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Facile synthesis of novel fluorescent distamycin analogues

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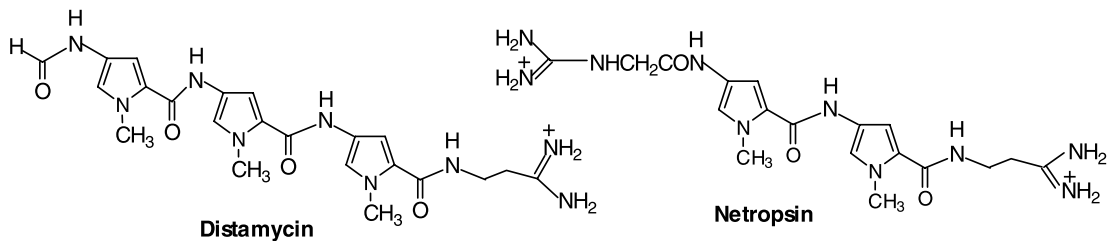
Abstract—A facile synthesis of four distamycin analogues that bear the dansyl fluorophore is described. The nature of the linkage between the fluorophore and the sequence recognition element had a dramatic effect on the fluorescence properties of these ligands upon DNA binding. © 2001 Published by Elsevier Science Ltd.

The natural products distamycin (Dst) and netropsin (Net) belong to a class of DNA binding antiviral antibiotics that bind to the minor groove of AT-rich sequences of double-stranded (*ds*)-DNA. Based on several thermodynamic and structural studies, efforts over the past 20 years have resulted in the development of 'lexitropsins' (information reading polyamides) that can bind a stretch of ca. 5–13 bp of DNA.[1]

In the light of the striking demonstrations of biological activity by such molecules,[2] a detailed study of the kinetics of polyamide–DNA interactions and monitoring of their cellular distribution becomes imperative for evaluating their future biological applications. For instance, a fluorescence-based stopped flow technique has recently been employed for determining the kinetic parameters of binding of Dst to *ds*-DNA, which exploited the fluorescence enhancement during the binding process.[3] For hairpin polyamides, however, this strategy was not very effective on account of the modest enhancement (ca. 30%) in the fluorescence on binding to *ds*-DNA.[4] It occurred to us that conjugation of Dst-like oligopeptides to a suitable fluorophore, which does not alter the DNA recognition properties, might result in substantially enhanced fluorescence emission on binding to *ds*-DNA. Such fluorescent polyamides could also be used for the massive screening of oligonucleotide libraries immobilized on microchips for their affinity for polyamides.[5] Sequence-dependent affinities in such cases could be estimated from the increase in the melting temperature (T_m) of the oligonucleotides, which could be monitored effectively by the ligand fluorescence. Additionally, development of fluorescent lexitropsins would also be useful in tracking their cellular distribution in vivo, employing techniques such as fluorescence microscopy. In this respect, a recent report by Lown et al. is noteworthy.[6]

Herein we report the synthesis and DNA binding properties of four novel Dst analogues, which bear the dansyl fluorophore either at the N-terminus (**1**–**3**) or at the C-terminus (**4**). It should be pointed out that the choice of the fluorophore in this case remained critical since systems with fused aromatic rings such as anthracene, acridine, pyrene etc. bind intercalatively to *ds*-DNA and wouldn't therefore faithfully report the minor groove environment of the *ds*-DNA by the sequence recognition moiety. Based on these considerations, the 5-dimethylaminonaphthalenesulfonyl (dansyl) group was chosen as the reporter fluorophore. Three of the four analogues synthesized contained the fluorophore *directly* attached to the N-terminus of the



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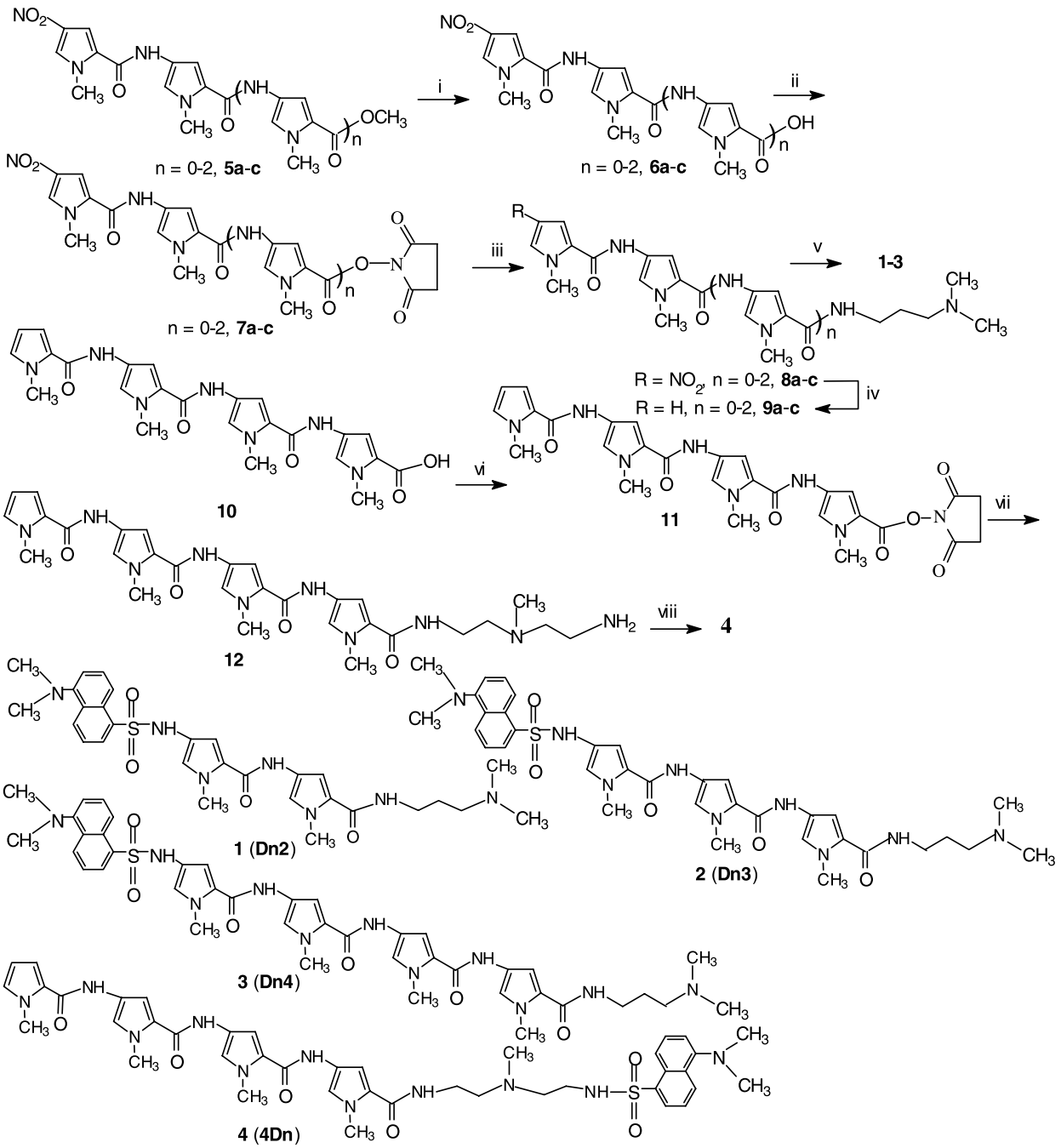
conjugated *N*-methylpyrrole carboxamide units. The fourth analogue contained the dansyl group at the C-terminus and was connected to the conjugated *N*-methylpyrrolecarboxamide units via a flexible linker.

The synthesis of the Dst derivatives, **1**–**3**, began with the preparation of the di-, tri- and tetra-peptide nitro-esters, **5a**–**c**. The starting material for the synthesis of **5a**–**c** was obtained by the nitration and subsequent esterification of *N*-methylpyrrole-2-carboxylic acid, according to a published procedure.[7] The methyl 4-nitro-*N*-methylpyrrole-2-carboxylate thus obtained was reduced using H_2–Pd/C in DMF to obtain the corresponding amine, which was coupled with the 4-nitro-*N*-methylpyrrole-2-carboxylic acid chloride, in the presence of Et_3N in DMF to obtain the nitro-dipeptide in 87% yield. Elongation of the peptide chain to the desired length was achieved by repetition of the reduction and amide coupling steps.[8] The methyl esters were saponified to obtain the corresponding acids, **6a**–**c**, as shown in Scheme 1.



Scheme 1. *Reagents*, *conditions and yields*: (i) 0.25 M NaOH (10 equiv.), 1:1 EtOH/H_2O, reflux, 1 h, then 0.5 M HCl, 50°C (90% each); (ii) DCC, HOSu, DMF, 0°C, 1 h, then rt, 6 h; (iii) *N*,*N*-dimethyl-1,3-diaminopropane, DMF, rt, 1 h (70, 65 and 65%); (iv) H_2/Pd/C, 1 atm rt, 20 h; (v) dansyl chloride, Et_3N, DMF, 0°C, 30 min, rt, 30 min (70, 70 and 65%); (vi) DCC, HOSu, DMF, 0°C, 1 h, then rt, 6 h; (vii) *N*-methyldiethylenetriamine (2 equiv.) rt, 1 h (65%); (viii) dansyl chloride, Et_3N, dry $CHCl_3$, 0°C, 30 min, rt, 30 min (70%).

Each of the acids, **6a**–**c** was converted to the respective *N*-hydroxysuccinimide ester, **7a**–**c** employing *N*-hydroxysuccinimide (HOSu) in the presence of dicyclohexylcarbodiimide (DCC) in dry DMF. Dicyclohexylurea (DCU) formed during the reaction was removed by filtration. *N*,*N*-Dimethyl-1,3-diaminopropane (2 equiv.) was added directly to the above filtrates containing the HOSu esters **7a**–**c**. The resulting solutions were allowed to stir at rt for 30 min. Solvent evaporation followed by column chromatography on silica gel using, first 4% MeOH in $CHCl_3$, followed by MeOH/$CHCl_3$/aq. NH_3 (20:78:2) gave **8a**–**c** as yellow, sticky solids in 70, 70 and 65% yields, respectively. These were dissolved in dry DMF and hydrogenated over Pd/C (5%) for 20 h. The catalyst was removed from the reaction mixture by filtration and washed twice with dry DMF. The combined filtrate and washings were mixed with Et_3N (1.5 equiv.) and cooled to 0°C. To this solution, 1.2 equiv. of solid dansyl chloride was added in batches with stirring. This reaction mixture was stirred for 30 min at 0°C and for another 30 min at rt. The solvent was subsequently removed under reduced pressure and the gummy residue so obtained was purified first by chromatography on silica gel column (6–20% MeOH/$CHCl_3$) followed by a second chromatographic purification on neutral alumina (4–6% MeOH/$CHCl_3$) to obtain **1**–**3** as a light yellow solids in 70, 65 and 65% isolated yields, respectively.

Similarly, the synthesis of the other Dst derivative, **4**, began with the preparation of the carboxylic acid, **10** starting from methyl 4-nitro-*N*-methylpyrrole-2-carboxylate by the successive reduction and coupling of 4-nitro-*N*-methylpyrrole-2-carbonyl chloride to the growing peptide chain. The chain termination, achieved with *N*-methylpyrrole-2-carbonyl chloride, followed by saponification of the methyl esters afforded the acid **10**.[7] This was converted to the succinimide ester **11** employing HOSu in the presence of DCC in dry DMF as shown in Scheme 1. The DCU formed during the reaction was removed by filtration, after the addition of EtOAc. DMF was removed by aqueous work up and the solid obtained upon evaporation of the EtOAc layer was purified by column chromatography employing 4% MeOH in $CHCl_3$ to obtain **11** in 80% yield. This was dissolved in dry $CHCl_3$ and was then added dropwise to a solution of *N*-methyldiethylenetriamine (2 equiv.) in dry $CHCl_3$. The resulting solution was allowed to stir at rt until TLC indicated complete disappearance of the starting ester (**11**). Solvent evaporation followed by column chromatography on silica gel using, first 4% MeOH in $CHCl_3$, followed by MeOH/$CHCl_3$/aq. NH_3 (20:78:2) afforded the desired product (ninhydrin active) **12**, in 70% isolated yield. This was dissolved in $CHCl_3$ and dansyl chloride (1.2 equiv.) was added in portions to the solution in the presence of 1.5 equiv. of Et_3N, at 0°C. The resulting solution was stirred 0°C for 30 min and then at rt for 30 min.

The solvent was subsequently removed under reduced pressure and the gummy solid obtained was purified first by column chromatography on silica gel (6–20%MeOH/$CHCl_3$) followed by a second chromatographic purification on neutral alumina (4–6% MeOH/$CHCl_3$) to obtain **4** as a light yellow solid (70%). All the numbered intermediates and final compounds were characterized by IR, NMR and mass spectra.[9]

Compounds **1**–**3** exhibited about 10-fold enhancement in fluorescence on binding to poly d(AT) as exemplified

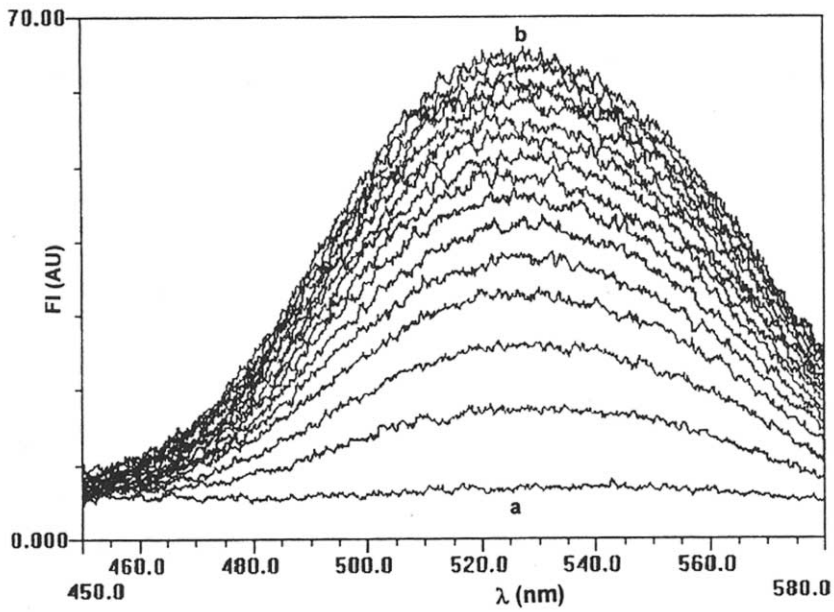


Figure 1. Fluorescence emission spectra of **Dn3** in the presence of poly d(AT) ($\lambda_{ex}=310$ nm) in 10 mM Tris–HCl buffer (pH 7.4) containing 40 mM NaCl. Curve 'a' represents free ligand, 0.97 μM. Subsequent curves correspond to the effect of addition of 5 μl each of 0.16 mM poly d(AT). At 'b' the [poly d(AT)] was 10.9 μM.

by Fig. 1, while no enhancement in fluorescence emission was observed in the case of poly d(GC). These observations clearly demonstrate the sequence specific binding character of **1**–**3** with *ds*-DNA. On the other hand, compound **4** failed to exhibit any such enhancement in fluorescence on addition of either of the above sequences, despite the fact that it had shown intense ICD signals in presence of poly d (AT) (not shown). Ligand **4** had also exhibited similar AT selective DNA foot-printing characteristics as the parent compound that carried the *N*,*N*-dimethyl-1,3-diaminopropionamide group at the C-terminus.[11] The lack of enhancement in fluorescence in the case of **4** may be a result of collisional quenching due to the solvent (water).

In conclusion, facile syntheses of four novel fluorescent distamycin analogues have been achieved and their fluorescence emission properties in presence of *ds*-DNA have been characterized. The nature of the linkage between the fluorophore and the sequence recognition element had a dramatic effect on the fluorescence properties of these ligands upon *ds*-DNA binding. Detailed characterization of the DNA binding properties of **1**–**4** is underway in our laboratory.

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9. Selected spectroscopic data for compounds **1**–**4**: (**1**) ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 1.76 (m, 2H, merging with the water peak), 2.31 (s, 6H), 2.47 (t, 2H, $J=6.62$ Hz); 2.90 (s, 6H); 3.43–3.46 (m, 2H), 3.74 (s, 3H); 3.88 (s, 3H), 6.26 (d, 1H, $J=1.5$ Hz), 6.32 (s, 1H), 6.39 (s, 1H), 7.11 (s, 1H), 7.19–7.26 (m, 2H), 7.43–7.48 (m, 2H), 7.55–7.61 (m, 2H), 8.12 (d, 1H, $J=7.2$ Hz), 8.32 (d, 1H, $J=7.2$ Hz), 8.53 (d, 1H, $J=8.1$ Hz). MALDI: calcd for $C_{29}H_{38}N_9O_4$ (MH^+) 580.4, obtd 580.4. (**2**): ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 1.75 (bs, 2H, merging with the water peak), 2.29 (s, 6H), 2.44 (bs, 2H), 2.88 (s, 6H), 3.43 (bs, 2H), 3.73 (s, 3H), 3.85 (bs, 6H), 6.27 (bs, 1H), 6.39 (bs, 1H), 6.47 (bs, 1H), 6.59 (s, 1H), 7.06 (bs, 1H), 7.17–1.19 (m, 2H), 7.44 (t, 1H, $J=7.8$ Hz), 7.55 (bs, 1H), 7.69 (bs, 2H), 8.11 (d, 1H, $J=6.9$ Hz), 8.34 (bs, 1H), 8.51 (d, 1H, $J=8.7$ Hz).[10] ESIMS: calcd for $C_{35}H_{44}N_9O_5S$ (MH^+) 702.5, obtd 702.3. (**3**): ^{1}H NMR (300 MHz, $CDCl_3$+acetone-d_6, δ ppm) 1.72–1.75 (m, 2H), 2.29 (s, 6H), 2.44 (t, 2H, $J=6.6$ Hz), 2.88 (s, 6H, merging with the water peak), 3.41–3.43 (m, 2H), 3.79 (s, 3H), 3.92 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 6.44 (s, 1H), 6.50 (s, 1H), 6.68 (s, 1H), 6.84 (s, 1H), 6.87 (s, 1H), 7.15 (s, 1H), 7.19 (s, 1H), 7.21– 7.24 (m, 2H), 7.45–7.56 (m, 2H), 7.77 (1H, merging with the $CHCl_3$ peak), 7.87 (bs, 1H), 8.11 (d, 1H, $J=7.2$ Hz), 8.37 (d, 1H, $J=9$ Hz), 8.51 (d, 1H, $J=8.4$ Hz), 9.02 (bs, 2H), 9.06 (s, 1H). Calcd for $C_{41}H_{50}N_{11}O_6S$ (MH^+) 824.5, obtd 824.3. (**4**): ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm) 1.99 (s, 3H), 2.45 (s, 2H), 2.53 (s, 2H), 2.74 (s, 6H), 2.96 (s, 2H), 3.43 (s, 2H), 3.79 (s, 3H), 3.83 (s, 3H), 3.89 (s, 3H), 4.01 (s, 3H), 5.95 (s, 1H), 6.18 (dd, 1H, $J1=4.1$ Hz, $J2=2.6$ Hz), 6.36 (s, 1H), 6.79 (bs, 2H), 6.90 (d, 1H, $J=1.8$ Hz), 6.97 (d, 2H, $J=7.2$ Hz), 6.96–6.99 (m, 1H), 7.12 (s, 1H), 7.21 (s, 1H), 7.29–7.32 (m, 2H), 7.38–7.44 (m, 2H), 8.15–8.24 (m, 3H), 8.40–8.45 (m, 2H). ESIMS: calcd for $C_{41}H_{50}N_{11}O_6S$ (MH^+) 824.5, obtd 824.3.
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