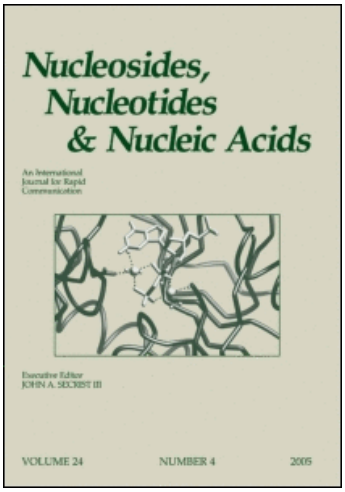


## Nucleosides, Nucleotides and Nucleic Acids

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# Nucleobase Modified Peptide Nucleic Acid

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# Nucleobase Modified Peptide Nucleic Acid

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## ABSTRACT

The $Pd^0/Cu^I$ catalyzed cross-coupling of terminal alkynes onto peptide nucleic acid monomers or submonomers bearing iodinated nucleobases has been utilized as a route to base-modified oligomers. Both 5-iodouracil and 5-iodocytosine derivatives undergo the cross-coupling to give the expected products in moderate to good yields. However, depending on the particular substrates and reaction conditions, the cross-coupling may be followed by a ring closing reaction to give the fluorescent furano- and pyrrolo-fused uracil and cytosine derivatives, respectively.

Since its introduction in 1991 by Nielsen and coworkers,[1] there have been over 600 publications involving peptide nucleic acid (PNA), from such diverse fields as chemistry, biochemistry, biotechnology, and medicine.[2] PNA is a synthetic mimic of DNA in which the sugar-phosphate backbone of the natural nucleic acid has been replaced with a polyamide backbone. Due to the lack of charge along the polymer, PNA is generally less soluble and exhibits more severe sequence-dependent aggregation as compared to DNA. We have been exploring the functionalization of pyrimi-

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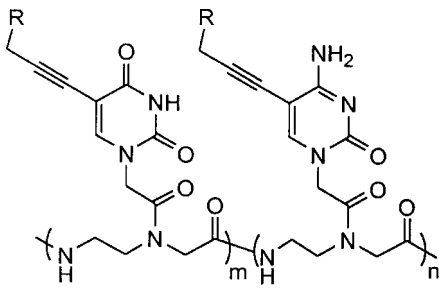


R = -OH, $-NH_2$, $-N(CH_3)_2$, etc.

*Figure 1.*

dine nucleobases on an otherwise standard PNA scaffold as a route to oligomers with modified properties.[3] The advantages of such an approach are that potentially diverse functional groups may be incorporated by well-established cross-coupling chemistry, the integrity of the Watson-Crick base pairing face is preserved, extensive functionalization may be achieved uniformly along the oligomer without introducing stereocentres (Fig. 1). Such oligomers are potentially interesting for reasons of increased water-solubility and increased hybrid stability as expected from intrastrand stacking[4] which would be sterically well tolerated based on molecular modeling studies (data not shown).

We recently reported the use of Sonogashira coupling for the derivatization of the PNA monomer containing 5-iodouracil in both solution- and solid-phase, as well as on a PNA oligomer bearing a single 5-iodouracil residue.[3] In general the findings were that solution-phase yields were modest (38–53%) while the solid-phase chemistry yielded the desired cross-coupled products as the only detectable products. Herein we report extension of this methodology to cytosine derivatives and progress towards the synthesis of PNA oligomers bearing pyrimidine nucleobases (Ura and Cyt), substituted at the 5-position with various groups that may modulate the biophysical properties of the polymer. The two approaches that we are pursuing are the synthesis of C5-alkynyl-pyrimidine derivatives for site specific incorporation of modified monomers and the synthesis of oligomers containing uniform subsitution of 5-iodo uracil and cytosine for a single global modification.

With regard to the first approach, we have done cross coupling on 5-iodouracil containing PNA monomers, but have found it cumbersome for method development. The preferred route is by the preparation of nucleobase acetic acid ester derivatives (Sch. 1, **1**- > **2**). In our hands, the cross-coupling reaction, even on simple substrates such as **1**, typically gives moderate chemical yields and sometimes produces many unidentified by-products or outright fails (e.g., Fmoc-protected propargylamine). However, we recently found that the known cyclization reaction to afford fluorescent furano-fused pyrimidine[5] derivatives readily occurs under standard work-up conditions.[6] The example illustrated in Sch. 1 occurred when a crude reaction mixture was accidentally overheated on a rotary evaporator. Product **3**





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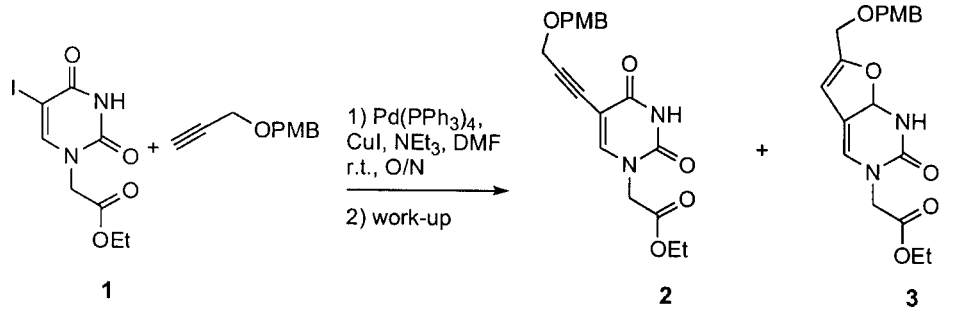
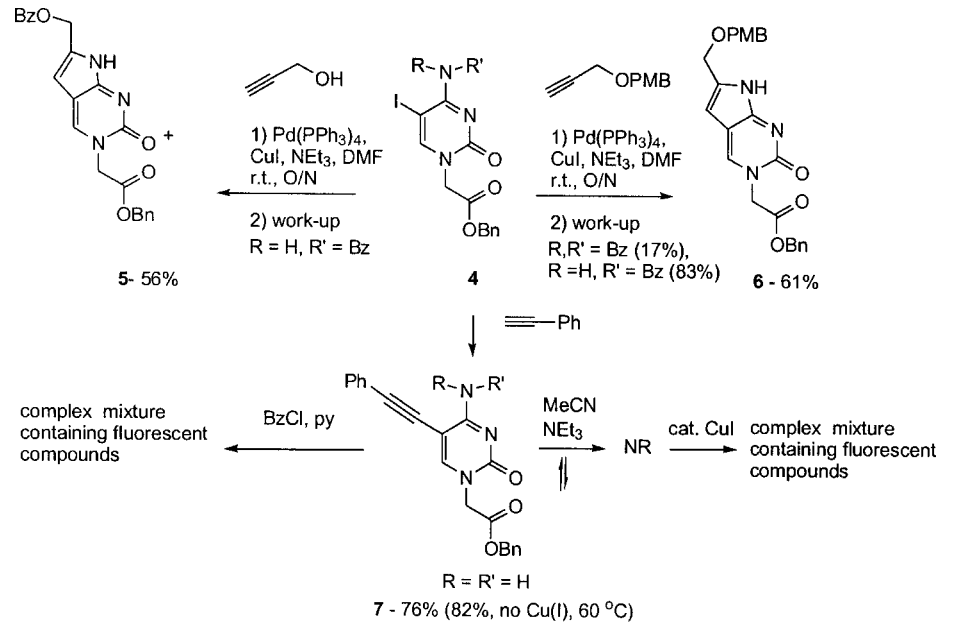



*Scheme 1.*

was produced in a 30% yield, the structure being confirmed by X-ray crystallography. This side reaction can be completely avoided by extracting copper salts from the crude reaction mixture with EDTA prior to evaporation of the solvent. Moreover, the cyclization is not catalyzed by protic acids such as trifluoroacetic acid, and should not occur during polyamide synthesis.

Initial studies on the cross-coupling of 5-iodocytosine derivatives were done with the $N^4$-group protected as the benzyl carbamate. It was found on numerous attempts that this cross-coupling could not be made to proceed cleanly. Given this difficulty, the benzoyl-protected compound **4** (R = Bz, R′ = H or Bz) was prepared. Again,




*Scheme 2.*





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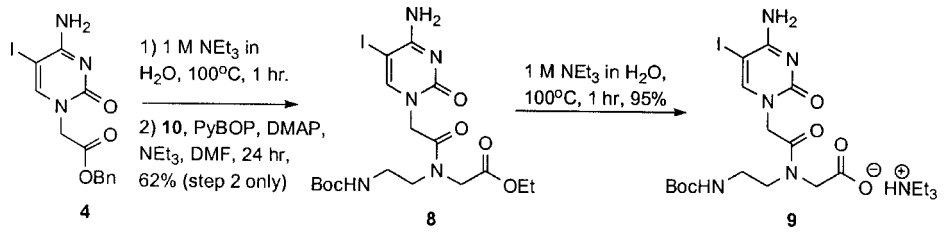


*Scheme 3.*

cross-coupling on this compound resulted in a complex mixture of products including the fluorescent pyrrolocytosine (Sch. 2, yields as shown). As illustrated, 5-iodo-$N^4$-benzoylcytosine undergoes cross coupling and deacylation. When reacted with *para*-methoxybenzyl-protected progargyl alcohol the annulation reaction occurs with ultimate loss of the acyl group. When propargyl alcohol is used, acyl group migration is observed. To our knowledge, this is the first report of a direct synthesis of pyrrolocytosine (from a cytosine derivative), although it has been formed by treatment of furanouracil with ammonia.[7]

The cross-coupled product was favoured by subjecting the unprotected derivative **4** ($R = R' = H$) to standard coupling conditions. Moreover, one attempt to acylate the product resulted in a similar complex mixture, suggesting that the difficulty arises from the instability of the $N^4$-acyl-5-alkynyl cytosine derivatives and not from the coupling reaction itself. Further studies are underway to better understand the nature of the side reactions occurring on the $N^4$-acyl derivatives. The conclusion for the time being is that PNA oligomerization must either proceed without $N^4$-acyl protection, or $N^4$-acyl groups must be removed prior to cross-coupling.

In order to pursue the second approach, the 5-iodo pyrimidine monomers were prepared by standard methods, the synthesis of the cytosine monomer is shown in Sch. 3. It is noteworthy that the acylation reaction to form **8** from triethylammonium 5-iodocytosine acetate and ethyl 2-(Boc-aminoethyl)glycinate **10** took place in the absence of $N^4$-protecting group without the occurrence of acylation or phosphorylation at $N^4$. This is taken as evidence that a PNA oligomer can be prepared using this monomer under the same amide bond-forming conditions.

We are also exploring the tandem coupling/annulation reaction to produce modified nucleobases such as 7-deazaadenine and subsequently from this tricyclic derivatives of adenine (1,5-dihydro-1,5,6,8,-tetraazaacenapthylenes).

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