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DNA recognition by the first *tail-to-tail* linked distamycin-like oligopeptide dimers†

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Sequence-specific bidentate binding to double-stranded (*ds*)-DNA by '*tail-to-tail*' linked dimeric distamycin analogues is described; compared to their monomeric analogues, these dimers exhibit greater affinity and longer binding site size and open up a novel avenue in the design of minor groove binders that overcome the phasing problem.

Design of molecules with low molecular mass (M_w *ca.* 10^3) that bind with high affinity and specificity to pre-determined DNA sequences that are 10–16 base pairs long is a key issue in chemical biology. For instance, a unique site within the 3 billion base pair human genome is defined by a minimum of 15–16 contiguous base pairs.[1] Natural products such as distamycin and netropsin which exert their biological activity by competing with the TATA box binding protein (TBP) for the target site on DNA serve as the 'lead compounds' for the design of such 'lexitropsins' (information reading oligopeptides).[2] Some of the compounds developed on the basis of these principles have indeed exhibited interesting biological properties.[3]

Distamycin (Dst)-type polyamides with $(n - 1)$ *N*-methylpyrrole rings and n amide groups bind preferentially to the minor groove sites containing $(n + 1)$ successive AT base pairs of *ds*-DNA. The repeating *N*-methylpyrrole-2-carboxamide unit, however, is ~20% longer than is required to match the base pair rise along the minor groove of the DNA helix. As a result longer polyamides ($n > 6$) are out of phase with DNA.[4,5] An over-wound curvature of longer polyamides is an added disadvantage. These factors result in a sub-optimal contact between DNA and the ligand leading to the manifestation of lower binding affinities. To reset an optimum fit of long polyamides with the DNA double-helix, flexible β-alanine or glycine residues have in fact been inserted.[4]

In an alternative strategy, two netropsin-like or distamycin-like cationic peptides have been connected in a 'head-to-head' (*N*-terminus-to-*N*-terminus) fashion using flexible or rigid linkers.[5] These bis(lexitropsins) were shown to adopt monodentate or bidentate modes of DNA binding depending upon the nature of the linker unit. Importantly those bound to DNA in a dimeric fashion also exhibited higher affinities.[1,6]

† Dedicated to the memory of Dr D. Ranganathan.

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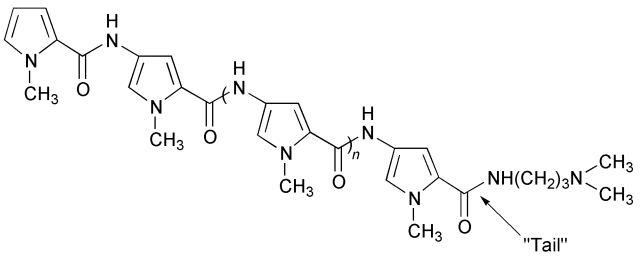
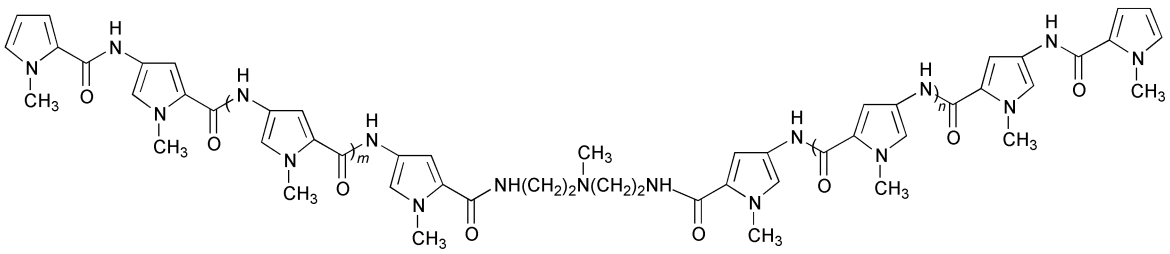
Herein we report a new approach to the design of dimeric lexitropsins such that optimum contacts between the successive amide units and the DNA helix are allowed. The following notable features characterize the present design. Firstly the dimers were constructed by linking the C-terminus of the individual monomers ('*tail-to-tail*' linkage). Secondly the positive charge necessary for optimizing the DNA affinity and water solubility is provided by the presence of a tertiary amine based linker which remains protonated at physiological pH.§ Significantly, these tail-to-tail linked *bis-Dst*s (**3**–**5**) bear only a single positive charge unlike the previously reported 'head-to-head' linked dimers which carry two positive charges. These bis-Dsts also lack the leading formamide unit at the *N*-termini.[7]

DNA binding abilities of the Dsts [**1**(**D3**) and **2**(**D4**)] and the corresponding bis-Dsts [**3**(**D33**), **4**(**D34**) and **5**(**D44**)] were examined for their ability to modify duplex DNA helix-to-coil transition temperature (T_m), induced circular dichroism (ICD) measurements, salt-dependence of ICD and fluorescence probe displacement assay.



1: n = 0 (**D3**); **2**: n = 1 (**D4**)

Dst and related minor groove binders are known to enhance the T_m of *ds*-DNA. This has been attributed to the stabilization of the double-helix.[8] Table 1 summarizes the results of the T_m measurements of DNA with the dimers **D33**, **D34** and **D44** along with those for the corresponding monomers, **D3** and **D4**. The ΔT_m is higher in the case of poly d(A.T) compared to calf thymus DNA (CT DNA, ~50% AT rich) indicating that these bis-Dsts have retained the AT-specific mode of DNA binding. The ΔT_m values are significantly higher for the complexes of the bis-Dsts compared to those of the corresponding monomers. This is notable, since the positive charge is not 'doubled' for the dimers compared with the monomers. The observed enhance-



3: m = 0, n = 0 (**D33**); **4**: m = 0, n = 1 (**D34**); **5**: m = 1; n = 1 (**D44**)

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Table 1 Summary of melting temperature measurements[a]

Oligopeptide	ΔT_m/°C	
	Poly d(A.T)	CT-DNA
D3	0.5	0
D4	11.0	1.6
D33	12.5	1.6
D34	21.5	5.0
D44	27.5	6.5

[a] 40 mM NaCl, 10 mM Tris·HCl (pH = 7.4), [D]/[P] = 0.2.

ment in T_m could be entirely due to specific H-bonding and van der Waals interactions. The ΔT_m was also found to increase as a function of the length of the oligopeptides. For instance, the $\Delta(\Delta T_m)$ between the complexes of **D34** and **D33** with poly d(A.T) was ~9 °C and that between the complexes of **D44** and **D34** was ~6 °C. These results would be possible only if both the 'arms' of the dimeric distamycins are in contact with *ds*-DNA implying the effectiveness of the bidentate mode of binding.

The induced Cotton effect (ICD) produced at 25 °C upon the binding of Dst and its analogues to DNA that appear in the 280–380 nm region is distinct from the intrinsic CD spectrum of DNA.[8] The intensity of the ICD signal is proportional to the strength of binding and also on the number of the *N*-methylpyrrolecarboxamide units present in the oligopeptides. All the three bis-Dsts produced intense ICD signals upon binding to poly d(A.T) and CT-DNA (not shown). The ICD was higher in the case of poly d(A.T) compared to CT-DNA and the ICD intensity increased in the order **D33** < **D34** < **D44**.

Monotonous increase was observed in the ICD signal with [ligand] until saturation at [D]/[P] ratios {ratio of [ligand] *vs.* [DNA] (in base molarity)} characteristic of each ligand/duplex type. Importantly, a single set of isodichroic points was seen in each case (not shown) implying the existence of equilibrium between the bound and the free forms of the ligand. Molar ellipticity (θ) observed in the case of the dimers was found to be at least twice as high as that for the monomers. Taken together these results suggest that both the 'arms' of the dimeric Dsts bind to DNA at all [D]/[P] ratios.

Salt-induced complex dissociation experiments are often used for comparing the relative binding affinities of ligands towards DNA.[8] The higher the binding affinity, the lower should be the complex dissociation with increasing salt concentration. Plots of the relative intensities of the positive ICD bands at the respective ICD λ_{max} as a function of [NaCl] are presented in Fig. 1. It is seen that the ICD spectra of the bis-Dsts are less susceptible to high [NaCl] compared to their respective monomers. The most dramatic difference was observed in the case of **D33**. These results clearly show that the dimers bind to AT-rich DNA stronger than their monomeric

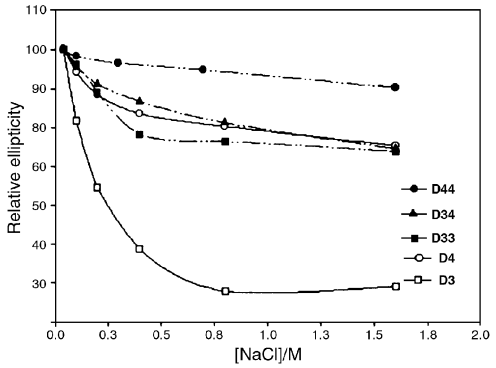

Fig. 1 Effect of NaCl on the ICD spectra of the complexes of **D33**, **D34**, **D44** and the monomers **D3** and **D4**.

counterparts, which would be possible only if the bidentate mode of binding is operational.

The binding site sizes of **1–5** were assessed from the saturation [D]/[P] values obtained from the CD titration plots. It was inferred that during DNA binding, each of the dimer arms overlap in a head-to-tail dimeric fashion with an equivalent arm from two neighboring dimers that lie on either side. The above arrangement is repeated throughout the DNA leading to 'multimeric' arrangement on the polymer. The *actual* number of bp covered by individual molecules of **3–5** may be assumed to be *ca*. 8–10 bp.

Finally, to estimate the relative binding constants (K_{app}) of these bis-Dsts and the corresponding monomers we also studied the competition between compounds **D3**, **D4**, **D33**, **D34**, **D44** and the well known AT specific DNA minor groove binder Hoechst-33258 for binding sites on poly d(A.T).[9] Displacement of Hoechst from poly d(A.T) was accompanied by a decrease in the fluorescence intensity measured at 460 nM (λ_{ex} = 355 nm). The apparent binding constants (K_{app}) were calculated from the concentration of the compounds required for 50% quenching of Hoechst fluorescence and the known values of [Hoechst] and binding constant[9] {[Hoechst-33258] = 230.4 nM, [poly d(AT)] = 4.6×10^{-6} M, $K_a = 5 \times 10^7$ M^{-1} (base molarity), 50 mM Tris·HCl buffer containing 100 mM NaCl)}. The K_{app} values for **D3**, **D4**, **D33**, **D34** and **D44** calculated this way were 6×10^5, 9.3×10^6, 2.0×10^7, 4.1×10^7 and 4.9×10^7 M^{-1} respectively. The highest enhancement in K_{app} compared to the corresponding monomer was observed in the case of **D33** (33-fold). The value of K_{app} for **D44** was 5 times that for the corresponding monomer **D4**.

In summary the present systems, which represent the first examples of 'tail-to-tail' linked dimeric lexitropsins, form a novel class of minor groove binders that bind to *ds*-DNA in a bidentate fashion and exhibit significantly greater affinity for *ds*-DNA compared to the respective monomers. These bind poly d(A.T) in a nearly 2:1 overlapped fashion and individual molecules seem to cover 8–10 bp and provide a significant step forward in the design of minor groove binders towards overcoming the phasing problem.

Notes and references

§ All the compounds were characterized by FT-IR, ^{1}H-NMR, and mass spectroscopy.

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