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Synthesis and Reactions with DNA of a Family of DNA-DNA Affinity Cross-Linking Agents

Snorri Th. Sigurdsson and Paul B. Hopkins*

Department of Chemistry, University of Washington, Seattle, Washington 98195

Abstract: A family of DNA-DNA cross-linking agents has been synthesized, in which 2,3-bis-(hydroxymethyl)-pyrrole is linked to the oligopeptide distamycin. These substances were efficient, sequence selective, DNA-DNA interstrand and intrastrand cross-linking agents. Substance **1a** formed interstrand and intrastrand cross-links at the sequences 5'- d(CGAATT) and 5'- d(GGAATT), respectively. The lesions from hydrolysis of the phosphodiester backbones of inter- and intrastrand cross-linked DNA were identical, and possessed the structure **20**. Substance **1a** was 1000-fold more active as a cross-linking agent than 2,3-bis-(hydroxymethyl)-1-methylpyrrole (**19**). The cytotoxicity of **2a** was comparable to cis-DDP.

INTRODUCTION

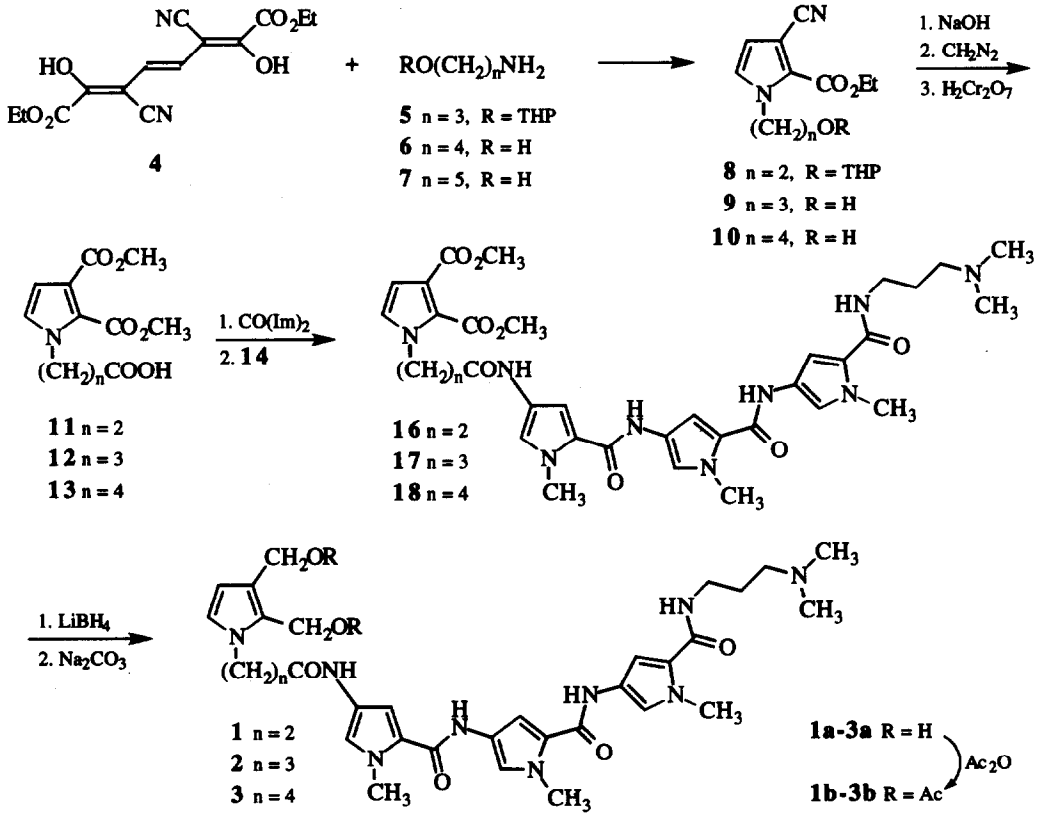
Bifunctional alkylating agents figure prominently among the anticancer drugs, including substances such as mitomycin C, cisplatin, the nitrosoureas and the mustards. It is widely accepted that DNA is the target of these alkylation reactions, and that double alkylation, possibly in the form of interstrand cross-links, is particularly critical in their biological action. Because the impact of genomic damage is no doubt a function of the precise location at which damage occurs, and chemical reactions of DNA can occur with considerable sequence selectivity, it is natural to ask whether the antitumor activity of the bifunctional alkylating agents is in any way related to the sequences of DNA at which these substances form bifunctional lesions. The answer seems likely to be no, because for those compounds which have been studied, it appears that the reactive sequences are far too short to impart appreciable selectivity within any genome. For example, mitomycin C forms interstrand and intrastrand cross-links which bridge deoxyguanosine at the dinucleotide sequences 5'-d(CG) and 5'-d(GG), respectively.[1] While the reactivity of these sequences is modestly modulated by the flanking bases, this increases the recognized sequences to only the tetranucleotide level. The probable high occurrence of these short sequences in the genome make it doubtful that this limited selectivity is the basis for the therapeutic value of these compounds as antitumor agents. The therapeutic utility of the bifunctional alkylating agents would be greatly enhanced if they could be more precisely targeted to defined genomic sites.

The targeting of drugs to a limited number of or even unique genomic sites has been the subject of extensive research.[2] In the case of targeted cross-linking agents, two approaches have been explored. In one, monofunctional alkylating agents capable of substantial DNA sequence recognition have been converted to bifunctional agents, most commonly by linking a pair of agents to one another by a bridging tether. This approach has been used with analogs of the pyrrolo[1,4]benzodiazepines (e.g. anthramycins) and CC-1065.[3] Alternatively, substances capable of non-covalent sequence recognition have been tethered to cross-linking agents of limited or low sequence specificity to create affinity cross-linking agents. These include oligopeptide-psoralen[4] and oligopeptide-chlorambucil[5] conjugates, both of which function by a triplex mechanism. The oligopeptide distamycin has previously been linked to a nitrogen mustard[6] and to a nitrosourea.[7]

We report herein the design, synthesis, and evaluation as targeted cross-linking agents of the family of distamycin-pyrrole conjugates **1-3**.[8,9] We show that these substances cross-link a plasmid with a ca. 1000-fold improvement in efficiency relative to substances which lack the distamycin moiety. Intra- and interstrand cross-links were formed in oligonucleotide duplexes at nucleotide sequences which link an AT-rich sequence, recognized by distamycin, with sequences prone to cross-linking by the pyrrole.

RESULTS AND DISCUSSION

Design. In the course of evaluating the origins of the sequence specificity with which reductively activated mitomycins and oxidatively activated pyrrolizidine alkaloids cross-link DNA, we have previously studied the reactivity toward DNA of 2,3-bis(acetoxymethyl)-1-methylpyrrole. Like mitomycin C and the pyrrolizidines, the simple pyrrole was found to be a DNA interstrand cross-linking agent and to express some selectivity for dG-to-dG cross-linking at 5'-d(CG), probably by linking the exocyclic N2 amino groups in the minor groove, but with very low efficiency (less than 1%).[10] We reasoned that this low efficiency relative to mitomycin C was likely the result of a very low non-covalent affinity of the pyrrole for the minor groove of DNA. Accordingly, the pyrrole

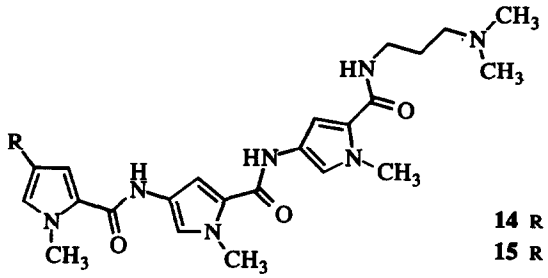


Scheme 1. Syntheses of cross-linking agents **1-3**.

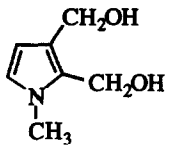
seemed well suited for delivery to DNA by a tethered auxiliary which independently recognizes DNA sequence information. We selected the minor groove binding agent distamycin with the goal of delivering the pyrrole to what we believed to be its preferred target, the minor groove amino groups of dG. This stands in contrast to the case of previously reported distamycin-mustard conjugates, which employ a cross-linking agent which prefers the major groove (N7 of dG), and are thus inherently "mismatched". The structural details of the pyrrole cross-linking reaction itself (i.e. the relevant transition states) remain largely unknown. To avoid the possiblity that the pyrrole would be constrained by the DNA-distamycin interaction to a conformation not conducive to cross-linking, we varied the tether lengths as illustrated in **1-3**.

Syntheses. The syntheses of conjugates **1-3** were achieved by coupling of **14**, a primary amine-containing analog of distamycin c, to **11-13**, carboxylic acid-bearing substituted pyrroles, followed by a series of functional group interchanges. The requisite pyrroles were prepared by the method of Huisgen and Laschtuvka, by condensation of triene **4** with primary amines **5-7**, to afford pyrroles **8-10**.[11] Sequential hydrolysis of the nitrile and ester functionalities, bisesterification of the carboxylic acids with diazomethane, and oxidation to the acid with Jones reagent afforded the pyrrolic acid diesters **11-13**, which were in turn converted to the corresponding imidazolides by treatment with 1,1'-carbonyldiimidazole.[12]

Amine **14** was prepared by hydrogenation of **15**,[13] and was coupled to the imidazolides of **11-13** to afford conjugates **16-18**. Reduction of the ester functions of **16-18** with $LiBH_4$ afforded compounds whose chromatographic and spectroscopic properties were consistent with complexation of the terminal, tertiary amine with borane. Treatment of these putative complexes with sodium carbonate in refluxing methanol afforded compounds **1a-3a**.[14] As described below, these diols were initially acetylated in situ to the corresponding diacetates (**1b-3b**). However, because the diols themselves were found to possess comparable DNA cross-linking activity, the acetylation step was subsequently omitted.



14 R = NH_2
15 R = NO_2



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Interstrand Cross-Linking of a Linearized Plasmid. To determine the efficiency with which one of these conjugates introduced interstrand cross-links into duplex DNA, we utilized the plasmid pBluescript II KS(-). The plasmid was linearized by digestion with EcoR1, then treated with varying concentrations of conjugate **1a**. Inspection of the nucleotide sequence of this linear duplex reveals 27 occurrences of a potential distamycin binding site, a run of 4 or more A or T residues, adjacent to the preferred site of interstrand cross-linking by the pyrrole unit, 5'-d(CG). The method of Hartley, which relies upon the ability of interstrand cross-linked DNA to renature anomalously rapidly, and thus be recovered as duplex rather than single strands following a denaturation

step, was employed to measure cross-linking.[15] At pH 5.0, it was found that **1a** was roughly 1000-fold more effective than the analog **19** which lacks the distamycin moiety. For example, ca. 30% cross-linking was observed at 10 nM concentration of **1a**, while a 10 μM concentration of **19** was required to achieve comparable cross-linking.[8]

Optimization of Cross-Linking Conditions. To set the stage for the study of conjugates **1-3** in synthetic DNA duplexes, a panel of four oligodeoxyribonucleotide duplexes was designed and synthesized (Table 1, **A-D**). These DNAs each contained a run of 4 or 5 contiguous dA residues intended to bind the distamycin analog. Adjacent to or separated by one intervening residue were 5'-d(CG) sequences at both the 5'- and 3'-ends which might be cross-linked through dG residues on opposite strands.

Table 1. Duplex DNAs studied herein.

A	5'CG**CG**AAAA**CG**CG GC**GC**TTTT**GC**GC	**C**	5'CG**CG**AAAAA**CG**CG GC**GC**TTTTT**GC**GC
B	5'G**CG**C**AAAA**G**CG**C C**GC**G**TTTT**C**GC**G	**D**	5'G**CG**C**AAAAA**G**CG**C C**GC**G**TTTTT**C**GC**G

We judged it impractical to study each of DNAs **A-D** separately with compounds **1-3** under a variety of experimental conditions. Instead, substance **2b** was arbitrarily selected for evaluation of the impact of DNA:agent ratio, pH, time, and temperature on interstrand cross-linking yield. Furthermore, DNAs **A-D** were assayed as a ca. 1:1:1:1 mixture. This mixture of ^{32}P-radiolabeled DNAs was incubated at pH 5 with the diacetate **2b** under the conditions shown in Table 2. The DNA was precipitated and analyzed by denaturing polyacrylamide gel electrophoresis (DPAGE) followed by autoradiography. Interstrand cross-linked DNAs migrate at roughly half the rate of the single strands, and are thus easily quantified. For the case of the admixed DNAs **A-D**, 4 bands differing slightly in electrophoretic mobility were observed for the starting DNA. At least 5 low mobility interstrand cross-linked products were detected, and it is the total of these that is summarized in Table 2. Analogous reactions conducted at pH 7 (data not shown) gave analogous results, but the extent of cross-linking was much lower. All subsequent experiments were thus conducted at pH 5.0.

As shown in Table 2, there was no increase in interstrand cross-link yield when the agent:DNA ratio exceeded 5:1, suggesting that under these conditions the distamycin binding sites were saturated. This is consistent with a binding constant for the conjugate to DNA of ca 10^8-10^9 M^{-1}.[16] Both prolonged time and temperature elevation above ambient enhanced the yield of interstrand cross-links. Consistent with the results which follow, reactions in which extensive interstrand cross-linking was observed did not return an equimolar mixture of single strand **A-D**. Instead, two of these were selectively consumed; their identities were not determined.

Influence of Tether Length on Interstrand Cross-Link Yield. Based upon the preliminary study of **2b** in admixed **A-D**, we studied conjugates **1b-3b** separately with each of DNAs **A-D** at pH 5.0, 25°C, agent:DNA duplex ratio of 5:1 (8 μm duplex DNA), for 18.5 h. Analysis was again by DPAGE, with the yield of interstrand cross-links quantified by phosphorimagery.

Table 2. Relative efficiency of DNA interstrand cross-linking using **2b** at pH 5.0 and the admixed DNAs in Table 1.

agent:DNA ratio	Time (h)	Temperature (°C)	Relative yield of cross-linked DNA
1:1	1	25	+
5:1	1	25	++
10:1	1	25	++
50:1	1	25	++
5:1	0.17	25	+
5:1	13	25	++++
5:1	1	4	+
5:1	1	37	+++

The DNA **A** containing the sequence 5'-d(CGAAAACG) was most efficiently interstrand cross-linked, to a low mobility product which migrated as predominantly a single band, in 73-81% yield. This yield is far superior to that for comparable reactions of many simple cross-linking agents (nitrogen mustard, nitrous acid, formaldehyde),[17] being instead comparable to those found with the best cross-linkers (psoralen, mitomycin C).[18] The results were similar for DNA **C**, bearing the central sequence 5'-d(CGAAAAACG), but the yield was reduced to ca. 50%. In contrast, the yields of interstrand cross-linked DNA were much lower for the DNAs **B** and **D** containing a 5'-d(GC) sequence adjacent to an oligopeptide binding site, dropping below 10% except for the case of conjugate **2b**, where DNAs **B** and **D** afforded a ca. 40% yield of interstrand cross-linked DNA. We speculate that the site of cross-linking is the 5'-d(CG) sequence, with the propylene tether leaping over the intervening base pair. Why conjugate **3b** is incapable of the same remains unknown. Perhaps this tether is too long, or geometrically reorients the reactive functional groups unproductively.

Our past experience with the solvolysis of acetoxymethylpyrroles suggested that the half life of these substances in water near neutral pH is on the order of minutes. Given that the observed cross-linking reactions were occurring on the time scale of hours (Table 2), it seemed reasonable that diols **1a-3a** might be intermediates in this process. Diols **1a-3a** were incubated separately with each of the DNAs **A-D**. The yields of interstrand cross-linked DNA were only ca. 10% lower than what was observed for the corresponding diacetates when the reaction was allowed to proceed for more than 12 h. Accordingly, all subsequent experiments were conducted without the acetylation step. Furthermore, all three of conjugates **1a-3a** gave appreciable yields of interstrand cross-linked DNA. For this reason, we chose only one of the three for further study. Conjugate **1a** was arbitrarily selected.

Sequence Selectivity. To further define the sequence selectivity of compound **1a**, the panel of DNA duplexes illustrated in Table 3 was prepared. In these DNAs, both the non-covalent binding site and the covalent bonding sites were varied.[8] These DNAs were incubated with conjugate **1a** under the same conditions described above (1:5 DNA duplex:conjugate ratio, pH 5.0, 50 mM sodium acetate buffer, 25 °C, 100 mM NaCl, 5 mM $MgCl_2$, 13 h) and the products analyzed by DPAGE and phosphorimagery (Figure 1).

Consistent with the findings from DNAs **A-D**, DNA **H** in which the distamycin binding site abuts the 5'-d(CG) site favored for pyrrole interstrand cross-linking, was by a large margin the most efficiently interstrand

Table 3. Self-complementary synthetic oligodeoxyribonucleotides used for the evaluation of the sequence selectivity of compound **1a**.

E	5'GATCC**AATT**GGATC CTAGG**TTAA**CCTAG	**I**	5'GATCI**AATT**CGATC CTAGC**TTAA**ICTAG
F	5'GATGG**AATT**CCATC CTACC**TTAA**GGTAG	**J**	5'GATCG**AATT**CIATC CTAIC**TTAA**GCTAG
G	5'GATGC**AATT**GCATC CTACG**TTAA**CGTAG	**K**	5'GAT**CG**A**CG**T**CG**ATC CTA**GC**T**GC**A**GC**TAG
H	5'GAT**CG**AATTC**G**ATC CTA**GC**TTAA**GC**TAG	**L**	5'GAT**CGCGCGCG**ATC CTA**GCGCGCGC**TAG

cross-linked of these DNAs. Substitution of deoxyinosine, which has the same structure as deoxyguanosine save the absence of the N2 amino group which has previously been shown to be critical for cross-linking by 2,3-bis-(acetoxymethyl)pyrroles,[10] for either deoxyguanosine residue at the sequence 5'-d(CG) as in DNAs **I** and **J** abolished the formation of interstrand cross-links. This result suggested that the cross-linking agent was likewise linking the deoxyguanosine residues at the sequence 5'-d(CG) through the exocyclic amino groups. As is found in the diacetate of the simple pyrrole **19**, the order of nucleotides at the 5'-d(CG) site was critical. DNA **G** which inverts these residues relative to **H**, thus containing a 5'-d(GC) site, was not efficiently interstrand cross-linked. The importance of the oligopeptide binding site was demonstrated by the inefficient crosslinking of DNAs **K** and

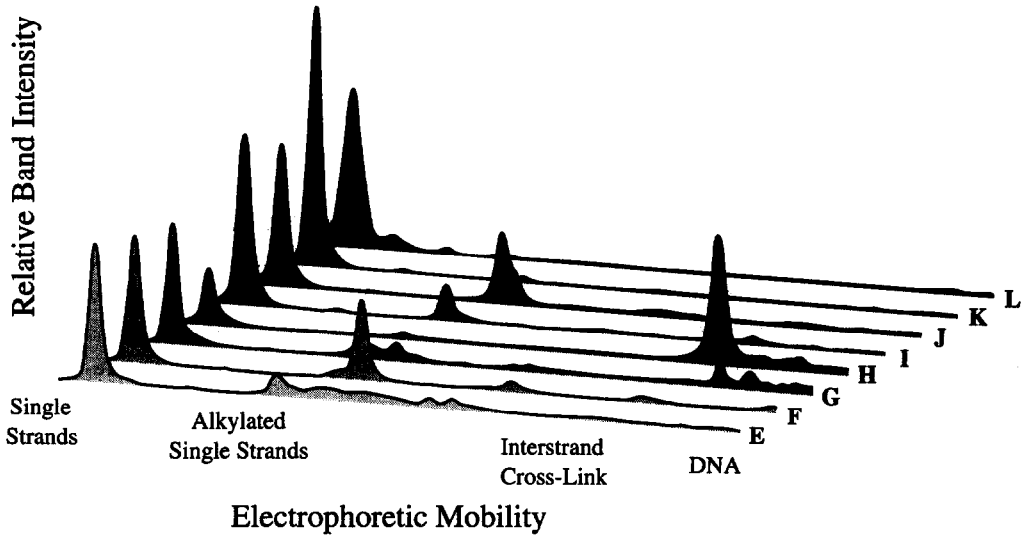


Figure 1. Reactions of conjugate **1a** with self-complementary, 5'-^{32}P-labeled synthetic oligodeoxyribonucleotides (Table 3). Electrophoretic mobility decreases to right. For purposes of clarity, single strand bands were aligned in this stacked plot; in reality these differ in mobility slightly from one another due to nucleotide composition differences. For purposes of comparison, the total area under each trace was normalized to a constant value.

L, in which two or all four of the central AT-pairs in **H** were changed to GC pairs, thus abolishing the distamycin binding site. Importantly, **K** and **L** were not efficiently cross-linked despite the presence of the 5'-d(CG) sequences which are the preferred interstrand cross-linking sites in the simple pyrrole. This is fully consistent with the very low efficiency with which the simple pyrroles create interstrand cross-links (less than 1%).[10]

Neither DNA **E** nor **F** in which a 5'-d(GG) sequence lies to the 3'- or 5'-side of the distamycin binding site, respectively, were interstrand cross-linked. Interestingly, however, DNA **F** did form in 33% yield a product intermediate in electrophoretic mobility between single strands and interstrand cross-links. Of the two reasonable candidate structures for this lesion, the conjugate bound through one or two covalent bonds to a single strand, studies described below demonstrated this product to be the latter.

Covalent Structure of 1a-Interstrand and Intrastrand Cross-Linked DNA. That deoxyguanosine was in fact the point of attachment between conjugate **1a** and DNA was demonstrated as follows. The duplex DNAs **F** and **H** were treated with compound **1a** and the major product was isolated from each reaction mixture using preparative DPAGE. The interstrand cross-linked DNA derived from DNA **H** was then subjected to enzymatic hydrolysis using snake venom phosphodiesterase (SVD) and calf intestinal alkaline phosphatase.[17a] RP-HPLC analysis suggested that this protocol left the DNA incompletely hydrolyzed even after prolonged periods of incubation. Increasing the amount of SVD and addition of the endonucleases DNase I and DNase II facilitated complete hydrolysis of the phosphodiester backbone of this interstrand cross-linked DNA. Analysis of the hydrolysates was performed using RP-HPLC with dual wavelength detection at 260 nm (primarily nucleotide absorbance) and 320 nm (absorbance by the pyrrole-oligopeptide conjugate only) (Figure 2). Quantitation of the released nucleotides of the interstrand cross-linked DNA (Figure 2, left) at 260 nm revealed the dC:dG:dT:dA molar ratio of 3.0:2.0:4.1:4.0 (calc'd 3.0:2.0:4.0:4.0 per single strand), two deoxyguanosine residues short (per duplex) of the original ratio of 3.0:3.0:4.0:4.0. The loss of two deoxyguanosine residues in the enzymatic digest of interstrand cross-linked DNA defined deoxyguanosines as the site of both covalent alkylations. In addition, there was a strongly retained peak which contained the long-wavelength chromophore of the oligopeptide moiety. This substance was collected and its positive ion electrospray ionization mass spectrum gave parent ions $M + H^+$, $M + Na^+$, and $M + K^+$ for M = m/e 1147, the mass expected for two deoxyguanosine residues plus one of pyrrole-oligopeptide conjugate **1a** less two moles of water. The fragmentation pattern was similar to that observed for other pyrrole-derived cross-link-lesions: fragment ions corresponding to loss of one and two sugar residues were observed as well as loss of one and two deoxyguanosine residues.[10] These data, along with the fact that substituting deoxyinosine for deoxyguanosine resulted in elimination of interstrand cross-link formation, are uniquely consistent with the formulation of the lesion as **20**.

The modified digestion conditions were also employed to hydrolyze the phosphodiester backbone of the most abundant product from DNA **F**. Quantitation of the released nucleotides in this case (Figure 2, right trace) at 260 nm revealed the dC:dG:dT:dA molar ratio of 2.8:1.1:3.9:4.0 (calc'd. 3.0:1.0:4.0:4.0), two deoxyguanosine residues short of the original ratio (per single strand) of 3.0:3.0:4.0:4.0. The loss of two deoxyguanosine residues in the enzymatic digest of alkylated single stranded DNA is again indicative of a cross-linked adduct between the adjacent deoxyguanosine residues at the sequence 5'-d(GGAATTCC). The strongly retained substance was collected and its positive ion electrospray ionization mass spectrum gave the same parent- and fragment ions as the lesion obtained from interstrand cross-linked DNA, suggesting that they both had structure **20**. The identity of **20**

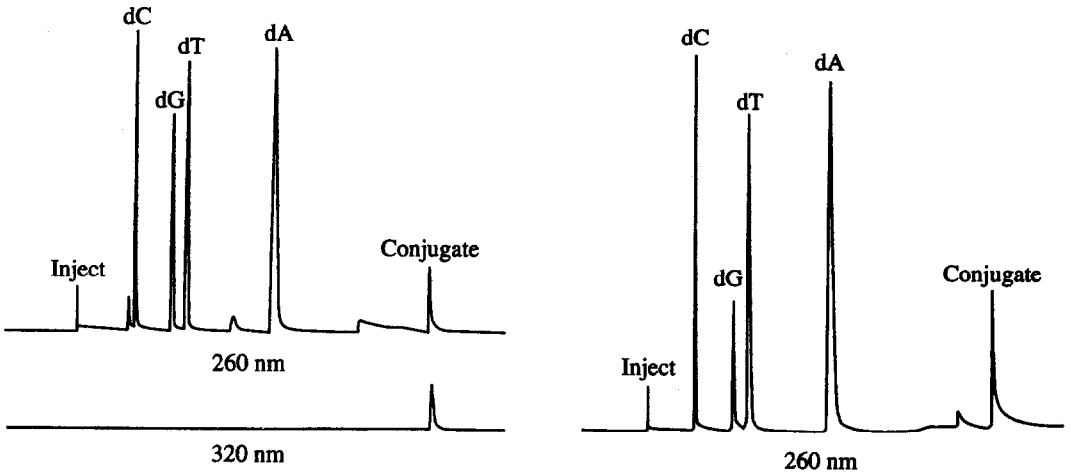
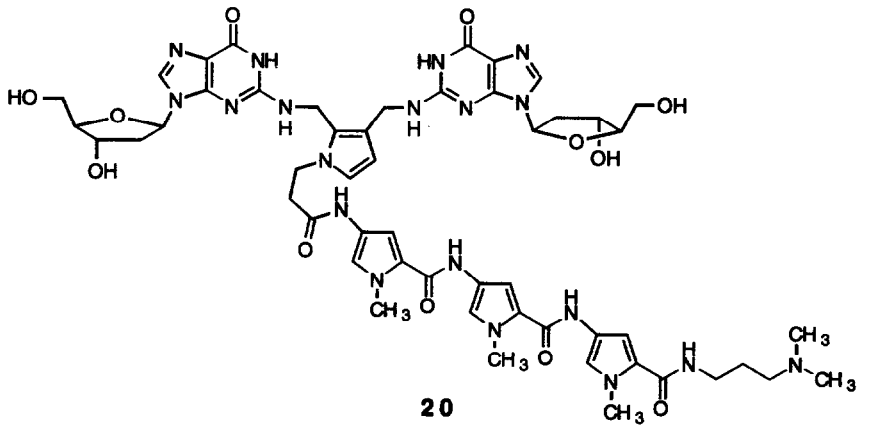


Figure 2. RP-HPLC analysis of hydrolyzed **1a**-inter- and intrastrand cross-linked DNA (left and right trace, respectively).

derived from **1a**-treated DNAs **H** and **F** was further confirmed by their coelution in RP-HPLC and the identity of their UV spectra in methanol.

In vitro toxicity of compound 2a. The conjugate **2a** was tested for toxicity in an L1210 cell line, and had an IC_{50} of 350 nM and IC_{90} of 910 nM. These values are comparable to those seen in this assay with cisplatin (IC_{50} 300 nM), but are 5 orders of magnitude higher than the corresponding values for another designed, targeted DNA cross-linking agent, bizelesin (IC_{50} 1 pM).[19] Conjugate **2a** is presently undergoing further evaluation as an antitumor and anti-AIDS candidate.



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CONCLUSIONS

We have developed rationally designed agents capable of efficiently interstrand or intrastrand cross-linking DNA with substantial nucleotide sequence selectivity. These agents couple an analog of distamycin, which binds non-covalently to the minor groove of duplex DNA at runs of four or more AT-pairs, to a bis-(hydroxymethyl)pyrrole which is capable of cross-linking the N2 amino groups of deoxyguanosine residues present at the sequence 5'-d(CG) (interstrand) or 5'-d(GG) (intrastrand). These studies were inspired by and the results find strong analogy in the work of Dervan and coworkers. Dervan et al. converted the inefficient iron(II) EDTA-catalyzed H_2O_2/ascorbic acid cleavage reaction of DNA into an efficient one by tethering the metal chelator to the DNA-binding element methidium.[20] In this way, the efficient DNA cutting action of the important antitumor substance bleomycin was mimicked. In our case, it is the action of reductively activated mitomycin C which is emulated: the simple pyrrole **19** shares some degree of interstrand DNA cross-linking sequence selectivity with reductively activated mitomycin C, presumably due to common structural elements.[10] However, like the inefficient cleavage of DNA by iron(II) EDTA, the simple pyrrole **19** is, on its own, a highly inefficient DNA cross-linking agent. Restoration of the DNA binding domain by tethering the pyrrole to distamycin causes the conjugates not only to rival mitomycin itself in interstrand cross-linking efficiency, but now reveals an *intrastrand* cross-linking reactivity of the pyrrole not previously observed, but recently documented for mitomycin C.[21] More direct analogy is found in the chemistry of distamycin-EDTA:Fe[22] and the haloacetyldistamcyins,[23] which efficiently target strand cleavage and alkylation reactions of DNA, respectively, to sequences bearing the distamycin binding site.

Our results stand in contrast to reports of both a distamycin-nitrosourea conjugate[7] and a distamycin-mustard conjugate.[6] In the latter case, the mustard *lacking* distamycin was comparably efficient as an interstrand cross-linking agent at a ten fold lower concentration than the conjugate! This result is easily understood, as at least the parent nitrogen mustard mechlorethamine cross-links major groove functional groups in duplex DNA. As such these conjugates are "mismatched". A "matched" pairing of the mustard chlorambucil with a homopyrimidine oligonucleotide which recognizes duplex DNA by a triplex mechanism has been reported.[5]

The finding that interstrand and intrastrand cross-linking reactions, which are believed to figure prominently in the action of therapeutically useful antitumor agents, can be sequence targeted as described here and elsewhere[4,5] raises hopes that this technique may find application in the development of highly specific therapeutic agents. Unfortunately, a fundamental limitation remains the lack of a general solution to the problem of designing agents which bind non-covalently to any DNA sequence of interest. These results highlight the urgency of that need, encouraging current efforts along these lines.

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EXPERIMENTAL

General. Materials and their sources were as previously described[17a] except as follows: DNase I, DNase II, Sigma. Gels were dried under vacuum on a Bio-Rad Model 583 Gel Dryer, at 80 °C, using cycle 2. Dried gels were autoradiographed on Amersham Hyperfilm-MP. RP-HPLC detection utilized sequential SSI 500 UV/VIS

(output to both an HP 3390A electronic integrator and a GOW-MAC model 70-150 recorder) and RAININ Dynamax model UV-1 (output to a Linear Model 255/mm recorder) detectors. The agarose used in gel electrophoresis was BRL Ultrapure Electrophoresis Grade Agarose. Agarose gels were run on a BRL Model H5 Horizontal System for Submerged Gel Electrophoresis (27 cm between electrodes). Solvent gradients for analytical separations and coinjections were run at 1 mL/min as follows: gradient A - solvent A, 100 mM ammonium acetate (pH 7.0); solvent B, acetonitrile; isocratic 94% A for 4 min, 11 min linear gradient to 70% A, 15 min linear gradient to 60 % A, 5 min linear gradient to initial conditions. Solvent gradients for preparative separations were run at 2 mL/min as follows: gradient B - solvent A, 10 mM ammonium acetate (pH 7.0); solvent B, acetonitrile; isocratic 94% A for 4 min, 11 min linear gradient to 70% A, 15 min linear gradient to 60 % A, 5 min linear gradient to initial conditions.

Air- or water-sensitive reactions were conducted under a positive pressure of argon. Commercial reagents were used as received, except for the following: acetonitrile, CH_2Cl_2, and pyridine were distilled under argon from calcium hydride; deuterated chloroform, DMF, DMSO, and dioxane were dried over 4 Å molecular sieves; diethyl ether and tetrahydrofuran were distilled under argon from sodium benzophenone ketyl; methanol was dried over 3 Å molecular sieves. Column chromatography was performed under slight positive pressure on Merck silica gel 60 (230-400 mesh); thin-layer chromatography was performed on precoated Merck silica gel 60 plates (0.25 mm). Infrared (IR) spectra were recorded as a thin film on a NaCl plate on a Perkin-Elmer Series 1600 FTIR spectrophotometer. Proton nuclear magnetic resonance spectra (^{1}H NMR) were determined on a Bruker AC200 (200 MHz) or a Bruker AMX500 (500 MHz) spectrometer and, unless otherwise noted, are reported in parts per million downfield from internal tetramethylsilane (0.00 ppm). Coupling constants (J) are given in Hertz. Low resolution mass spectra (LRMS) were measured on a KRATOS Profile HV3 mass spectrometer using direct sample insertion and electron impact (EI) ionization; high resolution FAB mass spectra (FAB-HRMS) and low resolution mass spectra (FAB-LRMS) were measured on a VG70SEQ double-focusing mass spectrometer. Electrospray mass spectra (ESMS) were recorded on a Sciex Atmospheric Pressure Ionization (API) triple quadrapole mass spectrometer. Ultraviolet (UV) spectra were measured on a Hewlett-Packard Model 8452A UV/VIS spectrophotometer and are reported as wavelength in nanometers.

Radiolabeling of DNA. DNA was ^{32}P-radiolabeled at the 5'-terminus using 0.1 OD_{260} units of DNA (4 nmole of base pairs) in 16 μL of water, 2 μL of 10X kinase buffer [resulting in a solution that was 50 mM Tris (pH 7.5), 10 mM $MgCl_2$, 0.1 mM spermidine, 5 mM DTT, 0.1 mM EDTA], 20 μCi, (2 μL) [γ-^{32}P] ATP and 10 units (1 μL) of T4 polynucleotide kinase at 37 °C for 30 min. Radiolabeling was stopped by addition of 2 μL of 3 M aqueous sodium acetate (pH 5.2), followed by 1.2 mL of cold ethanol (-20 °C). After 0.5 h at -20 °C, the resulting precipitate was collected by centrifugation and suspended in 90% EtOH (aq) at -20 °C. After 0.5 h at -20 °C, the precipitate was collected by centrifugation, dried, and used in the following experiments.

Analytical cross-linking reactions. One-fifth of the previously 5'-^{32}P-radiolabeled DNA (~0.02 OD_{260} units) was placed in a microfuge tube. To the tube was added 100 μL of a salt solution [33.3 mM $MgCl_2$, 667 mM NaCl] containing 1 OD_{260} unit of the unradiolabeled, complementary 14-mer DNA. The samples were heated to 90 °C and cooled to 25 °C over 3 h. To 15 μL of this solution were added 100 mM acetate buffer (50 μL, pH 5.0) (for experiments performed at pH 7.0, 100 mM MOPS was used) and H_2O (35 μL) [final concentrations: 8 mM duplex DNA, 100 mM NaCl, 5 mM $MgCl_2$, 50 mM acetate buffer]. This solution was

treated with 1-6 μL of **1b-3b** in CD_3CN [5 eq. agent per duplex or 40 μM in the final solution] at 25 °C. After vortexing, the mixture was allowed to stand the specified time and temperature (see text). The reaction was stopped by addition of 1.2 mL of cold ethanol (-20 °C). After 1 h at -20 °C, the resulting precipitate was collected by centrifugation and rinsed with 90% cold EtOH (aq) (1.2 mL) (-20 °C) and dried. Each sample was analyzed using 25% DPAGE.

DPAGE Analysis of Cross-link Yield. Pelleted DNA was dissolved in 10 μL of loading buffer [90% deionized formamide, 10 mM Tris (pH 7.5), 0.1% xylene cyanol, 0.1 mM EDTA]. The samples were subjected to 25% PAGE (19:1 acrylamide:bisacrylamide, 50% urea, 0.35 mm thick, 41 x 37 cm). Gels were prepared as follows: 23.75 g acrylamide, 1.25 g bisacrylamide, and 48 g urea were dissolved in 10 mL of 10 x TBE and 20 mL of water. The volume was brought to 100 mL with water and 350 μL of 20% aqueous ammonium persulfate was added prior to filtering through Whatman #5 filter paper. To the solution was added 25 μL of TEMED to induce polymerization, and the gel was poured and allowed to polymerize for 1 h. The gel was equilibrated on a Hoeffer thermojacketed Poker Face gel stand for 1 h at 45 °C. Samples were loaded using flat sequencing tips (Marsh Biomedical) on a Rainin P-200 Pipetman, and the loaded gel was run at 65 Watts with a Bio-Rad Model 3000 XI power supply until the xylene cyanol dye traveled 14-16 cm (ca. 4 h). The gel, covered on one side with Saran Wrap®, was transferred onto filter paper and dried for 1 h, and products were visualized by autoradiography or phosphorimagery.

Preparative Inter- and Intrastrand Cross-linking of DNA. DNAs containing the sequences 5'-d(CGAATTCG) or 5'-d(GGAATTCC) (Table 3) (10 OD_{260} units, 0.2 μmol duplex) were placed in separate microfuge tubes and independently treated as described here. To the tube was added 200 μL of a salt solution [33.3 mM $MgCl_2$, 667 mM NaCl], the sample was heated to 90° C, and cooled to 25 °C over 3 h. The resulting solution was divided into two equal portions placed in separate microfuge tubes. To each tube were added 100 mM acetate buffer (333 μL, pH 5.0) and H_2O (233 μL) [final concentrations: 8 μM duplex DNA, 100 mM NaCl, 5 mM $MgCl_2$, 50 mM acetate buffer]. This solution was treated with a solution of **1a** (0.042 μmol) in CH_3OH (20 μL) [2 eq agent per duplex] at 25 °C. After vortexing, the mixture was allowed to stand for 44 h (166 h for the DNA containing the sequence 5'-d(GGAATTCC)) at 25 °C after which the volume of the solution was reduced to ca. 100 μL in a vacuum centrifuge. and the reaction stopped by addition of 1 mL of cold ethanol (-20 °C). After 1 h at -20 °C, the resulting precipitate was collected by centrifugation, rinsed with 90% cold EtOH (aq) (-20 °C), and dried. This sample was purified using 25% DPAGE as described for the purification of DNA above, yielding 3.32 OD_{260} units of **1a**-*inter*strand cross-linked DNA and 1.68 OD_{260} units of **1a**-*intra*strand cross-linked DNA.

Quantitation of nucleosides in 1a-Interstrand Cross-Linked DNA. Cross-linked (0.1 OD_{260} unit) or the unmodified DNA containing the sequence 5'-d(CGAATTCG) (1 OD_{260} unit) in 82 μL of aqueous 10 mM $MgCl_2$, 50 mM Tris (pH 8.9) was treated with 1 μL (25 units) of DNase I, 2 μL (28.4 units) of DNase II, 5 μL (5 units) of alkaline phosphatase, and 5 μL (5 units) of phophodiesterase I at 37 °C for 38 h (1 h for unmodified DNA). RP-HPLC analysis was carried out on 30 μL of the above reaction mixtures using gradient A. Peaks were identified by comparison of retention times to those of authentic, commercial samples. Quantitation was based on the peak area ratios obtained from the RP-HPLC analysis of the hydrolysate of unmodified DNA which gave the following response factors: dC, 1.00 : dG, 1.83 : dT, 1.23 : dA, 2.11. Using these response

factors, the **1a**-interstrand cross-linked DNA was analyzed as 3.0(dC) : 2.0(dG) : 4.1(dT) : 4.0(dA) (calc'd: 3.0 : 2.0 : 4.0 : 4.0).

Isolation of 20 from 1a-Interstrand Cross-Linked DNA. Interstrand cross-linked DNA containing the sequence 5'-d(CGAATTCG) (4 OD_{260} units) was divided equally into each of four microfuge tubes and to each tube was added 82 μL of aqueous 10 mM $MgCl_2$, 50 mM Tris (pH 8.9). Each solution was treated with 1 μL (25 units) of DNase I, 2 μL (28.4 units) of DNase II, 5 μL (5 units) of alkaline phosphatase, and 5 μL (5 units) of phophodiesterase I at 37 °C for 36 h. An equal volume of DMSO was added to the aforementioned hydrolysates, the mixture was sonicated, and **20** was isolated by RP-HPLC using gradient B. The solvent was removed in vacuo and **20** was characterized as described below.

Quantitation of nucleosides in 1a-Interstrand Cross-Linked DNA and Isolation of 20. Cross-linked (2 x 1 OD_{260} unit) or unmodified DNA containing the sequence 5'-d(GGAATTCC) (1 OD_{260} unit) in 82 μL of aqueous 10 mM $MgCl_2$, 50 mM Tris (pH 8.9) (1 OD_{260} unit per microfuge tube) was treated with 1 μL (25 units) of DNase I, 2 μL (28.4 units) of DNase II, 5 μL (5 units) of alkaline phosphatase, and 5 μL (5 units) of phophodiesterase I at 37 °C for 23 h (2 h for unmodified DNA). RP-HPLC analysis was carried out on 10 μL of the above reaction mixtures using gradient A. Peaks were identified by comparison of retention times to those of authentic, commercial samples. Quantitation was based on the peak area ratios obtained from the RP-HPLC analysis of the hydrolysate of unmodified DNA which gave the following response factors: dC, 0.483 : dG, 0.872 : dT, 0.585 : dA, 1.00. Using these response factors, the **1a**-intrastrand cross-linked GGAATTCC was analyzed as 2.8(dC) : 1.1(dG) : 3.9(dT) : 4.0(dA) (calc'd: 3.0 : 1.0 : 4.0 : 4.0). An equal volume of DMSO was added to the aforementioned hydrolysate, the mixture was sonicated, and **20** was isolated by RP-HPLC using gradient B. After removing the solvent in vacuo, **20** was characterized as described below.

Comparison of 20 from 1-Interstrand and 1-Intrastrand Cross-Linked DNA. ESMS (methanol, inlet voltage 180 V, needle voltage 5000 V, 3 μL/min) m/e: **20** from interstrand cross-linked DNA: 1148, 1170, 1186 ($M+H^+$, Na^+ and K^+), 1032, 1054, 1070 (M-deoxyriboglycal+H^+, Na^+ and K^+), 916, 938, 954 (M-(2 x deoxyriboglycal)+H^+, Na^+ and K^+), 881 (M-deoxyguanosine+H+), 765, 787, 803 (M-deoxyriboglycal-deoxyguanosine+H^+, Na^+ and K^+), and 614, 636, 652 (M-(2 x deoxyguanosine)+H^+, Na^+ and K^+). **20** from intrastrand cross-linked DNA: 1148, 1170, 1186 ($M+H^+$, Na^+ and K^+), 1032, 1054, 1070 (M-deoxyriboglycal+H^+, Na^+ and K^+), 916, 938, 954 (M-(2 x deoxyriboglycal)+H^+, Na^+ and K^+), 881 (M-deoxyguanosine+H^+), 765, 787, 803 (M-deoxyriboglycal-deoxyguanosine+H^+, Na^+ and K^+), and 614, 636, 652 (M-(2 x deoxyguanosine)+H^+, Na^+ and K^+). UV (methanol): **20** from interstrand cross-linked DNA: λ_{max} 253 nm, 287 nm; **20** from intrastrand cross-linked DNA: λ_{max} 253 nm, 287 nm. **20** from inter- and intrastrand cross-linked DNA coeluted on HPLC using gradient A (26.4 min).

3-(2-Carboxethyl-3-cyano-1-pyrrolo)-O-tetrahydropyranyl-1-propanol (8). A stirred suspension of triene **4** (1.01 g, 3.5 mmol) in refluxing EtOAc (10 mL) was treated dropwise with 3-amino-O-(2-tetrahydropyranyl)-1-propanol (0.88 g, 5.5 mmol) in EtOAc (5 mL) over 0.5 h after which the mixture was refluxed for 1.2 h. The reaction mixture was cooled to 25 °C, the solvent removed in vacuo and the residue chromatographed on silica gel (CH_2Cl_2) to afford 0.56 g (56%) of **8** as a pale yellow oil: ^1H NMR (200 MHz,

$CDCl_3$): d 1.43 (3H, t, J=7, CH_3), 1.70 (6H, m, $CHCH_2CH_2CH_2$), 2.05 (2H, quin, J=6, $NCH_2CH_2CH_2$), 3.33 (1H, dt, J=10,6, NCH_2CH_2CHH'), 3.50 (1H, m, $CHOCHH'$), 3.72 (1H, dt, J=10,6, NCH_2CH_2CHH'), 3.82 (1H, m, $CHOCHH'$), 4.39 (2H, q, J=7, CH_2CH_3), 4.50 (3H, m, NCH_2 and $CHOCH_2$), 6.49 (1H, d, J=3, ArH), 6.87 (1H, d, J=3, ArH); HRMS (FAB, Thiogl.): observed: m/e 307.1655 ($M + H^+$); calc'd. for $C_{16}H_{23}N_2O_4$: 307.1658.

4-(2-Carboxethyl-3-cyano-1-pyrrolo)-1-butanol (9). A stirred suspension of triene **4** (4.40 g, 14.4 mmol) in refluxing EtOAc (30 mL) was treated dropwise with 4-amino-1-butanol (1.92 g, 21.6 mmol) in EtOAc (10 mL) over 1.25 h, after which the mixture was refluxed for 2 h. The reaction mixture was cooled to 25 °C, EtOAc (30 mL) and H_2O (20 mL) were added, and the organic phase separated. The organic phase was washed successively with 1 N HCl (10 mL), 10% aqueous NaOH (4 x 20 mL), brine (5 mL), and dried ($MgSO_4$) in the presence of activated charcoal. The solid was removed by filtration through Celite and the solvent was removed in vacuo and the resultant residue chromatographed on silica gel (2% $MeOH/CH_2Cl_2$) to afford 1.06 g (31%) of **9** as a pale yellow oil: ^1H NMR (200 MHz, $CDCl_3$): δ 1.44 (3H, t, J=7, CH_3), 1.58 (2H, m, CH_2), 1.89 (2H, m, CH_2), 3.70 (2H, q, J=6, CH_2OH), 4.38 (2H, t, J=7, NCH_2), 4.40 (2H, q, J=7, CH_2CH_3), 6.51 (1H, d, J=3, ArH), 6.84 (1H, d, J=3, ArH); HRMS (FAB, Thiogl.): observed: m/e 237.1239 ($M + H^+$); calc'd. for $C_{12}H_{17}N_2O_3$: 237.1239.

5-(2-Carboxethyl-3-cyano-1-pyrrolo)-1-pentanol (10). A stirred suspension of triene **4**[11] in refluxing EtOAc (30 mL) was treated dropwise with 5-amino-1-pentanol (2.22 g, 21.6 mmol) in EtOAc (10 mL) over 0.75 h, after which the mixture was refluxed for 2.6 h. The reaction mixture was cooled to 25 °C, EtOAc (30 mL) and H_2O (20 mL) was added, and the organic phase separated. The organic phase was washed successively with 1 N HCl (10 mL), 10% aqueous NaOH (4 x 20 mL), brine (5 mL), and dried ($MgSO_4$) in the presence of activated charcoal. The solid was removed by filtration through Celite, the solvent was removed in vacuo, and the residue chromatographed on silica gel (2% $MeOH/CH_2Cl_2$) to afford 1.10 g (30%) of **10** as a pale yellow oil: ^1H NMR (200 MHz, $CDCl_3$): δ 1.41 (2H, m, CH_2), 1.46 (3H, t, J=7, CH_3), 1.62 (2H, m, CH_2), 1.84 (2H, m, CH_2), 3.69 (2H, q, J=6, CH_2OH), 4.39 (4H, m, NCH_2 and CH_2CH_3), 6.52 (1H, d, J=3, ArH), 6.83 (1H, d, J=3, ArH); HRMS (FAB, Thiogl.): observed: m/e 251.1398 ($M + H^+$); calc'd. for $C_{13}H_{19}N_2O_3$: 251.1396.

3-(2,3-Dicarboxyl-1-pyrrolo)-O-(2-tetrahydropyranyl)-1-propanol. A stirred suspension of **8** (0.30 g, 0.97 mmol) in H_2O (3 mL) containing NaOH (0.55 g, 13.8 mmol) was refluxed for 5 h. The reaction mixture was cooled to 0 °C and stirred vigorously in the presence of CH_2Cl_2 (5 mL) while the aqueous layer was acidified (pH 2-3) by addition of 1 N HCl. The organic phase was separated and the aqueous phase was exhaustively extracted with CH_2Cl_2 (5 x 4 mL). The combined organic phases were then washed with brine and dried (Na_2SO_4). The solvent was removed in vacuo to afford 0.19 g (65%) of a pale yellow oil: ^1H NMR (200 MHz, $CDCl_3$): δ 1.70 (6H, m, $CHCH_2CH_2CH_2$), 2.12 (2H, quin, J=7, $NCH_2CH_2CH_2$), 3.37 (1H, m, NCH_2CH_2CHH'), 3.55 (1H, m, $CHOCHH'$), 3.74 (1H, m, NCH_2CH_2CHH'), 3.88 (1H, m, $CHOCHH'$), 4.62 (3H, m, NCH_2 and $CHOCH_2$), 6.78 (1H, d, J=3, ArH), 6.88 (1H, d, J=3, ArH).

4-(2,3-Dicarboxyl-1-pyrrolo)-1-butanol. A stirred suspension of **9** (0.90 g, 3.8 mmol) in refluxing H_2O (4 mL) was treated with a solution of NaOH (1.62 g, 40.5 mmol) in H_2O (5.2 mL) over 5 min. The reaction

mixture was refluxed for 6 h, cooled to 0 °C, H_2O added (25 mL), and the mixture was acidified (pH 2-3) by addition of conc. HCl. After storing the reaction mixture at 4 °C for 15 h, the solid was collected by filtration, washed with cold water (13 mL) and dried in vacuo to give 0.81 g (93%) of a white solid: ^{1}H NMR (200 MHz,DMSO-d_6): δ 1.34 (2H, m, CH_2), 1.69 (2H, m, CH_2), 3.37 (2H, t, J=6, $C\underline{H}_2OH$), 4.38 (2H, t, J=7, NCH_2), 6.63 (1H, d, J=3, ArH), 7.20 (1H, d, J=3, ArH).

5-(2,3-Dicarboxyl-1-pyrrolo)-1-pentanol. A stirred suspension of **10** (0.752 g, 3.01 mmol) in refluxing H_2O (4 mL) was treated with a solution of KOH (1.79 g, 32.0 mmol) in H_2O (4 mL) over 5 min. The reaction mixture was refluxed for 10 h, cooled to 0 °C, H_2O added (10 mL), and the pH was adjusted to ca. pH 8 by addition of conc. HCl. The solid was removed by filtration, washed with H_2O (5 mL), and discarded. The filtrate was cooled to 0 °C and acidified (pH 2-3) by the addition of conc. HCl. After storing the reaction mixture at 4 °C for 17 h, the solid was collected by filtration, washed with cold water (5 mL), and dried in vacuo to give 0.602 g (83%) of a white solid: ^{1}H NMR (200 MHz,DMSO-d_6): δ 1.24 (2H, m, CH_2), 1.39 (2H, m, CH_2), 1.66 (2H, m, CH_2), 3.36 (2H, t, J=6, $C\underline{H}_2OH$), 4.36 (2H, t, J=7, NCH_2), 6.64 (1H, d, J=3, ArH), 7.21 (1H, d, J=3, ArH).

3-(2,3-Dicarboxymethyl-1-pyrrolo)-O-tetrahydropyranyl-1-propanol. A THP-related impurity from the previous step was carried through this step. Therefore, the quantity of the starting material used and the yield of the product are only approximate. A solution of 3-(2,3-dicarboxyl-1-pyrrolo)-O-(2-tetrahydropyranyl)-1-propanol (1.15 g, 3.9 mmol) in Et_2O (20 mL) at 0 °C was treated dropwise with a freshly prepared solution of diazomethane until the yellow color of the diazomethane persisted. The excess diazomethane was destroyed by the addition of a few drops of dilute aqueous AcOH, the organic phase washed with sat'd. aqueous $NaHCO_3$ (8 mL), dried (Na_2SO_4), and the solvent removed in vacuo to afford 1.0 g (80%) of a pale yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.68 (m, $CHC\underline{H}_2C\underline{H}_2C\underline{H}_2$ and impurity), 2.03 (2H, quin, J=7, $NCH_2C\underline{H}_2CH_2$), 3.31 (1H, m, $NCH_2CH_2CH\underline{H}'$), 3.52 (m, $CHOCH\underline{H}'$ and impurity), 3.74 (1H, m, $NCH_2CH_2CH\underline{H}'$), 3.82 (3H, s, CH_3), 3.88 (3H, s, CH_3), 3.89(m, $CHOC\underline{H}H'$ and impurity), 4.28 (2H, t, J=7, NCH_2), 4.54 (1H, m, $C\underline{H}OCH_2$), 4.96 (impurity), 6.48 (1H, d, J=3, ArH), 6.76 (1H, d, J=3, ArH).

4-(2,3-Dicarboxymethyl-1-pyrrolo)-1-butanol. A solution of 4-(2,3-dicarboxyl-1-pyrrolo)-1-butanol (0.50 g, 2.2 mmol) in Et_2O (5 mL) at 0 °C was treated dropwise with a freshly prepared solution of diazomethane until the yellow color of the diazomethane persisted. The excess diazomethane was destroyed by the addition of a few drops of dilute aqueous AcOH and the solvent removed in vacuo to afford 0.52 g (92%) of a pale yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.54 (2H, m, CH_2), 1.85 (2H, m, CH_2), 3.64 (2H, m, $C\underline{H}_2OH$), 3.82 (3H, s, CH_3), 3.87 (3H, s, CH_3), 4.19 (2H, t, J=7, NCH_2), 6.48 (1H, d, J=3, ArH), 6.73 (1H, d, J=3, ArH).

5-(2,3-Dicarboxymethyl-1-pyrrolo)-1-pentanol. A solution of 5-(2,3-dicarboxyl-1-pyrrolo)-1-pentanol (0.500 g, 2.07 mmol) in Et_2O (5 mL) at 0 °C was treated dropwise with a freshly prepared solution of diazomethane until the yellow color of the diazomethane persisted. The excess diazomethane was destroyed by the addition of a few drops of dilute aqueous AcOH and the solvent removed in vacuo to afford 0.587 g (100%) of a pale yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.78 (2H, m, CH_2),

3.63 (2H, m, $C\underline{H}_2OH$), 3.82 (3H, s, CH_3), 3.88 (3H, s, CH_3), 4.16 (2H, t, J=7, NCH_2), 6.48 (1H, d, J=3, ArH), 6.70 (1H, d, J=3, ArH).

3-(2,3-Dicarboxymethyl-1-pyrrolo)propionic acid (11). A THP-related impurity from the previous step was carried through this step; therefore, the quantity of the starting material used and yield of **11** were estimated. A solution of 3-(2,3-Dicarboxymethyl-1-pyrrolo)-O-tetrahydropyranyl-1-propanol (0.1 g, 0.31 mmol) in acetone (3 mL) at 0 °C was treated dropwise with a solution of the Jones reagent until the yellow color of the chromic acid persisted. The solution was warmed to 25 °C, the solvent removed in vacuo, the residue partitioned between H_2O and CH_2Cl_2, and the aqueous phase extracted with CH_2Cl_2 (4 x 5 mL). The combined organic phases were extracted with sat'd. $NaHCO_3$ (3 x 4 mL). The combined aqueous phases were extracted with CH_2Cl_2 (3 mL) and the organic phase was reextracted with sat'd. $NaHCO_3$ (1 mL). The combined aqueous phases were acidified (pH ca. 2 to 3) with conc. HCl and exhaustively extracted with CH_2Cl_2 (6 x 3 mL). The combined organic phases were dried (Na_2SO_4) and the solvent removed in vacuo to afford 0.05 g (70%) of **11** as a pale yellow oil: ^1H NMR (200 MHz, $CDCl_3$): δ 2.90 (2H, t, J=7, $C\underline{H}_2COOH$), 3.84 (3H, s, CH_3), 3.88 (3H, s, CH_3), 4.48 (2H, t, J=7, NCH_2), 6.47 (1H, d, J=3, ArH), 6.84 (1H, d, J=3, ArH); HRMS (FAB, Thiogl.): m/e 256.0807 ($M + H^+$); calc'd. for $C_{11}H_{14}NO_6$: 256.0821.

4-(2,3-Dicarboxymethyl-1-pyrrolo)butyric acid (12). A solution of 4-(2,3-Dicarboxymethyl-1-pyrrolo)-1-butanol (0.080 g, 0.31 mmol) in acetone (3 mL) at 0 °C was treated dropwise with a solution of the Jones reagent until the yellow color of the chromic acid persisted. The solution was warmed to 25 °C, the solvent removed in vacuo, the residue partitioned between H_2O and CH_2Cl_2, and the aqueous phase extracted with CH_2Cl_2 (5 x 3 mL). The combined organic phases were washed with brine (5 mL), dried (Na_2SO_4), and the solvent removed in vacuo. The residue was chromatographed on silica gel (5% MeOH/CH_2Cl_2) to afford 0.052 g (62%) of **12** as a pale yellow oil: ^1H NMR (200 MHz, $CDCl_3$): δ 2.07 (2H, quin, J=7, $CH_2C\underline{H}_2CH_2$), 2.33 (2H, t, J=7, $C\underline{H}_2COOH$), 3.81 (3H, s, CH_3), 3.86 (3H, s, CH_3), 4.23 (2H, t, J=7, NCH_2), 6.48 (1H, d, J=3, ArH), 6.72 (1H, d, J=3, ArH), 9.81 (1H, bs, COOH); HRMS (FAB, Thiogl.): m/e 270.0967 ($M + H^+$); calc'd. for $C_{12}H_{16}NO_6$: 270.0978.

5-(2,3-Dicarboxymethyl-1-pyrrolo)valeric acid (13). A solution of 5-(2,3-Dicarboxymethyl-1-pyrrolo)-1-pentanol (0.518 g, 1.93 mmol) in acetone (10 mL) at 0 °C was treated dropwise with a solution of the Jones reagent (1.5 mL) over 20 min. The solution was stirred for 1 h after which iPrOH (1 mL) was added to quench the excess Cr(III). The solvent was decanted from an insoluble residue and saved. The residue was washed with CH_2Cl_2 (5 x 2 mL) and discarded. The combined organic phases were added to the solution from the reaction and the solvent removed in vacuo. The resulting residue was dissolved in CH_2Cl_2 (20 mL) and extracted with sat'd. $NaHCO_3$ (3 x 7 mL). The combined aqueous phases were extracted with CH_2Cl_2 (5 mL) and the organic phase was reextracted with sat'd. $NaHCO_3$ (1 mL). The combined aqueous phases were acidified (pH ca. 2 to 3) with conc. HCl and exhaustively extracted with CH_2Cl_2 (8 x 5 mL). The combined organic phases were dried (Na_2SO_4) and the solvent removed in vacuo to afford 0.378 g (69%) of **13** as a pale yellow oil: ^1H NMR (200 MHz, $CDCl_3$): δ 1.62 (2H, m, CH_2), 1.80 (2H, m, CH_2), 2.37 (2H, t, J=7, $C\underline{H}_2COOH$), 3.83 (3H, s, CH_3), 3.87 (3H, s, CH_3), 4.17 (2H, t, J=7, NCH_2), 6.48 (1H, d, J=3, ArH), 6.72 (1H, d, J=3, ArH), 11.05 (1H, bs, COOH). ^{13}C NMR (50 MHz, $CDCl_3$,$CDCl_3$ at 77 ppm): δ 21.5, 30.7, 33.3, 48.7, 51.7, 52.0,

110.4, 120.7, 123.5, 125.3, 161.7, 165.1, 179.0; HRMS (FAB, Thiogl.): m/e 284.1140 (M + H+); calc'd. for $C_{13}H_{18}NO_6$: 284.1134.

3-(1-Methyl-4-(1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido)pyrrole-2-carboxamido)dimethylaminopropane (14).

Compound **14** was freshly prepared for coupling reactions with imidazolides of acids **11-13**. The following procedure is a representative example of its preparation and was used in a reaction with imidazolide of acid **11**. A solution of **15**[13] (0.297 g, 0.595 mmol) in MeOH (4 mL) was hydrogenated with H_2 (1 atm) over 5% Pd(C) (ca. 20 mg) at 25 °C. After 6 h the catalyst was removed by filtration through Celite which was washed with MeOH (3 mL). The solvent from the filtrate was removed in vacuo. The residue was dissolved in DMF (0.4 mL) and the solvent removed in vacuo to remove traces of MeOH. This residue was dissolved in DMF (0.4 mL) and used directly in a reaction with **11**, in the preparation of **16**.

Compounds 16-18. The following procedure for the preparation of compound **18** is also representative of the procedures for the preparation of compounds **16** and **17**. A solution of **13** (0.113 g, 0.398 mmol) in DMF (0.5 mL), at 25 °C, was treated with 1,1'-carbonyldiimidazole (0.065 g, 0.402 mmol) in DMF (0.4 mL) over 5 min. The reaction mixture was kept at 25 °C for 6 h, after which **14** (prepared as described above from **15** (0.297 g, 0.595 mmol)) was added in DMF (0.5 mL) over 5 min. After stirring at 25 °C for 3.5 h, the solvent was removed in vacuo, the residue partitioned between sat'd. aqueous $NaHCO_3$ (4 mL) and CH_2Cl_2 (8 mL), and the aqueous phase extracted with CH_2Cl_2 (2 x 3 mL). The combined organic phases were dried (Na_2SO_4), the solvent removed in vacuo, and the residue was chromatographed on silica gel (2% Et_3N/EtOH) to give 0.186 g (64%) of **18** as a pale yellow oil.

16: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.71 (2H, m, $NHCH_2C\underline{H}_2$), 2.26 (6H, s, $N(CH_3)_2$), 2.44 (2H, t, J=6, $C\underline{H}_2N(CH_3)_2$), 2.78 (2H, t, J=6, CH_2CO), 3.39 (2H, m, $NHC\underline{H}_2$), 3.78 (3H, s, CH_3), 3.80 (3H, s, CH_3), 3.82 (6H, s, CH_3), 3.84 (3H, s, CH_3), 4.50 (2H, t, J=6, NCH_2), 6.40 (1H, d, J=3, ArH), 6.49 (1H, d, J=2, ArH), 6.57 (1H, d, J=2, ArH), 6.66 (1H, d, J=2, ArH), 6.80 (1H, d, J=3, ArH), 7.00 (1H, d, J=2, ArH), 7.21 (1H, d, J=2, ArH), 7.23 (1H, d, J=2, ArH), 7.66 (1H, m, NH), 8.06 (1H, bs, NH), 8.63 (1H, bs, NH), 8.74 (1H, bs, NH); HRMS (FAB, Thiogl.): m/e 706.3294 (M + H+); calc'd. for $C_{34}H_{43}N_9O_8$: 706.3312.

17: ^{1}H NMR (500 MHz,DMSO-d_6, DMSO-d_5 at 2.49 ppm): δ 1.62 (2H, m, J=7, $NHCH_2C\underline{H}_2$), 1.96 (2H, m, $NCH_2C\underline{H}_2$), 2.16 (6H, s, $N(CH_3)_2$), 2.18 (2H, m, CH_2CO), 2.21 (2H, t, J=7, $C\underline{H}_2N(CH_3)_2$), 3.19 (2H, q, J=7, $NHC\underline{H}_2$), 3.72 (3H, s, CH_3), 3.79 (3H, s, CH_3), 3.80 (3H, s, CH_3), 3.83 (3H, s, CH_3), 3.84 (3H, s, CH_3), 4.16 (2H, t, J=7, NCH_2), 6.44 (1H, d, J=3, ArH), 6.83 (1H, d, J=2, ArH), 6.87 (1H, d, J=2, ArH), 7.03 (1H, d, J=2, ArH), 7.09 (1H, d, J=3, ArH), 7.16 (1H, d, J=2, ArH), 7.18 (1H, d, J=2, ArH), 7.24 (1H, d, J=2, ArH), 8.07 (1H, m, NH), 9.81 (1H, s, NH), 9.83 (1H, s, NH), 9.84 (1H, s, NH); HRMS (FAB, Thiogl.): m/e 720.3434 (M + H+); calc'd. for $C_{35}H_{46}N_9O_8$: 720.3469.

18: ^{1}H NMR (500 MHz,DMSO-d_6, DMSO-d_5 at 2.49 ppm): δ 1.49 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.68 (2H, m, CH_2), 2.14 (6H, s, $N(CH_3)_2$), 2.24 (4H, m, CH_2CO and $C\underline{H}_2N(CH_3)_2$), 3.19 (2H, m, $NHC\underline{H}_2$), 3.71 (3H, s, CH_3), 3.79 (3H, s, CH_3), 3.80 (3H, s, CH_3), 3.83 (3H, s, CH_3), 3.85 (3H, s, CH_3), 4.13 (2H, t, J=7, NCH_2), 6.42 (1H, d, J=3, ArH), 6.83 (1H, s, ArH), 6.87 (1H, s, ArH), 7.04 (1H, s, ArH), 7.10 (1H, d, J=3, ArH), 7.16 (1H, s, ArH), 7.19 (1H, s, ArH), 7.24 (1H, s, ArH), 8.07 (1H, m, NH), 9.79 (3H, m, NH); HRMS (FAB, Thiogl.): m/e 734.3619 (M + H+); calc'd. for $C_{36}H_{48}N_9O_8$: 734.3626.

Compounds 1a-3a. The following procedure for the preparation of compound **3a** is also representative of the procedures for the preparation of compounds **1a** and **2a**. A solution of **18** (0.027 g, 0.037 mmol) in THF (3 mL) containing $LiBH_4$ (2.0 M in THF, 6.0 mmol) was heated at 60 °C for 25.5 h. The reaction mixture was cooled to 0 °C, and diluted with both CH_2Cl_2 (13 mL) and MeOH (2.0 mL). After 20 min the mixture was warmed to 25 °C, stirred at 25 °C for 40 min, and acetone (ca 0.02 mL) added. After additional 20 min, the solid was removed by filtration, washed with 5% MeOH/CH_2Cl_2 (3 mL), and then discarded. The filtrate was washed with H_2O:sat'd. aqueous K_2CO_3 (2:7) (7 mL and 2 x 3.5 mL) and the combined aqueous extracts were reextracted with CH_2Cl_2 (5 mL). The combined organic phases were dried (Na_2SO_4), the solvent removed in vacuo, and the residue was chromatographed on silica gel (10% MeOH/CH_2Cl_2) to give 0.020 g (76%) of the borane complex of compound **3a** (**3a•BH$_3$**) as a pale yellow solid. **1a•BH$_3$:** ^{1}H NMR (500 MHz, 9:1 $CDCl_3$:CD_3OD): δ 2.02 (2H, bm, $NHCH_2C\underline{H}_2$), 2.57 (6H, s, $N(CH_3)_2$), 2.75 (2H, m, CH_2CO), 2.79 (2H, m, $C\underline{H}_2N(CH_3)_2$), 3.35 (2H, bm, $NHC\underline{H}_2$), 3.80 (3H, s, NCH_3), 3.86 (6H, bs, 2 x NCH_3), 4.25 (2H, bt, NCH_2), 4.49 (2H, s, $C\underline{H}_2OH$), 4.61 (2H, s, $C\underline{H}_2OH$), 6.05 (1H, bs, ArH), 6.56 (1H, bs, ArH), 6.58 (1H, bs, ArH), 6.69 (2H, bs, ArH), 7.15 (1H, bs, ArH), 7.18 (1H, bs, ArH), 7.31 (1H, bs, ArH). **2a•BH$_3$:** ^{1}H NMR (500 MHz, CD_3OD, CHD_2OD at 3.30 ppm): δ 1.98 (2H, m, J=7, CH_2), 2.10 (2H, m, J=7, CH_2), 2.29 (2H, t, J=7, CH_2CO), 2.52 (6H, s, $N(CH_3)_2$), 2.78 (2H, m, $C\underline{H}_2N(CH_3)_2$), 3.29 (2H, t, J=7, $NHC\underline{H}_2$), 3.84 (3H, s, NCH_3), 3.85 (3H, s, NCH_3), 3.86 (3H, s, NCH_3), 4.00 (2H, t, J=7, NCH_2), 4.48 (2H, s, $C\underline{H}_2OH$), 4.59 (2H, s, $C\underline{H}_2OH$), 6.07 (1H, d, J=3, ArH), 6.66 (1H, d, J=3, ArH), 6.80 (1H, d, J=2, ArH), 6.82 (1H, d, J=2, ArH), 6.90 (1H, d, J=2, ArH), 7.11 (1H, d, J=2, ArH), 7.15 (2H, m, ArH). **3a•BH$_3$:** ^{1}H NMR (500 MHz, CD_3OD, CHD_2OD at 3.30 ppm): δ 1.68 (2H, m, J=7, CH_2), 1.81 (2H, m, J=7, CH_2), 2.00 (2H, m, J=7, CH_2), 2.31 (2H, t, J=7, CH_2CO), 2.54 (6H, s, $N(CH_3)_2$), 2.80 (2H, m, $C\underline{H}_2N(CH_3)_2$), 3.32 (2H, m, $NHC\underline{H}_2$), 3.85 (3H, s, NCH_3), 3.87 (3H, s, NCH_3), 3.88 (3H, s, NCH_3), 3.98 (2H, t, J=7, NCH_2), 4.47 (2H, s, $C\underline{H}_2OH$), 4.58 (2H, s, $C\underline{H}_2OH$), 6.05 (1H, d, J=3, ArH), 6.65 (1H, d, J=3, ArH), 6.80 (1H, d, J=2, ArH), 6.81 (1H, d, J=2, ArH), 6.91 (1H, d, J=2, ArH), 7.12 (1H, d, J=2, ArH), 7.16 (2H, m, ArH).

A solution of **3a•BH$_3$** (0.0084 g, 0.012 mmol) in MeOH (3 mL) was treated with anhydrous Na_2CO_3 (0.045 g, 0.43 mmol) and heated at 60 °C for 95 h. The reaction mixture was cooled to 25 °C and the solvent removed in vacuo. The residue was partitioned between H_2O (0.5 mL) and CH_2Cl_2 (5 mL) and the aqueous phase washed with CH_2Cl_2 (7 x 3 mL). The combined organic phases were dried (Na_2SO_4) and the solvent was removed in vacuo to afford 0.0058 g (70%) of **3a** as a pale yellow solid.

1a: ^{1}H NMR (500 MHz, 9:1 CD_3CN:CD_3OD,CD_2HCN at 1.93 ppm): δ 1.68 (2H, m, J=7, $NHCH_2C\underline{H}_2$), 2.22 (6H, s, $N(CH_3)_2$), 2.37 (2H, t, J=7, $C\underline{H}_2N(CH_3)_2$), 2.74 (2H, t, J=7, CH_2CO), 3.29 (2H, t, J=7, $NHC\underline{H}_2$), 3.82 (3H, s, NCH_3), 3.83 (3H, s, NCH_3), 3.86 (3H, s, NCH_3), 4.24 (2H, t, J=7, NCH_2), 4.40 (2H, s, $C\underline{H}_2OH$), 4.55 (2H, s, $C\underline{H}_2OH$), 6.00 (1H, d, J=3, ArH), 6.62 (1H, d, J=3, ArH), 6.64 (1H, d, J=2, ArH), 6.69 (1H, d, J=2, ArH), 6.86 (1H, d, J=2, ArH), 7.08 (1H, d, J=2, ArH), 7.18 (2H, m, ArH). UV (MeOH, nm): 236, 304 nm. HRMS (FAB, 3-NBA): m/e 650.3380 (M + H$^+$); calc'd. for $C_{32}H_{44}N_9O_6$: 650.3414.

2a: ^{1}H NMR (500 MHz, CD_3OD, CD_2HOD at 3.30 ppm): δ 1.75 (2H, m, J=7, CH_2), 2.11 (2H, m, J=7, CH_2), 2.24 (6H, s, $N(CH_3)_2$), 2.31 (2H, t, J=7, CH_2CO), 2.38 (2H, t, J=7, $C\underline{H}_2N(CH_3)_2$), 3.31 (2H, t, J=7, $NHC\underline{H}_2$), 3.85 (3H, s, NCH_3), 3.87 (3H, s, NCH_3), 3.88 (3H, s, NCH_3), 4.00 (2H, t, J=7, NCH_2), 4.48 (2H, s, $C\underline{H}_2OH$), 4.59 (2H, s, $C\underline{H}_2OH$), 6.07 (1H, d, J=3, ArH), 6.67 (1H, d, J=3, ArH), 6.77 (1H, s, ArH),

6.81 (1H, s, ArH), 6.91 (1H, s, ArH), 7.12 (1H, s, ArH), 7.16 (1H, s, ArH), 7.17 (1H, s, ArH); HRMS (FAB, Thiogl.): m/e 664.3523 ($M + H^+$); calc'd. for $C_{33}H_{46}N_9O_6$: 664.3571.

3a: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.52 (2H, bm, CH_2), 1.65 (2H, bm, CH_2), 2.07 (2H, bm, CH_2), 2.18 (6H, s, $N(CH_3)_2$), 2.23 (2H, bm, CH_2CO), 2.32 (2H, bm, $CH_2N(CH_3)_2$), 3.32 (2H, bm, $NHCH_2$), 3.72 (3H, s, NCH_3), 3.78 (3H, s, NCH_3), 3.80 (3H, s, NCH_3), 3.83 (2H, bm, NCH_2), 4.46 (2H, s, CH_2OH), 4.48 (2H, s, CH_2OH), 6.02 (1H, bs, ArH), 6.47 (1H, bs, ArH), 6.57 (2H, bs, ArH), 6.60 (1H, bs, ArH), 6.94 (1H, bs, ArH), 7.16 (1H, bs, ArH), 7.20 (1H, bs, ArH), 7.56 (1H, bm, $NHCH_2$), 8.49 (1H, bs, NH), 8.67 (1H, bs, NH), 8.80 (1H, bs, NH); HRMS (FAB, Thiogl.): m/e 678.3688 ($M + H^+$); calc'd. for $C_{34}H_{48}N_9O_6$: 678.3728.

Compounds 1b-3b. The following procedure for the preparation of compound **1b** is also representative of the procedures for the preparation of compounds **2b** and **3b**. A solution of **1a** (0.00105 g, 0.0016 mmol) (as measured by comparison to a known amount of DMAP by ^{1}H NMR) in $CDCl_3$ (0.4 mL) containing DMAP (0.000080 g, 0.00065 mmol) was treated sequentially with Et_3N (0.0065 g, 0.0644 mmol) and Ac_2O (0.0032 g, 0.0322 mmol) at 25 °C. ^{1}H NMR spectra were recorded before and 0.5 h after the addition of the Ac_2O and revealed that the acetylation was complete as judged by a ca. 0.5 ppm downfield shift of the methylene resonances adjacent to the alcohols being acylated. The solvent was removed in vacuo, and the residue dissolved in CD_3CN, and a ^{1}H NMR spectrum recorded. This solution was used directly in interstrand cross-linking reactions.

1b: ^{1}H NMR (500 MHz, 9:1 CD_3CN:CD_3OD,CD_2HCN at 1.93 ppm): δ 1.79 (2H, m, $NHCH_2CH_2$), ca. 2.0 ($COCH_3$ and $N(CH_3)_2$), 2.32 (2H, m, $CH_2N(CH_3)_2$), 2.77 (2H, t, J=7, CH_2CO), 3.34 (2H, m, $NHCH_2$), 3.84 (3H, s, NCH_3), 3.85 (3H, s, NCH_3), 3.87 (3H, s, NCH_3), 4.25 (2H, t, J=7, NCH_2), 4.96 (2H, s, CH_2OAc), 5.18 (2H, s, CH_2OAc), 6.09 (1H, d, J=3, ArH), 6.69 (1H, s, ArH), 6.75 (2H, m, ArH), 6.90 (1H, s, ArH), 7.13 (1H, s, ArH), 7.22 (2H, s, ArH).

2b: ^{1}H NMR (500 MHz, 9:1 CD_3CN:CD_3OD,CD_2HCN at 1.93 ppm): δ 1.34 (2H, m, CH_2), 1.72 (2H, m, CH_2), ca. 2.0 ($COCH_3$ and $N(CH_3)_2$), ca. 2.3 (2H, m, CH_2), 2.65 (2H, m, CH_2CO), 3.35 (2H, m, $NHCH_2$), 3.87 (3H, s, NCH_3), 3.88 (3H, s, NCH_3), 3.90 (3H, s, NCH_3), 3.99 (2H, t, J=7, NCH_2), 5.01 (2H, s, CH_2OAc), 5.15 (2H, s, CH_2OAc), 6.13 (1H, s, ArH), 6.63 (1H, s, ArH), 6.75 (2H, m, ArH), 6.90 (1H, s, ArH), 7.14 (1H, s, ArH), 7.19 (2H, m, ArH), 7.50 (1H, m, NH), 8.21 (1H, s, NH), 8.40 (2H, s, NH). FAB-LRMS (thioglycerol): m/e 748 $(M+H)^+$, 603, 532, 515 (B), 391, 347.

3b: ^{1}H NMR (500 MHz, 9:1 CD_3CN:CD_3OD,CD_2HCN at 1.93 ppm): δ 1.62 (2H, m, CH_2), 1.74 (2H, m, CH_2), 1.81 (2H, m, CH_2), ca. 2.0 ($COCH_3$ and $N(CH_3)_2$), 2.25 (2H, m, $CH_2N(CH_3)_2$), 2.70 (2H, m, CH_2CO), 3.33 (2H, m, $NHCH_2$), 3.85 (6H, s, NCH_3), 3.87 (3H, s, NCH_3), 3.91 (2H, t, J=7, NCH_2), 4.96 (2H, s, CH_2OAc), 5.08 (2H, s, CH_2OAc), 6.09 (1H, d, J=3, ArH), 6.74 (3H, m, ArH), 6.90 (1H, s, ArH), 7.13 (1H, s, ArH), 7.21 (1H, s, ArH), 7.22 (H, s, ArH).

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