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Solution Phase Synthesis of Potential DNA-Binding Molecules Based on the PNA Backbone

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Abstract: The *N*-(2-aminoethyl)glycine backbone unit of PNA has been derivatized with pyreneacetic acid and acetic acid moieties to produce monomers for the synthesis of potential polyintercalators. Short oligomers containing these residues have been assembled using solution phase coupling reactions.

Intercalators are molecules that bind to DNA by insertion of an aromatic moiety between the stacked base pairs of double helical DNA.[1] Significantly, these compounds have exhibited a variety of biological effects. Since the elucidation of the binding mode of the natural bisintercalator echinomycin,[2] numerous groups have sought to improve the biological activities of these drugs by synthesizing polyintercalators.[3,4] While many examples of bisintercalators have been reported,[3] far fewer accounts have described tris- or higher intercalators.[4] The design of these molecules presents a difficult problem because they must embody a two-dimensional array of aromatic moieties linked in a synthetically accessible fashion. Furthermore, a backbone of intermediate flexibility is desirable to balance the advantages of preorganization with the need to maintain sufficient flexibility to follow changes in the DNA conformation that ensue upon complex formation.

This laboratory has begun to explore the synthesis of polyintercalators using the oligomeric backbones of molecules related to nucleic acids. One attractive approach is to utilize the pseudopeptide backbone of a peptide nucleic acid (PNA).[5,6] The repetitive nature of the PNA backbone allows for the incorporation of nucleobases at specific locations on a two-dimensional array. Significantly, Schuster and coworkers[7] have recently reported the replacement of a single nucleobase in a PNA oligomer with anthraquinone. It should be possible to extend this scheme to the replacement of every nucleobase to produce materials with different DNA-binding abilities. For example, a PNA oligomer containing alternating aromatic and abasic residues could theoretically bind to double-stranded DNA by polyintercalation. The use of abasic spacers should position the intercalating aromatic groups approximately 10 Å apart, the optimal distance for neighbor exclusion binding of a polyintercalator.[2,4,8] Furthermore, a PNA-based backbone should possess moderate flexibility due to the mixture of rigid amide linkages and flexible methylene groups.[9]

Herein, we report the synthesis of 9-fluorenylmethoxycarbonyl (Fmoc)-protected PNA monomers containing the potential intercalator pyrene. Pyrene is an attractive chromophore for initial studies due to its reported ability to intercalate into DNA,[10] its intense fluorescence, and the lack of functional groups that

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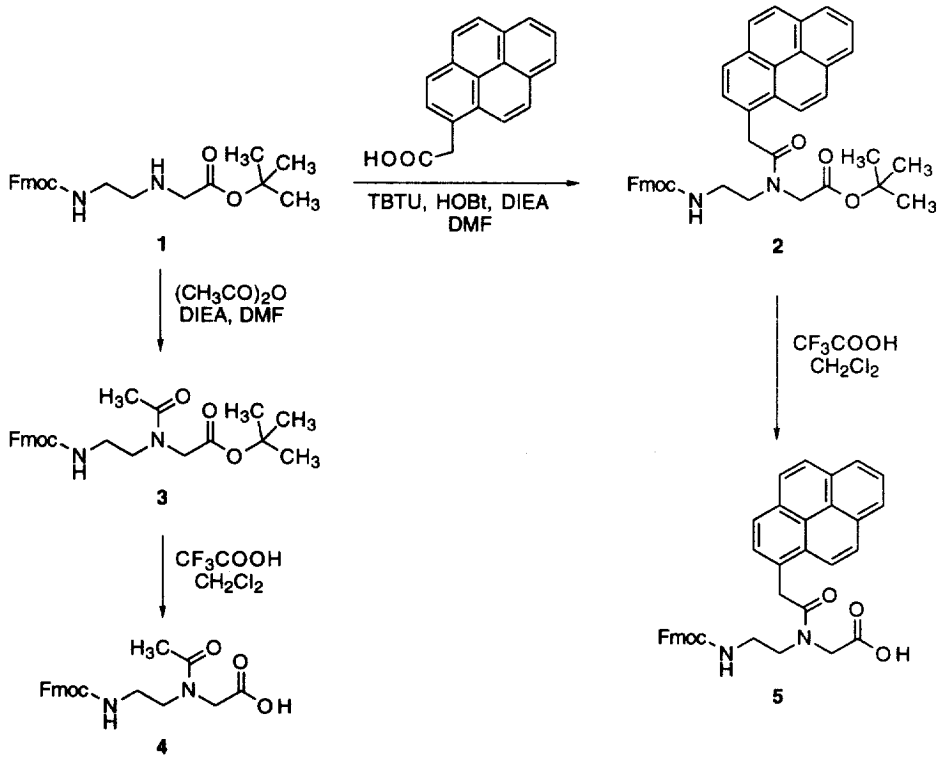
might require protection or otherwise complicate the synthesis. We also describe the synthesis of a Fmoc-protected acetyl monomer that serves as the PNA equivalent of an abasic residue.[11] Finally, we describe the solution phase synthesis of potential DNA-binding compounds containing mixtures of these residues .

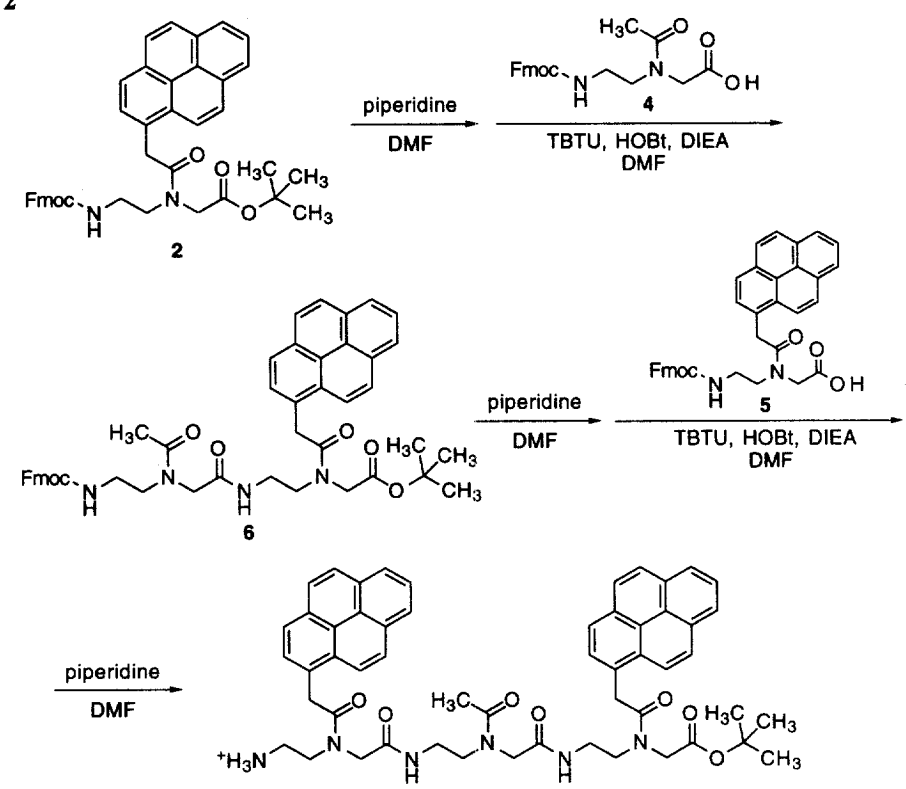
The starting material for the syntheses of the required monomers, *tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl] glycinate (**1**), was synthesized by the method of Thomson and co-workers.[12] Condensation of **1** with commercially available 1-pyreneacetic acid produced the fully protected monomer Fmoc-Py-tBu (**2**) in 77% yield (Scheme 1). Similarly, synthesis of the fully blocked acetyl monomer Fmoc-Ac-tBu (**3**) was accomplished in 59% yield by treatment of **1** with acetic anhydride and diisopropylethylamine (DIEA). Both of these compounds were purified by chromatography and produced satisfactory NMR and mass spectra.[13] Selective deprotection of the carboxy termini of *tert*-butyl (tBu) esters **2** and **3** was accomplished by brief treatment with trifluoroacetic acid in dichloromethane.

In order to demonstrate that the pseudopeptide monomers could be used to assemble oligomeric structures, the synthesis of a putative bisintercalator was undertaken. The most direct approach to the assembly of the heterotrimer was to utilize solution phase techniques. Surprisingly, these methods have not been successfully applied to the synthesis of PNAs using fully functionalized monomers.[14] The only report of a successful solution phase coupling of the pseudopeptides involved monomers lacking the base substituents. However, after some preliminary success in the solution phase coupling of appropriately deprotected acetyl monomers,[15] the synthesis of a bispyrenyl compound was attempted.

Scheme 1



Scheme 2



H-Py-Ac-Py-tBu (7)

The coupling reactions utilized typical conditions for Fmoc peptide synthesis (Scheme 2).[16,17] Thus, the amino group of the fully protected **2** was unmasked by treatment with piperidine. Condensation with acetyl monomer **4** was performed using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as the coupling agent in the presence of 1-hydroxybenzotriazole (HOBt) and DIEA. Using 1.2 equivalents of the free acid component, the coupling reactions proceeded in approximately 80% yield after purification using flash column chromatography on silica gel. The putative bisintercalator H-Py-Ac-Py-tBu (**7**) was synthesized in 73% overall yield following removal of the amino-terminal Fmoc group; the *tert*-butyl ester was left intact to produce a molecule with a net positive charge. The composition of **7** was confirmed using fast atom bombardment (FAB) mass spectrometry.[18] The solution phase method appears to be generally applicable to the syntheses of short oligomers. Using this procedure,[17] bispyrenyl molecules with differing numbers of acetyl spacers (Fmoc-Py-Py-tBu and Fmoc-Py-Ac-Ac-Py-tBu) and a trispyrenyl compound (Fmoc-Py-Ac-Py-Ac-Py-tBu) have also been synthesized.

This work has demonstrated that novel monomers based on the *N*-(2-aminoethyl)glycine unit of PNA can be efficiently synthesized. In contrast to the results reported for the Z-protected monomers bearing BOC-protected nucleobase substituents,[14] the solution phase syntheses of oligomers of **4** and **5** can be readily accomplished using standard conditions for Fmoc-mediated peptide synthesis. The reasons for this difference in reactivity are unclear, but could arise from the presence of a more hydrophobic substituent or from the use

of different N-protecting groups and coupling reagents. With the potential bisintercalator **7** in hand, experiments are currently underway to determine its DNA-binding characteristics.

References and Notes:

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13. ***tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(pyrene-1-yl)acetyl] glycinate (2):** MS (FAB, MeOH/NBA) 639 $(M+H)^+$; ^1H NMR (360 MHz, $CDCl_3$) δ 8.20–7.96 (m, 9H), 7.74 (d, 2H), 7.58 (t, 2H), 7.38 (m, 2H), 7.28 (m, 2H), 4.43–3.97 (m, 7H), 3.66–3.33 (m, 4H), 1.50 & 1.47 (rotomer s, 9H).
***tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-(acetyl) glycinate (3):** MS (FAB, MeOH/NBA) 439 $(M+H)^+$; ^1H NMR (360 MHz, $CDCl_3$) δ 7.75 (d, 2H), 7.59 (d, 2H), 7.40 (t, 2H), 7.31 (t, 2H), 4.39 & 4.34 (rotomer d, 2H), 4.21 (t, 1H), 3.94 & 3.90 (rotomer s, 2H), 3.54 & 3.48 (rotomer t, 2H), 3.36 (m, 2H), 1.51 & 1.49 (rotomer s, 9H).
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17. *Typical coupling procedure*: To a DMF solution of the amine component (generated by removal of the Fmoc group by treatment with 20% piperidine in DMF followed by evaporation *in vacuo*) was added sequentially a solution of the carboxylic acid (1.2 equiv) in DMF, TBTU (1.2 equiv), HOBT (1.2 equiv), and DIEA (2.0 equiv). The reaction mixture was stirred for 4 h at room temperature and was then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with brine. The aqueous wash was back-extracted with CH_2Cl_2, and the combined organic phases were washed with 0.1 N aqueous HCl, sat. aqueous $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography.
18. **H-Py-Ac-Py-*t*-Bu (7)**: MS (FAB, MeOH/NBA) 901.9 $(M+H)^+$.