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An Fmoc Solid-Phase Approach to Linear Polypyrrole-Peptide Conjugates

Eugenio Vázquez, Ana M. Caamaño, Luis Castedo and José L. Mascareñas*

Departamento de Química Orgánica y Unidad Asociada al CSIC, Universidad de Santiago de Compostela, 15706 Santiago de Compostela, Spain.

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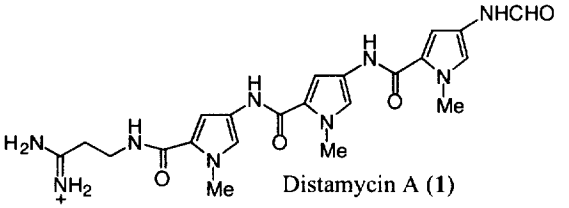
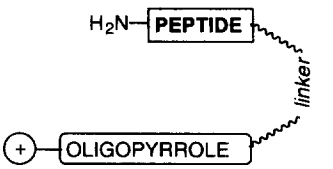
Abstract: A practical method for the synthesis of medium-sized peptides incorporating several N-methylpyrrole units is described. These peptides might prove useful as novel sequence-specific DNA binders.

Keywords: oligopyrrole; distamycin; bZIP proteins; solid-phase peptide synthesis.

Sequence-specific DNA-binding proteins play a central role in regulating DNA metabolism and gene expression in all organisms [1]. The growing amount of high-resolution structural data on protein-DNA complexes shows that the aminoacid residues that directly contact the DNA are often located in quite a short stretch of the protein sequence [2], however, the isolation of these regions from the rest of the protein suppresses their ability for sequence-specific recognition. Prominent examples are the bZIP family of transcription factors, which contact the DNA major groove exclusively in their basic domain, but cannot execute the folding-binding process without the assistance of a leucine zipper dimerizing unit [3]. It has recently been shown that covalent tethering of just one of the basic regions of a bZIP protein (GCN4) to an appropriate DNA site allows tight, specific binding [4], which suggests that this relatively short peptide may suffice for sequence-specific DNA recognition if it could be appropriately delivered near its consensus DNA site [5]. Therefore, it was envisioned that linking such domain to a molecule that binds with moderate-to-good affinity to adjacent DNA sequences could elicit a reasonable binding capability. Awareness of the existence of DNA-binding proteins that use both major and minor groove recognition segments [6], led us to focus our initial efforts on the preparation of covalent conjugates between the GCN4 basic region and oligopyrroles related to distamycin A (**1**), a well-known antibiotic that recognizes the minor groove of AT-rich sequences [7,8]. Here we report a rapid, practical Fmoc-based solid-phase approach to linear hybrids of the type of **2**, in which the N-terminus of the oligopyrrole is linked to the peptide C-terminus.



Distamycin A (**1**)



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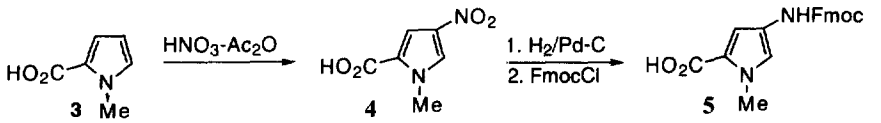
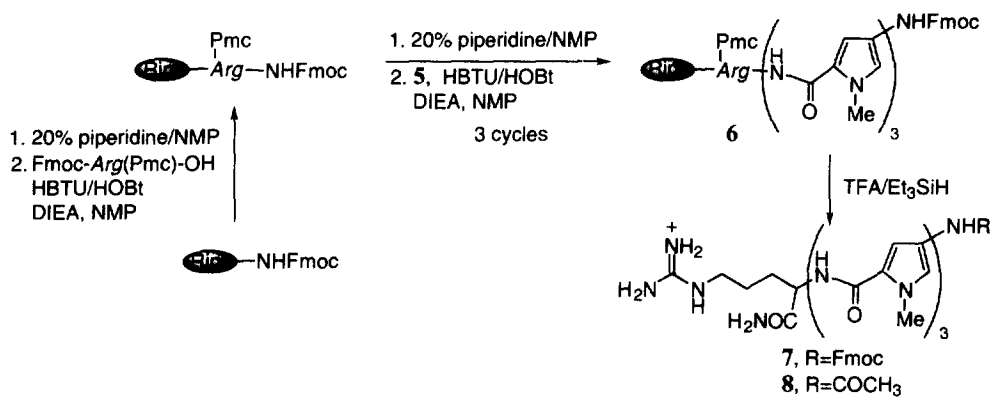
The oligopeptide nature of distamycin has recently inspired a truly practical synthetic approach to N-methylpyrrole polyamides based on the use of standard Boc solid-phase peptide chemistry [9a]. A major advantage of this method derives from the possibility of preparing the required Boc-aminopyrrole monomer in multigram amounts, albeit using a procedure involving seven steps. We found that the homologous Fmoc unit (**5**) can be synthesized in only two operational steps and 23% overall yield starting from commercially available N-methylpyrrolecarboxylic acid (**3**). The nitration step was accomplished by a slight modification of previously described procedures [10]. The transformation of **4** into the desired Fmoc derivative **5** was carried out in a one-pot procedure: hydrogenation under a slightly positive pressure of H_2 (5% Pd/C, 1M aqueous Na_2CO_3), removal of the catalyst by filtration through celite, with dioxane washings, and vigorous stirring of the filtrate with FmocCl (1.2 equiv) [11].



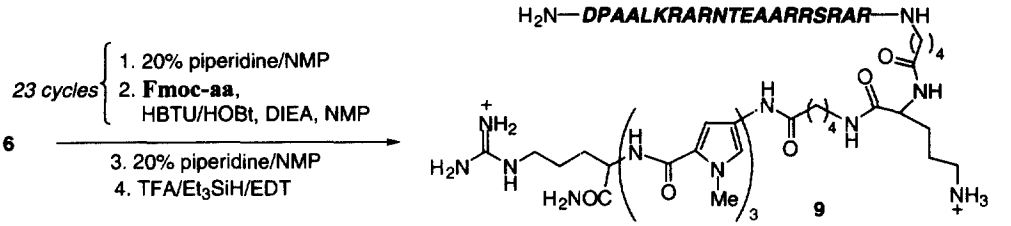
Scheme 1

With **5** in hand, implementation of an Fmoc solid-phase synthesis of targets like **2** would first require the introduction of a positively charged carboxy terminal tail that could mimic the amidinium group of distamycin. In most synthetic distamycin analogs, this function is carried out by a dimethylamino group [7b,12], but we envisaged that the amidinium might be better imitated by the guanidinium side chain of arginine, with the concurrent advantage of an easy incorporation of this aminoacid under solid-phase conditions. The synthesis of the oligopyrrole was carried out on a Rink-MBHA amide resin (0.55 mmol/g) using standard piperidine deprotection and HBTU coupling protocols (Scheme **2**) [13]. Qualitative ninhydrin tests after each cycle revealed that coupling of the Fmoc-pyrrole aminoacid **5** requires longer reaction times than common aminoacids [14]. After the assembly of the resin-linked tetrapeptide **6**, cleavage from the solid support and deprotection of the arginine side-chain was accomplished by treatment of the resin with a 9:1 mixture of TFA/Et_3SiH. Subsequent removal of the TFA *in vacuo* afforded a product that was purified by RP-HPLC [15] and shown by FAB-MS to be the Fmoc derivative **7** (approx. 46 % recovery) [16]. Alternatively, Fmoc removal and standard acetylation of the resulting amine (Ac_2O, DMAP, DIEA, NMP) provided, after deprotection/cleavage and purification, the N-acetylated tripyrrole **8** [16]. Preliminary studies indicate that **8** shares the AT-rich binding characteristics of distamycin, so it may prove to be an immediately available distamycin surrogate [17].



Scheme 2

Synthesis of the target hybrid peptide required incorporating the necessary Fmoc-amino-acids to the solid-supported tripyrrole **6**. Preliminary molecular modeling based on structural information from X-Ray data for GCN4 [18] and distamycin A [19] bound to their respective consensus DNA sequences, suggested that a link between the C-terminal Arg-245 of GCN4 and the N-terminus of the tripyrrole, consisting of one ornithine and two aminovaleric units, might be appropriate to span across the DNA phosphate backbone. This target hybrid (**9**) was successfully assembled from **6** using standard solid-phase Fmoc/tBu chemistry [20, 21].



Scheme 3

In summary, we have developed a practical method for the rapid synthesis of oligopyrrole-peptide conjugates that may lead to the discovery of novel sequence-specific DNA binders. Details on DNA-binding by **9** and related derivatives will be reported in due course [22].

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Abbreviations used:
Fmoc: 9-fluorenylmethoxycarbonyl; Boc: *t*-butyloxycarbonyl; MBHA: 4-methylbenzhydrylamine; HBTU: 2-(1H-benzotriazol-1-yl)-oxy-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt: hydroxybenzotriazole; DIEA: N,N-diisopropylethylamine; NMP: N-methylpyrrolidone; TFA: trifluoracetic acid; EDT: ethanedithiol; DMAP: N,N-dimethylaminopyridine; Pmc: 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Trt: triphenylmethyl.

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[11] After stirring overnight at room temperature the reaction mixture was poured into 1:1 1M aqueous Na_2CO_3/Et_2O solution. The aqueous phase was removed, carefully acidified with HCl, and extracted with EtOAc. Standard drying and concentration of the combined organic extracts gave a residue which crystallized from hexanes-dioxane (62 % yield, white solid, mp 200-202 °C). ^1H NMR (d_6-acetone, 300 MHz) δ 8.65 (s, 1H), 7.83 (d, 2H, J = 4 Hz), 7.6 3 (d, 2H, J = 4 Hz), 7.37 (m, 4H), 7.17 (s, 1H), 6.7 (s, 1H), 4.47 (d, 2H, J = 5 Hz), 4.26 (t, 1H, J = 5 Hz), 3.8 (s, 3H). HRMS (FAB): m/z calcd for $C_{21}H_{18}O_4N_2$: 363.1294, found 363.1305.

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[14] Protocol: After conventional anchoring of the first arginine and subsequent removal of its Fmoc group, the resin was successively washed with NMP, i-PrOH and NMP. DIEA (2M in DMF, 0.56 mmol) was added to a solution of **5** (0.6 mmol) and HBTU/HOBt (1:1, 0.6 mmol) in NMP (3 mL). After 2 min of activation, this mixture was added to the resin bearing the free amine group (0.2 mmol). The suspension was shaken for three hours, and the resin washed thoroughly with NMP and CH_2Cl_2 and dried under vacuum.

[15] The tripyrroles **7** and **8** were purified using a semipreparative RP C-18 column, eluting at a flow rate of 3 mL/min using a linear gradient of buffer B (0.1 % TFA/CH_3CN) in buffer A (0.1 % TFA/H_2O), from 5% to 95% over 15 min, with UV detection at 303 nm.

[16] **7**: FAB-MS m/z (relative intensity) 762 (M+1, 30), 563 (7), 345 (9), 282 (100); HRMS (FAB): m/z calcd. for $C_{39}H_{44}O_6N_{11}$: 762.3480, found 762.3487. **8**: HRMS (FAB): m/z calcd. for $C_{26}H_{36}O_5N_{11}$: 582.2901, found 582.2904.

[17] These studies are being carried out using circular dichroism techniques and will be reported in due course.

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[20] The Fmoc-aminoacids had the following protecting groups: Arg(Pmc), Asp(O*t*Bu), Cys(Trt), Glu(O*t*Bu), Lys(*t*-Boc), Ser(*t*Bu), Thr(*t*Bu).

[21] The tripyrrole oligopeptide **9** was isolated after the cleavage-deprotection step (0.8mL TFA-0.5 mL Et_3SiH-1.5 mL EDT/100 mg resin) by cold ethylether precipitation. Purification was carried out in an analytical RP C-18 column, eluting at a flow rate of 1 mL/min using a linear gradient of buffer B (0.1 % TFA/CH_3CN) in buffer A (0.1 % TFA/H_2O), from 5% to 95% over 30 min, with UV detection at 303 nm (approx. 16% overall recovery). The identity of **9** was corroborated by FAB-MS analysis (MW calcd for $[M+H]^+$ $C_{130}H_{224}O_{34}N_{55}$: 3099.74; found 3098.9, and ES-MS analysis: $[M+H^+]$ m/z = 3100.18.

[22] Preliminary circular dichroism studies of binding to the DNA fragment ds-(5'-AGGATTTTATGACGTTCG-3') suggest that compound **9** binds only through the minor groove counterpart.