CDPI$_3$-Enediyne and CDPI$_3$-EDTA Conjugates: A New Class of DNA Cleaving Agents

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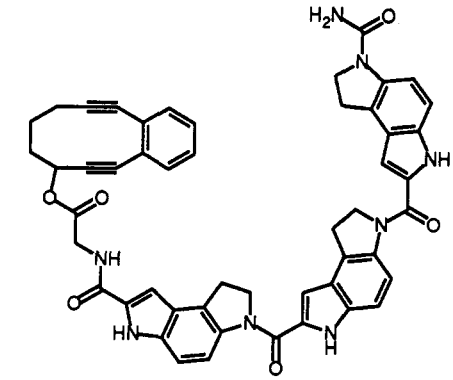
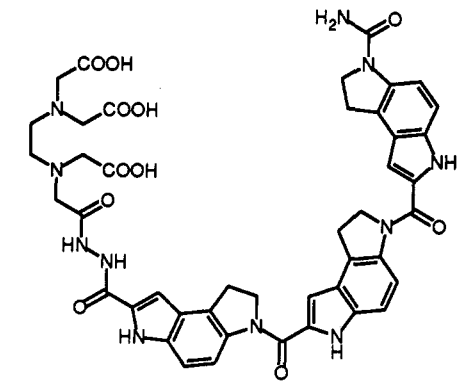
The synthesis and preliminary evaluation of **1a** and **1b**, prototypical CDPI$_3$-enediyne and CDPI$_3$-EDTA conjugates constituting a new class of DNA cleaving agents, are detailed.

The emergence of footprinting[1,2] and the complementary technique of affinity cleavage[2,3] for the determination of DNA binding selectivity coupled with structural detail within identified sequences obtained through X-ray crystallography, NMR spectroscopy, or molecular modeling have advanced the understanding of DNA–agent molecular recognition. The application of such techniques to the definition of the sequence selective alkylation of duplex DNA by (+)-CC-1065[4,5] and the duocarmycins[6] and the sequence-selective cleavage of duplex DNA by the enediyne antitumor antibiotics[7] including the esperamicins,[8] calicheamicins,[9] neocarzinostatin,[10] and dynemicin[11] continue to contribute to an understanding of the structural basis of the agents properties.

Herein, we detail the linkage of CDPI$_3$, a *synthetic* and effective noncovalent minor groove DNA binding agent based on the CC-1065 structure,[12–14] with EDTA and a simple enediyne capable of productive Bergman cyclization[15] in initial studies designed to explore the potential ramifications of incorporation of a nonselective DNA cleaving agent into the structure of a selective minor groove binding agent related to CC-1065.[16]



1a



1b

Synthesis of a CDPI$_3$-Enediyne and CDPI$_3$-EDTA Conjugate. Recognizing that the requisite enediyne for linkage with CDPI$_3$ should exhibit managable but productive Bergman cyclization reactivity, the enediyne **6** was selected for examination. Ideally, the enediyne should exhibit significant chemical stability at room temperature but possess productive reactivity at approximately 40 °C. On the basis of the Bergman cyclization rates of a series of previously disclosed enediynes,[17–36] **6** was anticipated to approach this appropriate window of chemical reactivity.

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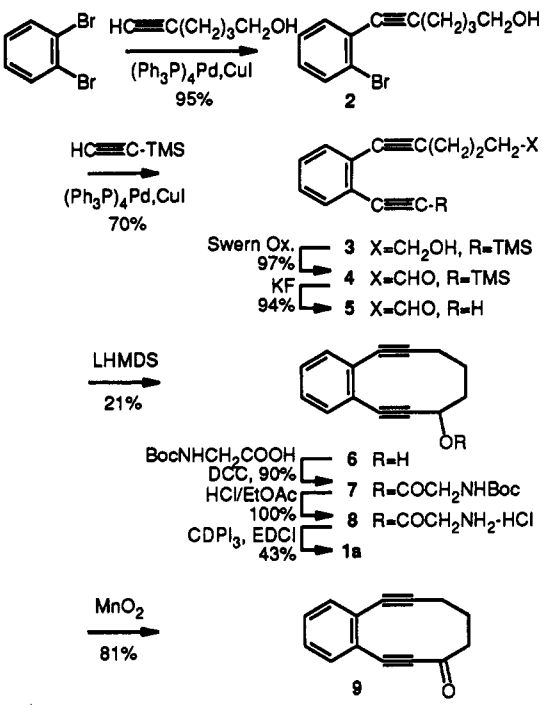
Palladium(0)-catalyzed coupling of *o*-dibromobenzene with 5-hexyn-1-ol cleanly provided **2** (95%). Subsequent direct palladium(0)-catalyzed coupling of **2** with (trimethylsilyl)acetylene provided **3** (70%) without deliberate protection of the primary alcohol and was accompanied by significant amounts of recovered, unreacted **2** (26%). Swern oxidation of **3** to the corresponding aldehyde **4** (97%) followed by fluoride-induced deprotection of the terminal acetylene provided **5** (94%). Attempts to promote the cyclization of **5** to provide **6** under the conditions of desilylation with in situ acetylide generation and cyclization have not proven successful.[36]

The key 10-membered ring cyclization was most effectively accomplished by slow addition (5 h) of LHMDS (4 equiv, THF, 25 °C) to a solution of **5** under dilute reaction conditions (0.04–0.005 M) and provided **6** in optimized conversions of 20–25%. The examination of a range of additional bases including KHMDS failed to improve on this conversion and many (NaH) failed altogether. Acylation of **6** with BOC-Gly (2 equiv, 1.1 equiv DCC, 0.1 equiv DMAP, CH_2Cl_2, 25 °C, 4 h, 90%) provided **7**.[37] Acid-catalyzed *N*-BOC deprotection followed by coupling of the intermediate amine hydrochloride salt **8** with CDPI$_3$[12] following the protocol developed in the prior studies (3.0 equiv EDCI, 3.0 equiv Et_3N, 1.0 equiv DMAP, DMF, 25 °C, 36 h) provided **1a** (43%). By necessity, the coupling reaction was run as a suspension in DMF and the crude product was isolated in essentially pure form by simple trituration with aqueous acid, aqueous base, and tetrahydrofuran. Analytically pure samples of **1a** were obtained by conventional chromatography (SiO_2, 0–5% DMF–THF gradient elution).

The preparation and evaluation of the DNA affinity cleavage agent derived from the linkage of EDTA[2,3,42] to CDPI$_3$ was examined with the agent **1b** (CDPI$_3$-EDTA) which was anticipated to derived DNA cleavage capabilities from Fenton chemistry when presented with Fe(II) in the presence of appropriate reducing (thiol) and oxidizing (O_2) agents.[1-3,38-43] CDPI$_3$-EDTA (**1b**) was prepared as outlined in Scheme II. As a result of the solubility properties of CDPI$_3$ hydrazide, **11** and **1b**, the direct and slow carbodiimide-promoted coupling of CDPI$_3$-$NHNH_2$ with triethyl ethylenediaminetetraacetate[44] proved technically challenging to conduct effectively and the separation of resulting mixtures of unreacted starting materials and **11** proved difficult. This coupling proved to proceed in much higher conversions (73% versus 30–40%) and the subsequent purification proved much simpler if triethyl ethylenediaminetetraacetate was first converted to the isolable mono-*N*-hydroxysuccinimide active ester **10** and subsequently coupled with CDPI$_3$ hydrazide.

Scheme I



Bergman Cyclization of 6 and 9. Pertinent to the projected properties of **1a** was the event and rate of Bergman cyclization. Important to its projected utility was a useful stability at 25 °C coupled with productive reactivity at 40 °C. Consistent with expectations, **6** proved stable at 25 °C and displayed a reasonable, albeit not rapid, rate of cyclization at 40–80 °C in 1,4-cyclohexadiene, Table I. In agreement with past observations, **12** was the major thermal product presumably derived from cyclization to the diyl followed by hydrogen atom abstraction, Scheme III. Similarly, **9** provided **13** (92%) upon heating at 82 °C in 1,4-cyclohexadiene presumably derived from Bergman cyclization and at a rate that was established qualitatively to be slightly faster than that of **6** (4 h vs 10 h for complete reaction, 82 °C). Notably, both **6** and **9** undergo Bergman cyclization at a rate substantially slower than the parent 10-membered enediyne ($t_{1/2}$ = 18 h, 37 °C)[18] and at rates

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Scheme II

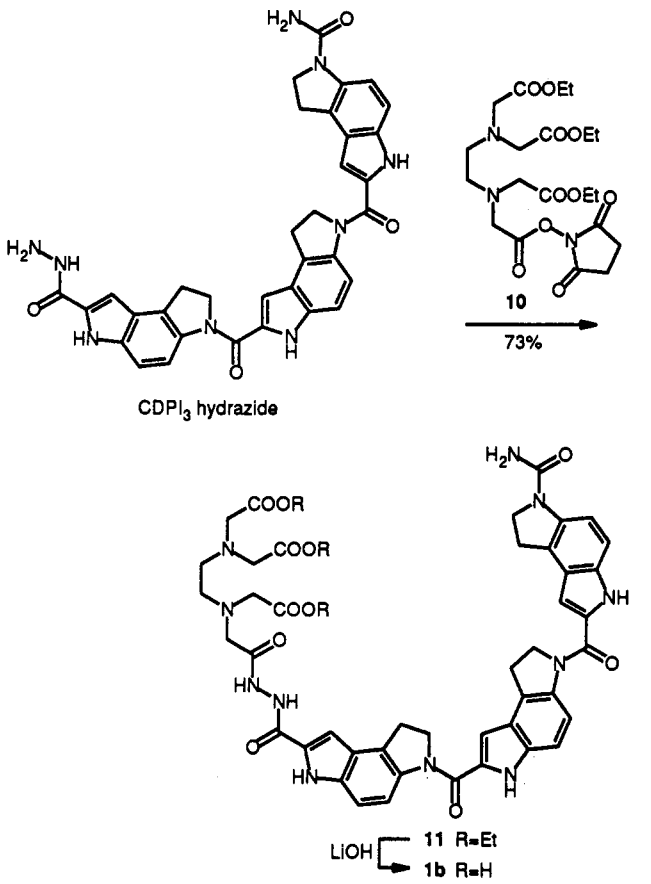
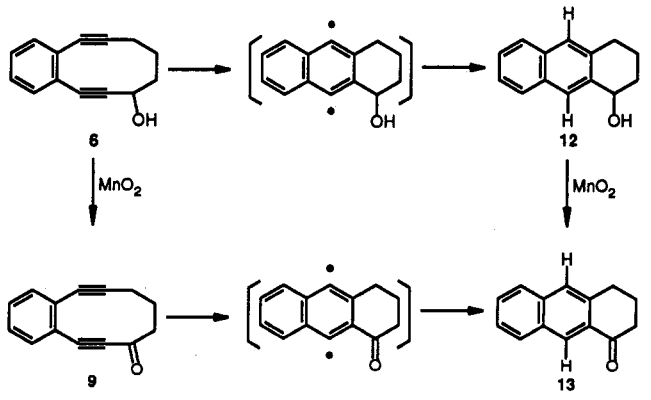


Table I. Bergman Cyclization of 6[a]

temp (°C)	k, min^{-1}	$t_{1/2}$, min
40	7.50×10^{-5}	9240 (154 h)
60	5.00×10^{-4}	1390 (23.2 h)
80	2.54×10^{-3}	273 (4.6 h)

[a] $E_a(6)$ = 19.4 kcal/mol, R = 0.9999.

Scheme III



comparable to those qualitatively established for related benzo[3,4-*b*]cyclodec-3-ene-1,5-diynes recently disclosed.[23,24]

DNA Cleavage Studies. The ability of the agents **1a**, **6**, **7**, and **9** to cleave duplex DNA was determined through examination of single-strand and double-strand cleavage of supercoiled ϕX174 DNA (form I) to produce relaxed (form II) and linear (form III) DNA, respectively. The thermal DNA cleavage reactions were run at 40 °C for 30 h. Under such conditions, each of the four agents produced DNA cleavage principally to provide relaxed (form II) DNA resulting from single-strand cleavage and presumably derived from Bergman cyclization of the enediyne. Each of the three simple agents **6**, **7**, and **9** proved comparable in their ability to cleave the duplex DNA and under the conditions detailed at concentrations of $4 \times 10^{-3} - 2 \times 10^{-5}$ M. Small distinctions in the relative efficiency of DNA cleavage were observed and follow the order of **9** > **6** ≥ **7** and the distinctions in **9** versus **6** and **7** may be related to the relative rates of Bergman cyclization (**9** > **6**, **7**), Table II. In contrast, **1a** proved to be a much more effective DNA cleavage agent and under the conditions examined (40 °C, 30 h) the agent exhibited detectable DNA cleavage at concentrations as low as 4×10^{-9} M, a concentration 4 orders of magnitude lower than that required of **6**, **7**, and **9**. Despite the detectable DNA cleavage at 4×10^{-9} M with **1a**, complete cleavage of the supercoiled ϕX174 DNA was not observed except at the high agent concentrations indicating that the efficiency of DNA cleavage under the reaction conditions may not be high. This may be related to a number of contributing factors including a slow Bergman cyclization rate that permits only a small percentage of agent to have reacted after 30 h at 37 °C, unproductive Bergman cyclization that does not lead to DNA strand scission, tight agent binding that inhibits subsequent effector agent binding and potential productive DNA cleavage, and nonoptimal binding orientation of the enediyne subunit. Although this inefficient cleavage of DNA may be perceived as a potential shortcoming of the agent, it does offer the advantage of a controlled rate and selectivity of DNA cleavage not accessible to more reactive DNA cleaving reactions. Such potential applications of **1a** are under current investigation. In addition, the naturally occurring enediynes as well as agents like bleomycin produce both single- and double-strand DNA lesions and the latter have been considered the more significant biological event.[45] Unlike the naturally occurring enediynes, **1a** failed to provide a substantial percentage of double-strand versus single-strand DNA cleavage events.

The efficiency of DNA cleavage by CDPI$_3$-EDTA (**1b**) in the presence of Fe(II) similarly was assessed with the single-strand and double-strand cleavage of supercoiled ϕX174 DNA (form I) to produce relaxed (form II) and linear (form III) DNA, respectively.[50] The Fe(II) complex of **1b** in the presence of O_2 and 2-mercaptoethanol produced both single- and double strand cleavage of the supercoiled ϕX174 DNA at concentrations significantly lower than that of background iron, and the agent alone in the absence of Fe(II) failed to produce DNA cleavage, Table III. In addition, the ratio of double-strand to single-strand cleavage events was established in kinetic measurements of the relative generation of linear versus relaxed ϕX174 DNA and typical results are presented in Table IV. A study of the time dependence of the relative amount of circular versus linear DNA generated in the DNA cleavage reaction suggests that the linear DNA generated by **1b** is not the consequence of random single-strand cleavage events that eventually provides DNA cleavage within 15 base-pairs on complementary strands required for the generation of linear DNA. The DNA cleavage reaction exhibits initial fast kinetics in the first 5 min and the subsequent decreasing rate may reflect conversion of the agent to a less active or inactive form in the course of the assay or metal complex reactivation kinetics. We assumed a Poisson distribution for the formation of single-strand and double-strand cleavage in order to calculate

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Table II. Cleavage of Supercoiled φX174 RFI DNA by 1a, 6, 7 and 9[a]

	1a (% form)			6 (% form)			7 (% form)			9 (% form)		
concentrations (μM)	I	II	III	I	II	III	I	II	III	I	II	III
0	95.0	5.0	0	95.0	5.0	0	95.0	5.0	0	95.0	5.0	0
4000	12.1	87.9	0	21.9	78.1	0	38.4	61.6	0	3.3	89.0	7.7
2000				38.5	61.5	0	40.3	59.7	0	15.3	84.7	0
400	50.2	49.8	0	60.8	39.2	0	57.4	42.6	0	55.6	44.4	0
200				72.4	27.6	0	72.6	27.4	0	67.6	32.4	0
40	61.8	38.2	0	86.6	13.4	0	77.2	22.8	0	78.5	21.5	0
20				92.0	8.0	0	80.8	19.2	0	87.6	12.4	0
4	62.7	37.3	0									
0.4	71.1	28.9	0									
0.04	75.2	24.8	0									
0.004	79.8	20.2	0									

[a] Solutions contained 1.0 μg of supercoiled φX174 RFI DNA (1.4×10^{-8} M) in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). The DNA cleavage reactions were run for 30 h at 40 °C and the electrophoresis was conducted at 50 V for 2.5 h on a 0.7% agarose gel containing 0.1 μg/mL ethidium bromide. Form I = supercoiled DNA, form II = relaxed DNA (single-stranded cleavage), form III = linear DNA (double-stranded cleavage). Direct fluorescence quantitation of the DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system visualized on a UV (312 nm) transilluminator.

Table III. Cleavage of Supercoiled φX174 RFI DNA by Fe(II)-1b[a]

concentrations (μM)		% form		
1b	Fe(II)	I	II	III
0	0	95.0	5.0	0
50	50	0	84.0	16.0
40	40	0	87.6	12.4
20	20	11.5	84.7	3.8
10	10	15.4	81.4	3.2
5	5	34.0	66.0	0
1	1	47.6	52.4	0
0	1	87.2	12.8	0
0	5	78.0	22.0	0
0	10	76.9	23.1	0

[a] Solutions contained 0.25 μg of supercoiled φX174 DNA (1.4×10^{-8} M) in 50 mM Tris-HCl buffer solution (pH 8) containing 10 mM 2-mercaptoethanol. The DNA cleavage reactions were run for 30 min at 37 °C and electrophoresis was conducted at 50 V for 2.5 h on a 1% agarose gel containing 0.1 μg/mL ethidium bromide. Form I = supercoiled DNA, form II = relaxed DNA (single-stranded cleavage), form III = linear DNA (double-stranded cleavage). Direct fluorescence quantitation of the DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system visualized on a UV (312 nm) transilluminator.

Table IV. Kinetics of Supercoiled φX174 RFI DNA Cleavage by Fe(II)-1b[a]

	% form		
time (min)	I	II	III
0	95.0	5.0	0
1	55.1	44.9	0
2	47.4	51.3	1.3
4	35.7	60.0	4.3
6	24.3	66.4	9.3
8	20.1	69.9	10.0
10	17.3	72.1	10.6
12	14.0	74.6	10.4
15	10.8	77.1	12.1
18	9.3	78.0	12.7
20	8.5	78.5	13.0
30	6.9	79.5	13.6
40	2.7	81.6	15.7

[a] The concentration of the Fe(II)-1b complex was 50 μM. Solutions contained 0.25 μg of supercoiled φX174 DNA (1.4×10^{-8} M) in 50 mM Tris-HCl buffer (pH 8) containing 10 mM 2-mercaptoethanol. The DNA cleavage reactions were run at 37 °C and electrophoresis was conducted at 50 V for 2.5 h on a 1% agarose gel containing 0.1 μg/mL ethidium bromide. The quantitation of percentage of forms I, II, and III present at each time point of Fe(II)-1b was conducted by direct fluorescence quantitation of the DNA in the presence of ethidium bromide using a Millipore Bio Image 60S RFLP system visualized on a UV (312 nm) transilluminator. Form I = supercoiled DNA, form II = relaxed DNA (single-stranded cleavage), form III = linear DNA (double-stranded cleavage).

the average number of single- and double-strand cuts per DNA molecule using the Freifelder–Trumbo equation.[46] The data for the first 1-5 min could be fitted to a linear equation with a ratio of 1:30 double-strand to single-strand cuts observed with **1b**. The theoretical ratio of approximately 1:100 would be expected if the accumulation of linear DNA were the result of random single-strand breaks within the 5386 base-pair size of φX174 DNA assuming that sequential cleavage in the complementary strands within 15 base-pairs is required for linearization of the hybridized DNA. Experimentally, Fe(II) alone produced a ratio of 1:98 under our conditions consistent with this theoretical ratio. For comparison, the ratio of double-strand to single-strand DNA cleavage by a number of natural products include: bleomycin A_2 (1:6–1:9),[47,48] deglycobleomycin A_2 (1:12),[48] calicheamicin γ^I (1:2),[9] neocarzinostatin (1:6–1:41),[49] and P-3A (1:30).[48]

Though effective DNA cleavage is observed with **1a** at low agent concentrations, clear improvements in the efficiency of DNA cleavage, the rate of DNA cleavage, and enhancements in the ratio of double-stranded to single-stranded DNA cleavage[45] remain to be addressed. The comparative properties of additional enediyne-CDPI$_n$ conjugates under present consideration will be disclosed in due course.[51]

Experimental Section

6-(2-Bromophenyl)hex-5-yn-1-ol (2). A solution of *o*-dibromobenzene (3.54 g, 15 mmol, 1.8 mL) in Et_3N (30 mL) under Ar at 25 °C was treated with 5-hexyn-1-ol (1.45 g, 15 mmol, 1.7 mL), $(Ph_3P)_4Pd$ (346 mg, 0.3 mmol), and CuI (85 mg, 0.45 mmol) and the reaction mixture was stirred under Ar at 70–80 °C for 12 h. The Et_3N was removed in vacuo, and the residue was treated with a solution of saturated aqueous NH_4Cl (60 mL) and extracted with Et_2O (4 × 50 mL). The combined Et_2O extracts were washed with saturated aqueous NH_4Cl (3 × 40 mL), dried ($MgSO_4$), and

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(50) That binding to duplex DNA was not inhibited through the linkage of EDTA to CDPI$_3$ was established in thermal denaturization studies of poly[dA]-poly[dT], ΔT_m = +15, +20, and +13 °C for **11**, **1b**, and CDPI$_3$, respectively. Incubation conditions: 3 d, 25 °C, agent-base-pair ratio 1:7 (see refs 13 and 14).
(51) In vitro cytotoxic activity, L1210 (μg/mL): **1a**, 2; **1b**, 2; **11**, 2; **6**, 7; **7**, 3.

concentrated in vacuo. Flash chromatography (SiO_2, 15% EtOAc–hexane) afforded **2** (3.59 g, 3.80 theoretical, 95%) as a pale yellow oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.55 (dd, 1 H, J = 8, 2.4 Hz, C3′-H), 7.41 (dd, 1 H, J = 8, 2.4 Hz, C6′-H), 7.06–7.26 (m, 2 H, C4′ and C5′-H), 3.70 (q, 2 H, J = 7.0 Hz, C1-H_2), 2.51 (t, 2 H, J = 7 Hz, C4-H_2), 1.62–1.88 (m, 4 H, C2 and C3-H_2), 1.54 (t, 1 H, J = 6 Hz, OH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 134.0, 133.0, 129.1, 127.5, 126.8, 126.0, 95.7, 80.2, 62.8, 32.1, 25.1, 19.8; IR (neat) ν_{max} 3350, 2928, 2232, 1585, 1551, 1465, 1425, 1328, 1152, 1049, 1020, 992, 941 cm^{-1}; EIMS, m/e (rel inten) 252/254 (M^+, 1/1), 129 (base); CIMS (2-methylpropane), m/e (rel inten) 253/255 (M^+ + H, 89/96), 235/237 (94/100); FABHRMS (NBA), m/e 253.0231 (M^+ + H, $C_{12}H_{13}BrO$ requires 253.0228).

6-[2-[(Trimethylsilyl)ethynyl]phenyl]hex-5-yn-1-ol (3). A solution of **2** (1.95 g, 7.7 mmol) in Et_3N (15 mL) under Ar at 25 °C was treated with (trimethylsilyl)acetylene (1.13 g, 11.6 mmol, 1.63 mL), $(Ph_3P)_4Pd$ (178 mg, 0.15 mmol), and CuI (44 mg, 0.23 mmol) and the reaction mixture was stirred under Ar at 50–55 °C for 48 h. The Et_3N was removed in vacuo, and the residue was treated with saturated aqueous NH_4Cl (30 mL) and extracted with Et_2O (4 × 30 mL). The combined Et_2O extracts were washed with saturated aqueous NH_4Cl (3 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 10% EtOAc–hexane) afforded **3** (1.45 g, 2.08 g theoretical, 70%) as a pale yellow oil and recovered **2** (507 mg, 26%). For **3**: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.34–7.46 (m, 2 H, C4′ and C5′-H), 7.14–7.26 (m, 2 H, C3′ and C6′-H), 3.70 (q, 2 H, J = 7 Hz, C1-H_2), 2.50 (t, 2 H, J = 7 Hz, C4-H_2), 1.62–1.84 (m, 4 H, C2 and C3-H_2), 1.54 (t, 1 H, J = 6 Hz, OH), 0.25 (s, 9 H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 132.8, 132.4, 128.8, 127.8, 127.2, 126.0, 104.4, 98.4, 94.8, 80.0, 62.8, 32.2, 25.2, 19.8, 0.34; IR (neat) ν_{max} 3378, 2943, 2226, 2155, 1631, 1590, 1472, 1437, 1325, 1249, 1208, 1155, 1096, 1055, 850, 756, 691 cm^{-1}; EIMS, m/e (rel inten) 270 (M^+, 7), 73 (base); CIMS (2-methylpropane), m/e 271 (M^+ + H, base); EIHRMS, m/e 270.1441 (M^+, $C_{17}H_{22}SiO$ requires 270.1440).

6-[2-[(Trimethylsilyl)ethynyl]phenyl]hex-5-yn-1-al (4). A stirred solution of $(COCl)_2$ (1.43 mmol, 0.13 mL) in dry CH_2Cl_2 (4 mL) under Ar at −78 °C was treated dropwise with a solution of DMSO (2.86 mmol, 0.24 mL) in CH_2Cl_2 (2 mL). The resulting mixture was stirred at −78 °C for an additional 15 min before a solution of **3** (348 mg, 1.3 mmol) in CH_2Cl_2 (4 mL) was added dropwise over 5 min at −78 °C. The reaction mixture was stirred for an additional 1 h at −78 °C. Et_3N (6.5 mmol, 0.94 mL) was added and the reaction mixture was stirred at −78 °C for 15 min before being allowed to warm to 25 °C. H_2O (10 mL) was added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (3 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 10% EtOAc–hexane) afforded **4** (335 mg, 345 mg theoretial, 97%) as a pale yellow oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 9.85 (t, 1 H, J = 1.0 Hz, CHO), 7.35–7.5 (m, 2 H, C4′ and C5′-H), 7.16–7.28 (m, 2 H, C3′ and C6′-H), 2.70 (dt, 2 H, J = 7, 1 Hz, C2-H_2), 2.55 (t, 2 H, J = 7 Hz, C4-H_2), 1.95 (p, 2 H, J = 7 Hz, C3-H_2), 0.25 (s, 9 H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 202.6, 133.0, 132.6, 128.8, 128.2, 127.4, 126.2, 104.4, 98.8, 93.6, 80.8, 43.2, 21.8, 19.6, 0.34; IR (neat) ν_{max} 3028, 2960, 2900, 2232, 2158, 1720, 1602, 1478, 1442, 1410, 1300, 1250, 1208, 1160, 1102, 1040, 952, 844, 764, 644 cm^{-1}; CIMS (2-methylpropane), m/e 269 (M^+ + H, base); EIHRMS 267.1211 (M^+ − H, $C_{17}H_{20}SiO$ requires 267.1205).

6-(2-Ethynylphenyl)hex-5-yn-1-al (5). A solution of **4** (180 mg, 0.67 mmol), KF (57 mg, 1.0 mmol), and H_2O (0.15 mL) in DMF (4 mL) was stirred under Ar at 25 °C for 5 h. The reaction mixture was poured onto ice (10 g) and the product was extracted with CH_2Cl_2 (4 × 10 mL). The combined organic layers were washed with H_2O (4 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 10% EtOAc–hexane) afforded **5** (124 mg, 132 mg theoretical, 94%) as a pale yellow oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 9.85 (t, 1 H, J = 1.0 Hz, CHO), 7.36–7.52 (m, 2 H, C4′ and C5′-H), 7.18–7.32 (m, 2 H, C3′ and C6′-H), 3.20 (s, 1 H, C≡CH), 2.72 (dt, 2 H, J = 7, 1 Hz, C2-H_2), 2.55 (t, 2 H, J = 7 Hz, C4-H_2), 1.45 (p, 2 H, J = 7 Hz, C3-H_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 203.0, 133.4, 132.4, 129.4, 128.4, 127.4, 125.4, 94.0, 83.2, 81.2, 80.8, 43.4, 21.4, 19.6; IR (neat) ν_{max} 3280, 3062, 2938, 2232, 2106, 1718, 1594, 1508, 1476, 1438, 758 cm^{-1}; EIMS, m/e (rel inten) 196 (M^+, 3), 152 (base); CIMS (2-methylpropane), m/e (rel inten) 197 (M^+ + H, 99), 155 (base); EIHRMS, m/e 195.0801 (M^+ − H, $C_{14}H_{12}O$ requires 195.0810).

Benzo[4,5]cyclodec-4-ene-2,6-diyn-1-ol (6). A solution of **5** (74 mg, 0.38 mmol) in dry THF (10 mL) was added slowly to a solution of LHMDS (1.5 mmol, 1.5 mL of 1.0 M in THF) in dry THF (65 mL) over 5 h under Ar at 25 °C. The reaction mixture was stirred at 25 °C for an additional 1 h before a solution of saturated aqueous NH_4Cl (20 mL) was added. The aqueous layer was extracted with Et_2O (3 × 30 mL), and the combined organic layers were washed with saturated aqueous NH_4Cl (2 × 20 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, 10% EtOAc–hexane) afforded **6** (15.6 mg, 74 mg theoretical, 21%) as a colorless oil and 17 mg (23% recovery) of **5**. For **6**: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.20–7.40 (m, 4 H, aromatic CH), 4.58–4.68 (m, 1 H, C1-H), 2.47 (t, 2 H, J = 7 Hz, C8-H_2), 2.08–2.23 (m, 3 H, C9-H_2 and C10 α-H or C10 β-H), 2.01 (d, 1 H, J = 7 Hz, OH), 1.70–1.90 (m, 1 H, C10 α-H or C10 β-H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 129.7, 128.9, 128.3, 128.1, 128.0, 127.3, 100.3, 98.5, 85.2, 82.0, 63.4, 38.0, 23.7, 21.3; IR (neat) ν_{max} 3406, 2930, 2862, 2224, 2194, 1596, 1468, 1450, 1356, 1332, 1228, 1176, 1104, 1056, 1038, 1014, 974, 760 cm^{-1}; EIMS, m/e (rel inten) 196 (M^+, 23), 139 (base); CIMS (2-methylpropane), m/e (rel inten) 197 (M^+ + H, 82) 181 (base); EIHRMS, m/e 196.0883 (M^+, $C_{14}H_{12}O$ requires 196.0888).

Bergman Cyclization Kinetics for 6. Kinetic studies on the cyclization of **6** at 40, 60, and 80 °C were carried out in degassed C_6H_6 solutions at 0.01 M concentration of **6** in the presence of 1,4-cyclohexadiene (100 equiv). The reactions were followed by HPLC (SiO_2) with 1-cyanonaphthalene as internal standard. The HPLC analysis was started immediately after addition of 1,4-cyclohexadiene (90.1 μg, 94.6 μl, 1 mmol, 100 equiv) and 1-cyanonaphthalene (1.5 mg) to 1 mL of a 0.01 M solution of **6** (1.96 mg, 0.01 mmol) in degassed C_6H_6 with EtOAc–hexane (2:98) as eluent at a 1 mL/min flow rate. The retention time for the solvent (C_6H_6), internal standard, and **6** was 4.42, 9.75, and 18.96 min, respectively. The remaining percentage of **6** in the reaction mixture during the reaction process was obtained by recording and calculating the ratio of the area of **6** to internal standard periodically (every 10 h, 2 h, and 20 min for 40, 60, and 80 °C, respectively). Linear regression analysis (r > 0.996, 0.997, and 0.998 for 40, 60, and 80 °C, respectively) of the linear plots of Ln (**6**, %) versus time revealed the Bergman cyclization rate constants and $t_{1/2}$ at different temperatures. The energy of activation (E_a) for the reaction was determined by an Arrhenius plot (r > 0.999).

***N*-(*tert*-Butyloxycarbonyl)glycine Benzo[4,5]cyclodec-4-ene-2,6-diyn-1-yl Ester (7).** A solution of **6** (9 mg, 0.046 mmol) in CH_2Cl_2 (2 mL) was treated with *N*-BOC-glycine (16.1 mg, 0.092 mmol, 2 equiv), DCC (10.5 mg, 0.0506 mmol, 1.1 equiv), and DMAP (0.6 mg, 0.0046 mmol, 0.1 equiv) under Ar at 25 °C and the reaction mixture was stirred at 25 °C for 4 h. Filtration of the reaction mixture, removal of the solvent in vacuo, and purification of the residue by flash chromatography (SiO_2, 10% EtOAc–hexane) afforded **7** (15.7 mg, 16.2 mg theoretical, 97%) as a white, waxy solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.21–7.36 (m, 4 H), 5.63 (m, 1 H, C1-H), 5.00 (br s, 1 H, NH), 3.88–4.01 (m, 2 H, CH_2NHBOC), 2.45–2.49 (m, 2 H, C8-H_2), 2.28–2.35 (m, 1 H, C10 α-H or C10 β-H), 2.10–2.15 (m, 2 H, C9-H_2), 1.84–1.89 (m, 1 H, C10 α-H or C10 β-H), 1.45 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.0, 156.2, 130.4, 129.7, 129.2, 128.6, 128.1, 127.9, 100.4, 94.6, 87.0, 82.5, 80.5, 66.2, 42.8, 35.0, 28.5, 24.0, 21.4; IR (neat) ν_{max} 3441, 2940, 2876, 2204, 2165, 1646, 1509, 1451, 1370, 1264, 1164, 1043, 984, 737 cm^{-1}; EIMS, m/e (rel inten) 353 (M^+, 0.3), 57 (base); CIMS (2-methylpropane), m/e (rel inten) 354 (M^+ + H, 0.4), 120 (100); FABHRMS (NBA-CsI), m/e 486.0681 (M^+ + Cs, $C_{21}H_{23}NO_4$ requires 486.0681).

***N*-[3-[[3′-[[3″-Carbamoyl-1″,2″-dihydro-3″*H*-pyrrolo[3″,2″-*e*]indol-7″-yl]carbonyl]-1′,2′-dihydro-3′*H*-pyrrolo[3′,2′-*e*]indol-7″yl]carbonyl]-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carbonyl]glycine Benzo[4,5]cyclodec-4-ene-2,6-diyn-1-yl Ester (1a).** A solution of 3 N HCl–EtOAc (3 mL) was added to a cooled solution of **7** (51 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) under Ar at 0 °C. The mixture was stirred at 0 °C for 10 min and allowed to warm to 25 °C where it was stirred for an additional 20 min. The volatile materials were removed in vacuo and the white hydrochloride salt of **8** was taken up in DMF (3 mL). The

solution was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 41.5 mg, 0.22 mmol), DMAP (9 mg, 0.072 mmol), CDPI$_3$[12] (44 mg, 0.072 mmol), and Et$_3$N (22 mg, 0.22 mmol) at 0 °C under Ar and the resulting reaction mixture was stirred at 0 °C for 30 min and 25 °C for 48 h. The reaction suspension was concentrated in vacuo and the dry paste was washed with H_2O (4 × 5 mL), 10% aqueous HCl (4 × 5 mL), and H_2O (2 × 5 mL) and dried in vacuo. The brown solid was dissolved in DMF, SiO_2 (300 mg) was added to form a paste, and the DMF was removed in vacuo. The SiO_2 containing absorbed sample was placed on top of a flash chromatography column (SiO_2) and eluted with 0–5% DMF–THF to afford **1a** (26 mg, 61 mg theoretical, 43%) as a light yellow solid: mp > 240 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.77 (s, 2 H, NH), 11.56 (s, 1 H, NH), 9.02 (s, 1 H, NH), 8.29 (dd, 2 H, J = 9.0 Hz, C4′ and C4″-H), 7.96 (d, 1 H, J = 9.0 Hz, C4-H), 6.91–7.55 (m, 10 H), 6.19 (s, 2 H, $CONH_2$), 5.64 (m, 1 H, C1-H), 4.68 (t, 4 H, J = 7.8 Hz, C2′ and C2″-H_2), 4.05 (m, 2 H, glycine CH_2), 3.98 (t, 2 H, J = 8.0 Hz, C2-H_2), 3.20–3.60 (m, 6 H, C1, C1′ and C1″-H_2 partially obscured by H_2O), 2.10–2.50 (m, 5 H, C8, C9-H_2 and C10 α-H or C10 β-H), 1.75–1.85 (m, 1 H, C10 α-H or C10 β-H); IR (KBr) ν_{max} 3415, 2922, 2218, 2166, 1653, 1605, 1578, 1433, 1409, 1365, 1342, 1285, 1258, 1216, 1185, 1146, 801, 760 cm^{-1}; FABHRMS (DTT/DTE), m/e 849.3176 (M$^+$ + H, $C_{50}H_{40}N_8O_6$ requires 849.3149).

Benzo[4,5]cyclodec-4-ene-2,6-diyn-1-one (9). A solution of **6** (9 mg, 0.041 mmol) in CH_2Cl_2 (1 mL) was treated with activated MnO_2 (35.5 mg, 0.41 mmol) at 0 °C and the reaction mixture was warmed to 25 °C and stirred for an additional 1 h. Filtration of the reaction mixture through Celite and removal of solvent in vacuo followed by purification of the residue by flash chromatography (SiO_2, 5% EtOAc–hexane) afforded **9** (6.4 mg, 7.9 mg theoretical, 81%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.25–7.58 (m, 4 H), 2.89 (t, 2 H, J = 7 Hz, C8-H_2), 2.60 (t, 2 H, J = 7.0 Hz, C10-H_2), 2.19 (p, 2 H, J = 7.0 Hz, C9-H_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 188.6, 132.0, 131.4, 131.4, 129.0, 128.2, 126.5, 101.0, 95.5, 85.5, 82.4, 46.7, 25.7, 22.2; IR (neat) ν_{max} 2978, 2866, 2232, 2092, 1646, 1598, 1448, 1381, 765 cm^{-1}; EIMS, m/e 194 (M$^+$, base); CIMS (2-methylpropane), m/e 195 (M$^+$ + H, base); EIHRMS, m/e 194.0728 (M$^+$, $C_{14}H_{10}O$ requires 194.0732).

1-Hydroxy-1,2,3,4-tetrahydroanthracene (12). A solution of **6** (13.8 mg, 0.07 mmol) in 1,4-cyclohexadiene (2 mL) was warmed at reflux (82 °C) for 10 h. Removal of the solvent in vacuo and purification of the residue by flash chromatography (SiO_2, 10% EtOAc–hexane) afforded **12** (8.7 mg, 13.9 mg theoretical, 63%, 60–73%) as a colorless, waxy solid: mp 87–88 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.94 (s, 1 H, C9-H), 7.72–7.84 (m, 2 H, C6 and C7-H), 7.63 (s, 1 H, C10-H), 7.38–7.46 (m, 2 H, C5 and C8-H), 5.00 (br s, 1 H, C1-H), 2.92–3.06 (m, 2 H, C4-H_2), 1.80–2.18 (m, 5 H, C2-H_2, C3-H_2 and OH); ^{13}C NMR ($CDCl_3$, 50 MHz), δ 138.6, 135.8, 133.5, 132.7, 128.2, 127.5, 127.5, 127.4, 126.5, 125.7, 69.2, 32.8, 29.7, 19.4; IR (neat) ν_{max} 3443, 3054, 2986, 1598, 1495, 1423, 1265, 895, 740 cm^{-1}; EIMS, m/e 198 (M$^+$, base); CIMS (2-methylpropane), m/e (rel inten) 199 (M$^+$ + H, 3), 181 (base); FABHRMS (NBA), m/e 198.1050 (M$^+$, $C_{14}H_{14}O$ requires 198.1045).

3,4-Dihydro-1(2*H*)-anthracenone (13). Method A: a solution of **9** (4 mg, 0.02 mmol) in 1,4-cyclohexadiene (0.5 mL) was warmed at reflux for 4 h. Removal of the solvent in vacuo and purification of the residue by flash chromatography (SiO_2, 5% EtOAc–hexane) afforded **13** (3.7 mg, 4.0 mg theoretical, 92%) as white solid: mp 94–96 °C, lit.[52] mp 95–96 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.62 (s, 1 H, C10-H), 7.99 (d, 1 H, J = 6.0 Hz, C9-H), 7.78 (d, 1 H, J = 6.0 Hz, C6-H), 7.67 (s, 1 H, C5-H), 7.39–7.60 (m, 2 H, C7 and C8-H), 3.12 (t, 2 H, J = 7.0 Hz, C4-H_2), 2.84 (t, 2 H, J = 7.0 Hz, C2-H_2), 2.18 (p, 2 H, J = 7.0 Hz, C3-H_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 199.5, 139.8, 136.4, 132.2, 131.2, 130.5, 129.3, 129.1, 127.6, 127.2, 126.5, 40.0, 30.3, 23.5; IR (neat) ν_{max} 3051, 2942, 2860, 1684, 1554, 1498, 1449, 895, 742 cm^{-1}; EIMS, m/e 196 (M$^+$, base); CIMS (2-methylpropane), m/e (rel inten) 197 (M$^+$ + H, base); FABHRMS (NBA), m/e 197.0966 (M$^+$ + H, $C_{14}H_{12}O$ requires 197.0966).

Method B: a solution of **12** (5.0 mg, 0.025 mmol) in CH_2Cl_2 (1 mL) at 0 °C was treated with activated MnO_2 (22 mg, 0.25 mmol) and the resulting mixture was warmed to 25 °C and stirred for 2 h. Filtration of the reaction mixture through Celite, removal of the solvent in vacuo, and purification of the residue by flash chromatography (SiO_2, 5% EtOAc–hexane) afforded **13** (4.6 mg, 5.0 mg theoretical, 93%) as a white solid identical in all respects with the Bergman cyclization product of **9** (method A).

Triethyl Ethylenediaminetetraacetate. This intermediate was prepared according to the procedure by Hay:[44] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.15 (q, 2 H, J = 9 Hz, $COCH_2$), 4.13 (q, 4 H, J = 9 Hz, CO_2CH_2), 3.53 (s, 4 H, NCH_2CO), 3.48 (s, 2 H, NCH_2CO), 3.46 (s, 2 H, NCH_2CO), 2.84 (s, 4 H, NCH_2CH_2N), 1.24 (t, 3 H, J = 9 Hz, CH_3), 1.23 (t, 6 H, J = 9 Hz, CH_3); IR (neat) ν_{max} 3854, 3822, 3802, 3712, 3690, 3650, 2984, 1734, 1670, 1654, 1636, 1558, 1540, 1522, 1508, 1398, 1208, 1022 cm^{-1}.

***N*-Hydroxysuccinimide Ester of Triethyl Ethylenediaminetetraacetate (10).** A solution of triethyl ethylenediaminetetraacetate (178 mg, 0.47 mmol) in THF (4 mL) was treated with *N*-hydroxysuccinimide (55 mg, 0.47 mmol) and DCC (104 mg, 0.47 mmol) at 0 °C under Ar. The resulting solution was stirred for 6 h at 0 °C and filtered. The filtrate was concentrated and purified by flash chromatography (SiO_2, 30–50% EtOAc–hexane gradient elution) to afford **10** (195 mg, 224 mg theoretical, 88%) as a colorless, waxy solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.14 (q, 2 H, J = 9 Hz, CO_2CH_2), 4.12 (q, 4 H, J = 9 Hz, $COCH_2$), 4.03 (s, 2 H, NCH_2CO), 3.60 (s, 2 H, NCH_2CO), 3.57 (s, 4 H, NCH_2CO), 2.90 (s, 4 H, NCH_2CH_2N), 2.81 (s, 4 H, $OCCH_2CH_2CO$), 1.25 (t, 3 H, J = 9 Hz, CH_3), 1.24 (t, 6 H, J = 9 Hz, CH_3).

Triethyl CDPI$_3$-EDTA (11). A solution of **10** (32 mg, 0.067 mmol) in DMF (1.5 mL) was treated with DMAP (8 mg, 0.067 mmol) and CDPI$_3$-hydrazide[5] (28 mg, 0.045 mmol) and the reaction mixture was stirred for 24 h at 25 °C under Ar. The solvent was removed under vacuum and the residual solid was washed with H_2O (3 mL × 2), a mixture of THF–CH_2Cl_2–MeOH (1:1:1, 3 mL × 2), and dried in vacuo. The yellow solid was dissolved in DMF (1.5 mL), SiO_2 (200 mg) was added to form a paste, and the DMF was removed in vacuo. The SiO_2 containing absorbed sample was placed on top of a flash chromatography column (SiO_2) and eluted with 4–15% MeOH–CH_2Cl_2 gradient elution to afford **11** (32 mg, 44 mg theoretical, 73%) as a pale yellow solid: mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.81 (s, 1 H, NH), 11.79 (s, 1 H, NH), 11.59 (s, 1 H, NH), 10.37 (s, 1 H, NH), 9.88 (s, 1 H, NH), 8.28 (d, 2 H, J = 8 Hz, C4′-H and C4-H), 7.99 (d, 1 H, J = 8.8 Hz, C4″-H), 7.38 (d, 1 H, J = 8.1 Hz, C5′-H), 7.35 (d, 1 H, J = 8.1 Hz, C5-H), 7.25 (d, 1 H, J = 8.1 Hz, C5″-H), 7.20 (s, 1 H, C8′-H), 7.13 (s, 1 H, C8-H), 6.99 (s, 1 H, C8″-H), 6.13 (s, 2 H, $CONH_2$), 4.69 (m, 4 H, C2″-H_2 and C2′-H_2), 4.07 (m, 8 H, C2-H_2 and CO_2CH_2), 3.58 (s, 2 H, NCH_2CO), 3.50 (s, 2 H, NCH_2CO), 3.40–3.70 (m, 6 H, C1″-H_2, C1′-H_2 and C1-H_2, partially obscured by H_2O), 3.34 (s, 4 H, NCH_2CO), 2.84 (s, 4 H, NCH_2CH_2N), 1.23 (t, 3 H, J = 9 Hz, CH_3), 1.18 (t, 6 H, J = 9 Hz, CH_3); IR (KBr) ν_{max} 3854, 3840, 3802, 3752, 3736, 3690, 3676, 3650, 3408, 2926, 1745, 1740, 1654, 1578, 1560, 1542, 1508, 1426, 1364, 1342, 1284, 1204, 1146, 1026, 804, 759 cm^{-1}; HRFABMS (NBA), m/e 1008.4029 (M$^+$ + Na, $C_{50}H_{55}N_{11}O_{11}$ requires 1008.3980).

CDPI$_3$-EDTA (1b). A solution of **11** (15 mg, 0.015 mmol) in THF–MeOH–H_2O (3:2:1, 1.5 mL) was treated with LiOH–H_2O (19 mg, 0.45 mmol) and the reaction mixture was stirred for 48 h at 60 °C. The solvent was removed in vacuo, and the residual solid was suspended in H_2O (2 mL) and acidified to pH 4 with the addition of 10% aqueous HCl. The solid was collected by centrifugation, washed with H_2O (2 mL × 3) and a mixture of CH_2Cl_2–THF–MeOH (1:1:1, 2 mL × 3), and dried in vacuo to afford **11** (10.4 mg, 13.7 mg theoretical, 76%) as a dark green solid: mp > 230 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.81 (s, 1 H, NH), 11.79 (s, 1 H, NH), 11.59 (s, 1 H, NH), 10.37 (s, 1 H, NH), 9.86 (s, 1 H, NH), 8.28 (br s, 2 H, C4′-H and C4-H), 7.99 (d, 1 H, J = 8.8 Hz, C4″-H), 7.38 (t, 2 H, J = 8.1 Hz, C5′-H and C5-H), 7.25 (d, 1 H, J = 8.1 Hz, C5″-H), 7.21 (s, 1 H, C8′-H), 7.13 (s, 1 H, C8-H), 6.99 (s, 1 H, C8″-H), 6.13 (s, 2 H, $CONH_2$), 4.68 (m, 4 H, C2″-H_2 and C2′-H_2), 4.00 (t, 2 H, J = 7.8 Hz, C2-H_2), 3.40–3.55 (m, 14 H, C1″-H_2, C1′-H_2, C1-H_2 and NCH_2CO, partially obscured by H_2O), 2.86 (s, 4 H, NCH_2CH_2N); IR (KBr) ν_{max} 3884, 3802, 3752, 3740, 3690, 3650, 3420, 2926, 1710, 1642, 1578, 1562, 1542, 1436, 1366, 1024, 996, 828, 768 cm^{-1}; HRFABMS (NBA), m/e 902.3211 (M$^+$ + H, $C_{44}H_{43}N_{11}O_{11}$ requires 902.3222).

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DNA Cleavage Studies for 1a, 6, 7, and 9. Solutions containing 1.0 μg of supercoiled φX174 DNA (1.4×10^{-8} M) in 23 μL TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6) were treated with 1 μL of a DMSO solution of the agents **1a**, **6**, **7**, and **9** at the 25× specified concentration. Consequently, the actual agent concentration in the incubation mixture was that specified in the gel legends. The solutions were incubated at 40 °C for 30 h. Electrophoresis of the resulting DNA was conducted on a 0.7% agarose gel containing 0.1 μg/mL ethidium bromide with loading buffer (pH 7.9) at 50 V, 2.5 h. The electrophoresis gels were immediately visualized on a UV transilluminator and photographed using black and white instant film (Polaroid T667).

DNA Cleavage Studies for CDPI$_3$-EDTA (1b). Efficiency of cleavage: all reactions were run with freshly prepared **1b**-Fe(II) complexes. The **1b**-Fe(II) complexes were prepared by combining 1 μL of a DMSO solution of **1b** at the 10× specified concentration with 1 μL of a freshly prepared equimolar aqueous ferrous ammonium sulfate solution followed by vortex mixing. Each of the **1b**-Fe(II) complex solutions was treated with 7 μL of a buffered DNA solution prepared by diluting 1 μg of supercoiled φX174 DNA (1.4×10^{-8} M) with 27 μL of a 50 mM Tris-HCl buffer solution (pH 8). The thoroughly mixed incubation solutions were placed in a 37 °C water bath for 2 h. The DNA cleavage reactions were initiated by adding 1 μL of an aqueous 10 mM 2-mercaptoethanol solution. The solutions, which have the concentrations specified on the gel legands, were thoroughly mixed by vortexing and incubated at 37 °C for 30 min. Electrophoresis was conducted on a 1% agarose gel containing 0.1 μg/mL ethidium bromide with loading buffer (pH 7.9) at 50 V for 2.5 h and the gel was immediately visualized on a UV transilluminator and photographed using black and white instant film (polaroid T667).

Kinetic DNA Cleavage Studies: CDPI$_3$-EDTA (1b). Quantification of double-stranded and single-stranded cleavage: the **1b**-Fe(II) complexes were formed by mixing 1 μL of a 500 μM solution of **1b** in DMSO with 1 μL of a freshly prepared, 500 μM aqueous ferrous ammonium sulfate solution. A volume of 7 μL of a buffered DNA solution, which was prepared by adding 1 μg of supercoiled φX174 DNA (1.4×10^{-8} M) to 27 μL of a 50 mM Tris-HCl buffer solution (pH 8), was added to each of the **1b**-Fe(II) complex solutions. The solutions were incubated in a 37 °C water bath for 2 h. The DNA cleavage reactions were initiated by adding 1 μL of an aqueous 10 mM 2-mercaptoethanol solution to each of the reaction mixtures. The solutions, which have the same 50 μM final concentrations, were thoroughly mixed and incubated at 37 °C for 40, 30, 20, 18, 15, 12, 10, 8, 6, 4, 2, and 1 min, respectively. The reactions were quenched with the addition of 7 μL of loading buffer (pH 7.9) formed by mixing Keller buffer (0.4 M Tris-HCl, 0.05 M NaOAc, 0.0125 M EDTA, pH 7.9) with glycerol (40%), sodium dodecyl sulfate (0.4%), and bromophenol blue (0.3%), and electrophoresis was run on a 1% agarose gel containing 0.1 μg/mL ethidium bromide at 50 V for 2.5 h. The gel was immediately visualized on a UV transilluminator and photographed using black and white instant film (Polaroid T667). Direct fluorescence quantification of the DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 605 RFLP System visualized on a UV (312 nm) transilluminator. The ratio of the double-strand to single-strand cuts was calculated by the Freifelder–Trumbo equation.[46]

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Supplementary Material Available: ^{1}H NMR spectra of **1a,b**, **2–7**, **9–13** and photographs of the gels from which the data for Tables II–IV were taken (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.