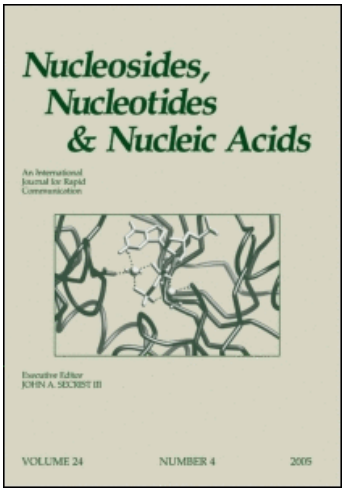

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SYNTHESIS OF A FLUORESCENT PNA MONOMER CONTAINING 5-((9*H*-FLUOREN-2-YL)ETHYNYL)URACIL

Filip Wojciechowski and Robert H. E. Hudson □ *Department of Chemistry, The University of Western Ontario, London, Ontario, Canada*

□ *Pyrimidine nucleobases bearing 5-phenylethynyl substitution represent compact and intrinsically fluorescent nucleobases. Such nucleobases are capable of selective recognition of a complementary base and may fluorimetrically report on hybridization events. Our past work has demonstrated that the fluorescence of 5-phenylethynyluracils is sensitive to substitution on the phenyl ring, however these are relatively weak fluorophores. We currently are pursuing the functionalization of the phenyl group of these modified nucleobases in order to further improve their fluorescence response, increase their aqueous solubility and stabilize hybrids formed with complementary nucleic acids. As an example of this work, we have synthesized the 5-((9*H*-fluoren-2-yl)ethynyl)uracil PNA monomer that will be incorporated into oligomers using Fmoc-based chemistry. Initial evaluation of the fluorescence of the 5-((9*H*-fluoren-2-yl)ethynyl)uracil derivative shows that the fluorescence intensity is approximately 50 times greater than a similar 5-phenylethynyluracil derivative when under identical conditions.*

Keywords Fluorescent nucleobases; 5-(9*H*-fluoren-2-yl)ethynyl)uracil; PNA

INTRODUCTION

Recently we have described the synthesis, properties and incorporation of fluorescent nucleobases derived from 5-phenylethynylpyrimidines into PNA.[1,2] The 5-alkynyl-substituted nucleobases show weaker fluorescence than the cyclized pyrrolocytosines or furanouracils.[1] Since the cross-coupled phenylethynylpyrimidines show substituent-dependent fluorescence we have decided to introduce a larger aromatic group, such as fluorene, which is expected to be more emissive and corroborated by the work of others. For example, Kim et al. have synthesized a fluorene-labeled phosporamidite followed by incorporation into a central position of an oligodeoxyribonucleotide. Their results indicate that a fully matched duplex exhibits a 3.4-fold enhancement in fluorescence intensity

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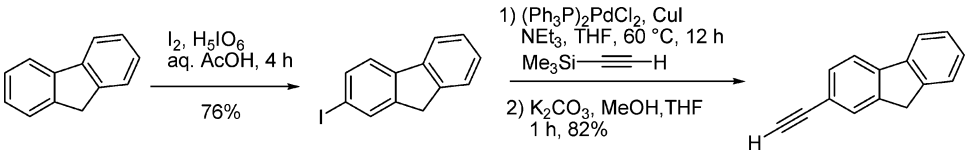


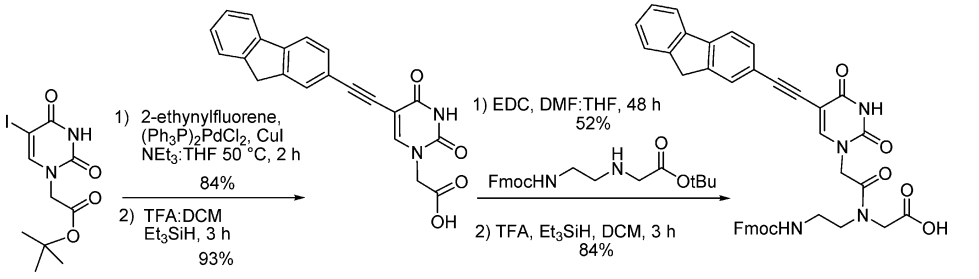
SCHEME 1

(λ_{max} = 425 nm) relative to the single strand.[3] The attachment of fluorene to a PNA backbone using a novel lysine PNA monomer was recently accomplished by Appella and coworkers. The modified oligomers showed comparable thermal stability to the unmodified aegPNA oligomer. A 4-fold increase in fluorescence intensity was observed when hybridized to the complementary strand compared to the unbound PNA.[4] Our aim is to introduce fluorene-containing pyrimidines into PNA in order to avoid introducing chirality into the backbone, increase the fluorescence response of the oligomer, stabilize the hybrids formed and study the fluorescence response of fluorene incorporated into oligonucleotides and PNAs.

RESULTS AND DISCUSSION

The synthesis of 2-ethynylfluorene began with commercially available fluorene which was monoiodinated at the 2 position with periodic acid dihydrate and iodine according to a literature procedure.[5] Next, Sonogashira coupling between TMS-acetylene and 2-iodofluorene followed by removal of the TMS group (K_2CO_3/MeOH) gave 2-ethynylfluorene in 82% over two steps, as shown in Scheme 1.

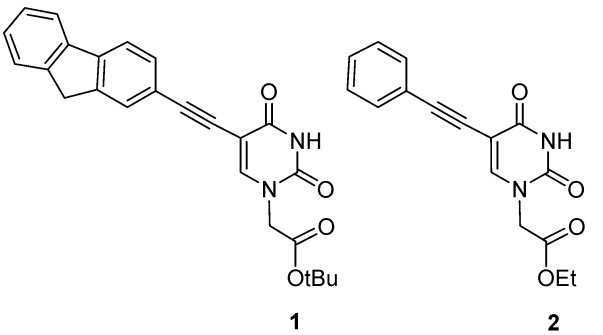
The synthesis of the 5-((9*H*-fluoren-2-yl)ethynyl)uracil PNA monomer was accomplished by palladium catalyzed cross-coupling of 2-ethynylfluorene with *tert*-butyl (5-iodouracil-1-yl)acetate to give *tert*-butyl (5-((9*H*-fluoren-2-yl)ethynyl)uracil-1-yl)acetate in 84% yield, after chromatographic purification. The *tert*-butyl group was conveniently removed with trifluoroacetic acid in DCM containing triethylsilane to yield (5-((9*H*-fluoren-2-yl)ethynyl)uracil-1-yl)acetic acid in 93%, Scheme 2. Finally, the



SCHEME 2

5-((9*H*-fluoren-2-yl)ethynyl)uracil PNA monomer was achieved by 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride (EDC) mediated coupling of the base acetic acid derivative to the Fmoc-protected backbone and removal of the *tert*-butyl ester, Scheme 2.

In order to judge our success at creating a more emissive fluorophore, we have chosen a practical approach. Probe strands are commonly used in the micromolar concentration range concentration when observing by UV-vis spectrophotometry. Since bimolecular duplex stability is concentration dependent, we have often characterized thermal denaturation by observing changes in fluorescence with identical solutions (μM concentration) to allow easy comparison of results between the two techniques. In using this approach, we get a qualitative, operational ranking of the sensitivity of a particular fluorophore. By defining "brightness" as the fluorescence emission under identical instrumental conditions and concentration we can qualitatively rank similar fluorophores. Applying this approach to the present case, we studied *tert*-butyl (5-((9*H*-fluoren-2-yl)ethynyl)uracil-1-yl)acetate (**1**) and ethyl (5-phenylethynyl)uracil-1-yl acetate (**2**) at 2.5 μM in dichloromethane.



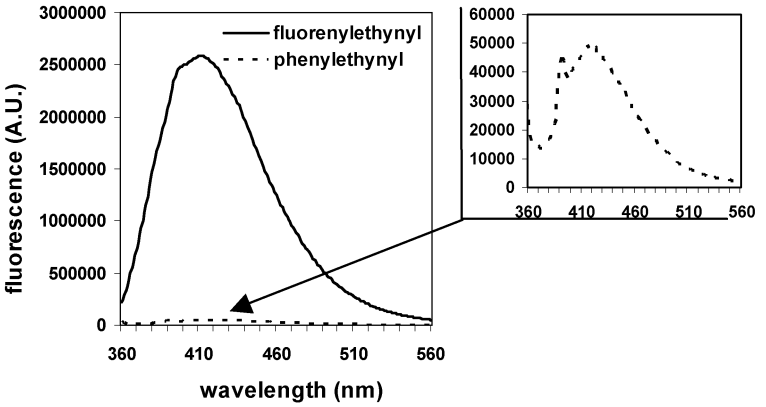


FIGURE 1 Comparison of the fluorescence of *tert*-butyl (5-((9*H*-fluoren-2-yl)ethynyl)uracil-1-yl)acetate **1** and ethyl (5-phenethynyl)uracil-1-yl acetate **2**.

Each fluorophore has a peak absorption band near 350 nm which when irradiated leads to blue fluorescence ($\lambda_{max} = 412$ nm for **1**; $\lambda_{max} = 420$ nm for **2**). As illustrated in Figure 1, the peak emission intensity of **1** is approximately 2.5×10^6 while that of **2** is 5×10^4 (inset).

The above monomer will be incorporated into a PNA oligomer and its hybridization properties and fluorescence response evaluated. We also are currently pursuing the synthesis of pyrrolocytosine containing fluorene via cyclization of 5-((9*H*-fluoren-2-yl)ethynyl)uracil. Since PNA suffers from low aqueous solubility introduction of the nonpolar fluorene ring will further decrease the solubility. Therefore, derivatization of fluorene with cationic groups is underway with the expectation that this will increase aqueous solubility and favourably affect the hybridization properties.

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