Synthesis of novel DNA binding agents: indole-containing analogues of bis-netropsin

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J. Chem. Research (S), 2000, 264–265
J. Chem. Research (M), 2000, 0751–0770

Molecular modelling studies showed that indole dicarboxylic acids are potential linkers for the synthesis of bis-netropsin analogues with a good fit to the minor groove of DNA. To test this hypothesis, 2-carboxyindole-6-acetic acid, indole-2,6-dicarboxylic acid, 6-(2-carboxyethyl)indole-2-carboxylic acid, 6-(2-carboxy-1-ethenyl)indole-2-carboxylic acid were prepared and coupled to 3-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]dimethylaminopropane. Similarly indole-2,5-dicarboxylic acid was prepared and coupled to 3-[1-methyl-4-(1-methyl-4-aminopyrrple-2-carboxamido)pyrrole-2-carboxamido]propionamidine hydrochloride. The derivatives of **26–28** showed especially strong binding to AT rich regions as shown by footprinting studies.

Oligomers of 3-amino-N-methyl-5-carboxylic acid such as the natural products distamycin and netropsin have cytotoxic properties because of their ability to bind to AT rich regions of the minor groove of DNA. In order to extend the available reading frame, several groups have prepared head to head linked dimers, known as bis-netropsins.[1–4] Detailed molecular modelling studies[7] showed that indole dicarboxylic acids would be good linking molecules. The selection of compounds for synthesis was made to include two substitution patterns and variation in length and rigidity of one of the side chains. This paper describes the synthesis of four new bis-netropsins of this type[9–11] the structures of which are shown in Fig. 1.

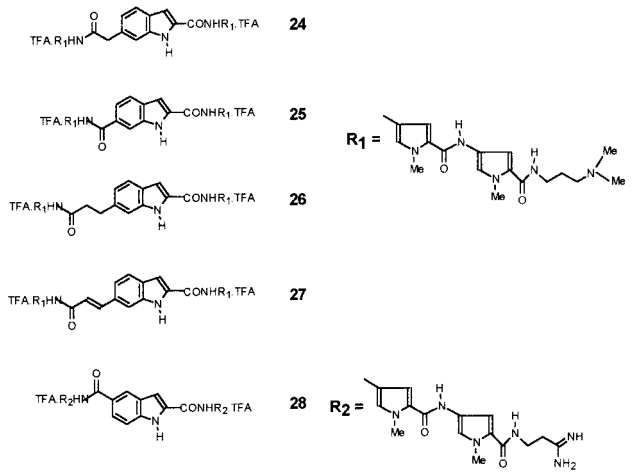
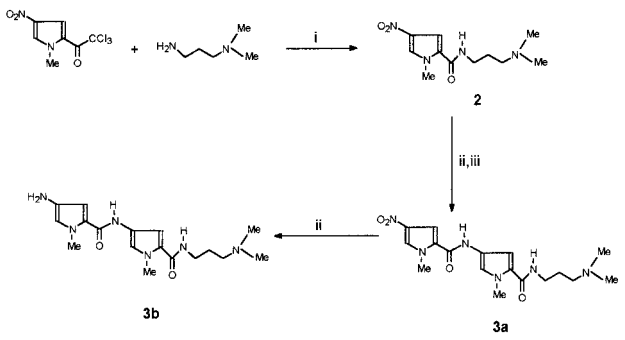


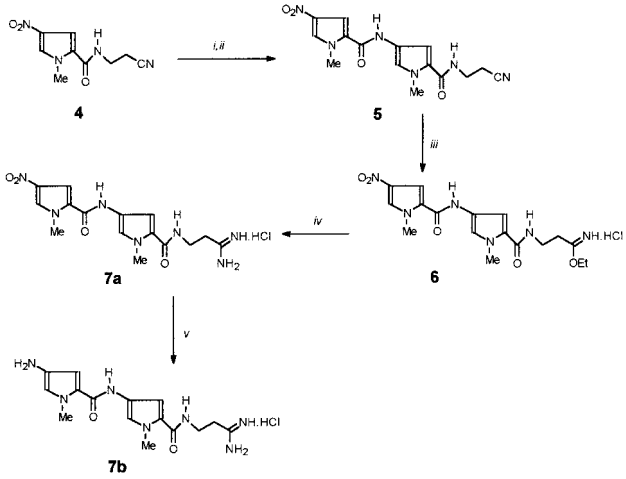
Fig. 1 Indole-linked head to head netropsin analogues prepared in this work

The pyrrole oligomers (R_1 and R_2, Fig. 1) were prepared by adaptations of published methods.[12–15] The indole dicarboxylic acids were obtained from side-chain modification of products of Fischer indole synthesis as shown in Schemes 3 and 4.

3a (Scheme 1) was hydrogenated in the presence of palladised charcoal at room temperature and atmospheric pressure to give the amine **3b** which was both air and light sensitive. Therefore this amine was used in the next reaction without further purification. 2-Carboxyindole-6-acetic acid (**12**) was treated with the amine (**3b**) using DCC as a coupling agent and DMAP as a catalyst. After purification by reverse phase HPLC the fractions containing the required material were freeze-dried immediately. The desired product **24** was obtained in a poor yield (13%) therefore an alternative coupling agent was sought. HBTU was generally found to be superior to DCC in this series, as shown by the following reactions. Coupling of the amine **3b** with the indole **20** gave rise to **25** in 52% yield, while coupling of the dicarboxylic acid **18**



Scheme 1 *Reagents: i* $CHCl_3$, RT.; *ii* N-methyl-4-2-trichloroacetylpyrrole, $CHCl_3$



Scheme 2 *Reagents: i* Pd–C, H2, DME, 40°C, *ii* 4-nitro-N-methylpyrrole-2carbonyl chloride, Et_3N, DME, *iii* EtOH (dry), HCl (g); *iv* NH_3 (dry), EtOH; Pd-C, H_2, EtOH, 60°C.

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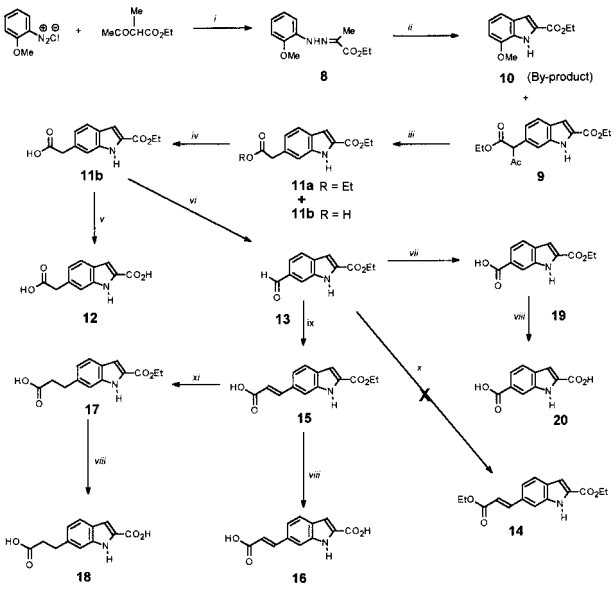

Scheme 3 *Reagents: i* NaOAc, H_2O, EtOH, 5–7°C; *ii* $CH_3COCH_2CO_2Et$, TsOH, benzene; *iii* NaOH, EtOH, reflux; *iv* HCl in HOAc; *v* 1. KOH, EtOH, 2. HCl; *vi* pyridine *N*-oxide. Ac_2O; *vii* $KMnO_4$, actone, H_2O, 50°C; *viii* KOH, EtOH; *ix* pyridine, piperidine, malonic acid, 50°C; *x* (carbethoxymethyl)triphenylphosphonium bromide, *n*-BuLi; *xi* Pd-C, H_2.

produced **26** in 61% yield. Coupling of **16** gave **27** in 61% yield also. Compounds **24–27** were obtained as bis-TFA salts. Similar chemistry was used with the nitropyrrole **7a** (Scheme 2) to obtain **28**, after purification by HPLC, in 29% yield as a bis-TFA salt with no distinct melting point. In this case, the DCC route was superior.

As described elsewhere, several of these indole-containing netropsin analogues showed high affinity for AT rich regions of DNA. Compound **24** and compounds **26–28** all bound particularly strongly as shown by footprinting studies at 1 to 3 μM concentrations in the presence of similar concentrations of DNA. Compound **25**, however, was a poor ligand.[10] This result was not predicted by the design calculations[7,10] and might be explained by a different conformation that does not fit the double helix well.

Received 15 February 2000; accepted 4 April 2000
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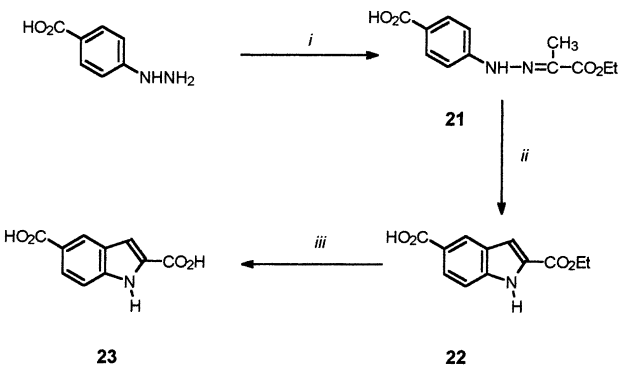

Scheme 4 Reagents: i CH_3COCO_2Et, HOAc, H2O; *ii* $ZnCl_2$ heat; *iii* 1. KOH, aq. EtOH; 2. HCl.

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