Synthesis of DNA-binding heteroaromatic oligoamides on liquid solid support

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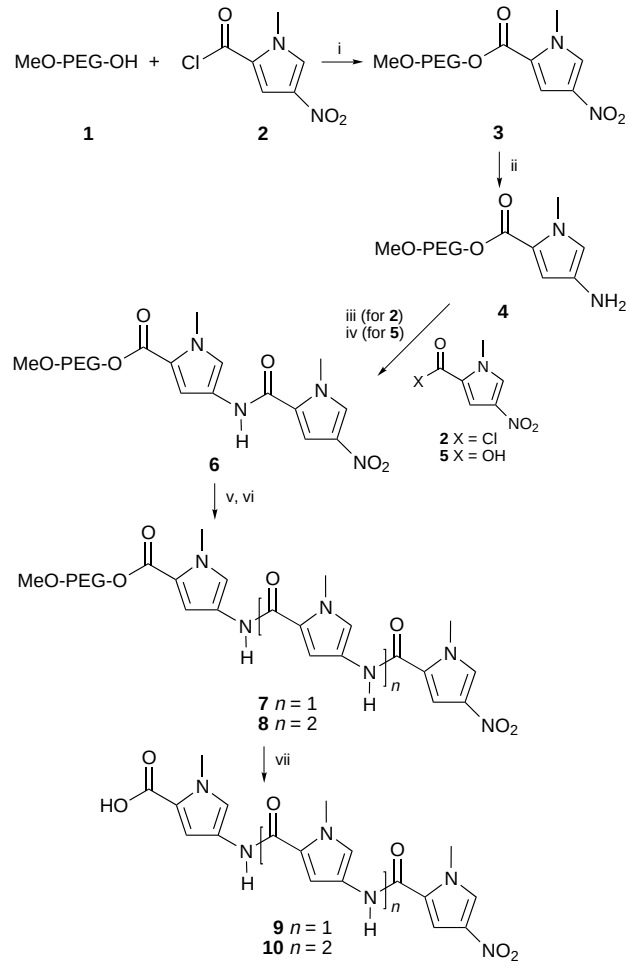
Heteroaromatic oligoamides as building blocks for the synthesis of sequence specific DNA binding molecules are obtained from nitro carboxylic acids on polyethylene glycol without the use of protecting groups

Mimicking the sequence specific DNA recognition of gene regulation proteins[1] with small molecules is not only an academic challenge: such molecules are highly desirable as regulatory factors in biotechnology or as gene specific drugs.[2] Several sequence-specific DNA-binding compounds[3] have been discovered over the last 10 years, such as triplex-forming oligonucleotids, peptide nucleic acids and minor groove binding heteroaromatic oligoamides.[3b] The stability and simple structure of the latter compounds renders them especially suitable for most applications. However, compared to the synthesis of aliphatic peptides, that of heteroaromatic imidazole/pyrrole oligoamides is difficult: the heteroaromatic amines are unstable and the carboxy group is less reactive in amide formation. To address these problems, Baird and Dervan recently reported a solid phase synthesis protocol for the synthesis of even complex hairpin oligoamides.[4] Unfortunately, the employed solid support together with the required protecting groups now make the synthesis of larger quantities of heteroaromatic oligoamides an expensive venture. In addition the current protocol is restricted to *N*-methylpyrrole and *N*-methylimidazole, so that new building blocks and coupling conditions must be developed for the introduction of each new heterocycle.[5] To remedy this situation we report here a versatile 'protecting group free' synthesis of short heteroaromatic oligoamides on polyethylene glycol 'liquid' solid support.[6]

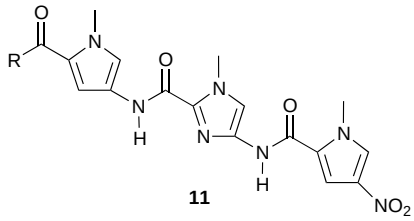
Methoxypoly(ethylene glycol) (MeO-PEG-OH) with an average molecular weight of 5000 g mol^{-1} was used as an inexpensive solid support. By the reaction of 1-methyl-4-nitro-1*H*-pyrrole-2-carbonyl chloride **1** with MeO-PEG-OH under standard conditions the first heterocyclic unit of the peptide synthesis is introduced quantitatively *via* ester linkage (Scheme 1). A sample of 100 g of MeO-PEG-OH can so accommodate *ca.* 2.5 g of the first heterocycle. Using the advantage of a soluble solid support, the nitro group is then reduced with NH_4HCO_2–Pd/C in MeOH over 1 h at room temperature. For work-up the heterogeneous catalyst is removed by filtration and the polymer-bound amine is precipitated by the addition of Et_2O. The solid is dissolved in CH_2Cl_2, rendering it ready for the next coupling step and leaving behind excess NH_4HCO_2. Either the reaction of **2** in the presence of pyridine or the coupling of the corresponding carboxylic acid **5** with DCC–HOBt can be used to introduce the next heterocycle in excellent yield.[7] Repetition of the reduction-coupling cycle gives trimers and tetramers. Finally, treatment with base[8] allows the quantitative cleavage of the oligoamide from the polymer.‡ The so obtained molecules§ are most suitable starting materials for the construction of DNA recognizing structures: solution phase peptide chemistry allows the large scale synthesis of hairpin structures,[9] immobilization leads to new stationary chromatography phases[10] and further functionalization gives DNA markers.[11]

The described procedure allows the large scale synthesis of heteroaromatic oligoamides without the use of protecting groups and chromatography. On top of that it facilitates reaction monitoring when new analogs are prepared. Using available nitro carboxylic acids[12] a variety of carbo- or hetero-cyclic units can be introduced, whereby the successful incorporation is confirmed by NMR spectroscopy.¶ With aromatic nitro isocyanates as building blocks the amide linkage is replaced by a urea moiety. Such peptidomimetic heteroaromatic compounds may also have interesting DNA-binding properties, but this remains to be established. Examples of structures that were prepared by the reported route, including yields, are: **11** (61%), **12** (56%) and **13** (62%).

In conclusion we have shown that heteroaromatic oligoamides can be prepared in a most economical way on MeO-PEG polymer support avoiding the use of protecting groups. The procedure facilitates both large scale synthesis and reaction optimization for known and new heteroaromatic oligoamides. This will make this interesting class of compounds more easily available for the further investigation of specific molecular recognition of biological structures.



Scheme 1 *Reagents and conditions*: i, CH_2Cl_2, Py, quant.; ii, NH_4HCO_2, Pd/C, MeOH, room temp., 1 h; iii, CH_2Cl_2, Py, quant.; iv, DCC, HOBt, quant.; v, NH_4HCO_2, MeOH, Pd/C; vi, DCC, HOBt, 83%; vii, 2 M, NaOH, 50 °C, 6 h, quant.



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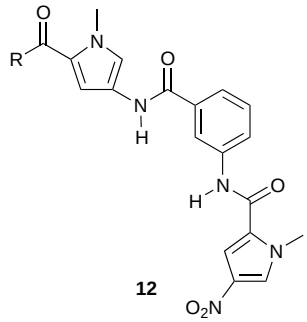

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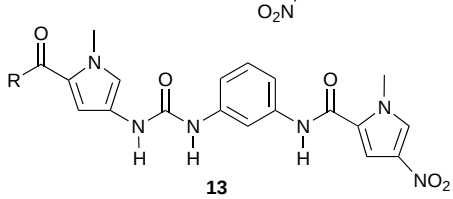

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Notes and References

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‡ *General procedure* for the reduction-coupling cycle on MeO–PEG-support: A mixture of **3** (5 g, 1 mmol), Pd/C (10%, 100 mg, 9 mol%) and NH_4HCO_2 (1 g, 16 mmol) in MeOH (50 ml) was stirred for 1 h at room temp. The catalyst was removed by filtration, Et_2O (400 ml) was added to the solution and the precipitate was collected by filtration. The white solid was dissolved in CH_2Cl_2 (25 ml), leaving behind excess NH_4HCO_2 that was filtered of. Either **2** (570 mg, 3.3 mmol) and pyridine (1 ml) or a solution of **5** (560 mg, 3.3 mmol), $HOBt·H_2O$ (510 mg, 3.3 mmol) and DCC (680 mg, 3.3 mmol) in DMF (25 ml) were added and the mixture was stirred for 12 h. The reaction mixture was filtered, the polymer-bound product was precipitated by addition of Et_2O (400 ml) and collected by filtration. The crude product was redissolved, precipitated twice and dried *in vacuo*.

§ *Selected data* for **9**: δ_H(400 MHz, [$^2H_6$]DMSO) 3.82 (s, 3 H), 3.86 (s, 3 H), 3.96 (s, 3 H), 6.85 (d, ^4J 1.9, 1 H), 7.05 (d, ^4J 1.8, 1 H), 7.26 (d, ^4J 1.6, 1 H), 7.42 (d, ^4J 1.8, 1 H), 7.59 (d, ^4J 1.9, 1 H), 8.18 (d, ^4J 1.8, 1 H), 9.94 (s, 1 H), 10.28 (s, 1 H); 12.16 (s, 1 H); δ_C(100 MHz, [$^2H_6$]DMSO) 36.1 (+), 36.2 (+), 37.5 (+), 104.6 (+), 107.6 (+), 108.4 (+), 118.7 (+), 119.6 (C_{quart}), 120.3 (+), 121.5 (C_{quart}), 122.6 (C_{quart}), 122.9 (C_{quart}), 126.3 (C_{quart}), 128.3 (+), 133.8 (C_{quart}), 156.9 (C_{quart}), 158.3 (C_{quart}), 162.0 (C_{quart}); *m*/*z* (70 eV) 370 (74%) [$M^+ - CO_2$], 275 (100).

¶ For NMR analysis, 100 mg of the polymer bound product were dissolved in 0.5 ml of $CDCl_3$. The terminal methoxy group of MeO-PEG was used to calibrate polymer loading and reaction yields. *Selected data* for **11**: δ_H(400 MHz, [$^2H_6$]DMSO) 3.52 (3 H), 4.05 (3 H), 4.10 (3 H), 6.89 (1 H), 7.44 (1 H), 7.48 (1 H), 7.49 (1 H), 7.64 (1 H), 9.05 (1 H), 9.16 (1 H). For **12**: 3.92 (3 H), 4.07 (3 H), 6.90 (1 H), 7.44 (1 H), 7.57 (1 H), 7.65 (1 H), 7.70 (1 H), 8.09 (1 H), 8.15 (1 H), 9.01 (1 H), 9.14 (1 H). For **13**: 3.87 (3 H), 4.02 (3 H), 6.69 (1 H), 7.2–7.3 (3 H), 7.42 (1 H), 7.53 (1 H), 7.60 (1 H), 7.68 (1 H), 7.73 (1 H), 7.92 (1 H), 8.73 (1 H). Signals of the resin are omitted. All integrals are relative to the methoxy group of the resin.

1 There are five main peptide structural motifs for DNA-binding that have been identified: helix-turn-helix, zinc finger, leucine zipper, helix-loop-helix and antiparallel β-sheets (β-ribbon). D. S. Johnson and D. L. Boger, in *Comprehensive Supramolecular Chemistry*, ed. J. L. Atwood, J. E. Davies, D. D. Macnicol, F. Vögtle and J.-M. Lehn, Elsevier Science, Oxford, 1996, vol. 4, p. 77.

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7 The coupling with DCC–DMAP gave **6** in a lower yield of 92%.

8 M. A. Tilak and C. S. Hollinden, *Tetrahedron Lett*., 1968, 1297.

9 M. Mrksich, M. E. Parks and P. B. Dervan, *J. Am. Chem. Soc*., 1994, **116**, 7983. MeO-PEG support is not suitable for a direct synthesis of extended structures due to the known alteration of its physical properties with growing oligoamide chain length. This leads to incomplete precipitation.

10 B. König, H. Buchholz, M. Rödel and M. Schulze, patent pending.

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