Synthesis of Distamycin Analogs and Their Interactions with Calf Thymus DNA

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Two distamycin analogs (PyPyPy-γ-Dp and PyPyPyPy-γ-Dp) were synthesized by a haloform reaction and the DCC/HOBT coupling reaction in a simple and fast way without amino protection. By using calf thymus DNA, the interaction between the analogs and DNA duplex was studied by CD, and ITC.

Keywords Synthesis, distamycin analogs, calf thymus DNA, molecular recognition

The design of synthetic ligands that read the information in the DNA double helix has been a central goal at the interface of chemistry and biology.[1] Syntheses of DNA-binding molecules, such as triplex-forming oligonucleotides,[2] peptide nucleic acids,[3] oligosaccharide[4] and oligopeptides[5] have been exploited. Distamycin containing *N*-methylpyrrolecarboxamides belongs to a well known class of oligopeptide antibiotics called "Lexitropsins".[6] Preferential binding of distamycin at specific AT-rich regions in the minor groove of synthetic DNA has been demonstrated by X-ray crystallographic,[7] NMR,[8] footprinting and affinity cleaving studies.[9] Our interest in the design and synthesis of DNA-binding molecules has led us to modify distamycin and introduce γ-aminobutyric acid to increase the binding size of distamycin analogs. Here, we report an efficient synthesis of distamycin analogs without amino protection and the interaction between the analogs and calf thymus DNA studied by circular dichroism spectropolarimetry (CD), and isothermal titration calorimeter (ITC).

To construct two distamycin analogs, PyPyPy-γ-Dp and PyPyPyPy-γ-Dp (where Py = *N*-methylpyrrole, γ = γ-aminobutyric acid, Dp = *N*,*N*-dimethylaminopropylamide), 4-nitro-*N*-methyl-2-trichloroacetylpyrrole was used as a key intermediate, which was easily prepared from commercial *N*-methylpyrrole (**1**).[6,10] Compound **7a** was synthesized by a haloform reaction between **3** and ethyl γ-aminobutyricate without chromatographic purification. Compound **5** was prepared in good yield by the DCC/HOBT mediated coupling reaction between amino pyrrol and 1-methylpyrrole-2-carboxylic acid. After the saponification and the neutralization, acid **6** was obtained, which was coupled in the presence of DCC/HOBT, with the amino pyrrol formed *in situ* from **7a**, **7b** to give **9a**, **9b**. Finally, using the same coupling method, compounds **10a**, **10b** were converted to **11** and **12**, respectively (Scheme 1). The structures of **11** and **12**[11] were confirmed by IR, NMR and HRMS.

CD[12] provides means for detecting and characterizing the DNA binding of the analogs **11** and **12**. As an example, Fig. 1 shows the CD spectra by incremental titration of **12** into a solution of calf thymus DNA (pH 7.4, buffer: KH_2PO_4-NaOH (100 mmol·L^{-1}), EDTA (1 mmol·L^{-1})) at room temperature. Neither the distamycin analog sole (not shown) nor the DNA sole (Fig. 1) exhibits CD signals in 300—380 nm wavelength region. However, upon addition of **12** to a solution of the DNA, a substantial CD signal ([θ]) in 300—380 nm

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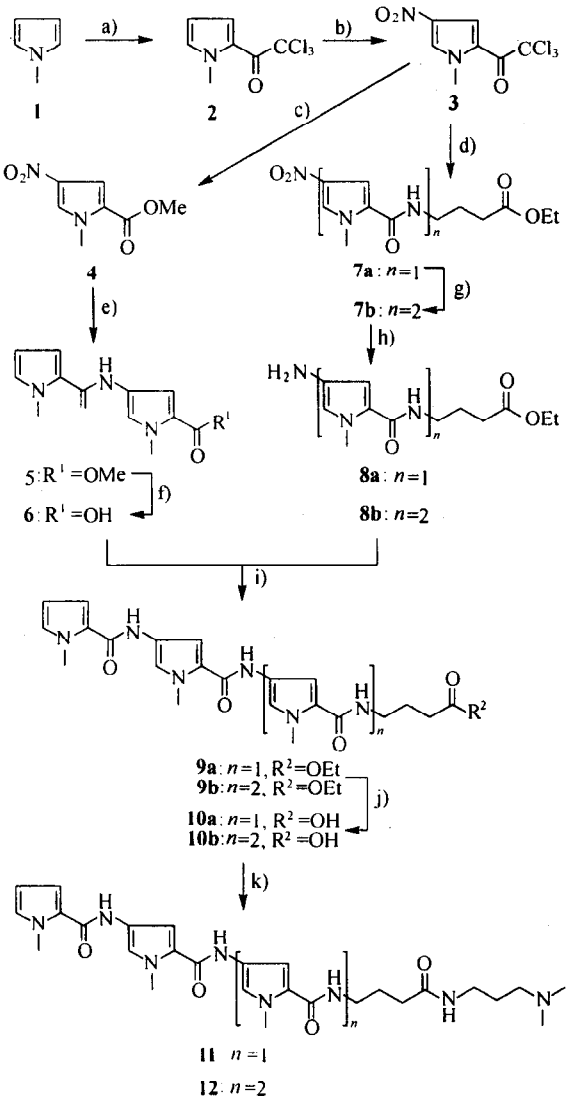
appeared, which is indicative of the interactions between **12** and the DNA duplex. Further inspection of the CD spectrum shows that the maximum CD signal in this region is 6.0×10^2 deg·M^{-1}·cm^{-1} for **12** and 0.5×10^2 deg ·$^{-1}$· cm -1 for **11**. This difference of the maximum CD signal indicated that the binding affinity of **12** to calf thymus DNA is higher than that of **11**.

Scheme 1



Reagents: a) CCl_3COCl, Et_2O; b) HNO_3, H_2SO_4, Ac_2O; c) MeOH,. NaH; d) Ethyl γ-aminobutyric acid, EtOAc; e) I. H_2, Pd/C, DMF; II. 1-methylpyrrole-2-carboxylic acid, DCC/HOBT, DMF; f) I. NaOH, EtOH/H_2O; II. 6 mol/L HCl; g) I. H_2, Pd/C, EtOAc; II. **3**, EtOAc; h) H_2, Pd/C, DMF; i) DCC/HOBT, DMF; j) I. NaOH, EtOH/H_2O; II. 6 mol/L HCl; k) *N*, *N*-dimethylaminopropylamine, DCC/HOBT, DMF/*N*-methyl-2-pyrrolidinone.

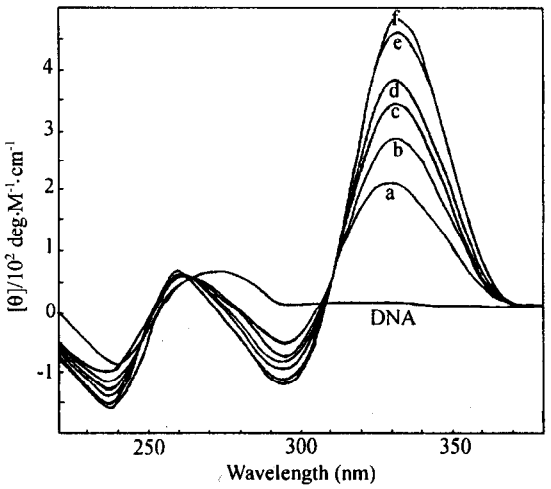


Fig. 1 CD titration of calf thymus DNA with the analog **12**. [DNA (4.0×10^{-5} mol·L^{-1}), analog **12** [a (0.54×10^{-5} mol·L^{-1}), b (1.1×10^{-5} mol·L^{-1}), c (1.6×10^{-5} mol·L^{-1}), d (2.2×10^{-5} mol·L^{-1}), e (2.7×10^{-5} mol·L^{-1}), f (3.2×10^{-5} mol·L^{-1})]. Molar ellipticities, [θ], are in units of deg·M^{-1}·cm^{-1}.

In order to provide a quantitative explanation for above observations, the molar enthalpy changes of **11** and **12** when binding to calf thymus DNA were measured on ITC. The values of the molar enthalpy change are -3.4 ± 0.4 kJ/mol for **11** and -15.5 ± 0.8 kJ/mol for **12**. These thermodynamic data indicate that **11** and **12** can form hydrogen bonds with calf thymus DNA, and the strength of the interaction between the synthetic compounds and DNA was related to the number of the hydrogen bonds. The protons at amides, which took part in the formation of hydrogen bonds with the acceptors on the base pair of DNA, increase the binding affinity; the aromatic protons, which were buried deeply in the minor groove to interact with the wall of the minor groove though extensive Van der Waals forces, also enhance the binding affinity.

In conclusion, these experimental results have showed that the designed **11** and **12** are a kind of low molecular weight DNA-binding molecule and can efficiently binding to DNA duplex.

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11 The spectral data of **11** are: IR υ: 3302, 2941,1640, 1541, 1466, 1438,1415, 1255, 1207, 1114, 1063, 740 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 10.85 (s, 1H), 8.97 (s, 1H), 8.88 (s, 1H), 8.15 (s, 1H), 7.40 (s, 1H), 7.34 (s, 1H), 7.06 (s, 1H), 6.97 (s, 1H), 6.91 (s, 1H), 6.71 (s, 1H), 6.05 (s, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 3.80 (s, 3H), 3.36—3.29 (m, 4H), 3.03 (s, 2H), 2.70 (s, 6H), 2.45—2.37 (m, 4H), 1.95—1.90 (m, 2H), 1.28—1.25 (m, 2H). HRMS $(C_{27}H_{39}N_8O_4)^+$ Calcd *m/z*: 539.3089 (M+H). Found *m/z*: 539.3072. The spectral data of **12** are: IR υ: 3290, 2936, 1642, 1538, 1466, 1435, 1406, 1254, 1204, 1109, 1061, 739 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 8.90 (s, 2H), 8.51 (s, 1H), 7.52 (s, 1H), 7.31 (s, 1H), 7.27 (s, 2H), 7.16 (s, 1H), 6.91 (s, 1H), 6.84 (s, 1H), 6.73 (s, 1H), 6.68 (s, 2H), 6.03 (s, 1H), 3.92 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 3.73 (s, 3H), 3.38—3.24 (m, 2H), 3.21—3.10 (m, 2H), 3.08—2.90 (m, 2H), 2.40—2.28 (m, 2H), 2.19 (s, 6H), 1.85—1.72 (m, 2H), 1.62—1.50 (m, 2H). HRMS $(C_{33}H_{45}N_{10}O_5)^+$ Calcd *m/z*: 661.3569 (M+H). Found *m/z*: 661.3590 (M+H).

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