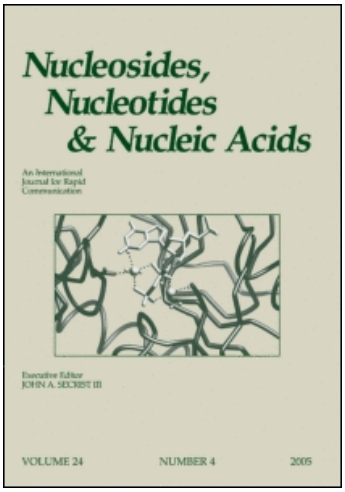

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A DIRECT SYNTHESIS OF PYRROLOCYTOSINE FROM 5-IODOCYTOSINE

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▫ *We have employed a tandem Sonogashira/annulation reaction between 5-iodocytosine derivatives and terminal alkynes to yield the fluorescent bicyclic nucleobase pyrrolcytosine. Pyrrolocytosine bearing substituents only on the pyrrole ring are conveniently synthesized from 5-iodocytosine. Water soluble pyrrolocytosines are being investigated as reporter groups in SNP analysis.*

INTRODUCTION

Since its introduction in 1991 by Nielsen and coworkers,[1] peptide nucleic acid (PNA) has been investigated for a wide variety of applications ranging in biochemistry, biotechnology and medicine. One of the most attractive properties of PNA is its avid and highly sequence selective binding to complementary nucleic acids. These properties, combined with its chemical and biological stability, make the use of PNA as a sequence probe very attractive.[2]

During our studies concerning the functionalization of pyrimidine nucleobases by cross-coupling of 5-iodonucleobases with terminal alkynes,[3–6] we noted, under mildly forcing conditions, that the 5-alkynyl substituents underwent a cyclization reaction with the ortho-positioned heteroatom to yield furanouracils or pyrrolocytosines (pC).*,† The accepted reaction sequence involves sequential, metal-catalyzed cross-coupling (step 1) and annulation (step 2) (Scheme 1). We recently reported reaction conditions for complete control over partitioning between the 5-alkynyl and annulated products with respect to cytosine derivatives,[4] partly antedated by the work of Ohtsuka.† Cytosine derivatives lacking a protecting group on the exocyclic amino group react cleanly to give the 5-alkynyl products, whereas the N4-acyl or carbamate protected cytosines spontaneously cyclize under the

*First described for uridine derivatives: see Ref. [7].
†First described for cytidine derivatives: see Ref. [8].

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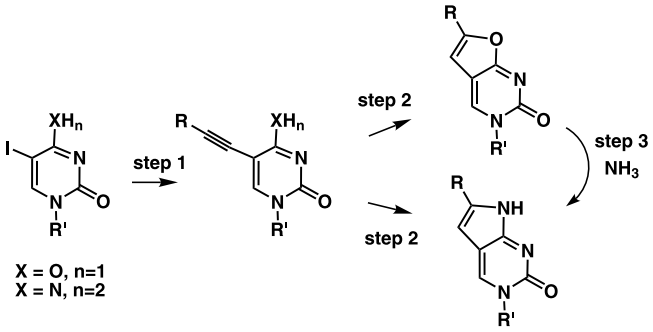
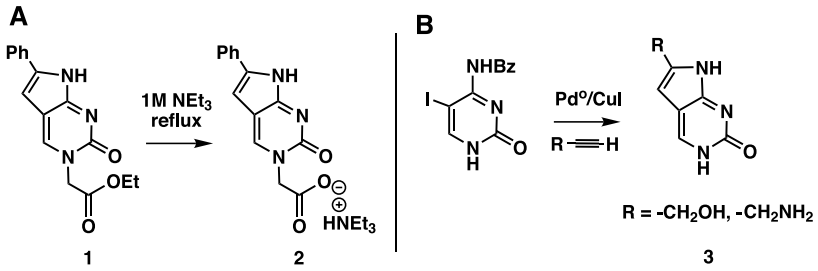


SCHEME 1



SCHEME 2

reaction conditions. During these studies we found that the fluorescence intensity and emission wavelength depend on the nature of the alkyne, with simple derivatives of phenylacetylene possessing increased fluorescence relative to alkyl-substituted alkynes.[4] Alternatively, the pyrrolocytosine heterocycle may be formed by the ammonolysis of furanouracil (Scheme 1, step 3).[9]

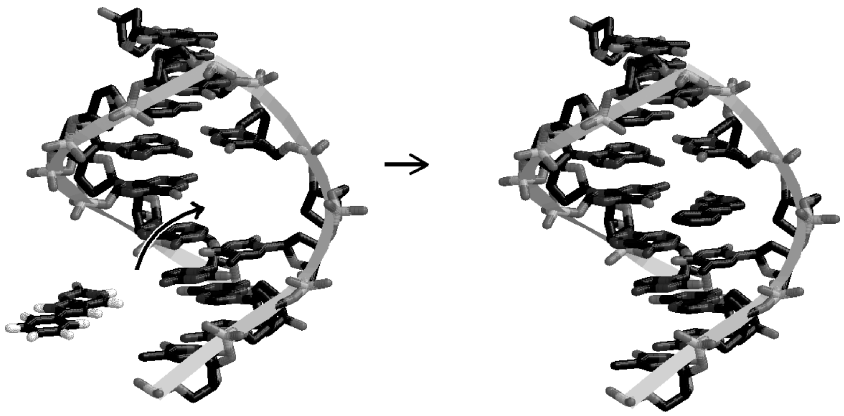


FIGURE 1

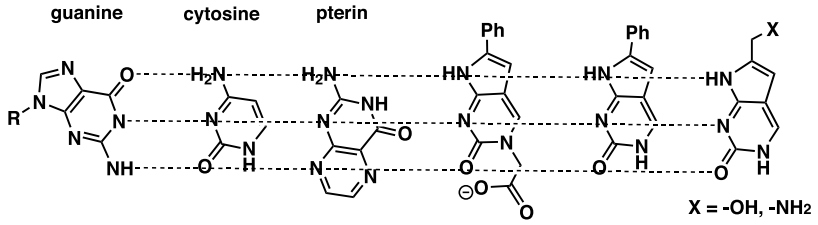


FIGURE 2

Of late, there has been interest in oligonucleotides that incorporate pyrrolocytidine for their ability to pair with guanosine and fluorimetrically report the state of hybridization, being more emissive in the single-stranded state.[10,11] Scheme 2A shows our approach to the synthesis of a nucleobase submonomer for incorporation into PNA. In order to avoid difficulties in isolating the water-soluble product, and to avoid damaging the heterocycle, we performed the conversion of **1**→**2** using aqueous triethylamine. After lyophilization, the product is obtained in near quantitative yield. Subsequent conversion to a PNA monomer compatible with solid phase synthesis proceeded smoothly and without the requirement for a nucleobase protecting group.

Another interest of ours' is to prepare and evaluate small, water-soluble pyrrolocytosines that may be used to detect single nucleotide polymorphisms in conjunction with a probe sequence containing an abasic site. The feasibility of this strategy was elegantly demonstrated by Teramae and coworkers who used pterin as the reporter molecule.[12] A change in the fluorescence signals the binding of a reporter groups to the abasic site provided by the probe sequence (Figure 1). Ideally, the reporter molecule should resemble cytosine as closely as possible so that it may intercalate into the abasic site without sterically clashing with the probe oligonucleotide backbone. The alignment of such molecules capable of binding to guanine is illustrated in Figure 2.†† A water soluble pyrrolocytosine bearing a methylenecarboxylate substituent at N1 has been synthesized, but it is not optimal in design because it possesses both a potential steric clash due to the N1-substituent and the negative charge which is unfavorable for binding to DNA. To overcome these perceived drawbacks, we have synthesized pyrrolocytosines without substitution at N1, Scheme 2B. Importantly, the binding of pyrrolocytosines to a duplex possessing an abasic site should be tolerant toward substitution on the pyrrole ring as these groups are directed into the major groove. Aqueous solutions of these molecules emit brightly at ca. 450 nm under illumination at 360 nm.

Currently we are pursuing the use of N1-unsubstituted pyrrolocytosines in the context of small molecules as well as pC-containing PNA as fluorimetric probes.

††Adapted from Ref. [12], with phenyl-substituted pC shown. The model was constructed using HyperChem ver. 5.1 and chromophore was docked into the abasic site using H-bond distant constraints and allowing the abasic residue to deform.

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