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SYNTHESIS AND DNA BINDING PROPERTIES OF TRYPTOPHAN LINKED MONOCATIONIC NETROPSIN ANALOGUES

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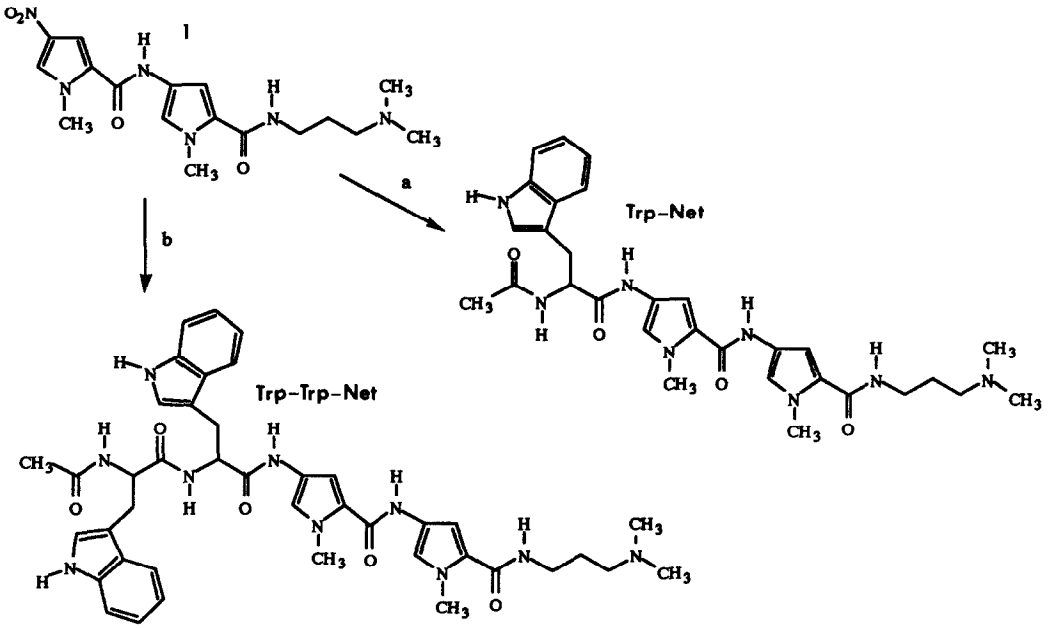
ABSTRACT: *One tryptophan (Trp-Net) and two tryptophan (Trp-Trp-Net) residues have been linked to the amino terminus of a minor groove binding Netropsin analogue. DNA melting measurements indicate that Trp-Trp-Net binds significantly stronger than Trp-Net to double helical DNA. Both compounds induce helix extension as measured by changes in DNA viscosity indicating the possibility of intercalation of the tryptophan indole ring.*

The oligopeptide Netropsin has been studied extensively for its ability to bind to specific regions (AT rich) of double helical DNA.[1] Amino acid analogues of Netropsin bearing various amino acids at the carboxy terminus have been synthesized to study the effect of size and charge on the overall binding strength.[2] We report the synthesis of two monocationic oligopeptides that contain either one tryptophan (Trp-Net) or two tryptophan residues (Trp-Trp-Net) linked at the amino terminus of a Netropsin analogue. Tryptophan residues residing in short polypeptides have been shown to bind to DNA at least in part via intercalation of the indole ring.[3] Trp-Net and Trp-Trp-Net were synthesized to test whether addition of an aromatic amino acid to the minor groove binding portion would give rise to a compound that could bind to DNA by a mixture of intercalation and groove binding in a fashion similar to known Netropsin-acridine conjugates.[4] It was expected that such substitution could lead to enhanced binding strength and possible changes in sequence specificity.

The oligopeptides Trp-Net and Trp-Trp-Net were synthesized according to scheme 1. The nitro group of nitro dipyrrole 1 [5], was reduced to the amine via catalytic transfer hydrogenation in refluxing 95 % ethanol.[6] The subsequent amine was not isolated but condensed directly with the imidazolide of N-Acetyl-L-tryptophan in dry dimethylformamide solution to afford, after flash chromatography, Trp-Net in 52 % yield.[7] In similar fashion condensation of the amine with N-Acetyl-L-tryptophanyl-L-tryptophan yielded, after chromatography, Trp-Trp-Net in 48 % yield. Peptide coupling with acylimidazoles can lead to significant amounts of racemization under certain conditions especially if base is present.[8] Proton NMR analysis (270 MHz) of Trp-Net in the presence of a chiral shift reagent (Aldrich "Resolve-Al") indicated that less than 5 % racemization had occurred in the coupling step. NMR and reverse phase HPLC analysis of Trp-Trp-Net also indicated less than 5 % racemization as well. The amino terminus of both peptides was acetylated to generate an amide NH that could be used to hydrogen bond to acceptors in the minor groove.

The DNA binding efficiencies of the new compounds were determined by their ability to raise the melting temperature of Calf Thymus (CT) or Salmon Testes (ST) DNA.[9] The peptides were allowed to bind to 114 μM sonicated DNA in 5 mM pH 7.5 Tris hydrochloride buffer containing 1 mM NaCl. Melting curves were obtained at different DNA phosphate to drug ratio's as shown in Table 1. At equimolar concentrations Trp-Net raised the melting temperature of CT DNA by 3.4 degrees whereas Trp-Trp-Net raised the melting temperature by 11.0 degrees celsius indicating a stronger binding interaction. Similar results were obtained with ST DNA.

Scheme 1



a. Cyclohexene / 10 % Pd-C then 1,1-carbonyldiimidazole / N-Acetyl-L-tryptophan, b. Cyclohexene / 10 % Pd-C then 1,1-carbonyldiimidazole / N-Acetyl-L-tryptophanyl-L-tryptophan.

Table 1

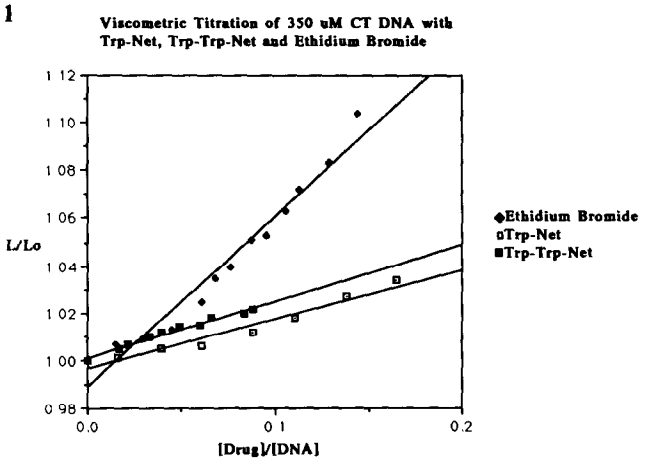
Effect of Trp-Net and Trp-Trp-Net on the Helix to Coil Transition Temperature of Sonicated Calf Thymus or Salmon Testes DNA

DNA [μM]	Peptide [μM]	T_m (°C)	Change in T_m
114 CT	none	54.3(0.7)	----
114 CT	82.9 Trp-Net	57.7(0.3)	3.4
114 CT	415 Trp-Net	59.6(0.8)	5.3
114 CT	27.6 Trp-Trp-Net	58.3(0.4)	4.0
114 CT	82.9 Trp-Trp-Net	65.3(0.6)	11.0
114 ST	none	55.5(0.3)	----
114 ST	82.9 Trp-Net	58.1(0.3)	2.6
114 ST	27.6 Trp-Trp-Net	58.2(0.9)	2.7
114 ST	82.9 Trp-Trp-Net	64.6(0.9)	9.0

This increase in binding by Trp-Trp-Net relative to Trp-Net may be due to fact that there are two more H-bonds that could be formed with the NH of the indole ring and the additional amino terminus amide NH. To determine the contribution, if any, of intercalation in the binding process DNA viscosity measurements were performed.[10] Data from viscometric titrations of 350 μM CT DNA with Trp-Net, Trp-Trp-Net and ethidium bromide when plotted as relative helix extension (L/Lo) versus drug to DNA phosphate ratio gave slopes of 0.21, 0.24 and 0.74 respectively as shown in Figure 1. As expected the strong intercalator ethidium bromide causes extensive helix

lengthening in the concentration ranges studied. Both Trp-Net and Trp-Trp-Net induced significantly less helix extension.

Figure 1



Trp-Trp-Net does show more helix extension than Trp-Net which correlates well with the DNA melting studies. The observation of some helix extension with both compounds is intriguing and may be due to the fewer number of binding sites available to an AT specific binding molecule relative to the larger number of sites available to the non-specific ethidium bromide. It is unlikely that both tryptophan residues of Trp-Trp-Net are intercalated since such a bis-intercalated complex would violate the "excluded site" principle.[13] It is more likely that Trp-Trp-Net binds to the helix in an extended conformation. We are currently performing DNA footprint experiments to determine if indeed these molecules do recognize AT rich regions. Such experiments will be reported in due course.

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7. Physical and Spectral Data, **Trp-Net**: 3-{1-Methyl-4- [1-methyl-4-(3-(3-indolyl)-2-aminoacetyl-propanamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-1-(N,N-dimethylamino)propane: M.p.: 75-77 °C, UV(MeOH): (220 nm, log ε = 4.84), (290 nm, log ε = 4.60), IR(KBr): 3285, 2948, 1666, 1645, 1584, 1531, 1462, 1486, 1406, 1260, 1098 cm^{-1}, ^{1}H-NMR(269.65 MHz, $DMSO_{d6}$): 10.79 (s, 1 H, CONH), 10.04 (s, 1 H, CONH), 9.86 (s, 1 H, CONH), 8.18 (d, 1 H, J=7.6, CH), 8.06 (s, 1 H, CONH), 7.66 (d, 1 H, J=1.2,

CH), 7.34 (d, 1 H, J=1.2, CH), 7.19 (s, 2 H, 2 CH's), 7.15 (d, 1 H, J=1.2, CH), 7.10 - 6.95 (m, 2 H, 2 CH's), 6.91 (d, 1 H, J=1.2, CH), 6.83 (d, 1 H, J=1.0, CH), 4.69 (q, 1 H, J=5.3, CH), 3.84 (s, 3 H, CH_3), 3.81 (s, 3 H, CH_3), 3.24 - 3.10 (m, 3 H, CH_2 and CH), 3.00 (m, 1 H, CH), 2.26 (t, 2 H, J=6.9, CH_2), 2.15 (s, 6 H, $N(CH_3)_2$), 1.84 (s, 3 H, CH_3), 1.62 (m, 2 H, J=6.8, CH_2), ^{13}C-NMR(67.8 MHz, $DMSO_{d6}$): (169.19, q), (169.07, q), (161.21, q), (158.39, q), (136.08, q), (127.32, q), (123.46, CH), (123.09, CH), (122.92, CH), (122.08, q), (121.62, q), (120.87, CH), (118.51, CH), (118.37, q), (118.22, CH), (117.79, q), (111.25, CH), (110.13, q), (104.34, CH), (104.03, CH), (57.06, CH_2), (53.72, CH), (45.08, CH_3), (37.14, CH_2), (36.01, CH_3), (35.90, CH_3), (28.04, CH_2), (27.09, CH_2), (22.54, CH_3), High-resolution FAB: m/z 575.3098 [$(M+H)^+$; calcd for $C_{30}H_{39}N_8O_4$ 575.3094]. **Trp-Trp-Net:** 3-{1-Methyl-4-{1-methyl-4-(3-(3-indolyl)-2-amino-3-[(3-indolyl)-2-aminoacetylpropanamido]propanamido]pyrrole-2-carboxamido-pyrrole-2-carboxamido}-1-(N,N-dimethylamino)propane: M.p.: 163 - 165 °C, UV(MeOH): (220 nm, log ε = 4.87), (289 nm, log ε = 4.47), IR(KBr): 3298, 2930, 1660, 1653, 1638, 1582, 1532, 1434, 1405, 1358, 1276, 1260, 1225, 1092 cm^{-1}, ^{1}H-NMR(269.65 MHz, $DMSO_{d6}$): 10.81 (m, 3 H, 3 CONH's), 9.93 (s, 1 H, CONH), 9.88 (s, 1 H, CONH), 8.08 (m, 2 H, 2 CH's), 7.57 (m, 2 H, 2 CH's), 7.40 - 6.80 (m, 12 H, 12 CH's), 4.60 - 4.54 (m, 2 H, 2 CH's), 3.84 (s, 3 H, CH_3), 3.81 (s, 3 H, CH_3), 3.30 - 2.80 (m, 6 H, 3 CH_2's), 2.29 (m, 2 H, J=4.7, CH_2), 2.17 (s, 6 H, $N(CH_3)_2$), 1.77 (s, 3 H, CH_3), 1.63 (m, 2 H, CH_2), ^{13}C-NMR(67.8 MHz, $DMSO_{d6}$): (171.73, q), (169.45, q), (168.62, q), (161.13, q), (158.31, q), (135.99, q), (127.27, q), (123.55, CH), (123.44, q), (122.98, q), (122.00, q), (121.51, q), (120.76, CH), (118.20, CH), (117.88, q), (117.71, q), (111.17, CH), (110.11, q), (109.96, q), (109.73, q), (104.29, CH), (103.97, CH), (56.92, CH_2), (53.78, CH), (53.61, CH), (44.92, CH_3), (37.00, CH_2), (35.96, CH_3), (35.82, CH_3), (27.55, CH_2), (27.41, CH_2), (26.95, CH_2), (22.46, CH_3). High-resolution FAB: m/z 761.3888 [$(M+H)^+$ calcd for $C_{41}H_{49}N_{10}O_5$ 761.3887].

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9. DNA Melting Studies: 5 mg of Calf Thymus DNA (Sigma Type I, highly polymerized) was suspended in 100 mL of 5 mM pH 7.5 Tris buffer and sonicated for 4 hours. Final DNA concentration as determined by UV absorption at 260 nm was 114 μM nucleotide assuming ε is 6600 M.[11] DNA melts were performed by following the UV absorption at 260 nm as a function of the cuvette temperature. Our DNA preparations gave and average melting temperature of about 55 C. Total hyperchromicity of this preparation was generally about 30% and provided a A_{260} to A_{280} ratio of 1.8. Each curve was run three times and the melting point was determined graphically. Melting points are reported as the average plus or minus the standard deviation.

10. Viscometric Titrations: 15 mg of Calf Thymus DNA (Sigma Type I, highly polymerized) was suspended in 100 mL of 5 mM pH 7.5 Tris, 1 mM NaCl buffer and sonicated for 4 hours. Final DNA concentration determined as before was 350 μM. Viscosity measurements were performed with a 10 mL Cannon Ostwald No. 80 - 100 viscometer. Solutions were filtered into the viscometer and the viscometer was immersed in a 37 °C water bath. Flow times were determined with a stopwatch and run in triplicate to give standard deviations of 0.1 seconds. The flow time of the buffer solution was 73.3 seconds and the buffer DNA solution 80.5 seconds. Flow times for buffer, buffered DNA and DNA-drug complexes were fitted to the equation for relative helix extension (L/Lo) and were plotted against the drug to DNA phosphate ratio.[12]

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