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Synthesis of Isochrysohermidin−Distamycin Hybrids

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The synthesis of the alkylating subunit of the DNA cross-linking agent, isochrysohermidin (**2**), and its subsequent incorporation into conjugates with distamycin A (**1**) are described. The DNA binding properties of these agents were compared to that of distamycin A, using a fluorescence intercalator displacement (FID) assay.

Introduction

Gene expression is regulated by a host of inhibitor and enhancer proteins that selectively bind to specific sequences of DNA. The selective disruption of this process by small molecules, which bind to DNA in a sequence-specific manner, may provide access to new therapeutics. Among this class of agents, distamycin A is one of the most widely studied. Distamycin A, originally isolated from *Streptomyces sp.*,[1] is a minor groove binding agent with sequence specificity toward A−T rich sites within duplex DNA. Its sequence specificity and high affinity is derived from a combination of interactions including hydrogen bonding, van der Waals contacts, and electrostatic interactions of the cationic amidine side chain with the phosphate backbone of DNA.[2] The more recent discovery of 2:1 complexes,[3] their elaboration into side-by-side antiparallel γ-hairpin polyamides, and the advent of Dervan's pairing rules with the template modifications to selectively recognize G (Im vs Py) or A (Hp vs Py) provides a powerful paradigm on which to design sequence-selective DNA binding agents.[4] A number of studies have examined the consequences of incorporating nonselective alkylating agents into the distamycin structure (e.g. α-haloacylamides, nitrogen mustards).[5] A more limited series of studies have examined conjugates with selective alkylating agents (e.g. CBI,[6] duocarmycin A[7]) that combine the noncovalent binding selectivity inherent in the distamycin conjugate with the alkylation selectivity to further enhance binding selectivity and affinity.

In recent studies, we described the total synthesis of isochrysohermidin (**2**) and disclosed the first report of its interstrand DNA cross-linking properties.[8] Isolated from *Mercurialis perennis* L., both *d,l*- and *meso*-forms were found to occur naturally with the *d,l*-diastereomer unambiguously identified by X-ray crystallography.[9] The dimeric *N*-methylcarbinolamides undergo a slow ring-opening event during the interconversion of *d,l*- and *meso*-**2**. This ring-opening reaction exposes an electrophilic carbonyl capable of trapping nucleophiles within the minor groove of duplex DNA. The only nucleophile readily accessible to minor groove bound isochrysohermidin is believed to be the C2 amine of guanine. By incorporation of a single carbinolamide subunit of isochrysohermidin into distamycin, we sought to establish whether it may be possible to direct a reversible (vs irreversible)[5−7] guanine alkylation near adjacent A−T rich sites within duplex DNA (Figure 1).

Results and Discussion

The distamycin analogues were prepared by solution-phase synthesis requiring only acid/base liquid−liquid extraction protocols for the isolation and purification of the distamycin subunits. The amidine side chain found in the natural product was replaced with a *N,N*-dimethylaminopropylamine side chain to facilitate the ease of synthesis.[10] This substitution is well-documented and

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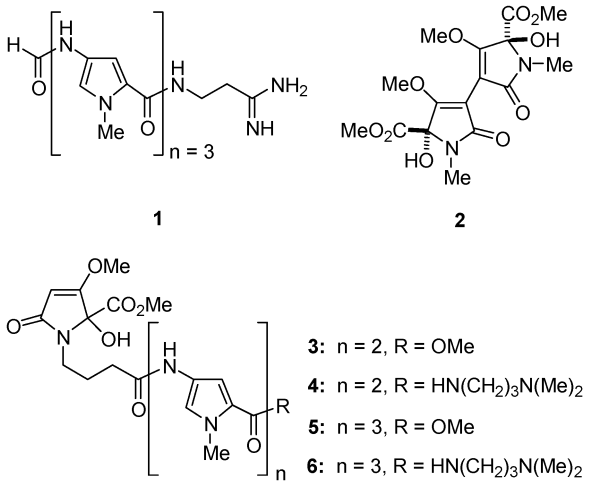
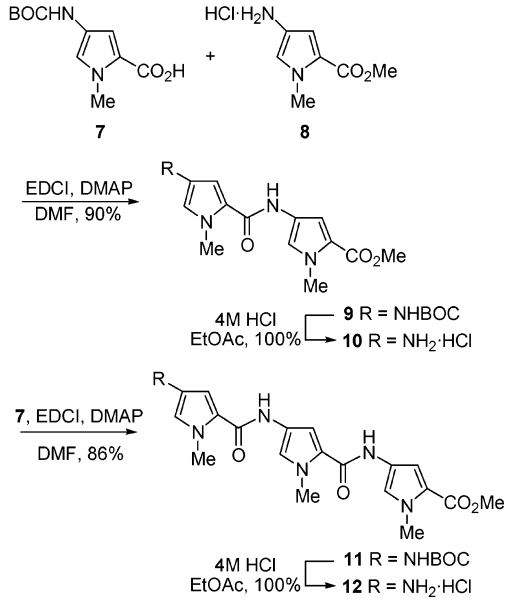


FIGURE 1. Distamycin A (**1**), isochrysohermidin (**2**), and hybrid agents (**3**−**6**).

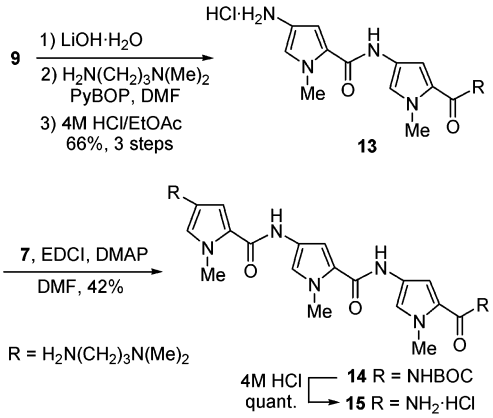
SCHEME 1



does not adversely affect minor groove binding affinity or selectivity. The distamycin subunits **10**, **12**, **13**, and **15** were prepared as previously detailed[10] by coupling **7** with **8** in the presence of EDCI and DMAP to afford **9** in 90% yield (Scheme 1). Subsequent treatment of **9** with anhydrous 4 M HCl/EtOAc followed by coupling with **7**, in the presence of EDCI/DMAP, provided the distamycin core **11** in 86% yield as detailed in our prior efforts. Subsequent treatment of peptides **9** and **11** with 4 M HCl/EtOAc removed the BOC group and provided the corresponding HCl salt of the methyl ester derivatives of the distamycin subunits (**10** and **12**).

Distamycin subunits **13** and **15**, incorporating the *N*,*N*-dimethylaminopropylamine side chain, were accessed from **9** as previously described.[10] Saponification with LiOH was followed by the addition of *N*,*N*-dimethylaminopropylamine and PyBOP to afford the corresponding adduct. Subsequent treatment with 4 M HCl/EtOAc provided **13** in 66% yield over three steps (Scheme 2). The tripeptide **14** was prepared by coupling dipeptide **13**

SCHEME 2



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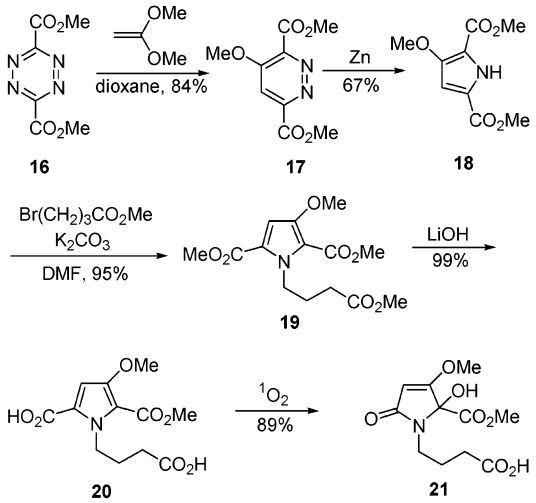

with **7**, in the presence of EDCI and DMAP, and provided the corresponding tripeptide 42% yield. Treatment of **14** with 4 M HCl/EtOAc removed the BOC group and provided the corresponding HCl salt of the distamycin subunit **15** in quantitative yield.

The trisubstituted pyrrole precursor to the isochrysohermidin alkylation subunit was prepared utilizing a 1,2,4,5-tetrazine → 1,2-diazine → pyrrole Diels−Alder strategy (Scheme 3).[11] An inverse electron demand Diels−Alder reaction of 1,2,4,5-tetrazine **16**[12] and dimethoxyketene acetal[13] provided the corresponding 1,2-diazine **17**[14] in 84% yield. A subsequent reductive ring contraction of the 1,2-diazine was achieved by using freshly activated zinc dust in glacial acetic acid to provide the trisubstituted pyrrole **18**[14] in 67% yield. The four-carbon tether was installed by *N*-alkylation of **18** with methyl

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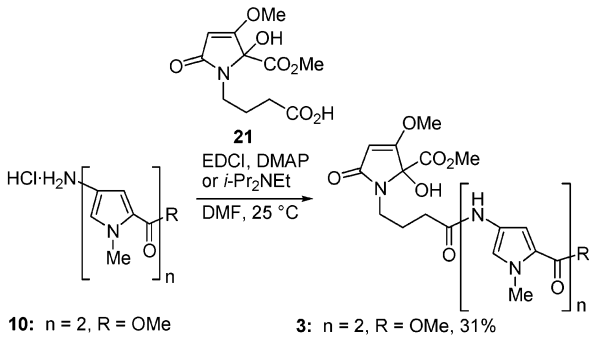
SCHEME 4



4-bromobutyrate (K_2CO_3, DMF) to furnish **19** in 95% yield. Highly selective saponification of the two sterically and electronically more accessible methyl esters provided the diacid **20** (99%). Initially, more elaborate, selective protections were anticipated to be necessary to cleanly provide **20** (e.g. use of benzyl 4-bromobutyrate). However, such selective protections/deprotections proved unnecessary and simple treatment of **19** with 2 equiv of LiOH at 25 °C provided **20** in superb conversions (99%). In the final step, a [4+2] cycloaddition of $^1O_2$ across the pyrrole followed by a low-temperature oxidative decarboxylation with fragmentation of the intermediate endoperoxide afforded the isochrysohermidin subunit **21** in 89% yield.[14c] In initial efforts, the $^1O_2$ was generated photochemically in the presence of the photosensitizer, Rose Bengal. However, since the photosensitizer was difficult to completely remove from the reaction mixtures, a resin-bound form of Rose Bengal was used, which was found to effect the desired transformation without any decrease in reactivity or product yields.[15]

With the isochrysohermidin subunit **21** in hand, the hybrid conjugates were prepared by coupling with the distamycin substructures incorporating either two or three *N*-methyl pyrrole subunits. Accessing isochrysohermidin−dipyrrole analogue **3** was achieved by treatment of **10** with **21** in the presence of EDCI/DMAP to provide **3** in 31% yield. Similarly, treatment of dipyrrole **13** followed by addition of **21** in the presence of EDCI/DMAP provided **4** in 32% yield after purification. The tripyrrole conjugates **5** and **6** were prepared from the corresponding tripyrroles **12** and **15**, respectively. The tripyrrole conjugate **5** was prepared from coupling **12** and **21** in the presence of EDCI and *i*-Pr_2NEt to provide **5** in 37% yield after purification by column chromatography. Similarly, the tripyrrole conjugate incorporating the *N*,*N*-dimethylpropylamine tail (**6**) was obtained from EDCI and *i*-Pr_2NEt mediated coupling of **15** and **21** to provide **6** in 32% yield (Scheme 4).

DNA Binding Affinity. The DNA binding properties of compounds **3**−**6** were first established by using a fluorescence intercalator displacement (FID) assay titration to establish a binding constant (*K*).[16] This method is based on the loss of fluorescence derived from the titration displacement of ethidium bromide from a DNA of interest. The agents were examined for their ability to bind a hairpin deoxyoligonucleotide containing a central five base pair AT-rich binding site (AAAAA) adjacent to capping GC base pairs relative to distamycin A and results are summarized in Table 1.

TABLE 1. DNA Binding Affinity of 3−6 Compared to Distamycin and Its Derivatives

Agent	Poly-d[A]–poly-d[T] K ($\times 10^6$ M^{-1})	5′-CGAAAAAC A A A 3′-GCTTTTTG A A A K ($\times 10^6$ M^{-1})
distamycin A	15.0	17.0
22	15.9	15.5
23	2.1	6.4
3	--	6.3
4	--	5.9
5	--	8.9
6	--	13.8

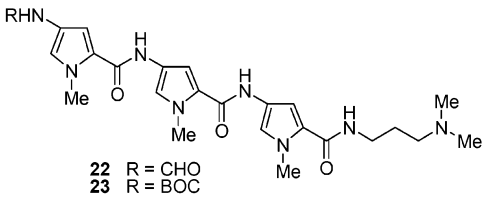


FIGURE 2. Distamycin analogues utilized in the FID assay.

Additionally, the binding affinities of several other distamycin derivatives (**22** and **23**, Figure 2) are included for comparison. Distamycin A binds to poly-d[A]-poly-d[T] and the 5′-AAAAA-3′ hairpin deoxyoligonucleotide with essentially the same affinity. Moreover, replacement of the amidine side chain on distamycin with *N*,*N*-dimethylpropylamine (**22**) simplifies the synthesis and does not adversely affect binding affinity. By contrast, substitution at the *N*-terminus of distamycin analogues has more of an impact on DNA binding affinity. Replacement of the *N*-formyl group with a sterically bulky BOC group with **23** lowers the binding affinity and suggests that large substituents at the *N*-terminus are not as well accommodated in the minor groove. Dipyrrole hybrids **3** and **4** show a 3-fold decrease in binding affinity for the hairpin deoxyoligonucleotide. These derivatives possess one less pyrrole subunit than distamycin and are therefore expected to be less effective noncovalent DNA binding agents. Interestingly, both **3** and **4** exhibited an affinity greater than expected and there is essentially no difference in binding affinity between **3** and **4** potentially representative of a DNA alkylation event. Tripyrrole derivative **5** lacks the C-terminal basic side chain and has half the binding affinity of the natural product. However, by incorporating three *N*-methylpyrrole subunits as well as the basic side chain into the hybrid **6**, it is possible to obtain a binding affinity close to that of distamycin and its closest analogue **22**. Disappointingly, **3**−**6** exhibited no time-dependent increase in binding affinity indicative of a slow, reversible covalent attachment to DNA. Thus, although the surprisingly effective

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behavior of **5** might suggest a covalent attachment to DNA, the behavior of **6** relative to **22** along with the lack of time-dependent binding affinity (data not shown) suggests it is not observed. Although it is possible that the covalent attachment is rapidly reversible, the intrinsic stability of the carbinolamide of isochrysohermidin ($t_{1/2}$ ca. 24−48 h, DMSO) suggests that is also unlikely. Thus, although we do not yet have a good explanation for the surprising behavior of **3**−**5**, we are confident that it is not derived from a stable, slowly reversible covalent attachment to DNA.[17]

Experimental Section

Dimethyl 3-Methoxy-1-[(3-methoxycarbonyl)propyl]-1*H*-pyrrole-2,5-dicarboxylate (19). Methyl 4-bromobutyrate (309 μL, 2.44 mmol) was added to a solution of **18**[14] (281 mg, 1.32 mmol) and K_2CO_3 (455 mg, 3.29 mmol) in anhydrous DMF (20 mL). The reaction mixture was warmed at 80 °C and stirred under N_2. After 4 h, the reaction mixture was cooled to 25 °C, poured into H_2O (100 mL), and extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under vacuum. Chromatography (SiO_2, 50% EtOAc−hexanes) afforded **19** (399 mg, 96%) as a white solid: mp 74−75 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.52 (1H, s), 4.81 (2H, t, J = 7.3 Hz), 3.85 (3H, s), 3.82 (6H, s), 3.64 (3H, s), 2.31 (2H, t, J = 7.6 Hz), 2.04 (2H, quint, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 161.1, 160.6, 152.5, 124.1, 112.8, 101.0, 57.9, 51.7, 51.5, 51.4, 45.4, 31.0, 26.7; MALDI-HRFTMS m/z 336.1056 (M + Na^+, $C_{14}H_{19}NO_7$ requires 336.1054).

1-(4-Butyric acid)-4-methoxy-5-methoxycarbonyl-1*H*-pyrrole-2-carboxylic Acid (20). LiOH·H_2O (117 mg, 2.80 mmol) was added to a stirred solution of **19** (399 mg, 1.27 mmol) in a 2:1:1 solution of THF:MeOH:H_2O (8 mL). After 20 h, the mixture was partitioned between Et_2O and H_2O. The aqueous layer was acidified with the addition of 5% aqueous HCl (pH 3.0) and extracted with EtOAc (4 × 20 mL). The combined EtOAc layers were dried (Na_2SO_4), filtered, and concentrated under vacuum to afford diacid **20** (358 mg, 99%) as a white solid: mp 193−195 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.61 (1H, s), 4.79 (2H, t, J = 7.3 Hz), 3.81 (3H, s), 3.79 (3H, s), 2.25 (2H, t, J = 7.4 Hz), 2.04 (2H, quint, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.7, 163.1, 162.8, 154.2, 126.3, 113.8, 102.5, 58.3, 51.6, 46.3, 31.9, 28.1; MALDI-HRFTMS m/z 308.0744 (M + Na^+, $C_{12}H_{15}NO_7$ requires 308.0741).

Isochrysohermidin Subunit 21. A 3:1 solution of CH_3CN−H_2O (40 mL) was added to a quartz flask charged with **20** (23 mg, 0.08 mmol) and Rose Bengal resin (7.0 mg, 0.0006 mmol).[18] The solution was irradiated under a Hanovia high-pressure mercury lamp (450 W) through a uranium yellow glass filter (transmits <330 nm) with a steady stream of O_2 bubbled through the solution. After 3 h, a small amount of activated charcoal was added to remove any solubilized Rose Bengal and the solution filtered through Celite and rinsed with MeOH. The solvent was concentrated under vacuum to afford **21** (21.8 mg, 86% yield) as a transparent glass: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.14 (1H, s), 4.62 (1H, s), 3.79 (3H, s), 3.77 (3H, s), 3.68 (1H, dt, J = 14.4, 6.6 Hz), 3.08 (1H, dt, J = 14.5, 6.6 Hz), 2.32 (2H, t, J = 7.2 Hz), 1.79 (2H, quint, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 170.9, 170.8, 167.3, 94.8, 63.6, 59.0, 53.4, 40.2, 25.2, 23.6; MALDI-HRFTMS m/z 296.0728 (M + Na^+, $C_{11}H_{15}NO_7$ requires 296.0741).

Compound 3. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (33 mg, 0.17 mmol) and DMAP (11 mg, 0.08 mmol) were added to a mixture of **21** (12 mg, 0.04 mmol) and **10** (29 mg, 0.08 mmol) in anhydrous DMF (0.5 mL) and the reaction mixture was stirred at 0 °C under N_2. After 16 h, the reaction mixture was diluted with 1:1 *i*-PrOH−$CHCl_3$ (10 mL) and washed with 10% aqueous $NaHCO_3$ (2 × 20 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated under vacuum. Chromatography (SiO_2, 15:1 $CHCl_3$–MeOH) afforded **3** (3.3 mg, 31%) as an off-white syrup: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.84 (1H, br s), 7.73 (1H, s), 7.41 (1H, s), 7.15 (1H, s), 6.77 (1H, s), 6.67 (1H s), 5.06 (1H, s), 3.90 (3H, s), 3.89 (3H, s), 3.85 (3H, s), 3.82 (3H, s), 3.81 (3H, s), 3.57 (1H), 2.36 (2H, t, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.6, 172.6, 165.0, 163.2, 161.5, 124.6, 123.9, 123.4, 122.6, 121.0, 110.5, 106.1, 94.2, 59.8, 54.1, 51.6, 39.4, 37.1, 37.0, 34.7, 26.5; MALDI-HRFTMS m/z 554.1862 (M + Na^+, $C_{24}H_{29}N_5O_9Na$ requires 554.1857).

Compound 4. EDCI (29 mg, 0.151 mmol) was added to a mixture of **21** (10 mg, 0.038 mmol), **13** (29 mg, 0.076 mmol), and DMAP (18 mg, 0.151 mmol) in anhydrous DMF (0.3 mL) and the reaction mixture was stirred at 25 °C under N_2. After 24 h, the reaction mixture was diluted with 1:1 *i*-PrOH−$CHCl_3$ (10 mL) and washed with H_2O (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated under vacuum. Chromatography (RPC_{18}-PTLC, 4:1 MeOH−50 mM HCO_2NH_4 buffer) afforded **4** (7 mg, 32%) as a clear syrup: ^{1}H NMR (2:1 CD_3OD−CH_2Cl_2, 500 MHz) δ 8.44 (1H, s), 7.15 (1H, d, J = 1.8 Hz), 7.11 (1H, d, J = 1.8 Hz), 6.87 (1H, d, J = 1.8 Hz), 6.83 (1H, d, J = 1.8 Hz), 5.18 (1H, s), 3.88 (3H, s), 3.87 (3H, s), 3.85 (3H, s), 3.78 (3H, s), 3.40 (4H, m), 3.20 (3H, m), 2.88, (6H, s), 2.32 (2H, t, J = 7.7 Hz), 1.97 (3H, m); ^{13}C NMR (2:1 CD_3OD−CH_2Cl_2, 125 MHz) δ 177.0, 176.9, 172.5, 159.6, 126.9, 123.3, 120.8, 120.5, 106.5, 105.9, 99.8, 94.0, 59.7, 56.6, 55.5, 43.7, 39.3, 36.6, 34.5, 30.7, 27.4, 27.3, 26.6, 26.3; MALDI-HRFTMS m/z 602.2937 (M + H^+, $C_{28}H_{39}N_7O_8$ requires 602.2933).

Compound 5. EDCI (35 mg, 0.18 mmol) was added to a mixture of **21** (12 mg, 0.04 mmol), **12** (39 mg, 0.09 mmol), and *i*-Pr_2NEt (16 μL, 0.09 mmol) in anhydrous DMF (0.5 mL). The reaction mixture was stirred under N_2 at 0 °C for 3 h and allowed to warm to 25 °C. After 18 h, the reaction mixture was diluted with 1:1 *i*-PrOH−$CHCl_3$ (10 mL) and washed with H_2O (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated under vacuum. Chromatography (SiO_2, 12:1 $CHCl_3$−MeOH) afforded **5** (11 mg, 37%) as an off-white syrup: ^{1}H NMR (1:1 CD_3OD−CD_2Cl_2, 500 MHz) δ 7.68 (1H, s), 7.34 (1H, d, J = 2.2 Hz), 7.18 (1H, d, J = 2.2 Hz), 7.11 (1H, d, J = 1.8 Hz), 6.91 (1H, d, J = 2.2 Hz), 6.87 (1H, d, J = 2.2 Hz), 6.80 (1H, d, J = 1.8 Hz), 5.12 (1H, s), 3.89 (3H, s), 3.88 (3H, s), 3.87 (3H, s), 3.84 (3H, s), 3.78 (3H,s), 3.77 (3H, s), 3.45 (1H, dt, J = 14.5, 7.0 Hz), 3.16 (1H, dt, J = 14.5, 7.0 Hz), 2.32 (2H, t, J = 7.5 Hz), 1.86 (2H, m); ^{13}C NMR (1:1, CD_3OD−CD_2Cl_2, 125 MHz) δ 174.7, 173.3, 172.0, 169.1, 162.7, 160.8, 124.2, 124.1, 123.2, 122.8, 122.6, 122.0, 120.5, 120.3, 120.1, 109.8, 105.8, 105.3, 93.7, 88.6, 78.8, 59.4, 51.4, 38.9, 36.9, 36.7, 34.1, 30.3, 26.0; MALDI-HRFTMS m/z 653.2459 (M^+, $C_{30}H_{35}N_7O_{10}$ requires 653.2440).

Compound 6. EDCI (31 mg, 0.180 mmol) was added to a mixture of **21** (11 mg, 0.04 mmol), **15** (39 mg, 0.08 mmol), and *i*-Pr_2NEt (14 μL, 0.090 mmol) in anhydrous DMF (0.5 mL). The reaction mixture was stirred under N_2 at 0 °C for 3 h and allowed to warm to 25 °C. After 24 h, the reaction mixture was diluted with 1:1 *i*-PrOH−$CHCl_3$ (10 mL) and washed with 10% aqueous $NaHCO_3$ (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated under vacuum. Chromatography (RPC_{18}-PTLC, 6:1 MeOH−50 mM HCO_2NH_4 buffer) afforded **4** (7 mg, 32%) as a clear syrup: ^{1}H NMR (2:1 CD_3OD−CD_2Cl_2, 500 MHz) δ 7.17 (1H, br s), 7.09 (1H, s), 6.92 (1H, s), 6.86 (1H, s), 6.91 (1H, br s), 6.82 (1H, s), 6.79 (1H, s), 5.09 (1H, s), 3.90 (3H, s), 3.88 (12H, br s), 3.39 (4H, br t, J = 6.3 Hz), 3.13 (2H, m), 3.05 (2H, br t, J = 7.7 Hz), 2.80 (6H, br s), 2.31 (2H, m), 1.96 (2H, m), 1.83 (2H, m), 0.877 (2H, br t, J = 6.6 Hz); ^{13}C NMR (1:1, CD_3OD−CD_2Cl_2, 125 MHz) δ 173.0, 170.3, 169.4, 168.1, 167.0, 164.9, 161.4, 124.6, 123.8, 123.4,

(17) Cytotoxic activity: L1210 IC_{50} = 67 (**1**), 119 (**3**), 165 (**4**), >200 (**5**), and >200 μM (**6**).

(18) Concentration of resin-bound Rose Bengal was determined according to ref 15 (0.09 mol of Rose Bengal/g of support).

123.3, 120.9, 120.7, 120.5, 106.5, 106.4, 105.9, 56.7, 47.4, 47.3, 43.8, 41.1, 37.1, 37.0, 36.7, 34.4, 30.7, 27.4, 27.3, 26.6, 25.3, 23.7, 23.1; MALDI-HRFTMS *m/z* 723.3337 ($M + H^+$, $C_{34}H_{45}N_9O_9$ requires 723.3340).

Determination of DNA Binding Constants. A 3-mL quartz cuvette was loaded with Tris buffer (0.1 M Tris, 0.1 M NaCl, pH 8) and ethidium bromide (0.44×10^{-5} M final concentration). The fluorescence was measured (excitation 545 nm, emission 595 nm, EtBr) and normalized to 0% relative fluorescence. The 5′-AAAAA-3′ hairpin deoxyoligonucleotide was added (1.5 μM, 12 μM in base pair final concentration), and the fluorescence measured again and normalized to 100% relative fluorescence. A solution of the agent (3 μL, 0.1 mM in DMSO) was added, and the fluorescence measured following 5 min of incubation at 23 °C. Subsequent addition of 3-μL aliquots of the agent was continued until the system reached saturation and the fluorescence remained constant with successive compound additions.

Scatchard Analysis of the Titration Curve. The ΔF was plotted versus molar equivalents of agent and the ΔF_{sat} was determined mathematically by solving the simultaneous equations representing the pre- and postsaturation regions of the titration curve. Utilizing eqs 1−3, a Scatchard plot was generated where ΔF/[free agent] was plotted vs ΔF. The slope of the region immediately preceding complete saturation of the system provided $-K$.[16]

$$\left(\frac{\Delta F_x}{\Delta F_{sat}}\right)\frac{1}{X} = \text{fraction of DNA} - \text{agent complex} \quad (1)$$

$$\left[1 - \left(\frac{\Delta F_x}{\Delta F_{sat}}\right)\frac{1}{X}\right] = \text{fraction of free agent} \quad (2)$$

$$[\text{DNA}]_T\left[X - \frac{\Delta F_x}{\Delta F_{sat}}\right] = [\text{free agent}] \quad (3)$$

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Supporting Information Available: ^{1}H NMR of all new compounds **3**−**6** and **19**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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