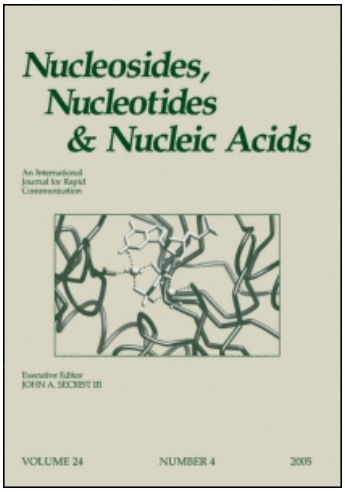


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# SYNTHESIS OF NEW, BASE-MODIFIED PNA MONOMERS

**Ferenc Sipos and Gyula Ságí** □ *Institute of Biomolecular Chemistry, Chemical Research Center of the Hungarian Academy of Sciences, 1025 Budapest, Hungary*

□ *A number of N-Boc-protected peptide nucleic acids (PNA) monomers containing 5-aryl- and 5-alkynyl-uracil bases have been synthesized using different palladium-catalyzed cross-coupling reactions. Starting from the base-unprotected 5-iodo-uracil PNA monomer, only the Stille couplings were accomplished successfully, while Suzuki couplings with boronic acids containing the same aryl groups failed. During Sonogashira couplings with terminal alkynes, significant amounts of unrequired furano[2,3-d]pyrimidine by-products were formed. Protection of the lactam function by p-methoxybenzylation prevented the opportunity for intramolecular cyclization as well as formation of a negative charge on the 4-O atom, making it possible to reach almost quantitative yields at Sonogashira couplings and acceptable conversions in Suzuki reactions.*

**Keywords** Peptide nucleic acids; Pd-catalyzed cross-couplings; 5-substituted-uracils



## INTRODUCTION

Peptide nucleic acids (PNAs), containing an *N*-(2-aminoethyl)glycine backbone instead of the sugar-phosphate moiety of the natural nucleic acids, belong to the most powerful and promising DNA mimics due to their strong hybridization ability with DNA and RNA and complete resistance to nucleases and proteases.[1] Due to these beneficial properties they are suitable for replacement of their corresponding DNA counterparts as therapeutic agents and have proved to be highly efficient diagnostic tools (e.g., PNA microarrays).

In oligonucleotides 5-C-substitution of uracil base, among other things, makes possible the introduction of protein recognition elements,[2] fluorophores,[3] and spin labels.[4] In addition, PNA-DNA chimeras[5] as well as homogenous oligodeoxynucleotides containing 5-propynyl-uracils in place of thymines have higher duplex stability[6] and increased mismatch recognizing ability.[7] The incorporation of different 5-heteroaryl-uracil moieties into short oligodeoxynucleotides also results in enhanced

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thermal stability of the modified DNA:RNA duplexes compared to that of the thymine-containing counterpart.[8] This is due to an increased $\pi - \pi$ stacking interaction between the neighboring bases. A similar incorporation of 5-aryl- and 5-alkynyl-uracils into PNAs is also expected to increase the stability of PNA:DNA and PNA:RNA complexes, which should also be accompanied by a higher mismatch penalty. Some hydrophobic aryl or arylethynyl substituents are likely to improve the cellular uptake, which is rather poor in the case of unmodified PNAs.

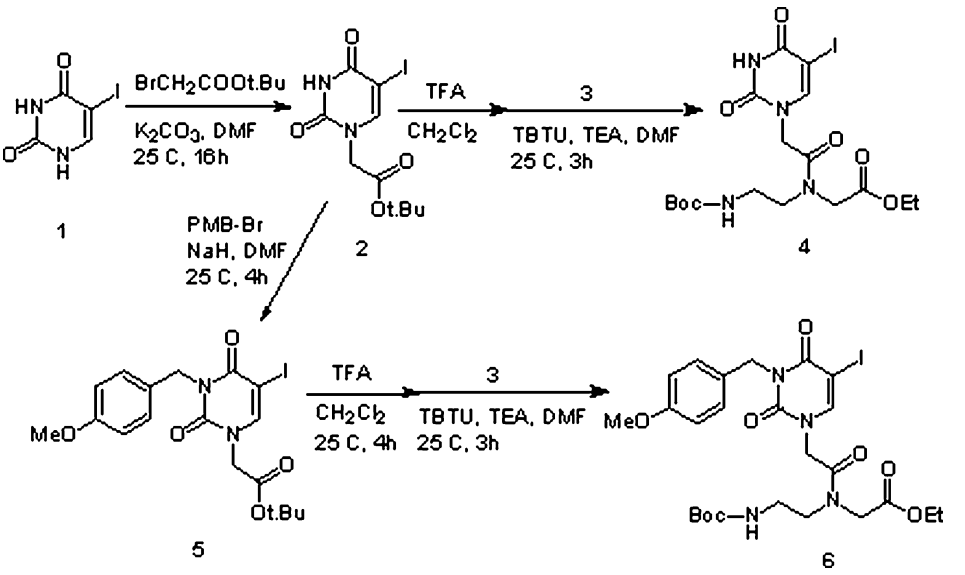
Syntheses of 5-*C*-substituted-uracil nucleosides were usually accomplished by different Pd-catalyzed cross-coupling (Heck, Sonogashira, Suzuki, and Stille) reactions, starting from 5-halo-, 5-HgCl-, or 5-OTf-uracil nucleosides.[9] However, in the synthesis of analogous 5-*C*-substituted PNAs, due to different solubility and altered chemical properties (e.g., base- and acid-sensitivity) of the *N*- and *C*-protected PNA monomers, it was necessary to change certain reaction conditions and to find the most convenient starting materials and reagents. Therefore, we aimed to elaborate on useful synthetic strategies for the preparation of N-Boc protected PNA monomers containing 5-alkynyl- and 5-aryl-uracil bases, by application of Pd-catalyzed cross-couplings. In order to study the stabilities of different PNA:DNA and PNA:RNA complexes incorporation of these building units into a short (11-mer) homopyrimidine PNA oligomer is in progress.



## RESULTS AND DISCUSSION

### Synthesis of Key Intermediates

For the synthesis of 5-*C*-substituted–uracil PNA monomers by different Pd-catalyzed cross-couplings 5-iodo-uracil **1** appeared to be the most convenient starting material (see Scheme 1). Alkylation of **1** with *tert*-butyl-bromoacetate afforded the required $N^1$-alkyl derivative **2** as a major product, beside the minor $N^3$-alkyl- and $N^1$, $N^3$-dialkyl compounds. Acidolysis of **2** with TFA followed by coupling of the free acid with *N*-(2-Boc-aminoethyl)glycine ethyl ester[10] **3** provided the $N^3$-unprotected 5-iodo-uracil PNA monomer **4**, which has been synthesized by Hudson et al.[11] in a similar way. Unfortunately, as it turned out later, starting from **4** only the Stille couplings can be executed successfully, while Suzuki couplings using free aryl-boronic acids failed. In addition, Sonogashira couplings of **4** with terminal alkynes resulted in significant amounts of unrequired, ring-closured furano[2,3-d]pyrimidine by-products in all cases. In order to prevent the opportunity of this intramolecular cyclization, which is due to the formation of a negative charge on the 4-*O*-atom in basic medium, synthesis of an appropriate lactam-protected intermediate was necessary. This was realized by introduction of the orthogonal *p*-methoxybenzyl



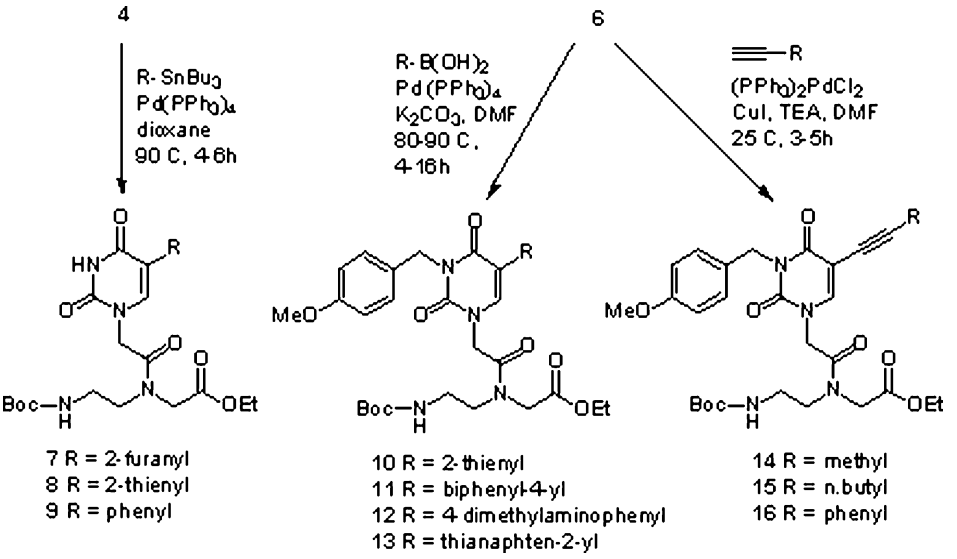

**SCHEME 1** Synthesis of key intermediates.



(PMB) protecting group into the $N^3$-position of **2**. (It must be noted that *p*-methoxybenzylation of **4** was not found to be selective for the lactam since it led to simultaneous benzylation of the Boc-carbamate NH too, even under mild (2–4°C) conditions.) The $N^3$-PMB-5-iodo-uracil-1-yl-acetic acid *tert*-butyl ester **5** thus obtained was then subjected to acidolysis and coupling to **3** to give the required $N^3$-protected 5-iodo-uracil analog **6**, as an alternative starting material.

## Stille Couplings

Due to lesser electronegativity and thus the stronger metallic character of tin relative to boron, aryl-trialkylstannanes are better aryl group donors than the corresponding aryl-boronic acids.[12] Therefore we first tried to couple the $N^3$-unprotected 5-iodo-uracil PNA monomer **4** with some aryl-tributylstannanes in the presence of the Pd(0) catalyst, at elevated temperature (see Scheme 2). All three reactions proceeded smoothly, leading to the formation of 5-(2-furanyl-, 2-thienyl-, and phenyl)-uracil PNA monomers **7, 8**, and **9**, respectively, with good yields. However, since only very few aryl-trialkylstannanes are commercially available and in addition most of them are toxic, synthesis of a wide variety of 5-aryl-uracil PNA monomers in order to carry out studies on alternative methods, such as Suzuki coupling, seemed reasonable.




**SCHEME 2** Synthesis of 5-substituted-uracil PNA monomers.



## Suzuki Couplings

Unfortunately, attempted couplings of **4** with the most reactive 2-thienyl-boronic acid—even despite variation of the solvents (DMF, 1,2-dimethoxyethane, EtOH), the bases ($K_2CO_3$, $Cs_2CO_3$, KOt.Bu, NaOEt) and the Pd-catalysts ($Pd(PPh_3)_4$, $(PPh_3)_2PdCl_2$, $Pd(OAc)_2$ + dppf)—remained unsuccessful. Since in the presence of a base, due to deprotonation of the imide NH, there must be a partial negative charge on the 4-*O* atom, which is in close proximity with the Pd atom and thus can lower its electrophilic character, thereby the transmetallation step is inhibited. This presumption was certified by application of the $N^3$-PMB-protected monomer **6**, as the only convenient starting material. Although the isolated yields were generally lower (37–65%) compared to those of Stille couplings (61–79%) but the conversions are still acceptable considering that—except for the 2-thienyl—these substituents are bulkier than aryl groups introduced by the aryl-tributylstannane reagents. Finally the following $N^3$-PMB-5-aryl-uracil PNA monomers were prepared: 5-(2-thienyl) **10**, 5-(biphenyl-4-yl) **11**, 5-(4-dimethylaminophenyl) **12**, and 5-(thianaphten-2-yl) **13**. As it was indicated by TLC in the two latter cases the coupling efficiency further increased when aryl-boronic acid pinacol esters were used instead of the free acids.

## Sonogashira Couplings

As previously mentioned, coupling of **4** with three different terminal alkynes gave high overall yields but proportions of the unrequired

furano[2,3-d]pyrimidine by-products were significantly higher (30–40%) than those found at analogous couplings (<10%) with sugar-protected 5-iodo- or 5-TfO-uridines. Although these fluorescent side-products can be separated completely from the 5-alkynyl-uracil analogs by silica gel chromatography but their unexpectedly high proportion still makes it preferable to start from **6**, when in the absence of imide NH intramolecular cyclizations cannot occur. Applying this strategy, the required $N^3$-PMB-5-alkynyl-uracil PNA monomers, such as 5-propynyl (**14**), 5-(1-hexyn-1-yl) **15** and 5-phenylethynyl **16**, as sole products, were isolated by nearly quantitative yields.

UV spectra of all the investigated 5-aryl- and alkynyl-uracil PNA monomers show large bathochromic shifts relative to that of the thymine-containing reference compound which refers to a near planar orientation between the uracil base and the 5-substituents. It is expected to enhance the base-stacking interactions of adjacent base pairs, leading to higher duplex stability.



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