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A Practical Approach to Orthogonally connected Oligopyrrole-Peptide Conjugates

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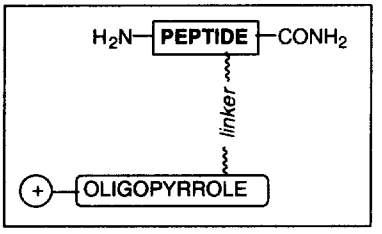
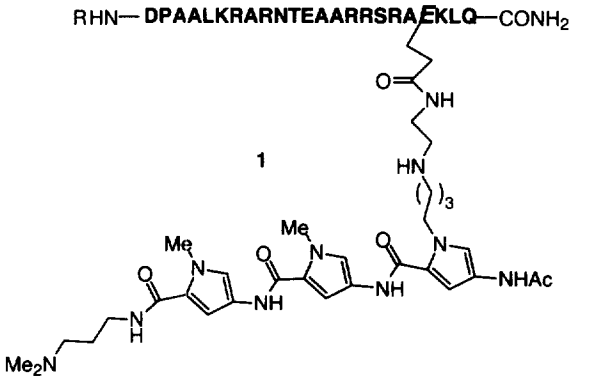
Abstract: A practical method for synthesizing orthogonally connected peptide-oligopyrrole conjugates by coupling the oligopyrrole fragment to a selectively deprotected glutamic acid side-chain of a resin-bound peptide is described. © 1999 Elsevier Science Ltd. All rights reserved.

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

In our preceeding communication [1] we described a solid-phase approach to N-methyloligopyrrole-peptide conjugates connected via the N-terminus of the oligopyrrole and the C-terminus of the basic region of GCN4, a bZIP protein that binds specific sequences of DNA as a non covalent dimer. It is our expectation that this type of hybrid, in which the pyrrole-peptide link is designed to crossover the phosphate backbone, may allow sequence-specific DNA recognition thanks to the cooperative action of their minor and major groove binding moieties [2]. Since the well-known sandwich-type fit of distamycin in the minor groove places its N-methyl units just outside the groove, we reasoned that these positions might be specially appropriate for linking the major groove binding peptide [3]. We therefore undertook the construction of orthogonally connected oligopyrrole-peptide conjugates in which a linker bridges a pyrrole nitrogen and a suitable side-chain of the basic region of GCN4. By superimposing the X-ray structures of GCN4 [4] and distamycin A [5] bound to their respective DNA sites, we concluded that a suitable target would be the hybrid **1**, that incorporates a linkage between the nitrogen of the N-terminal pyrrole and the side chain of a glutamic acid replacing the arginine 245 present in the basic region of the natural protein.





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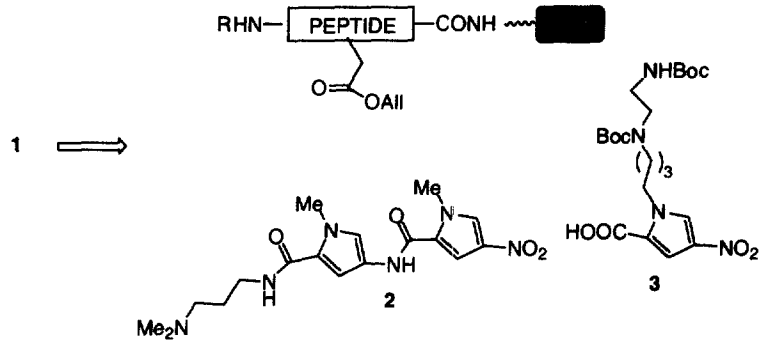
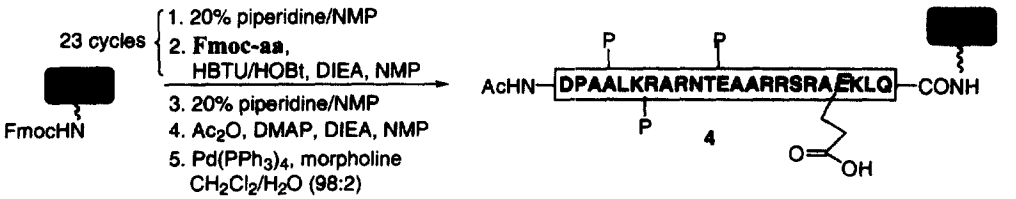
We reasoned that hybrids like **1** could be rapidly approached by orderly connection of the pieces shown in Scheme 1, with the more challenging step being the attachment of the oligopyrrole to the resin-linked peptide, that must therefore bear an orthogonal protecting group at the side-chain of the glutamic acid residue.



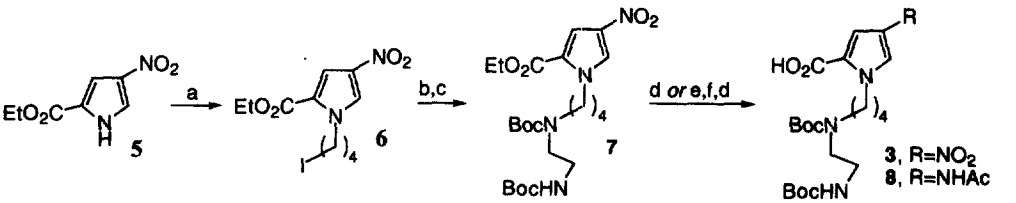
Scheme 1

The synthesis of the peptide was carried out using Fmoc/tBu chemistry on a Rink-MBHA amide resin (0.55 mmol/g), under typical piperidine deprotection and HBTU coupling protocols [6]. All Fmoc-aminoacids were introduced with the usual side-chain protecting groups [7], except the glutamic acid at position 4, which was protected as allyl to allow subsequent selective deprotection with the peptide still attached to the resin. The identity of the peptide was corroborated by deprotection/cleavage of samples before and after allyl deprotection [8].



Scheme 2

The synthesis of the N-alkylnitropyrrole **3** was carried out as indicated in Scheme 3. Alkylation of the known nitropyrrole **5** [9] with 1,4-diiodobutane under basic conditions was easily controlled to give iodide **6** in 81% yield. Coupling of this iodide with Boc-ethylenediamine and protection of the secondary amine [10] gave **7** in 58% yield. Hydrolysis of the ester led to the required nitroacid **3** in almost quantitative yield [11].

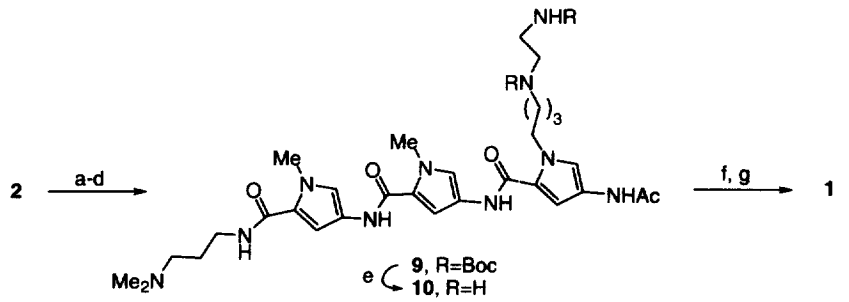


(a) I-$(CH_2)_2$-I, K_2CO_3, acetone, reflux; (b) $NH_2(CH_2)_2NHBoc$, K_2CO_3, CH_3CN; (c) $(Boc)_2O$, K_2CO_3, dioxane; (d) NaOH, EtOH, H_2O; (e) H_2, Pd/C, MeOH; (f) Ac_2O, Et_3N, DMF.

Scheme 3

Alternatively, the N-acetyl derivative **8** can be prepared by hydrogenation of **7** followed by acetylation and ester hydrolysis (65% for the three steps). Dipyrrole **2** was prepared from N-methylpyrrolecarboxylic acid following a recently described procedure [12]. Attempts to prepare tripyrrole **9** by hydrogenation of the nitrodipyrrole **2** and subsequent stirring of a DMF solution of the resulting unstable amine with the acid **8** in the presence of base and a coupling agent such as HBTU, EDC or DECP, all failed to give detectable amounts of the desired product. Remarkably, the coupling reaction did proceed efficiently using the electron-poor nitropyrrole **3** instead of the N-acetyl derivative **8**. Coupling of units **2** and **3** was best achieved (81% yield) by hydrogenation of **2** to the amine, filtration and vacuum removal of the MeOH, and stirring of a DMF solution of the residue with DECP (1.2 equiv), Et_3N (6 equiv) and acid **3** (1.5 equiv). Homologation of the terminal nitro group to the required acetamide, was then achieved by hydrogenation (which required a longer time than for **2**) followed by N-acetylation (68% yield) [13].

After removal of the Boc protecting groups, attachment of the diamine **9** to the resin-linked peptide was carried out under standard solid-phase amide formation conditions, using HATU as the coupling agent. RP-HPLC monitoring of the reaction, which required the cleavage/deprotection/precipitation of resin aliquots, revealed the appearance of a late-eluting compound that was identified as the desired hybrid **1** (50% conversion after 12h at rt) [14].



(a) H_2 (balloon pressure), Pd/C, MeOH, 2 h; (b) DECP, DMF, Et_3N, **3**; (c) H_2 (balloon pressure), Pd/C, MeOH, 12 h; (d) Ac_2O, Et_3N, DMF; (e) TFA, CH_2Cl_2; (f) HATU, DIEA, DMF, **4**; (g) TFA, EDT, thioanisole.

Scheme 4

Summing up, we have developed a synthetic approach to orthogonally connected N-methyloligopyrrole-peptide conjugates which should prove flexible enough to obtain a variety of derivatives with modified linkers and/or binding partners. It is our expectation that some of these compounds may reproduce the DNA binding properties of natural proteins.

Acknowledgements. Funding of the Xunta de Galicia and the Spanish Ministry of Education and Culture under the grants XUGA20907A96 and PB97-0524, respectively, is acknowledged. E.V. and A.M.C. thank the Universidad of Santiago de Compostela and The Xunta de Galicia for their respective fellowships. D.G. thanks the Roche Foundation and the Universidad de Santiago for a fellowship.

Abbreviations used:

Fmoc: 9-fluorenylmethoxycarbonyl; Boc: *t*-butyloxycarbonyl; 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; DECP: diethyl cianophosphonate; HATU: 2-(1H-azabenzotriazol-1-yl)-oxy-1,1,3,3-tetramethyluronium hexafluorophosphate.

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[2] Several proteins use major groove and minor groove binding motifs, see for instance: (a) Klemm JD, Rould MA, Aurora R, Herr W, Pabo CO Cell 1994; 77:21-32. (b) Feng JA, Johnson RC, Dickerson RE Science 1994; 263: 348-355. (c) Lo ML, Ha S, Pelczer I, Pal S, Walker S. Proc. Nat. Acad. Sci. USA 1998; 95:8455-8460.
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[7] The aminoacids were incorporated with 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).

[8] An aliquot of the N-acetylated resin (before removal of the allyl protecting group of the glutamic acid) was treated for 4h at rt with the cocktail TFA/anisole/thioanisole/EDT (9:0.2:0.5:0.3, 1 mL/100 mg resin), precipitated with cold diethyl-ether and purified by RP-HPLC using an analytical RP C-18 column, eluting at a flow rate of 1 mL/min with a linear gradient of buffer B (0.1 % TFA/CH_3CN) in buffer A (0.1 % TFA/H_2O), from 5% to 95% over 20 min, with UV detection at 220 nm. The resulting peptide was identified by ES-MS analysis: $[M+H^+]$ m/z = 2690.6, calcd: 2689.5. Application of the same protocol after the palladium-promoted removal of the allyl protecting group gave the fully deprotected peptide, ES-MS $[M+H^+]$ m/z = 2650.0, calcd: 2649.4.

[9] The nitropyrrole **5** was prepared by known procedures: Hale WJ, Hoyt WV J. Am. Chem. Soc. 1915; 37: 2538-2582.

[10] We preferred the protection of the secondary amine in order to avoid side reactions. Preliminary experiments involved protection as alloc, but we later found that this group is incompatible with the nitro reduction conditions.

[11] **3**, ^1H NMR ($CDCl_3$, 250 MHz) δ 7.64 (s, 1H), 7.35 (s, 1H), 4.33 (t, 2H, J = 6.9 Hz), 3.19 (m, 6H), 1.72-1.68 (m, 2H), 1.49-1.36 (m, 2H), 1.32 (s, 18H). FAB-MS: m/z 397.15 (M-OtBu).

[12] He GX, Browne KA, Groppe JC, Blaskó A, Mei HY, Bruice TC J. Am. Chem. Soc. 1993; 115: 7061- 7071.

[13] **9**, ^1H NMR ($CDCl_3$, 250 MHz) δ 8.9 (br s, 1H), 8.81 (br s, 1H), 8.21 (br s, 1H), 7.52 (br s, 1H), 7.06-7.20 (m, 3H), 6.51-6.70 (m, 3H), 4.16 (br s, 2H), 3.75 (s, 6H), 3.26 (br s, 2H), 3.01-3.18 (m, 6H), 2.21 (t, 2H, J = 6.1 Hz), 2.16 (s, 6H), 2.00 (s, 3H), 1.64 (m, 4H), 1.34 (s, 20H). ^1H NMR (CD_3OD, 250 MHz) δ 7.86 (br s, 1H), 7.80 (s, 1H), 7.31 (s, 1H), 7.15 (br s, 1H), 7.08 (br s, 1H), 6.87 (br s, 1H), 6.79 (br s, 1H), 4.37 (t, 2H, J = 7 Hz), 3.89 (s, 3H), 3.81 (s, 3H), 3.31 (t, 2H, J = 6.3 Hz), 3.21 (s, 3H), 3.20-3.01 (m, 8H), 2.81 (s, 6H), 1.90 (m, 2H), 1.63 (m, 2H), 1.41 (m, 2H), 1.31 (s, 18H). FAB-MS: m/z 810.47 (M+1).

[14] An aliquot of the resin was treated with the cleaving cocktail defined in ref. 8, precipitated with cold diethyl-ether and subjected to semipreparative RP-HPLC (UV detection at 304 nm) to afford as majority product the desired hybrid **1**: ES-MS $[M+H^+]$ m/z = 3242.3, calcd: 3241.8. Preliminary circular dichroism studies of binding to the DNA fragment ds-(5'-AGGATTTTATGACGTTCG-3') suggest that hybrid **1** binds weakly, and apparently only via its the minor groove counterpart. Detailed studies will be reported in due course.