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Porphyrin-Netropsin: a Potential Ligand of DNA

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Abstract : The synthesis of a water-soluble cationic porphyrin bearing a netropsin motif on a glycine side arm is reported. Molecular modelling revealed that this molecule should exhibit good sequence-specific binding to DNA.

The selective binding of a molecule to a particular sequence of nucleotides on DNA is the result of a combination of steric and electronic features which determine the length and sequence of the binding site. Studies of DNA-protein complexes for example, have shown that this specificity results from H-bonding, electrostatic and/or van der Waals interactions between specific atoms or groups of atoms of the base pairs and the ligand. Our strategy to design new molecules which are capable of specific binding to extended regions of DNA is to link together two different molecules which separately have shown some specificity towards limited (2-4) base pair sequences.

The netropsin molecule is known to bind in the minor groove of DNA with a marked preference for sequences of four AT nucleotides, e.g 5'- AATT- 3'.[1] Adducts of netropsin (or distamycin) to intercalating agents (phenoxazone, oxazolopyridocarbazole, acridine and ellipticine) have previously been reported, resulting in at least one instance in an increased affinity for DNA.[1b,2] We report here the synthesis, characterization and theoretical DNA-interaction computations of a hybrid molecule, combining the specific minor groove ligand netropsin to an intercalating cationic porphyrin. Cationic porphyrins, e.g. tetrakis(4-methylpyridinium)porphyrin, H_2TMPyP-4, have been shown to bind strongly to DNA either by intercalation or by external binding.[3] The former binding mode appears to be favored at G-C rich sequences, the latter at A-T rich sequences. Our hypothesis is that the linking of the two molecules should result in an increase in both the affinity for DNA and the nucleotide sequence specificity.

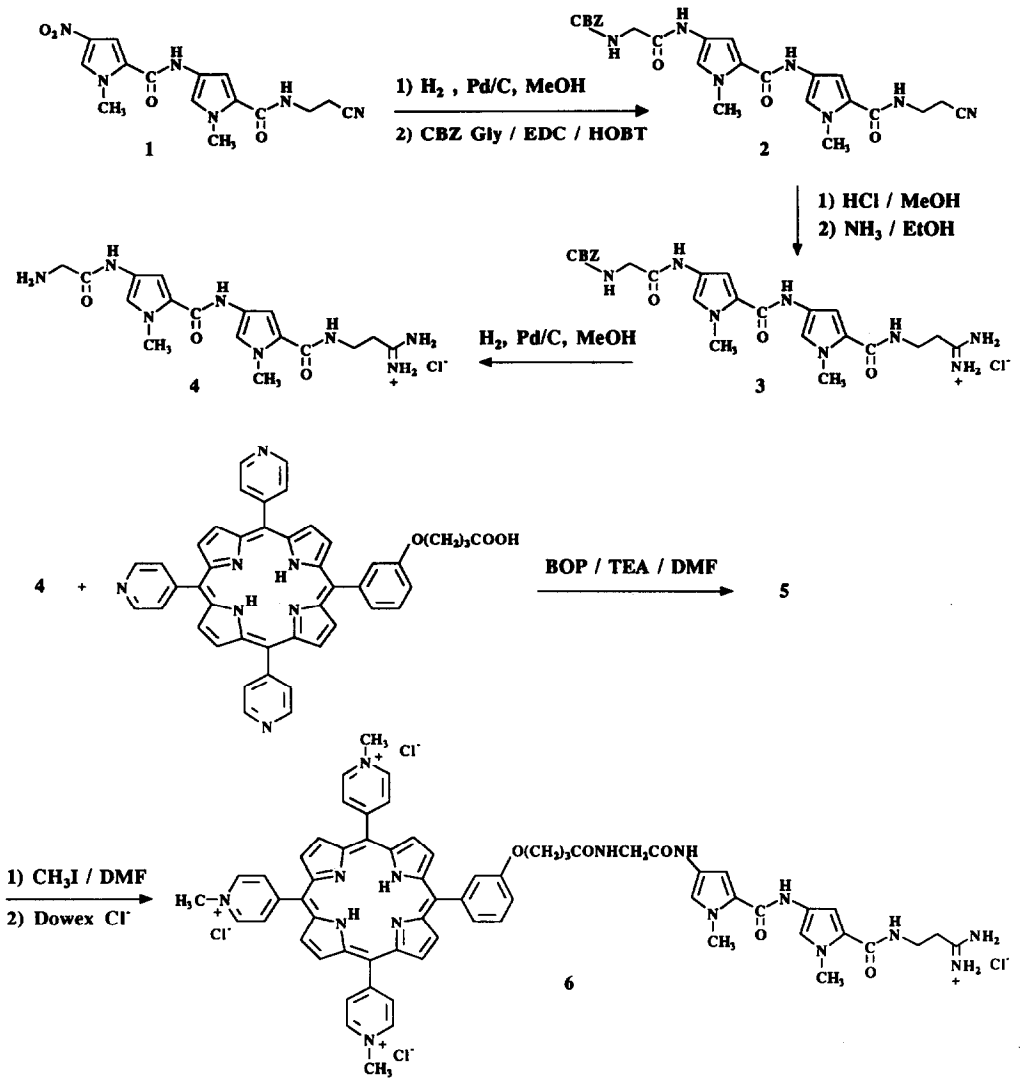
In order to optimize the intercalation of the porphyrin moiety, theoretical computations of porphyrin-netropsin DNA-interactions were performed with the JUMNA (JUnction Minimizations of Nucleic Acids) procedure.[4] We will restrict the present comparisons to complexes having an intercalation site in-between the

pyrimidine-purine sequence d(C^3pG^4)-d($C^{9'}pG^{10'}$) of the Dickerson dodecamer, d(CGCGAATTCGCG)$_2$.[5] As for netropsin, they show that the binding of the porphyrin-netropsin hybrid to DNA is stabilized by H-bonding interactions which involve the two DNA-strands. When comparing porphyrin-netropsin to porphyrin-glycine-netropsin (**6**, see Scheme), the present results indicate that introducing a glycine fragment between the polymethylenic chain and the netropsin should result in improved binding energetics to DNA as well as less penalizing DNA conformational rearrangement energy (see Table). Interestingly, a related conclusion was very recently reached in a study by Durnez *et al*, bearing on the binding to DNA of a netropsin-ellipticine molecule.[6] However, we observed lower values of the binding energetics of netropsin alone, compared to those of the porphyrin-netropsin molecule. Non-intercalative minor groove binding modes of **6** were found to be at least 10 kcal/mole less favourable than intercalation.[5]

Table : Energetic Values (in Kcal/mole) of the Binding of Netropsin and Porphyrin-Netropsin Molecules to d(CGCGAATTCGCG)$_2$.

Ligand	porphyrin-glycine-netropsin	porphyrin-netropsin	netropsin
$E_{interaction}$	-234.4	-216.5	-162.6
ΔE_{DNA}	45.2	76.4	14
ΔE_{ligand}	19.9	9.3	7.2
$\Delta E = E_{inter} + \Delta E_{DNA} + \Delta E_{lig}$	-169.3	-130.8	-141.4

The synthesis of the porphyrin-glycine-netropsin is summarized in Scheme. Synthesis of 1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-pyrrole-2-carboxamido propionitrile **1** was adapted from previously published procedures.[7] It was hydrogenated over Pd/C to give the corresponding amine, which was immediately coupled to CBZ-glycin in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) and hydroxybenzotriazole (HOBT) to give **2** in 50% yield. Pinner's reaction on **2** with HCl in ethanol, followed by ammonia gave **3** in 75% yield. After removal of the CBZ protecting group by hydrogenolysis over Pd/C in methanol, the coupling of the resulting amine **4** and the porphyrin (obtained from 4-pyridinecarboxaldehyde, 3-(ethoxycarbonyl)propyloxybenzaldehyde and pyrrole, followed by saponification to the free acid)[8] was performed using the BOP reagent to give **5** in 80% yield. Treatment of the latter with CH_3I in DMF (50°C, 3hr), followed by ion exchange with a Cl^- Dowex resin, yielded the desired water-soluble product **6**.[9]



Scheme : Synthesis of porphyrin-glycine-netropsin **6**

Acknowlegments

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9. **Spectroscopic data of 5 and 6** :

   ^{1}H NMR chemical shifts (ppm) in DMSO d_6 :

   5 : *porphyrinic protons* : 9.02 (d, J = 5.3 Hz, 6H, pyridyl), 8.85-8.93 (m, 8H, pyrrolic), 8.24 (d, J = 5.3 Hz, 6H, pyridyl), 7.75-7.78 (d + s, J = 7.5 Hz, 2H, phenolic), 7.70 (t, J = 7.5 Hz, 1H, phenolic), 7.41 (d, J = 7.5 Hz, 1H, phenolic), 4.18 (t, J = 7.2 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 2.04 (m, 2H), -3.05 (s, 2NH); *glycine protons* : 8.48 (m, 1NH), 3.30 (under HDO, 2H) ; *netropsin protons* : 9.84 (s, 2NH), 8.48 (m, 1NH), 8.21 (s, 2NH_2), 7.12, 7.06, 6.92 and 6.84 (d, J = 1.2 Hz, 1.2 Hz, 1.5 Hz, 1.5 Hz, 4x1H, pyrrolic), 3.77 and 3.70 (s, 2x3H), 3.30 (under HDO, 2H), 2.57 (t, J = 6.3 Hz, 2H).

   6 : *porphyrinic protons* : 9.51 (d, J = 5.9 Hz, 6H, pyridyl), 9.04-9.14 (m, 8H, pyrrolic), 9.00 (d, J = 5.9 Hz, 6H, pyridyl), 7.79 (t+d + s, J = 7.5 Hz, 3H, phenolic), 7.49 (d, J = 7.5 Hz, 1H, phenolic), 4.72 (s, 9H), 4.22 (t, J = 7.2 Hz, 2H), 2.40 (t, J = 7.2 Hz, 2H), 2.07 (m, 2H), -3.05 (s, 2H, NH); *glycine protons* : 8.29 (m, 1NH), 3.79 (d, J = 6.4 Hz, 2H) ; *netropsin protons* : 9.99 (s, 1NH), 9.90 (s, 1NH), 8.97 (s, 1NH_2), 8.73 (s, 1NH_2), 8.29 (m, 1NH), 7.16, 7.05, 6.91 and 6.84 (d, J = 1.2 Hz, 1.2 Hz, 1.5 Hz, 1.5 Hz, 4x1H, pyrrolic), 3.78 and 3.67 (s, 2x3H), 3.47 (m, 2H), 2.65 (t, J = 6.3 Hz, 2H).

   UV - Visible spectra : λ (nm) :

   5 (CH_2Cl_2) : 417 (100%), 514 (7,6%), 547 (3,8%), 588 (2,7%), 648 (1,6%).

   6 (H_2O) : 305 (14%), 428 (100%), 522 (8,6%), 561 (5,1%), 588 (4,9%), 649 (3,6%). The ratio of the optical densities at 428 nm and 305 nm is close to the ratio of the extinction coefficients at 422 nm and 296 nm for unbound tricationic porphyrins and netropsin respectively.

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