) derivatives were prepared in a similar manner to yield 59 and 67% of the alkyl triamines.

Transamination of d-TpC with L_6. The alkyl triamine, L_6 (0.42 g, 0.002 mol), was dissolved in 0.5 mL of water and the pH adjusted to between 8.5 and 9.0 with hydrochloric acid (concentrated). Sodium bisulfite (0.0012 mol) was added and the mixture shaken vigorously until most of the sodium bisulfite was dissolved, adding water if necessary. The pH was carefully adjusted to between 7.0 and 7.2 and the dinucleoside monophosphate (15 mg, 0.027 mmol) dissolved. The final volume of the solution was 1.4 mL. Nitrogen was bubbled through the solution for 1 min. The vial was sealed and incubated at 38–40 °C.

Ten microliters of solution was removed periodically to monitor the progress of the reaction, using Sephadex gel chromatography or electrophoresis. The reaction was terminated after 8–10 days.

The contents of the vial was rinsed into a small separatory funnel. The pH was adjusted (>11) with 10% sodium hydroxide (2.5 mL) and the mixture then extracted with chloroform (3 × 15 mL) to remove unreacted amine. One molar barium chloride solution (0.0012 mol) was added and the aqueous mixture centrifuged. The aqueous layer was filtered and concentrated to 1–2 mL at 35–40 °C under reduced pressure. The solution was placed on a Sephadex G-10 column (75 × 2.5 cm) and eluted with water. The major ultraviolet absorbing peak was collected, concentrated, and purified by preparative cellulose TLC (solvent B). The major ultraviolet absorbing band (R_f 0.80), dTpC–L_6, was isolated by elution with water. The product was concentrated and lyophilized to yield 0.0186 mmol (68%) of a white solid as judged by ultraviolet spectroscopy using $\lambda_{max}^{pH\ 7.0}$ = 272 nm (ϵ = 21 500). Transaminations using L_2 and L_4 were carried out by similar procedures.

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2. Binding Sites of Anions in Superoxide Dismutase[29]

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Abstract: The electronic absorption spectra in the range (10–25) × 10^3 cm^{-1} and the ESR spectra have been carefully remeasured for copper–zinc superoxide dismutase in the presence of increasing amounts of NCO^-, N_3^-, and F^-. The results have been compared with those obtained by multinuclear NMR spectroscopy regarding ^1H of the water solvent, ^{13}C of $N^{13}CO^-$, ^{19}F, and ^{14}N and ^{15}N of NCO^-, NCS^- and N_3^-. A model is proposed, which takes into account also the spectroscopic behavior of CN^- and NCS^-. Within this frame, the ligands bind the enzyme at the copper site in a 1:1 ratio, substituting an equatorial histidine nitrogen. The CN^- derivative is square planar, whereas the NCO^-, NCS^-, and F^- derivatives are five-coordinate, with an apical water molecule. Azide may behave like CN^-, although the possibility of a copper(II)–inihibitor (1:2) is also taken into consideration.

Bovine erithrocyte superoxide dismutase is a dimeric metalloenzyme containing a zinc(II) and a copper(II) ion in each subunit;[1-5] its biological role is to prevent the accumulation of the toxic O_2^- ion in tissues,[6] by catalyzing the reaction

$$2O_2^- + 2H^+ \rightleftarrows H_2O_2 + O_2$$

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