JOC The Journal of Organic Chemistry

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Pyrimidine-Fused Heterocyclic Frameworks Based on an N4-Arylcytosine Scaffold: Synthesis, Characterization, and PNA Oligomerization of the Fluorescent Cytosine Analogue 5,6-BenzopC

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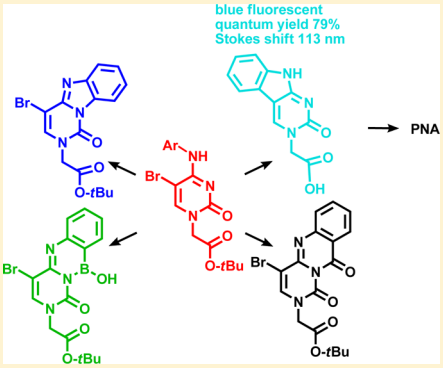
Supporting Information

ABSTRACT: A synthesis of an intrinsically fluorescent cytosine analogue 5,6-benzopC has been developed utilizing the reductive Ni-mediated cyclization of an N4-aryl,N4-(Boc)cytosine intermediate as a key step. 5,6-BenzopC was found to possess interesting fluorescence properties (Φ = 0.79, EtOH; Stoke's shift 113 nm). Peptide nucleic acid (PNA) oligomerization of the 5,6-benzopC monomer was carried out, followed by hybridization studies with complementary deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) which showed the modification to be well tolerated in the sequence contexts examined. Initial attempts to synthesize the heterocyclic skeleton present in 5,6-benzopC resulted in the discovery of routes to the pyrimido[1,6-*a*]benzimidazole, pyrimido[1,6-*a*]quinazoline, and pyrimido[1,6-*a*]benzo[*b*]6-bora-1,3-diazine heterocyclic frameworks.



INTRODUCTION

6-Phenylpyrrolocytosine (6-PhpC, 1, Figure 1) is an intrinsically blue fluorescent nucleobase analogue recently developed in our laboratory for use in peptide nucleic acids.[1] The key step in the synthesis of 6-PhpC is a one-pot domino Sonogashira cross-coupling/5-*endo*-*dig* annulation reaction between the corresponding 5-iodocytosine analogues and phenylacetylene.[2] The good emissivity of the 6-PhpC chromophore (Φ 0.61, EtOH)[2b] along with the fact that 6-PhpC scaffold obeys Watson−Crick base-pairing rules with guanine (Figure 1) make this nucleobase analogue an excellent probe for biological applications.

Introduction of 6-PhpC into PNA (peptide nucleic acid) sequences is well tolerated, resulting in increased melting temperature and reasonable discrimination against binding to mismatched sequences.[2b,c] A PNA oligomer containing a modified 6-PhpC subunit has been shown to inhibit mutant huntingtin protein (a hallmark of Huntington's disease) in an allele-selective manner.[3] In a more recent study, 6-PhpC-modified siRNA was found to have gene silencing potency and immunostimulatory properties mimicking those of natural short interfering RNA (siRNA).[4] The intrinsic fluorescence of 6-PhpC has also been exploited to monitor cellular uptake and distribution of PNA[3,5] and RNA[4] in the aforementioned studies.

In order to gain a better understanding of fluorescence and hybridization properties of 6-PhpC, we chose to prepare a fused-ring analogue (5,6-benzopC acetic acid, **2a**, Figure 1) suitable for incorporation into PNA. We envisioned that a transition-metal-mediated cyclization of N4-aryl-5-bromocytosine analogues (**3**, Figure 1), synthesized here for the first time, would provide access to the pyrimido[5,4-*b*]indole framework present in *tert*-butyl 5,6-benzopC acetate (**2b**). We have successfully synthesized 5,6-benzopC acetic acid (**2a**) and conjugated the corresponding nucleobase analogue with a Boc-chemistry compatible PNA backbone. The fluorescence properties associated with this analogue[6] have been evaluated. PNA oligomerization was carried out, followed by hybridization studies with corresponding DNA and RNA complements. Unfortunately, a complete quenching of the fluorescence was observed during these studies even upon binding to a complementary DNA strand, and possible reasons for this unexpected behavior are discussed below.

In addition to the preparation, characterization, and oligomerization of 5,6-benzopC-containing monomer **2a**, we have also found that *tert*-butyl 1-oxo-4-bromopyrimido[1,6-*a*]benzimidazol-2-ylacetate (**4**, Figure 1) and *tert*-butyl 1,10-dioxo-4-bromopyrimido[1,6-*a*]quinazolin-2-ylacetate (**5**, Figure 1) can be obtained in good yields from *tert*-butyl N4-aryl-5-bromocytosin-1-ylacetates **3b**, **3d**, and **3f** (Figure 1) by simple variation of the reaction conditions. As discussed later, there are several methodologies available for the preparation of the heterocyclic skeleton present in compound **4**, while the highly oxidized heterocyclic framework of **5** has not been described in the literature. These unexpected observations have been made when the substrates **3a**−**f** have been subjected to Cu- and Pd-catalyzed intramolecular cross-coupling reactions with the intention to prepare 5,6-benzopC-containing PNA monomer **2a**.

Received: December 27, 2013
Published: March 25, 2014

   dx.doi.org/10.1021/jo402873e | *J. Org. Chem.* 2014, 79, 3336−3347

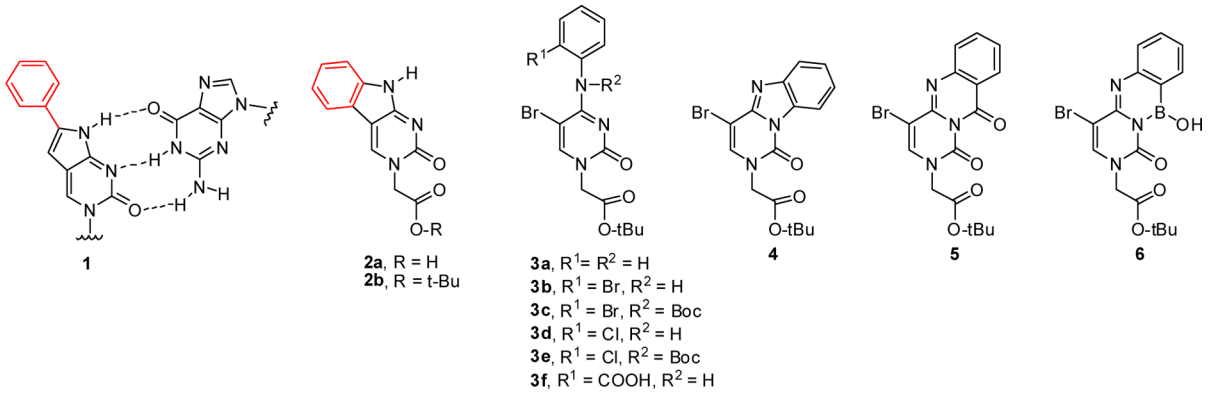
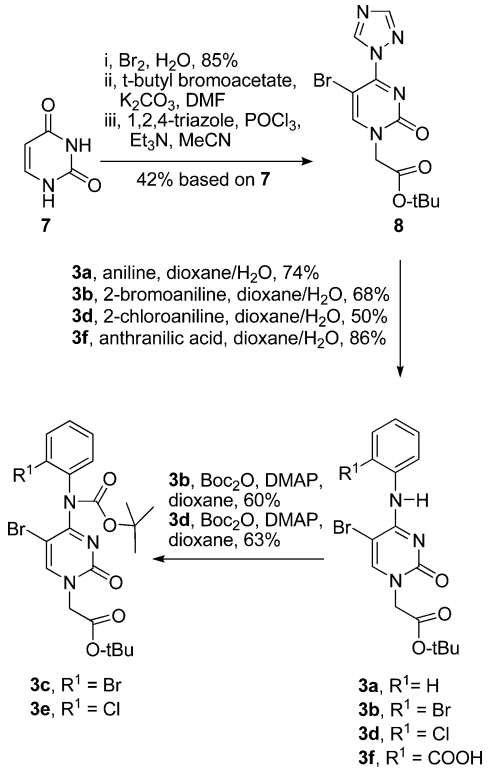


Figure 1. Structures of 6-PhpC (**1**) and 5,6-benzopC (**2**) showing a Watson−Crick base pairing with guanine. Structures of N4-arylcytosines **3** and heterocycles **4**−**6** described in this study.

An unprecedented heterocyclic skeleton present in *tert*-butyl 1-oxo-10-hydroxy-4-bromopyrimido[1,6-*a*]benzo[*b*]6-bora-1,3-diazin-2-yl-acetate (**6**, Figure 1) has been obtained in a similar fashion by reacting *tert*-butyl 4-(1,2,4-triazol-1-yl)-5-bromocytosin-1-ylacetate (**8**, Scheme 1) with 2-aminobenzeneboronic acid pinacol ester. As described herein, our overall findings imply that N4-arylcytosine scaffold is a versatile source of the diversity in the chemistry of nitrogen-containing heterocycles.

Scheme 1. Synthesis of 4-Arylaminocytosine Analogues 3a−f



RESULTS AND DISCUSSION

Preparation of *tert*-Butyl N4-Aryl-5-bromocytosin-1-yl Acetates as Substrates for Cyclization. The substrates for cyclization reactions, *tert*-butyl N4-aryl-5-bromocytosin-1-ylacetates **3a**, **3b**, **3d**, and **3f**, were prepared in four steps from uracil (**7**). Bromination of uracil (**7**),[7] followed by alkylation with *tert*-butyl bromoacetate and subsequent reaction with 1,2,4-triazole/$POCl_3$, provided the triazolyl-modified nucleobase **8** in 42% overall yield (Scheme 1).[8] Compound **8** was purified by flash column chromatography (FCC) and crystallization. The ^{1}H NMR spectrum of **8** was found to be in agreement with that reported by Pedroso et al.,[8] and the ^{13}C NMR spectrum of **8** was completely consistent with the structure.

An aromatic nucleophilic substitution of **8** with aniline was attempted following the procedures reported by Pedroso et al.[8] We have found that rigorous exclusion of water was undesirable, and the addition of a small amount of water facilitates the nucleophilic substitution of **8** with aniline (Scheme 1). The reaction was performed at room temperature (rt) in dioxane containing 10% water, in the absence of any additional base, and the product cytosine analogue **3a** conveniently precipitated from the reaction mixture and required no further purification (74% yield, Scheme 1).

A similar methodology was employed to prepare N4-arylcytosine analogues **3b**, **3d**, and **3f**. These reactions involved less nucleophilic anilines (2-bromoaniline, 2-chloroaniline, and anthranilic acid) and were therefore performed at 100 °C using 10% water in dioxane as a solvent without additional base. Upon cooling to rt, compounds **3b** (68% yield) and **3d** (50% yield) crystallized from the reaction mixture, whereas **3f** formed a gel and was subsequently dried and recrystallized from MeOH/water (1:1) in 86% yield (Scheme 1). Finally, cyclization substrates **3c** and **3e** were prepared in ca. 60% yield by treatment of **3b** and **3d** with Boc_2O in the presence of DMAP (Scheme 1).

Preparation of 5,6-BenzopC Acetic Acid (2a) and Its Conjugation with a Boc-OEt PNA Backbone. Several transition-metal-based protocols were considered for the cyclization of cytosine analogues **3** to *tert*-butyl 5,6-benzopC acetate (**2b**). Pd-mediated approaches[9] (substrates **3b** and **3d**) did not prove to be successful and led only to decomposition. An interesting discovery was made when elemental copper was used to promote the cyclization of **3b** and **3d**. This approach resulted yielded pyrimido[1,6-*a*]benzimidazol-2-ylacetate (**4**, Figure 1), as discussed below. Unfortunately, the desired *tert*-butyl 5,6-benzopC acetate (**2b**) could not be prepared by Cu-mediated cyclization. We have also investigated an intramolecular decarboxylative coupling[10] of substrate **3f**; however, this approach only led to intractable mixtures devoid of the desired product.

Since we observed the unwanted cyclization involving N3 of cytosine, a reasonable explanation was that the large groups

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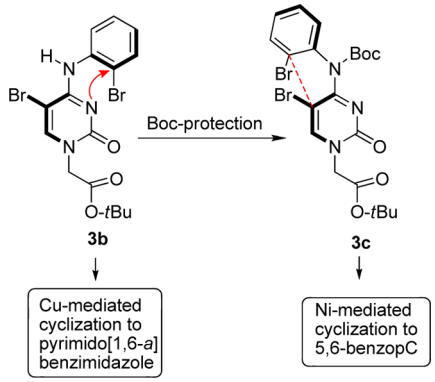
present at C5 of cytosine (Br) and in the *ortho* position on the *N*-arylamine moiety (Br, Cl, COOH) forced substrates **3b**, **3d**, and **3f** to adopt an anti conformation, thus disfavoring the formation of the desired pyrimido[5,4-*b*]indole heterocycle **2b**. We envisioned that use of the bulky *tert*-butyloxycarbonyl (Boc) protecting group on the exocyclic amine may favorably bias the conformation for the desired cyclization and simultaneously mask the reactivity of the N3 of the cytosine ring (Scheme 2).

Scheme 2. Presumed Conformations and Cyclization Patterns of Substrates 3b and 3c

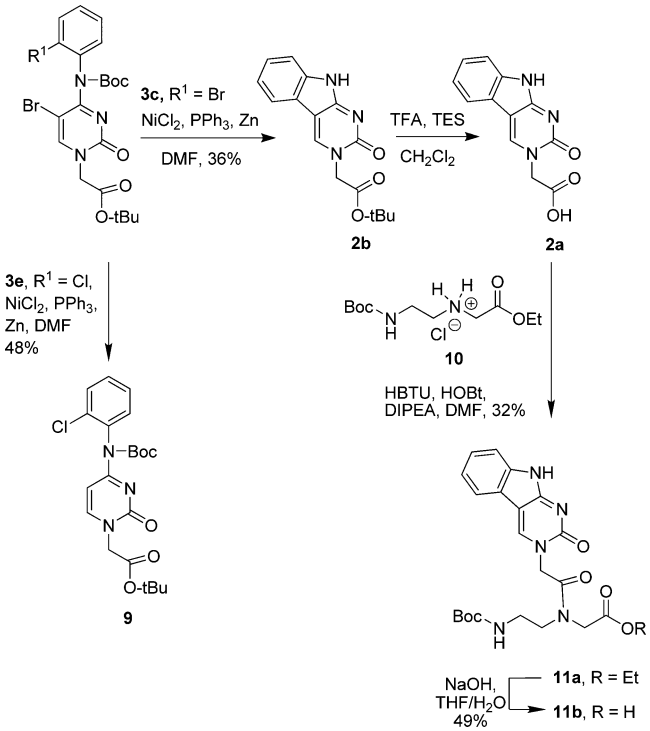


With the substrate **3c** in hand, we turned our attention to a Ni-mediated cyclization of **3c** ($NiCl_2/PPh_3/Zn^0$).[11] This reaction was performed by in situ preparation of the nickel catalyst (indicated by the solution turning deep red)[11b] and then slowly adding a solution of the cyclization substrate **3c**. As the reaction progressed, the red color slowly disappeared. After extractive workup, the desired *tert*-butyl 5,6-benzopC acetate (**2b**) was isolated by FCC (blue fluorescent spot on the TLC plate). Interestingly, the protecting Boc group was not retained during the cyclization reaction, and attempts to isolate the intermediate containing the Boc group were not successful. The reaction was initially executed using the proportions of reagents as described[11b] (0.2 equiv of $NiCl_2$, 3.8 equiv of PPh_3, and 15 equiv of Zn); however, the yields of the isolated product **2b** were not higher than 10−15%. The yield of **2b** was improved (to 36%) when an equimolar amount of $NiCl_2$ was used (Scheme 3). A further increase of the amount of $NiCl_2$ did not provide **2b** in higher yield.

We were able to prepare sufficient amounts of *tert*-butyl 5,6-benzopC acetate (**2b**) to permit detailed spectroscopy characterization and characterization of its fluorescence properties and for the conjugation with a Boc-OEt PNA backbone via the corresponding carboxylic acid derivative **2a** as described below. The formation of *tert*-butyl 5,6-benzopC acetate (**2b**) was not observed when a chlorine-containing substrate **3e** was exposed to the conditions of Ni-mediated cyclization. Debromination on the cytosine ring took place instead; corresponding product **9** was isolated in 48% yield (Scheme 3). We have also attempted the Ni-mediated cyclization of unprotected substrates **3b** and **3d**; however, only products of decomposition were detected as indicated by TLC analysis.

The PNA monomer containing the 5,6-benzopC nucleobase was then accessed via standard transformations. TFA-mediated *tert*-butyl group deprotection provided the 5,6-benzopC derived acid **2a** (Scheme 3), which was fully characterized (see the Supporting Information), followed by HBTU/HOBt[12]

Scheme 3. Synthesis of a PNA Monomer 11b



coupling to the backbone submonomer **10**[13] and a final saponification,[14] Scheme 3. Although the synthesis of **11b** from uracil (**7**) involved nine steps and furnished the final product in 1.1% overall yield, this was sufficient material to subsequently incorporate into oligomers to characterize their hybridization and fluorescence properties.

Ni-Mediated Cyclization on the Boc-OEt and Boc-OBn PNA Backbones. Although the 5,6-benzopC-containing PNA monomer **11b** was successfully prepared, our initial synthetic route suffered from low yields for the Ni-mediated cyclization[11b] to give **2b**, the subsequent coupling[12] of **2a** with the PNA backbone submonomer **10**, and the final saponification of the monomer ester (**11a**→**11b**, Scheme 3). Since we had already explored optimization of the Ni-mediated cyclization, we aimed for improving the latter two steps in the aforementioned triad of reactions.

The inefficient coupling of nucleobase acetic acid derivative **2a** to the Boc-OEt PNA backbone was partly ascribed to its poor solubility. Thus, we explored the possibility of coupling the cyclization substrate to the Boc-OEt PNA backbone and then performing the Ni-mediated reactions. Happily, the coupling of the cytosine **3b** derivative with carboxyl protecting group removed proceeded in a much improved yield leading to intermediate **12a**, Scheme 4. The substrate was prepared for cyclization by installation of the Boc group and then underwent the Ni-mediated reaction[11b] to give the Boc-OEt PNA backbone conjugated 5,6-benzopC **11a** in a yield similar to that achieved earlier for the N4-arylcytosine (**3c**).

Having established that the Ni-mediated cyclization was possible on a protected PNA monomer, we addressed the low yield of isolated product after saponification of **11a** to give **11b**. The hydrolysis proceeded smoothly, but the aqueous extractive workup was found to be somewhat troublesome and unavoidable, and prolonged exposure of **11b** to the aqueous acid (pH ∼ 3) resulted in partial removal of the Boc group. To

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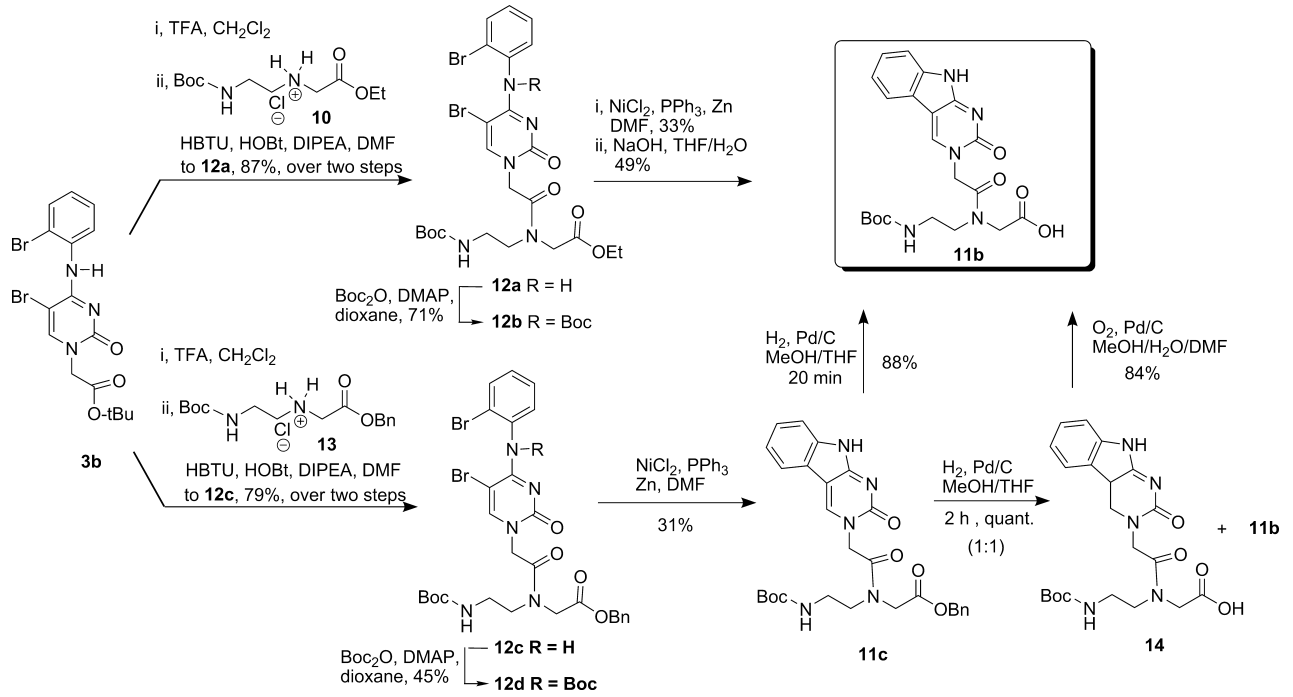
Scheme 4. Alternative Routes Converge to the PNA Monomer 11b

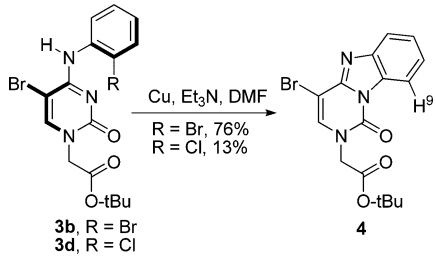


circumvent this issue, we turned to the Boc-OBn PNA backbone,[15] wherein the ester functionality can be removed by catalytic hydrogenation. The Boc-OBn PNA backbone (**13**) was prepared according to the literature procedures[16] and conjugation of the cytosine derivative **3b** proceeded well to give intermediate **12c**. Installation of the Boc group and Ni-mediated cyclization[11b] furnished **11c** (Scheme 4). Removal of the benzyl ester from **11c** was attempted by a catalytic hydrogenation[17] under an atmosphere of H_2 (10% Pd/C, 2 h). Even under these relatively mild conditions, in addition to the desired removal of the ester functionality (compound **11b**) an over-reduction was found to take place (^{1}H NMR and MS spectrometry) giving ca. 1:1 mixture in quantitative yield of the desired product **11b** and the 5,6-dihydropyrimidine product[18] (compound **14**, Scheme 4). While it has been reported that the cytosine ring can be restored by the oxidation with DDQ,[19] we were concerned about the compatibility of the rather harsh (DDQ, heat) reaction conditions with the benzopC PNA monomer **11b**. As an alternative approach we attempted catalytic oxygenation[20] (balloon filled with O_2, 10% Pd/C, 18 h) and were pleased to find that the desired 5,6-benzopC PNA monomer **11b** can be obtained in 84% yield (Scheme 4). To avoid over-reduction, we investigated the hydrogenation of **11c** using 1,4-cyclohexadiene as a hydrogen source.[21] This methodology failed to remove the benzyl ester; however, we later found that simply shortening the reaction time of the catalytic hydrogenation of **11c** to 20 min leads to the clean formation of the desired PNA monomer **11b** (88%, Scheme 4).

The alternative syntheses of **11b** discussed herein represents an overall improvement as the desired monomer **11b** can be prepared in 3.4% overall yield (via the Boc-OEt PNA backbone) in nine steps from uracil (**7**) or in 1.8% overall yield (via the Boc-OBn PNA backbone) in eleven steps from uracil (**7**) compared to the original approach that afforded the monomer **11b** in 1.1% overall yield.

Cu-Mediated Formation of Pyrimido[1,6-*a*]-benzimidazole Heterocyclic Framework. While exploring possible alternative methods to reductively couple the bis-aryl bromide cytosine analogue **3b**, we attempted a Cu^0-mediated cyclization (Cu^0, Δ in DMF, Et_3N; Scheme 5). TLC analysis of

Scheme 5. Cu-Mediated Cyclization of 3b and 3d to Form *tert*-Butyl 1-Oxo-4-bromopyrimido[1,6-*a*]benzimidazol-2-yl Acetate (4)



the reaction mixture indicated consumption of the starting material, and after standard extractive workup a single, unknown product was isolated by FCC in 76% yield. Key spectral evidence for determination of the structure came from the observation in ^{13}C NMR spectrum of a high-field aromatic signal (δ 91.3 ppm) consistent with the presence a C−Br bond. The presence of Br was further supported by HRMS analysis which displayed the typical isotopic pattern and allowed us to deduce the molecular formula $C_{16}H_{17}BrN_3O_3$. Although the presence of the bromine in the product was not consistent with the structure of *tert*-butyl 5,6-benzopC acetate (**2b**), this reaction was investigated in more detail. When substrate **3b** was treated under the same reaction conditions, except without Cu^0, no product was formed. Variation of the base did not have an influence on the outcome of the reaction. When a chlorinated cytosine analogue **3d** was subjected to the reaction conditions (Cu wire, Et_3N, DMF, heat), the same unknown product was obtained, albeit in lower yield (13%, Scheme 5). In addition to these observations, the Boc-protected substrates **3c** and **3e**

dx.doi.org/10.1021/jo402873e | J. Org. Chem. 2014, 79, 3336−3347

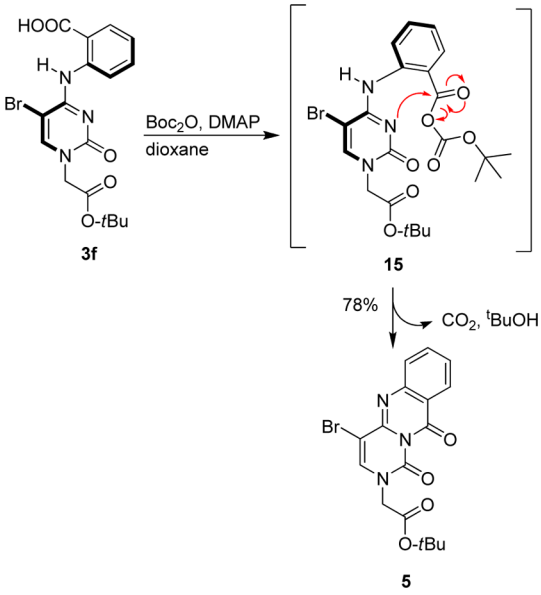
failed to undergo the Cu-mediated cyclization under various conditions, resulting in the recovery of starting materials **3c** and **3e**. Overall, these studies suggested the endocyclic N3 of cytosine ring is quite capable of nucleophilic displacement of a halogen atom present in the 2-haloarene moiety attached to the exocyclic N4. The unknown product was determined to be *tert*-butyl 1-oxo-4-bromopyrimido[1,6-*a*]benzimidazol-2-ylacetate (**4**, Scheme 5).

It is noteworthy that the heterocyclic skeleton present in **4** has been previously prepared in moderate yield (49%) by treatment of 3′,5′-di-*O*Ac-4-*O*-[(2,4,6-triisopropylphenyl)-sulfonyl]-5-bromo-2′-deoxyuridine with *o*-phenylenediamine in refluxing THF.[22] Only the ^{1}H NMR spectrum (80 MHz) of the product was provided in the original report;[22] thus, we were only able to carry out a tentative comparison with the spectral data acquired for heterocycle **4**. The chemical shift of the signal due to H9 (Scheme 5) was determined to be δ 8.31−8.49 ppm (m, 1H).[22] This value is in a good agreement with our data [δ 8.39 ppm (d, J = 8 Hz, 1H)]. The high chemical shift associated with this proton is attributed to the anisotropy of the nearby carbonyl group. The pyrimido[1,6-*a*]-benzimidazole heterocyclic skeleton has been well studied, especially with respect to its toxicology, and several different methods are available for its preparation.[23] Among these, the reaction of 2′-deoxycytidine with *p*-benzoquinone has been previously used to synthesize monomers suitable for DNA oligomerization.[24] Our methodology represents, to the best of our knowledge, the first methodology for the preparation of pyrimido[1,6-*a*]benzimidazole heterocyclic framework relying on the Cu-catalyzed reaction as a key step.[25]

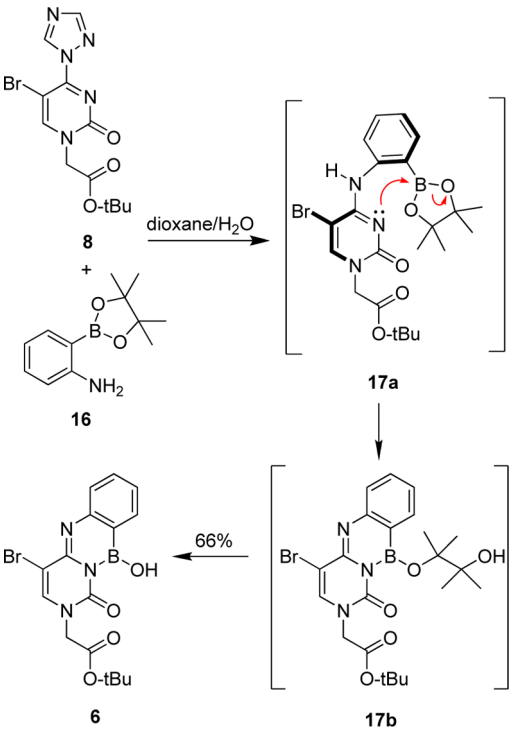
Formation of Pyrimido[1,6-*a*]quinazoline Heterocyclic Skeleton from Anthranilic Acid Modified Cytosine Analogue 3f. The anthranilic acid modified analogue **3f** was synthesized with the intention to investigate the possibilities of an intramolecular decarboxylative coupling[10] to form the heterocyclic skeleton present in *tert*-butyl 5,6-benzopC acetate (**2b**). This approach proved to be unsuccessful and failed to produce the desired product despite extensive experimentation. As done previously for **3b**, we attempted to protect the exocyclic N4 of the cytosine ring in **3f** with the Boc group. The reaction was carried out in warm dioxane as shown in the Scheme 6. After isolation of the product (heterocycle **5**), the ^{1}H NMR spectrum was not consistent with the expected structure as only one signal indicating the presence of *tert*-butyl moiety was observed [δ 1.51 ppm (s, 9H)]. In addition, five aromatic signals observed in the ^{1}H NMR spectrum were similar in chemical shifts and splitting patterns to those observed for *tert*-butyl 1-oxo-4-bromopyrimido[1,6-*a*]-benzimidazolylacetate (**4**). The HRMS spectrum of **5** revealed the molecular mass of 406 $(M + H)^+$, consistent with the molecular formula $C_{17}H_{17}BrN_3O_4$, and thus the unknown product was assigned the structure of *tert*-butyl 1,10-dioxo-4-bromopyrimido[1,6-*a*]quinazolinylacetate (**5**, Scheme 6). The formation of this heterocyclic compound is rationalized as depicted in Scheme 6. Presumably, the mixed anhydride **15** is formed in situ upon treatment with Boc_2O, followed by a nucleophilic attack of N3 of the cytosine ring. The product is obtained in good yield (78%), and this represents the first synthesis of the highly oxidized 1,10-dioxopyrimido[1,6-*a*]-quinazoline heterocyclic skeleton.

Formation of Pyrimido[1,6-*a*]benzo[*b*]6-bora-1,3-diazine Heterocyclic Framework. Suzuki−Miyaura coupling[26] between aryl halides and aryl boronic acids or aryl boronic acid esters represents an excellent methodology for the formation of a wide variety of biaryls. Due to some of the difficulties encountered in producing the 5,6-benzopC nucleus (Scheme 1), we decided to investigate the potential of an intramolecular Suzuki−Miyaura reaction on the substrate prepared by the reaction of 1,2,4-triazolyl-modified cytosine **8** and the commercially available 2-aminobenzeneboronic acid pinacol ester (**16**), Scheme 7. After 24 h of reaction, TLC analysis

Scheme 6. Cyclization of 3f to Form *tert*-Butyl 1,10-Dioxo-4-bromopyrimido[1,6-*a*]quinqzolin-2-yl Acetate (5)



Scheme 7. Reaction of Modified Cytosine 8 with Aniline Derivative 16 to Form *tert*-Butyl 1-Oxo-10-hydroxy-4-bromopyrimido[1,6-*a*]benzo[*b*]6-bora-1,3-diazin-2-yl Acetate (6)



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indicated consumption of the starting material **8**, and precipitation was induced adding a small amount of water and setting aside the reaction at 4 °C. The solid material collected by filtration was then analyzed. Neither the ^{1}H NMR nor HRMS spectrum of the product was consistent with the product of the replacement of 1,2,4-triazolyl moiety with *ortho*-substituted pinacol borane ester modified aniline. Only two aliphatic signals were observed in the ^{1}H NMR spectrum corresponding to the *tert*-butyl acetate moiety (δ 4.37 ppm, (s, 2H), 1.26 ppm, (s, 9H)); the signals attributable to the pinacol borane ester were not present. Mass spectrometry indicated the molecular mass of 405 $(M + H)^+$, along with an unusual isotope pattern which was attributed to the presence of both boron and bromine atoms in the molecule which was consistent with the molecular formula $C_{16}H_{17}BBrN_3O_4$. Additionally, a signal with δ 19.7 ppm was present in the ^{11}B NMR spectrum indicating the presence of tricoordinate boron atom.[27]

The structure and formation of the product can be rationalized as depicted in Scheme 7. Presumably, the intermediate **17a** (not detected) forms upon reacting the 1,2,4-triazolyl intermediate **8** with 2-aminobenzeneboronic acid pinacol ester (**16**). A nucleophilic attack of N3 subsequently takes place to form the intermediate **17b**, which further undergoes the hydrolysis under the conditions of the reaction with the formation of heterocycle **6** (*tert*-butyl 1-oxo-10-hydroxy-4-bromopyrimido[1,6-*a*]benzo[*b*]6-bora-1,3-diazin-2-ylacetate **6**, Scheme 7). The outcome of the reaction is consistent with the pattern of reactivity observed for N4-arylcytosines as described herein.

As the heterocyclic framework present in **6** is rather unusual and to the best of our knowledge unknown,[27] we confirmed the structural assignment by single-crystal X-ray analysis. Although the compound **6** is poorly soluble in a variety of solvents, crystals suitable for X-ray diffraction were grown by slow evaporation from a solution in AcOH. As a consequence of crystallization from AcOH, the solid-state structure of heterocycle **6** shows an acetate coordinated to the boron center. Thus, the solid-state structure possesses a zwitterionic character bearing the positive charge on the nitrogen in the position 5 (formerly N4 of cytosine), while the negative charge is located at the tetrahedral boron atom bearing the acetate (see the Supporting Information).

Fluorescence of *tert*-Butyl 5,6-BenzopC Acetate (2b). The steady-state fluorescence properties of *tert*-butyl 5,6-benzopC acetate (**2b**) have been investigated: the excitation maximum was found to be red-shifted from the natural nucleobases (λ_{excit} = 328 nm, EtOH), and the emission maximum at λ_{emiss} = 441 nm possessed a large Stokes shift of 113 nm (Figure 2). The quantum yield was determined (Φ = 0.79, EtOH) as described previously,[2b] using pyrene as a standard.[28] The large Stokes shift along with an excellent quantum yield associated with *tert*-butyl 5,6-benzopC acetate (**2b**) are superior among related compounds previously described in our laboratory.[2]

The solvatochromicity of the 5,6-benzopC chromophore was investigated by a examining comixtures of EtOH and H_2O. The fluorescence emission spectra of solutions of *tert*-butyl 5,6-benzopC acetate (**2b**) in EtOH with varying amounts of water (20, 40, 60, and 80%) were recorded (Figure 2).[2b] A substantial quenching of the fluorescence emission was observed with increasing water along with a noticeable bathochromic shift of the emission maxima. Severe quenching (>90%) of the fluorescence was detected in the solution containing 80% water. Under these conditions, no luminophore aggregation was evident; however, the limited solubility prevented using pure water. These results demonstrate that the chromophore present in *tert*-butyl 5,6-benzopC acetate (**2b**) is sensitive to its environment. The dramatic response to increasing polarity of the solvent comixtures was an encouraging indication that the 5,6-benzopC may behave as a microenvironmentally responsive nucleobase once incorporated into an oligomer.

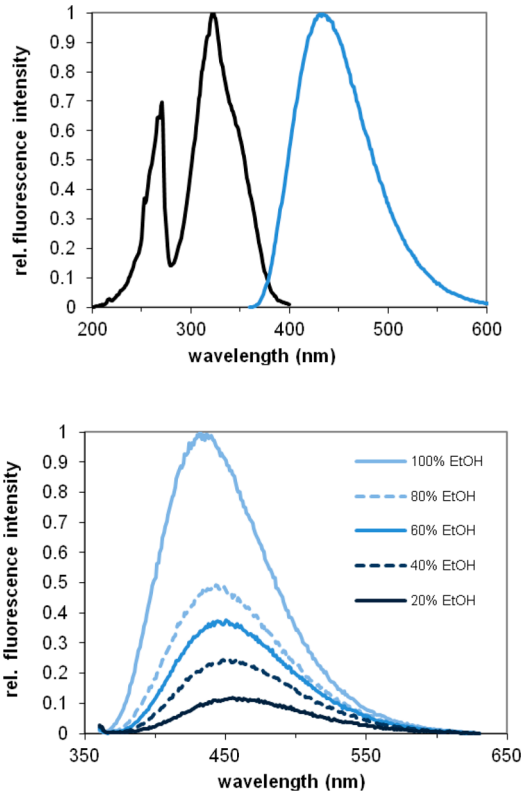

Figure 2. Steady-state fluorescence spectra of **2b** in EtOH (top). Excitation spectrum in black, emission in blue. Solvatochromicity study of **2b** in EtOH/water mixtures (bottom).

Oligomerization of the PNA Monomer 11b and Hybridization Studies. PNA oligomerization was carried out manually using the well-established protocols for Boc PNA chemistry.[29] Three oligomers were prepared as follows. The position of the unnatural nucleobase is indicated as X: GTA GAT CXC T-Lys (sequence 1A), Lys-GTA GAT CXC T-Lys (sequence 1B), or GTA GAT XAC T-Lys (sequence 2). Oligomers were purified by HPLC and were characterized by HR-ESI-MS. With the PNA oligomers in hand, their hybridization properties were investigated; the results are summarized in Table 1. The incorporation of the 5,6-benzopC nucleobase into sequences 1A and 1B moderately increases the stability of the corresponding DNA (ΔT_m +3 °C, 1A; ΔT_m +6 °C, 1B) and RNA (ΔT_m +5 °C, 1A; ΔT_m +1.5 °C, 1B) duplexes, while in sequence 2 it is tolerated in the DNA duplex showing no stabilization and a mild destabilization (ΔT_m −3 °C) toward complementary RNA. The differential stabilization of PNA:NA heteroduplexes based on sequence context is not unusual for PNAs containing modified bases and can be quite profound in some instances.[2b,30] Sequences 1 and 2 differ mainly by the flanking bases whereas −CXC− is more stabilizing versus −TXA−. Although it may be expected for 5,6-benzopC to gain additional stacking interactions over the purine, examination of

dx.doi.org/10.1021/jo402873e | J. Org. Chem. 2014, 79, 3336−3347

Table 1. Hybridization Studies with PNA Sequences 1A, 1B, and 2

sequence[a,b] (N→C)	X	DNA T_m (°C)	ΔT_m (°C)	RNA T_m (°C)	ΔT_m (°C)
GTA GAT C**X**C T-K	C	53.0		65.0	
GTA GAT C**X**C T-K	bpC	56.0	+3.0	70.0	+5.0
K-GTA GAT C**X**C T-K	C	53.0		63.0	
K-GTA GAT C**X**C T-K	bpC	59.0	+6.0	65.5	+1.5
GTA GAT **X**AC T-K	C	53.0		56.0	
GTA GAT **X**AC T-K	bpC	53.0	0.0	53.0	−3.0

[a]Measurements were carried out as described in the General Experimental Procedures. Each strand was present at 2 μM in a buffer of 100 mM NaCl, 10 mM NaH_2PO_4, 0.1 mM EDTA, pH 7.0. [b]No cooperative transition was observed for any case of mismatched nucleoside across from bpC. K = lysine (Lys); bpC = 5,6-benzopC.

the reported structure of a PNA:DNA duplex[31] suggests the geometry of the ring fusion rather favors interactions with the nucleobase on the pseudo 5′ side. One may speculate that this would result in less stacking (with the pyrimidine) or a clash with 5-methyl group of thymine thus leading to −TXA− being a less favored sequence.

The fluorescence properties of sequences 1A, 1B, and 2 upon binding to the DNA and RNA complementary strands and corresponding DNA mismatches have been also investigated. Unfortunately, nearly complete quenching of the fluorescence has been observed for all of the three sequences (see the Supporting Information for details) whether in the single-stranded or duplex state. This behavior can be attributed to the high sensitivity of the 5,6-benzopC fluorophore to the aqueous environment,[32] and even binding to a complementary DNA and RNA does not provide a sufficiently dehydrated environment to prevent the quenching of the fluorescence. This observation is also consistent with the results of dilution studies with the PNA oligomers. We have found that the fluorescence associated with the PNA oligomers containing 5,6-benzopC fluorophore is to some extent restored upon dissolving the oligomers in small amount of water and mixing the solutions with increasing amounts of dioxane or DMSO (see the Supporting Information). Our findings indicate that 5,6-benzopC fluorophore might operate in a hydrophobic environment and may be useful for binding of ligands/proteins to the oligomer for which a DNA-based scaffold would be preferred over that of PNA.

CONCLUSIONS

In conclusion, we have developed a methodology for the preparation of the pyrimido[5,4-*b*]indole heterocyclic skeleton[33] present in a fluorescent nucleobase analogue *tert*-butyl 5,6-benzopC acetate (**2b**). A Ni-mediated cyclization[11b] of *tert*-butyl N4-(Boc)-N4-(2-bromophenyl)-5-bromocytosin-1-ylacetate (**3c**) was employed as a key step. The chemistry is compatible with both Boc-OEt and Boc-OBn PNA backbones allowing for the conjugation at an earlier stage of the monomer synthesis followed by the cyclization to form the corresponding PNA monomers. The PNA oligomers have been successfully synthesized by means of manual Boc PNA chemistry.[29] The substitution was found to be well tolerated in the corresponding DNA and RNA duplexes, in the sequences examined.

The fluorescence properties of *tert*-butyl 5,6-benzopC acetate (**2b**) have been determined, revealing unprecedented values for the Stokes shift (113 nm) and quantum yield (Φ 0.79, EtOH) associated with pC analogs previously described in our laboratory.[2] Moreover, a strong solvatochromicity was also observed, indicating that the 5,6-benzopC chromophore is sensitive to its microenvironment. These properties make the 5,6-benzopC chromophore potentially an excellent complement to pC analogues previously reported.[2,32] Unfortunately, a complete quenching of the fluorescence was observed upon binding of the PNA oligomers to the DNA and RNA complements. This can be attributed to the high sensitivity of the 5,6-benzopC fluorophore to the aqueous environment.[32] Further synthetic modifications of the benzopC scaffold are underway to probe this phenomenon.

We have also found that a Cu-mediated cyclization of *tert*-butyl N4-(-Boc)-N4-(2-halophenyl)-5-bromocytosin-1-ylacetates provided an access to *tert*-butyl 1-oxo-4-bromopyrimido-[1,6-*a*]benzimidazol-2-ylacetate (**4**). The reaction was found to proceed in good yield in the case of bis-brominated cyclization substrate. In somewhat related cyclization, a treatment of anthranilic acid modified cytosine acetate with Boc_2O afforded *tert*-butyl 1,10-dioxo-4-bromopyrimido[1,6-*a*]quinqzolin-2-ylacetate (**5**) as a first example of the oxidized heterocyclic skeleton derived from he pyrimido[1,6-*a*]quinazoline framework. Finally, an unusual boron-containing heterocyclic framework pyrimido[1,6-*a*]benzo[*b*]6-bora-1,3-diazine has been prepared in a similar manner starting from *tert*-butyl 4-(1′,2′,4′-triazole)-1′-yl-5-bromocytosin-1-ylacetate (**8**) and 2-aminobenzeneboronic acid pinacol ester (**16**). In all three cases, the reactivity of the N3 of the cytosine ring played a crucial role. These unexpected reactions indicate that N4-arylcytosines represent an excellent source of diversity in heterocyclic chemistry and might serve as a template for other cyclization reactions involving various cytosine analogues.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents were commercially available, unless otherwise stated. Organic solvents were HPLC grade and used as such, except for DMF and dioxane (solvent purification system) and water (18.2 MΩ·cm^{-1} Millipore water). Organic extracts were dried with Na_2SO_4 and solvents were removed under reduced pressure in a rotary evaporator. Flash column chromatography (FCC) was carried out using silica gel, mesh size 230 − 400 Å. Thin layer chromatography (TLC) was carried out on Al backed silica gel plates, compounds were visualized by UV light. Preparative thin layer chromatography (PTLC) was carried out on glass backed silica gel plates (20 × 20 cm), layer thickness 1000 μm, compounds were visualized by UV light. Melting points were obtained on Fisher−Johns apparatus and are uncorrected. Ultra performance liquid chromatography (UPLC) was performed using a BEH C18 column (particle size 1.7 μm; 1.0 i.d. × 100 mm) and HR-ESI-MS detector. Mobile phase: Method A: 100% H_2O−100% MeCN (both solvents containing 0.1% HCOOH) over 5 min, linear gradient, flow rate 0.1 mL/min. HPLC analysis and purification was performed using a Microsorb-MV C_{18} 300 Å column (4.6 i.d. × 250 mm). The mobile phase for method B (sequences 1A and 1B) was 0−5 min, 99% H_2O−1% MeCN (both solvents containing 0.1% TFA) then 60% H_2O−40% MeCN over 25 min, 1 mL/min. The mobile phase for method C (sequence 2) was 0−5 min, 99% H_2O−1% MeCN (both solvents containing 0.1% TFA) then 100% MeCN over 20 min, 1 mL/min. NMR spectra were recorded on 400 MHz spectrometer; for ^{1}H (400 MHz), δ values were referenced as follows $CDCl_3$ (7.26 ppm),

dx.doi.org/10.1021/jo402873e | J. Org. Chem. 2014, 79, 3336−3347

CD_3OD (3.31 ppm), DMSO-d_6 (2.49 ppm) and for ^{13}C (100 MHz) $CDCl_3$ (77.0 ppm), CD_3OD (49.2 ppm), DMSO-d_6 (39.5 ppm). The ^{11}B NMR spectrum was acquired in dioxane. Mass spectra (MS) were obtained using an electron spray ionization (ESI) time-of-flight (TOF) instrument. Fluorescence and UV spectra measurements were carried out on respective instruments in spectrophotometric grade solvents. Quantum yield associated with compound **2a** was determined in EtOH as described previously[2b] using pyrene as a standard.[28] Hybridization and UV melting experiments were carried out in a buffer containing 100 mM NaCl, 10 mM Na_2HPO_4, and 0.1 mM EDTA at pH 7.0. T_m experiments were performed at 2×10^{-6} M for each strand concentration. Samples were heated to 95 °C and were allowed to cool to room temperature gradually (ca. 3 h). Denaturation was performed from 20 to 85 °C at a ramp of 0.5 °C/min. ΔT_m values are the difference between the T_m of sequences 1A, 1B, and 2 containing unnatural nucleobase and control sequences containing cytosine. The T_m values are an average of three measurements and are rounded to the nearest 0.5 °C. T_m values were estimated for cooperative transitions by the first derivative method. Temperature-dependent UV spectra that lacked upper and lower baselines lacked sigmoidal shape or were indistinguishable from ssPNA "self-melts" were deemed not to be cooperative transitions. Samples for the fluorescence measurements associated with the PNA oligomers were prepared in the same manner as those used for the determination of T_m values, using the same buffer, the same pH value and the same concentration.

Preparation of *tert*-Butyl-4-(1′,2′,4′-triazol-1′-yl)-5-bromocytosin-1-ylacetate (8). 5-Bromouracil was prepared from uracil (7) as described in ref 7. 5-Bromouracil (1.44 g, 7.56 mmol) was dissolved in dry DMF (13 mL), followed by the addition of K_2CO_3 (1.04 g, 7.56 mmol) and *tert*-butyl bromoacetate (1.12 mL, 7.56 mmol). The mixture was stirred at room temperature (rt) for 18 h. DMF was removed by coevaporation with $PhCH_3$ (3 × 200 mL), and the residue was partitioned between water (80 mL) and EtOAc (80 + 60 + 40 mL). The combined organic extract was dried and concentrated to leave crude *tert*-butyl 5-bromouracil-1-ylacetate (1.91 g, 83%) of sufficient purity for the next step.

1,2,4-Triazole (3.92 g, 56.71 mmol) was dissolved in dry MeCN (190 mL), and the solution was cooled to 0 °C followed by the addition of $POCl_3$ (1.17 mL, 12.6 mmol) and Et_3N (13.2 mL, 94.52 mmol). The mixture was stirred for 30 min at 0 °C, and then a solution of *tert*-butyl 5-bromouracil-1-ylacetate (1.91 g, 6.3 mmol) in dry MeCN (70 mL) was added in one portion. The cooling bath was removed, and the mixture was stirred for 18 h at rt. MeCN was evaporated; the residue was partitioned between water (60 mL) and EtOAc (60 + 2 × 40 mL). The combined organic extract was dried and concentrated; the residue was subjected to FCC on 70 g SiO_2, hexanes/acetone (2:1). Colorless needles started to form upon concentration of the eluate, hexanes were added, and the mixture was set aside for 2 h at −5 °C. The crystals were filtered off, washed with hexanes, and dried to leave *tert*-butyl 4-(1′,2′,4′-triazol-1′-yl)-5-bromocytosine-1-ylacetate (**8**, 1.36 g, 51%, based on 5-bromouracil): colorless needles; mp 118−120 °C; ^1H NMR ($CDCl_3$) δ 9.14 (s, 1H), 8.15 (s, 1H), 8.12 (s, 1H), 4.61 (s, 2H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.2, 156.0, 153.9, 153.4, 152.9, 145.1, 86.5, 84.3, 51.7, 27.9; HRMS (ESI) m/z found 356.0366 $[M + H]^+$ (calcd 356.0358 for $C_{12}H_{15}BrN_5O_3$).

Reaction of *tert*-Butyl 4-(1′,2′,4′-Triazole-1′-yl)-5-bromocytosin-1-ylacetate (8) with Anilines. A solution of *tert*-butyl 4-(1′,2′,4′-triazol-1′-yl)-5-bromocytosin-1-ylacetate (**8**, 712 mg, 2 mmol) in dioxane (7.2 mL) was treated with anilines as follows: aniline (365 μL, 4 mmol); 2-bromoaniline (447 mg, 2.6 mmol); 2-chloroaniline (270 μL, 2.6 mmol); and anthranilic acid (357 mg, 2.6 mmol). Water was added, and the mixtures were stirred at for 4 h at rt (aniline) or for 48 h at 100 °C. A white crystalline precipitate formed in the mixture containing the aniline; white crystalline precipitates formed upon cooling to rt in the case of 2-haloanilines. The mixtures were set aside for 3−4 h at 4 °C, and separated precipitates were filtered off with suction, were washed with water and cold (−10 °C) Et_2O, and were dried to provide analytically pure products. In the case of anthranilic acid, a gel formed upon cooling the reaction mixture to rt; the gel was redissolved in MeOH/H_2O (1:1, 50 mL), and the solution was concentrated to dryness. The residue was crystallized from MeOH/H_2O to afford white crystals which were filtered off and washed as described above.

*tert-Butyl N4-phenyl-5-bromocytosin-1-ylacetate (**3a**, 563 mg, 74%):* white crystalline solid; mp 204−206 °C; ^1H NMR ($CDCl_3$) δ 7.72 (m, 2H), 7.55 (s, 1H), 7.35 (m, 2H), 7.30 (br s, D_2O exch, 1H), 7.15 (m, 1H), 4.45 (s, 2H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 166.8, 157.6, 154.6, 145.5, 137.0, 129.0, 125.1, 121.6, 88.0, 83.2, 50.9, 28.0; HRMS (ESI) m/z found 380.0604 $[M + H]^+$ (calcd 380.0610 for $C_{16}H_{19}BrN_3O_3$).

*tert-Butyl N4-(2′-bromophenyl)-5-bromocytosin-1-ylacetate (**3b**, 628 mg, 68%):* white crystalline solid; mp 194−196 °C; ^1H NMR ($CDCl_3$) δ 8.76 (d, J = 8 Hz, 1H), 8.01 (br s, D_2O exch, 1H), 7.59 (s, 1H), 7.57 (dd, J = 8, 1.5 Hz, 1H), 7.36 (ddd, J = 8, 8, 1.5 Hz, 1H), 7.02 (ddd, J = 8, 8, 1.5 Hz, 1H), 4.48 (s, 2H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 166.7, 157.6, 154.5, 145.8, 135.2, 132.2, 128.5, 125.7, 123.1, 114.7, 88.6, 83.4, 51.0, 28.0; HRMS (ESI) m/z found 457.9717 $[M + H]^+$ (calcd 457.9715 for $C_{16}H_{18}Br_2N_3O_3$).

*tert-Butyl N4-(2′-chlorophenyl)-5-bromocytosin-1-ylacetate (**3d**, 415 mg, 50%):* white crystalline solid; mp 270−272 °C; ^1H NMR ($CDCl_3$) δ 8.80 (dd, J = 8, 1 Hz, 1H), 8.03 (br s, D_2O exch, 1H), 7.59 (s, 1H), 7.57 (dd, J = 8, 1 Hz, 1H), 7.32 (m, 1H), 7.08 (ddd, J = 8, 8, 1 Hz, 1H), 4.48 (s, 2H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 166.6, 157.6, 154.5, 145.8, 134.1, 128.9, 127.8, 125.2, 123.9, 122.8, 88.5, 83.4, 51.0, 28.0; HRMS (ESI) m/z found 414.0201 $[M + H]^+$ (calcd 414.0220 for $C_{16}H_{18}BrClN_3O_3$).

*tert-Butyl N4-(2′-oxycarbonylphenyl)-5-bromocytosin-1-ylacetate (**3f**, 729 mg, 86%):* white crystalline solid; mp 276−278 °C; ^1H NMR (CD_3OD) δ 9.14 (d, J = 8.5 Hz, 1H), 8.14 (s, 1H), 8.13 (m, 1H), 7.62 (m, 1H), 7.20 (m, 1H), 4.51 (s, 2H), 1.50 (s, 9H); ^{13}C NMR (CD_3OD) δ 171.4, 168.7, 160.4, 157.6, 148.8, 142.1, 135.1, 132.7, 124.6, 122.9, 118.7, 90.5, 84.1, 52.7, 28.4; HRMS (ESI) m/z found 424.0505 $[M + H]^+$ (calcd 424.0508 for $C_{17}H_{19}BrN_3O_5$).

Reaction of *tert*-Butyl N4-(2′-Halophenyl)-5-bromocytosin-1-ylacetates with Boc_2O. Separate mixtures containing *tert*-butyl N4-(2′-halophenyl)-5-bromocytosin-1-ylacetates (**3b** or **3d**, 1 mmol), Boc_2O (437 mg, 2 mmol), and DMAP (12 mg, 0.1 mmol) in dry dioxane (6 mL) were stirred at 60 °C for 90 min (**3b**) or 1 h (**3d**). The mixtures were cooled to rt, diluted with brine (50 mL), and extracted with EtOAc (2 × 30 mL). Combined organic extracts were dried and concentrated, and the residues were subjected to FCC purification on 50 g SiO_2, hexanes/EtOAc (1:1). Evaporation of eluates afforded white solid residue, which was crystallized from CH_2Cl_2/hexanes solution.

*tert-Butyl N4-Boc-N4-(2′-bromophenyl)-5-bromocytosin-1-ylacetate (**3c**, 333 mg, 60%):* white crystalline solid; mp 186−188 °C; ^1H NMR ($CDCl_3$) δ 7.76 (s, 1H), 7.60 (m, 1H), 7.33 (m, 2H), 7.18 (m, 1H), 4.49 (s, 2H), 1.50 (s, 9H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.6, 164.6, 154.3, 151.2, 149.1, 139.3, 133.4, 130.8, 129.3, 128.0, 123.1, 95.4, 83.8, 83.7, 51.3, 28.0, 27.9; HRMS (ESI) m/z found 580.0041 $[M + Na]^+$ (calcd 580.0059 for $C_{21}H_{25}Br_2N_3O_5Na$).

*tert-Butyl N4-Boc-N4-(2′-chlorophenyl)-5-bromocytosin-1-ylacetate (**3e**, 324 mg, 63%):* white crystalline solid; mp 176−178 °C; ^1H NMR ($CDCl_3$) δ 7.78 (s, 1H), 7.42 (m, 1H), 7.32 (m, 1H), 7.27 (m, 2H), 4.50 (s, 2H), 1.50 (s, 9H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 165.6, 164.8, 154.4, 151.2, 149.1, 137.6, 132.7, 130.5, 130.2, 129.1, 127.4, 95.4, 83.8, 83.7, 51.3, 27.9, 27.8; HRMS (ESI) m/z found 514.0740 $[M + H]^+$ (calcd 514.0744 for $C_{21}H_{26}BrClN_3O_5$).

Ni-Mediated Cyclization of *tert*-Butyl N4-Boc-N4-(2′-bromophenyl)-5-bromocytosin-1-ylacetate (3c). PPh_3 (258 mg, 0.99 mmol), $NiCl_2$ (34 mg, 0.26 mmol), and Zn dust (254 mg, 3.89 mmol) were placed into a round-bottom flask, and the flask was then charged with N_2. After the flask was flushed with N_2 gas for 10 min, dry DMF (1.5 mL) was added, and the mixture was stirred at 80 °C for 5−10 min (formation of dark red catalyst was visible).[11b] A solution of *tert*-butyl N4-Boc-N4-(2′-bromophenyl)-5-bromocytosin-1-ylacetate (**3b**, 145 mg, 0.26 mmol) in dry DMF (1 mL) was added dropwise over a period of ca. 3 min. The mixture was stirred for 1 h at 80 °C (N_2 atmosphere), cooled to room temperature, diluted with 4% EDTA

dx.doi.org/10.1021/jo402873e | J. Org. Chem. 2014, 79, 3336−3347

solution (40 mL), and extracted with EtOAc (2 × 20 + 10 mL). The combined organic extract was washed with brine (2 × 50 mL), dried, and concentrated. The residue was subjected to FCC purification on 30 g of SiO_2, CH_2Cl_2/MeOH (95:5). Evaporation of the eluate afforded *tert*-butyl pyrimido[5,4-*b*]indol-3-ylacetate or *tert*-butyl 5,6-benzopC acetate (**2b**) as white solid residue (28 mg, 36%): ^{1}H NMR (DMSO-d_6) δ 11.69 (br s, D_2O exch, 1H), 8.87 (s, 1H), 7.77 (d, J = 8 Hz, 1H), 7.33 (dd, J = 8, 7.5 Hz, 1H), 7.28 (d, J = 8 Hz, 1H) 7.18 (dd, J = 8, 7.5 Hz, 1H), 4.64 (s, 2H), 1.43 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 167.4, 162.3, 155.1, 142.4, 139.9, 126.6, 121.3, 120.3, 119.8, 111.3, 103.5, 81.6, 52.4, 27.7; HRMS (ESI) m/z found 300.1342 $[M + H]^+$ (calcd 300.1348 for $C_{16}H_{18}N_3O_3$).

Debromination of *tert*-Butyl N4-Boc-N4-(2′-chlorophenyl)-5-bromocytosin-1-ylacetate (3e) under the Conditions of Ni-Mediated Coupling. PPh_3 (199 mg, 0.76 mmol), $NiCl_2$ (5 mg, 0.04 mmol), and Zn dust (200 mg, 3 mmol) were placed into a round-bottom flask, and the Ni catalyst was prepared as described above. A solution of *tert*-butyl N4-Boc-N4-(2′-chlorophenyl)-5-bromocytosin-1-ylacetate (**3e**, 103 mg, 0.2 mmol) in dry DMF (1 mL) was added dropwise over a period of ca. 3 min. The mixture was stirred for 1 h at 80 °C (N_2 atmosphere), cooled to room temperature, diluted with 4% EDTA solution (40 mL), and extracted with EtOAc (2 × 20 mL). The combined organic extract was washed with brine (2 × 40 mL), dried, and concentrated. The residue was subjected to FCC purification on 30 g of SiO_2, hexanes/EtOAc (1:1). Evaporation of the eluate afforded *tert*-butyl N4-Boc-N4-(2′-chlorophenyl)cytosin-1-ylacetate (**9**) as a colorless solid (42 mg, 48%): ^{1}H NMR (DMSO-d_6) δ 8.08 (d, J = 7.5 Hz, 1H), 7.58 (m, 1H), 7.39 (m, 3H); 7.23 (d, J = 7.5 Hz, 1H), 4.48 (s, 2H), 1.40 (s, 9H); 1.34 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 166.9, 164.2, 154.5, 151.3, 149.8, 137.2, 131.8, 130.7, 129.4, 129.3, 127.8, 96.5, 83.1, 81.7, 51.1, 27.6, 27.3; HRMS (ESI) m/z found 436.1656 $[M + H]^+$ (calcd 436.1639 for $C_{21}H_{27}ClN_3O_5$).

BenzopC Acetic Acid (2a). TFA (300 μL) and TES (100 μL) were added to a suspension of the *tert*-butyl pyrimido[5,6-*b*]indole-3-ylacetate (**2b**, 27 mg, 0.09 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred for 18 h at rt, the solvent was evaporated, excess TFA was removed by coevaporation of the residue with CH_2Cl_2 (3 × 30 mL), and the residue was used for the conjugation with the Boc-OEt and Boc-OBn PNA backbones without further purification. Analytical samples of **2a** were obtained by subjecting the residue to the PTLC using CH_2Cl_2/MeOH/NH_4OH (80:19:1) as an eluent. The band with R_f 0.05 was carved off the plate, the silica was extracted with MeOH and filtered off, and the solvent was evaporated to leave pyrimido[5,4-*b*]indol-3-ylacetic acid or benzopC acetic acid (**2a**, 9 mg, 41%) as a slightly yellow solid: ^{1}H NMR (DMSO-d_6) δ 11.57 (br s, D_2O exch, 1H), 8.81 (s, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.29 (m, 2H), 7.14 (dd, J = 7.5, 6.5 Hz, 1H), 4.50 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 161.9, 155.6, 143.0, 139.8, 126.2, 121.1, 120.6, 119.7, 111.1, 103.0, 41.1; HRMS (ESI) m/z found 244.0714 $[M + H]^+$ (calcd 244.0722 for $C_{12}H_{10}N_3O_3$).

Conjugation of Benzo-PC Acetic Acid (2a) with Boc-OEt PNA Backbone Hydrochloride (10). Crude benzo-PC acetic acid (**2a**) prepared from 0.15 mmol of **2b** (by treatment with TFA/TES as described above) was dissolved in dry DMF (600 μL), diisopropylethylamine (DIPEA, 135 μL, 0.77 mmol) was added, and the mixture was cooled to 0 °C. HBTU (58 mg, 0.15 mmol) and HOBt (6 mg, 0.05 mmol) were added, and the mixture was stirred for 5 min at 0 °C. Boc-OEt PNA backbone hydrochloride (**10**, 43 mg, 0.15 mmol) and DIPEA (135 μL, 0.77 mmol) were added, the cooling bath was removed, and the mixture was stirred for 18 h at 50 °C. It was then cooled to rt and diluted with brine (40 mL). The aqueous phase was extracted with EtOAc (2 × 20 mL), and the combined organic extract was washed with brine (2 × 40 mL), dried, and concentrated. The residue was subjected to FCC on 20 g SiO_2, CH_2Cl_2/MeOH (95:5). Evaporation of the eluate afforded 5,6-benzopC PNA monomer **11a** as a white solid (23 mg, 32%): ^{1}H NMR (DMSO-d_6) δ 11.64 (br s, D_2O exch, 1H), 8.76 (s, 0.75H), 8.75 (s, 0.25H), 7.80 (d, J = 8 Hz, 0.25H), 7.78 (d, J = 8 Hz, 0.75H), 7.35 (m, 2H), 7.17 (m, 1H), 6.99 (t, D_2O exch, J = 5 Hz, 0.75H), 6.78 (t, D_2O exch, J = 5 Hz, 0.25H), 4.96 (s, 1.5H), 4.78 (s, 0.5H), 4.41 (s, 0.5H), 4.21 (q, J = 7 Hz, 0.5H), 4.08 (q, J = 7 Hz, 1.5H), 4.07 (s, 1.5H), 3.50 (m, 1.5H), 3.30 (m, 0.5H), 3.25 (q, J = 6.5 Hz, 1.5H), 3.04 (q, J = 6.5 Hz, 0.5H), 1.39 (s, 6.75H), 1.36 (s, 2.25H), 1.27 (t, J = 7 Hz, 0.75H), 1.18 (t, J = 7 Hz, 2.25H); ^{13}C NMR (DMSO-d_6) δ 169.4 (minor), 169.0 (major), 167.9 (minor), 167.7 (major), 162.2 (major + minor), 155.8 (minor), 155.1 (major), 142.8 (major + minor), 139.9 (major + minor), 128.8 (minor), 126.5 (major + minor), 121.3 (major + minor), 120.4 (major + minor), 119.7 (major + minor), 111.2 (major + minor), 103.4 (major + minor), 78.0 (major), 77.8 (minor), 61.2 (minor), 60.5 (major), 50.7 (minor), 50.4 (major), 49.3 (minor), 48.0 (major), 47.1 (major), 46.9 (minor), 38.4 (major), 37.7 (minor), 28.2 (major + minor), 14.0 (major + minor); HRMS (ESI) m/z found 472.2173 $[M + H]^+$ (calcd 472.2196 for $C_{23}H_{30}N_5O_6$).

Hydrolysis of 5,6-benzopC PNA Monomer 11a. 5,6-BenzopC PNA monomer **11a** (76 mg, 0.16 mmol) was dissolved in THF (2 mL), followed by the addition of NaOH solution (2 M, 320 μL, 0.64 mmol). The mixture was vigorously stirred for 40 min at rt and cooled to 0 °C, and the pH was adjusted to ca. 3 (1 M HCl). The mixture was diluted with water (5 mL) and extracted with EtOAc (5 × 10 mL). The combined organic extract was dried and concentrated to leave the Boc-OH 5,6-benzopC PNA monomer **11b** (35 mg, 49%) as a slightly yellow solid: ^{1}H NMR (DMSO-d_6) δ 11.66 (br s, D_2O exch, 1H), 8.78 (s, 0.75H), 8.77 (s, 0.25H), 7.81 (d, J = 7.5 Hz, 0.25H), 7.78 (d, J = 7.5 Hz, 0.75H), 7.31 (m, 2H), 7.17 (m, 1H), 6.98 (t, D_2O exch, J = 5.5 Hz, 0.75H), 6.78 (t, D_2O exch, J = 5.5 Hz, 0.25H), 4.96 (s, 1.5H), 4.78 (s, 0.5H), 4.31 (s, 0.5H), 4.01 (s, 1.5H), 3.40 (m, 2H), 3.25 (m, 1.5H), 3.04 (m, 0.5H), 1.39 (s, 6.75H), 1.37 (s, 2.25H); ^{13}C NMR (DMSO-d_6) δ 170.6 (major + minor), 168.0 (major), 167.4 (minor), 162.2 (major), 162.1 (minor), 155.2 (major), 155.1 (minor), 143.1 (minor), 142.9 (major), 139.9 (major), 139.8 (minor), 128.8 (minor), 128.7 (major), 126.5 (major), 126.4 (minor), 121.3 (major + minor), 120.5 (major), 120.4 (minor), 119.9 (minor), 119.8 (major), 111.3 (major), 11.2 (minor), 103.4 (major + minor), 78.0 (major), 77.7 (minor), 50.8 (major + minor), 50.5 (major + minor), 48.1 (major + minor), 47.1 (major + minor), 28.2 (major + minor); HRMS (ESI) m/z found 444.1877 $[M + H]^+$ (calcd 444.1883 for $C_{21}H_{26}N_5O_6$).

Conjugation of *tert*-Butyl N4-(2′-Bromophenyl)-5-bromocytosin-1-ylacetate (3b) with Boc-OEt PNA Backbone Hydrochloride (10) and Boc-OBn PNA Backbone (13). TFA (700 μL) was added to separate suspensions of *tert*-butyl N4-(2′-bromophenyl)-5-bromocytosin-1-ylacetate (**3b**, 358 mg, 0.78 mmol) in CH_2Cl_2 (2.1 mL). The mixtures were stirred for 18 h at rt, the solvents were evaporated, excess TFA was removed by coevaporation with CH_2Cl_2 (3 × 30 mL), and the residues were dried on the vacuum pump for 24 h. In separate flasks the residues were suspended in dry DMF (3 mL), followed by the addition of DIPEA (680 μL, 3.9 mmol). The resulting solutions were cooled to 0 °C, and HBTU (296 mg, 0.78 mmol) and HOBt (32 mg, 0.23 mmol) were added. The mixtures was stirred for 5 min at 0 °C, followed by the addition of Boc-OEt PNA backbone hydrochloride (**10**, 220 mg, 0.78 mmol) or Boc-OBn PNA backbone (**13**, 241 mg, 0.78 mmol) and DIPEA (in the case of hydrochloride **10**) (680 μL, 3.9 mmol). The mixtures were stirred for 24 h at 50 °C, were cooled to rt, and were diluted with brine (50 mL). The aqueous phases were extracted with EtOAc (2 × 30 mL), and the combined organic extracts were washed with brine (2 × 60 mL), dried, and concentrated. The residue was subjected to FCC on 60 g SiO_2, CH_2Cl_2/MeOH (95:5) (conjugate **12a**) or 45 g SiO_2, CH_2Cl_2/MeOH (98:2), later replaced with CH_2Cl_2/MeOH (95:5) (conjugate **12c**). Evaporation of the eluates afforded corresponding bis-brominated PNA monomers **12a** and **12c**.

*Boc-OEt PNA monomer **12a** (429 mg, 87%):* slightly yellow foam; ^{1}H NMR (DMSO-d_6) δ 8.85 (s, D_2O exch, 1H), 8.09 (s, 1H), 7.72 (d, J = 8 Hz, 1H), 7.65 (m, 1H), 7.44 (dd, J = 8, 8 Hz, 1H), 7.24 (dd, J = 8, 8 Hz, 1H), 6.94 (t, D_2O exch, J = 5.5 Hz, 0.75H), 6.75 (t, D_2O exch, J = 5.5 Hz, 0.25H), 4.73 (s, 1.5H), 4.55 (s, 0.5H), 4.31 (s, 0.5H), 4.18 (q, J = 7 Hz, 0.5H), 4.08 (q, J = 7 Hz, 1.5H), 4.04 (s, 1.5H), 3.41 (m, 1.5H), 3.32 (m, 0.5H), 3.18 (q, J = 6 Hz, 1.5H), 3.02 (q, J = 6 Hz, 0.5H), 1.38 (s, 9H); 1.24 (t, J = 7 Hz, 0.75H), 1.18 (t, J = 7 Hz, 2.25H); ^{13}C NMR (DMSO-d_6) δ 169.3 (minor), 169.0 (major), 167.7 (minor), 167.4 (major), 159.0 (major + minor), 155.7 (major), 155.5

dx.doi.org/10.1021/jo402873e | J. Org. Chem. 2014, 79, 3336−3347

(minor), 153.8 (major + minor), 148.2 (minor), 148.1 (major), 136.5 (major + minor), 132.6 (major + minor), 128.6 (major + minor), 128.2 (major), 128.0 (minor), 120.6 (major + minor), 85.5 (major + minor), 78.0 (major), 77.8 (minor), 61.1 (minor), 60.5 (major), 49.1 (minor), 48.8 (major), 47.9 (major + minor), 47.0 (major + minor), 38.2 (major + minor), 28.2 (major + minor), 14.0 (major + minor); HRMS (ESI) m/z found 630.0581 $[M + H]^+$ (calcd 630.0563 for $C_{23}H_{30}Br_2N_5O_6$).

Boc-OBn PNA monomer ***12c*** *(425 mg, 79%):* slightly yellow foam; ^{1}H NMR (DMSO-d_6) δ 8.87 (s, D_2O exch, 1H), 8.09 (s, 0.75H), 8.05 (s, 0.25H), 7.72 (d, J = 8 Hz, 1H), 7.66 (d, J = 8 Hz, 1H), 7.40 (m, 6H); 7.24 (dd, J = 8, 8 Hz, 1H), 6.97 (t, D_2O exch, J = 5.5 Hz, 0.75H), 6.77 (m, D_2O exch, 0.25H), 5.21 (s, 0.5 H); 5.13 (s, 1.5 H); 4.76 (s, 1.5H), 4.59 (s, 0.5H), 4.41 (s, 0.5H), 4.13 (s, 1.5H), 3.44 (m, 1.5H), 3.34 (m, 0.5H), 3.19 (m, 1.5H), 3.01 (m, 0.5H), 1.38 (s, 6.75H), 1.37 (s, 2.25 H); ^{13}C NMR (DMSO-d_6) δ 172.1 (major + minor), 169.3 (minor), 168.9 (major), 167.8 (minor), 167.5 (major), 164.6 (major + minor), 159.0 (major + minor), 155.8 (major), 155.6 (minor), 153.9 (major + minor), 148.1 (major + minor), 136.5 (major), 136.1 (minor), 135.8 (major), 135.6 (minor), 132.6 (major + minor), 128.6 (major + minor), 128.5 (2 × major + minor), 128.4 (minor), 128.2 (major + minor), 128.1 (major), 128.0 (2 × minor), 127.9 (major), 120.6 (major + minor), 85.6 (major + minor), 78.1 (major), 77.8 (minor), 66.0 (major), 65.4 (minor), 50.0 (major), 49.1 (minor), 48.8 (major), 48.3 (minor), 48.0 (major), 47.0 (minor), 38.2 (major + minor), 28.3 (minor), 28.2 (major); HRMS (ESI) m/z found 692.0710 $[M + H]^+$ (calcd 692.0719 for $C_{28}H_{32}Br_2N_5O_6$).

Boc-Protection of Bis-brominated PNA Monomers 12a and 12c. Separate solutions of bis-brominated PNA monomers **12a** (413 mg, 0.65 mmol) and **12c** (451 mg, 0.65 mmol), Boc_2O (286 mg, 1.31 mmol), and DMAP (8 mg, 0.07 mmol) in dry dioxane (**12a**, 3.5 mL; **12c**, 2.5 mL) were stirred for 18 h at 50 °C (monomer **12a**) or 48 h at 70 °C (monomer **12c**). The mixtures were cooled to rt and were diluted with brine (50 mL). The aqueous phases were extracted with EtOAc (2 × 30 mL), and combined organic extracts were dried and concentrated. The residue was subjected to FCC on 60 g SiO_2, CH_2Cl_2/MeOH (95:5) (conjugate **12b**) or 40 g SiO_2, CH_2Cl_2/MeOH (98:2) (conjugate **12d**). Evaporation of the eluates afforded Boc-protected bis-brominated PNA monomers **12b** and **12d**.

Boc-protected Boc-OEt PNA monomer ***12b*** *(338 mg, 71%):* slightly yellow foam; ^{1}H NMR (DMSO-d_6) δ 8.47 (s, 1H), 7.73 (dd, J = 8, 1.5 Hz, 1H), 7.45 (ddd, J = 8, 8, 1.5 Hz, 1H), 7.31 (m, 2H), 6.95 (t, D_2O exch, J = 6 Hz, 0.75H), 6.74 (t, D_2O exch, J = 6 Hz, 0.25H), 4.87 (s, 1.5H), 4.69 (s, 0.5H), 4.32 (s, 0.5H), 4.18 (q, J = 7 Hz, 0.5H), 4.08 (q, J = 7 Hz, 1.5H), 4.05 (s, 1.5H), 3.42 (m, 1.5H), 3.32 (m, 0.5H), 3.18 (q, J = 6 Hz, 1.5H), 3.01 (q, J = 6 Hz, 0.5H), 1.45 (s, 9H); 1.37 (s, 9H); 1.24 (t, J = 7 Hz, 0.75H), 1.18 (t, J = 7 Hz, 2.25H); ^{13}C NMR (DMSO-d_6) δ 169.2 (minor), 168.8 (major), 166.8 (minor), 166.4 (major), 163.7 (major + minor), 155.7 (major + minor), 153.7 (major + minor), 152.2 (minor), 152.1 (major), 150.5 (major + minor), 139.3 (major + minor), 133.2 (major + minor), 130.5 (major + minor), 129.7 (major + minor), 128.6 (major + minor), 122.6 (major + minor), 93.6 (major + minor), 83.2 (major + minor), 78.0 (major), 77.8 (minor), 61.2 (minor), 60.5 (major), 50.1 (minor), 49.8 (major), 49.2 (minor), 47.9 (major), 47.02 (major + minor), 38.9 (major), 38.2 (minor), 28.1 (major + minor), 27.5 (major + minor), 14.0 (major + minor); HRMS (ESI) m/z found 730.1107 $[M + H]^+$ (calcd 730.1087 for $C_{28}H_{38}Br_2N_5O_8$).

Boc-protected Boc-OBn PNA monomer ***12d*** *(232 mg, 45%):* slightly yellow foam; ^{1}H NMR (DMSO-d_6) δ 8.47 (s, 0.75H), 8.45 (s, 0.25H), 7.73 (d, J = 8 Hz, 1H), 7.38 (m, 8H); 6.97 (t, D_2O exch, J = 5.5 Hz, 0.75H), 6.76 (t, D_2O exch, J = 5.5 Hz, 0.25H), 5.21 (s, 0.5H), 5.13 (s, 1.5H), 4.90 (s, 1.5H), 4.74 (s, 0.5H), 4.42 (s, 0.5H), 4.14 (s, 1.5H), 3.44 (m, 1.5H), 3.34 (m, 0.5H), 3.20 (m, 1.5H), 3.02 (m, 0.5H), 1.45 (s, 9H); 1.37 (s, 6.75H); 1.36 (s, 2.25H); ^{13}C NMR (DMSO-d_6) δ 169.2 (minor), 168.8 (major), 166.8 (minor), 166.5 (major), 163.8 (major), 163.7 (minor), 162.3 (major + minor), 155.8 (major + minor), 153.7 (major + minor), 152.2 (minor), 152.1 (major), 150.6 (major + minor), 139.4 (major + minor), 135.7 (major), 135.5 (minor), 133.3 (major + minor), 130.5 (major + minor), 129.7 (major + minor), 128.6 (minor), 128.5 (minor) 128.4 (2 × major), 128.2 (minor), 128.1 (major + minor), 127.9 (major), 122.6 (major + minor), 93.7 (major + minor), 83.2 (major + minor), 78.1 (major), 77.8 (minor), 66.4 (minor), 66.0 (major), 49.8 (major + minor), 48.0 (major + minor), 47.1 (major + minor), 38.2 (major + minor), 28.2 (major + minor), 27.5 (major + minor); HRMS (ESI) m/z found 792.1244 $[M + H]^+$ (calcd 792.1222 for $C_{33}H_{40}Br_2N_5O_8$).

Ni-Mediated Cyclization of Boc-Protected Bis-brominated PNA Monomer 12b and 12d. PPh_3 (230 mg, 0.88 mmol), $NiCl_2$ (30 mg, 0.23 mmol), and Zn dust (227 mg, 3.47 mmol) in dry DMF (1 mL) were used to form the Ni catalyst as described above. Separate solutions of Boc-protected bis-brominated PNA monomers **12b** (169 mg, 0.23 mmol) and **12d** (185 mg, 0.23 mmol) in dry DMF (1 mL) were added dropwise over a period of ca. 3 min to the separate solutions of the freshly prepared Ni catalyst (N_2 atmosphere). The mixtures were stirred for 30 min at 80 °C (N_2 atmosphere), were cooled to room temperature, were diluted with 4% EDTA solution (40 mL), and were extracted with EtOAc (20 + 3 × 10 mL). Combined organic extracts were washed with brine (2 × 50 mL), dried, and concentrated. The residues was subjected to FCC purification on 25 g SiO_2, CH_2Cl_2/MeOH (95:5), later replaced with CH_2Cl_2/MeOH (9:1). Evaporation of the eluates afforded 5,6-benzopC PNA monomers **11a** and **11c** as white solid residue (36 mg, 33%).

Boc-OEt 5,6-benzopC PNA monomer ***11a*** *(36 mg, 33%):* colorless solid, for the complete spectral characterization see above.

Boc-OBn 5,6-benzopC PNA monomer ***11c*** *(38 mg, 31%):* colorless solid; ^{1}H NMR (DMSO-d_6) δ 11.65 (br s, D_2O exch, 1H), 8.76 (s, 0.75H), 8.73 (s, 0.25H), 7.80 (d, J = 8 Hz, 0.25H), 7.77 (d, J = 7.5 Hz, 0.75H), 7.37 (m, 7H); 7.17 (m, 1H), 7.01 (t, D_2O exch, J = 5.5 Hz, 0.75H), 6.80 (t, D_2O exch, J = 5 Hz, 0.25H), 5.24 (s, 0.5H), 5.13 (s, 1.5H), 4.99 (s, 1.5H), 4.82 (s, 0.5H), 4.51 (s, 0.5H), 4.17 (s, 1.5H), 3.53 (t, J = 6.5 Hz, 1.5H), 3.35 (m, 0.5H), 3.26 (q, J = 6 Hz, 1.5H), 3.05 (q, J = 6.5 Hz, 0.5H), 1.39 (s, 6.75H); 1.36 (s, 2.25H); ^{13}C NMR (DMSO-d_6) δ 169.5 (minor), 169.0 (major), 168.1 (minor), 167.8 (major), 162.2 (major + minor), 155.8 (major + minor), 155.1 (major + minor), 142.9 (major + minor), 139.9 (major + minor), 135.8 (major), 135.6 (minor), 128.5 (2 × minor), 128.4 (2 × major), 128.2 (minor), 128.1 (major + minor), 127.9 (major), 126.6 (major + minor), 121.3 (major + minor), 120.4 (major + minor), 119.9 (minor), 119.8 (major), 111.3 (major + minor), 103.5 (major + minor), 78.1 (major), 77.8 (minor), 66.6 (minor), 66.0 (major), 50.8 (minor), 50.5 (major), 49.3 (minor), 48.1 (major), 47.2 (major + minor), 38.4 (major + minor), 28.2 (major + minor); HRMS (ESI) m/z found 534.2330 $[M + H]^+$ (calcd 534.2353 for $C_{28}H_{32}N_5O_6$).

Hydrogenolytic Removal of the Bn Group from the Monomer 11c. 10% Pd/C (50 mg) was added to a solution of the Boc-OBn 5,6-benzopC PNA monomer **11c** (55 mg, 0.1 mmol) in MeOH (5.5 mL) and THF (1.5 mL). The mixture was vigorously stirred for 20 min at rt in an atmosphere of H_2 (balloon filled with H_2), the catalyst was filtered off using a Celite pad, the filter was washed with MeOH, and the filtrate was concentrated to leave the Boc-OH 5,6-benzopC PNA monomer **11b** (40 mg, 88%) as a slightly yellow solid. For the complete spectral characterization see above.

When the reaction mixture (using the same amount of starting material **11c**) was stirred for 2 h at rt under in H_2 atmosphere (balloon filled with H_2), a 1:1 mixture of the desired Boc-OH 5,6-benzopC PNA monomer **11b** and the product of the cytosine ring overreduction (compound **14**) was obtained in quantitative yield (44 mg) upon removal of the catalyst by filtration through a Celite plug. The mixture of **11c** and **14** (41 mg, 0.092 mmol) was dissolved in MeOH (3 mL), water (1 mL), and DMF (100 μL), and 10% Pd/C (25 mg) was added. The mixture was vigorously stirred for 18 h at rt in the atmosphere of O_2 (balloon filled with O_2), the catalyst was filtered off (Pasteur pipet with a cotton plug), and the filtrate was concentrated to leave the Boc-OH 5,6-benzopC PNA monomer **11b** (34 mg, 84%) as a slightly yellow solid. For the complete spectral characterization see above.

Cu-Mediated Cyclization of *tert*-Butyl N4-(2′-Halophenyl)-5-bromocytosin-1-ylacetates. Cu wire (46 mg, 0.72 mmol) and Et_3N (140 μL, 1 mmol) were added to a solution of *tert*-butyl N4-(2′-

dx.doi.org/10.1021/jo402873e | J. Org. Chem. 2014, 79, 3336−3347

bromophenyl)-5-bromocytosin-1-ylacetate (**3b**, 115 mg, 0.25 mmol) in DMF (1 mL). The mixture was stirred for 48 h at 70 °C, cooled to room temperature, diluted with 10% Na_2SO_3 solution (20 mL), and extracted with EtOAc (20 + 10 mL). The combined organic extract was washed with brine (2 × 30 mL), dried, and concentrated. The residue was subjected to FCC purification on 25 g SiO_2, hexanes/acetone (5:1). Evaporation of the eluate afforded *tert*-butyl 1-oxo-4-bromopyrimido[1,6-*a*]benzimidazol-2-ylacetate (4) as a slightly yellow solid (72 mg, 76%). Treatment of *tert*-butyl N4-(2′-chlorophenyl)-5-bromocytosin-1-ylacetate (**3d**, 104 mg, 0.25 mmol) was carried out for 48 h at 120 °C, affording 12 mg (13%) of the same product after the workup procedure described above: ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 8 Hz, 1H), 7.91 (d, J = 8 Hz, 1H), 7.51 (ddd, J = 8, 8, 1 Hz, 1H), 7.44 (ddd, J = 8, 8, 1 Hz, 1H), 7.36 (s, 1H), 4.60 (s, 2H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 166.1, 146.7, 146.3, 143.5, 135.4, 131.0, 126.2, 124.6, 120.0, 115.3, 91.3, 83.9, 50.2, 28.0; HRMS (ESI) m/z found 378.0455 $[M + H]^+$ (calcd 378.0453 for $C_{16}H_{17}BrN_3O_3$).

Boc_2O-Mediated Cyclization of *tert*-Butyl N4-(2′-Oxycarbonylphenyl)-5-bromocytosin-1-ylacetate (3f). A mixture of *tert*-butyl N4-(2′-oxycarbonylphenyl)-5-bromocytosin-1-ylacetate (**3f**, 212 mg, 0.5 mmol), Boc_2O (218 mg, 1 mmol), and DMAP (6 mg, 0.05 mmol) in dry dioxane (3 mL) was stirred for 1 h at 60 °C. The mixture was cooled to rt, diluted with brine (30 mL), and extracted with EtOAc (2 × 20 mL). The combined organic extract was dried and concentrated, and the residue was subjected to FCC purification on 30 g of SiO_2, hexanes/acetone (2:1). Concentration of the eluate resulted in the formation of crystals, hexanes were added, and the mixture was set aside for 2 h at −10 °C. The crystals were filtered off, washed with hexanes, and dried to give *tert*-butyl 1,10-dioxo-4-bromopyrimido[1,6-*a*]quinqzolin-2-ylacetate (**5**) as slightly yellow crystals (158 mg, 78%): mp 210−212 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (dd, J = 8, 1.5 Hz, 1H), 7.78 (m, 2H), 7.49 (m, 1H), 7.38 (s, 1H) 4.42 (s, 2H), 1.51 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.9, 159.6, 146.7, 145.7, 144.6, 138.1, 135.4, 127.9, 127.4 (2 × C), 120.6, 96.7, 83.8, 50.7, 28.0; HRMS (ESI) m/z found 406.0403 $[M + H]^+$ (calcd 406.0402 for $C_{17}H_{17}BrN_3O_4$).

Reaction of *tert*-Butyl-4-(1′,2′,4′-triazol-1′-yl)-5-bromocytosin-1-ylacetate (8) with 2-Aminobenzeneboronic Acid Pinacol Ester (16). A solution of *tert*-butyl 4-(1′,2′,4′-triazol-1′-yl)-5-bromocytosin-1-ylacetate (7, 178 mg, 0.5 mmol) and 2-aminobenzeneboronic acid pinacol ester (16, 126 mg, 0.58 mmol) in dioxane (1.8 mL) and water (200 μL) was stirred for 24 h at 95 °C. The mixture was cooled to room temperature, a small amount of water (ca. 1−2 mL) was added, and the mixture was set aside for 2 h at 3 °C. The precipitate which formed was filtered off with suction, consecutively washed with water and cold (−10 °C) Et_2O, and dried. The mother liquor was concentrated to ca. one-half of its original volume and set aside for 2 h at 3 °C, and the second crop of the product was treated as described above to afford *tert*-butyl 1-oxo-10-hydroxy-4-bromopyrimido[1,6-*a*]benzo[*b*]6-bora-1,3-diazin-2-ylacetate (**6**, 166 mg, 66%) as a slightly yellow solid: ^{1}H NMR (DMSO-d_6) δ 10.46 (s, D_2O exch, 1H), 8.48 (m, 1H), 8.26 (m, 1H), 8.02 (m, 1H), 7.45 (dd, J = 8, 8 Hz, 1H), 7.22 (dd, J = 7.5, 7.5 Hz, 1H) 4.37 (s, 2H), 1.26 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 166.5, 155.6, 152.1, 147.9, 141.9, 135.1, 130.4 (2 × C), 124.1, 118.9, 85.8, 81.8, 51.0, 27.4; ^{11}B NMR (dioxane) δ 19.7; HRMS (ESI) m/z found 405.0497 $[M + H]^+$ (calcd 405.0495 for $C_{16}H_{17}BBrN_3O_4$).

Synthesis of PNA Sequences 1A, 1B, and 2. PNA synthesis was performed using the established protocols for the manual Boc PNA synthesis.[29] A Boc-Lys(2-Cl-Cbz)-modified resin (200 mg) was downloaded with the T monomer to give the loading of 0.063 mmol/g.[34] The resulting resin was divided into two parts: the first part of the resin (110 mg, 6.89 × 10^{-6} mol) was used to prepare the PNA sequences 1A and 1B, and the second part of the resin (70 mg, 4.30 × 10^{-6} mol) was used to prepare the PNA sequence 2. Incorporation of the unnatural monomer **11b** required repeated (twice) coupling. After the synthesis was finished, the conjugates were cleaved off from the resin,[29] and crude PNA oligomers were obtained by the precipitation with cold (−10 °C) Et_2O, giving 15 mg of the crude PNA sequence 1A, 14 mg of the crude PNA sequence 1B, and 25 mg of the crude PNA sequence 2. Approximately 2−3 mg of the crude PNA sequences were dissolved in water (1 mL) and were subjected to HPLC purification as described in the General Experimental Procedures. The fractions containing the desired PNA sequences were frozen and lyophilized, and the pure PNA sequences were dissolved in water to achieve the final concentration of ca. 1 − 1.5 optimum density (OD) units. Resulting solutions have been used for the hybridization studies as described in the General Experimental Procedures.

PNA sequence 1A: HPLC (method B) t_R 23.0 min; HRMS (ESI) m/z found 2904.1881 $[M + H]^+$ (calcd 2904.1971 for $C_{119}H_{151}N_{58}O_{32}$).

PNA sequence 1B: HPLC (nethod B) t_R 23.5 min; HRMS (ESI) m/z found 3032.2772 $[M + H]^+$ (calcd 3032.2921 for $C_{125}H_{163}N_{60}O_{33}$).

PNA sequence 2: HPLC (method C) t_R 19.2 min; HRMS (ESI) m/z found 2928.2060 $[M + H]^+$ (calcd 2928.2084 for $C_{120}H_{151}N_{60}O_{31}$).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra, UPLC chromatograms, and HR-ESI mass spectra of compounds **2a,b**, **3a−f**, **4−6**, **8**, **9**, **11a,b**, and **12a−d**. ^{1}H NMR spectrum of 1:1 mixture of compounds **11b** and **14**. ^{11}B NMR spectrum of compound **6**. Detailed X-ray characterization of compound **6** (CIF). UPLC chromatograms and HR-ESI mass spectra of sequences 1A, 1B, and 2. Fluorescence studies with sequences 1A, 1B, and 2.This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank The Natural Sciences and Engineering Research Council (NSERC) of Canada for the financial support of this work. We thank Mr. Jonathan Dube, Dr. Mathew Willans, and Prof. Paul J. Ragogna for their help with the acquisition of ^{11}B NMR spectrum of compound **6** and for a fruitful discussion on this topic. Dr. Paul Boyle is acknowledged for performing the X-ray crystallographic analysis of compound **6** and subsequent structure solution.

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