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Synthesis of Amino- and Guanidino-G-Clamp PNA Monomers

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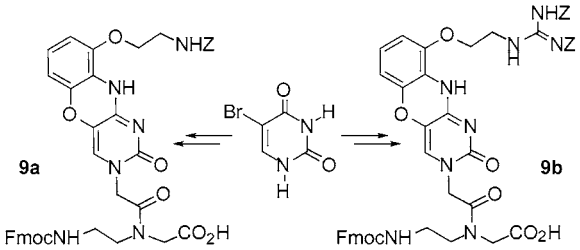
ABSTRACT



Syntheses of the protected amino- and guanidino-G-clamp PNA monomers, 9a and 9b, respectively, have been accomplished in eight steps from 5-bromouracil. Enhanced stacking interactions and additional hydrogen bonds with guanine should increase the affinity of PNAs incorporating these cytosine analogues for their complementary strands.

The therapeutic potential of antisense oligonucleotides (ODNs)[1] has stimulated studies aiming at chemically modifying the nucleic acids structure in order to increase their affinity toward their complementary strands and to improve their nuclease resistance and cell uptake.[2] Increase in affinity between nucleic acids strands is mainly achieved by improving stacking interactions and hydrogen bonding.[3] Enhanced stacking can be accomplished by introducing polycyclic base analogues, and an increase in the number of hydrogen bonds achieved by the simultaneous recognition of both the Watson−Crick and Hoogsteen binding faces of guanine and adenine bases. For this purpose, a tricyclic cytosine analogue, having the structure of an aminoethoxy-derivatized phenoxazine ring, was designed to bind to the guanine residue like a clamp (amino-G-clamp, Figure 1a), and incorporated into

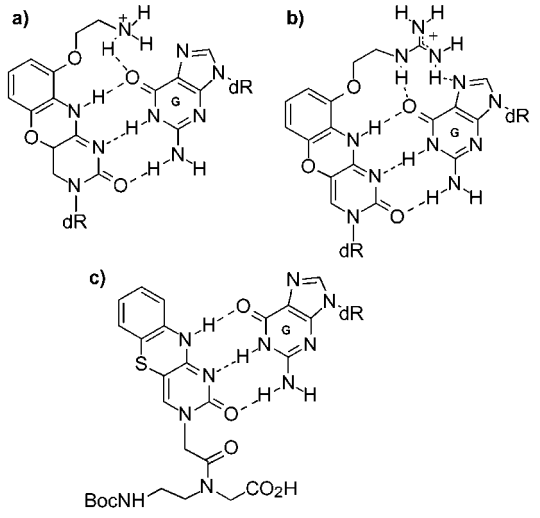


Figure 1. Hydrogen bonding in base-pairs of guanine with cytosine analogues: (a) amino-G-clamp, (b) guanidino-G-clamp, and (c) the fluorescent phenothiazine PNA monomer.

(1) Abbreviations: ACN = acetonitrile, DIEA = *N*,*N*-diisopropylethylamine, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, Fmoc = 9-fluorenylmethoxycarbonyl, ODN = oligodeoxynucleotide, PNA = peptide nucleic acid, TEA = triethylamine, Z = benzyloxycarbonyl.

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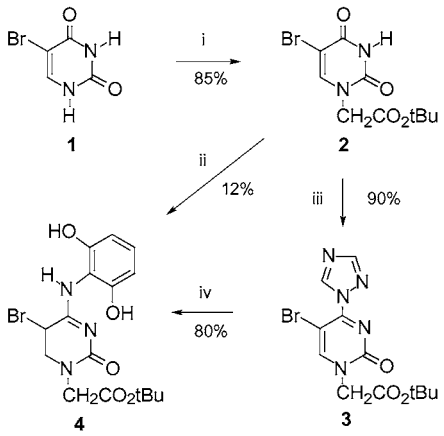
oligodeoxynucleotides.[4] A single incorporation of an amino-G-clamp was found to increase the T_m of a DNA decamer by 18 °C, presumably by the formation of four hydrogen bonds and enhanced stacking interactions. The amino-G-clamp conferred enhanced potency to a 15-mer phosphorothioate (PS) antisense oligonucleotide that showed sequence-specificity and RNase H activation.[5] Additionally, unaided cellular permeation was observed for a 7-mer PS-ODN incorporating the naked phenoxazine nucleobase analogue (without the aminoethoxy arm).[6] Furthermore, a single incorporation of an amino G-clamp at the 3′-end completely protects the oligonucleotides against 3′-exonuclease attack.[7] Recently, the X-ray diffraction analysis of a modified DNA decamer containing a nucleoside analogue, the guanidino-ethoxyphenoxazine derivative (Figure 1b), has shown that this guanidino-G-clamp forms five hydrogen bonds with guanine.[8]

The phenothiazine cytosine analogue has been incorporated into both oligonucleotides[4a] and peptide nucleic acids[9] (PNAs; the structure of the PNA monomer is shown in Figure 1c). The phenothiazine-containing oligomers formed more stable duplexes than the unmodified ones, and exhibited high fluorescent quantum yields.[9b] PNAs are nucleic acid analogues that contain a pseudo-peptide backbone to which the nucleobases are attached.[10] PNAs, which form sequence-specific and stable duplexes with DNA and RNA and are resistant to nuclease degradation, have been used for numerous therapeutic and biological applications.[11]

In this context, we were prompted to prepare the suitably protected amino- and guanidino-G-clamp PNA monomers, to introduce these base modifications into PNAs and examine their hybridizaton properties and other potential applications. Herein, for the first time we describe the synthesis of such PNA monomers via an independent route that is significantly different from the originally employed synthesis of the amino-G-clamp nucleoside derivative.[4b]

Since 5-bromodeoxyuridine had been employed for the synthesis of the nucleoside derivative,[4b] we chose 5-bromouracil **1** as the starting material for our route (Scheme 1).

Scheme 1[a]



[a] Reaction conditions: (i) $BrCH_2CO_2tBu$, K_2CO_3/DMF, rt, 21 h; (ii) Ph_3P, CCl_4/CH_2Cl_2, reflux, 3 h, and then 2-aminoresorcinol, DBU, rt, 13 h; (iii) 1,2,4-triazole, $POCl_3$, TEA/ACN, 0 °C, 30 min, and then addition of **2**, rt, 21 h; (iv) 2-aminoresorcinol, DBU/ACN, rt, 20 h.

However, attempts to directly introduce 2-aminoresorcinol at the C-4 position of bromouracil by the described method[4b] (activation of C-4 with Ph_3P-CCl_4 and then reaction with the aromatic amine in the presence of DBU) failed in our hands. Similarly, discouraging results were obtained when the starting material was N^1-trityl-5-bromouracil or N^1-methoxycarbonylmethyl-5-bromouracil. Only when N^1-*tert*-butoxycarbonylmethyl-5-bromouracil **2** was used were we able to obtain the N^4-dihydroxyphenyl derivative of 5-bromocytosine **4**, but in a low yield (12%).

The success came through the activation of the C-4 position of the uracil ring of **2** with $POCl_3$/1,2,4-triazole,[12] which allowed the isolation of the triazole derivative **3**. Subsequent reaction with 2-aminoresorcinol/DBU afforded the key intermediate **4** in 72% yield from **2**. It is worth mentioning that most of the difficulties found were associated with the low solubility of 2-aminoresorcinol, 5-bromouracil, and most of its derivatives. For instance, reactions iii and iv (Scheme 1) on N^1-methoxycarbonylmethyl-5-bromouracil, instead of **2**, provided the 2-aminoresorcinol-substituted derivative in low yield (8%).

The next two synthetic steps are the cyclization reaction to form the phenoxazine derivative **5** and the introduction of the Z-protected amino- or guanidino-arms by a Mitsunobu alkylation to afford either **6a** or **6b** (Scheme 2). Matteucci and co-workers[4b] employed a saturated solution of NH_3 in MeOH for the anchimerically assisted cyclization of the nucleoside derivative, but extremely low yields were obtained in our case using this reagent. Instead, a dilute solution of 10 equiv of KF in EtOH allowed us to isolate the desired phenoxazine **5** in reasonably good yield (76%). With respect to the Mitsunobu alkylation reaction, it was carried out using either *N*-Z-ethanolamine or *N*,*N*′-bis-Z-*N*″-(2-hydroxyethyl)-

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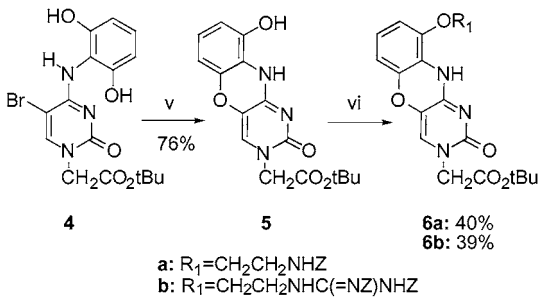
Scheme 2[a]



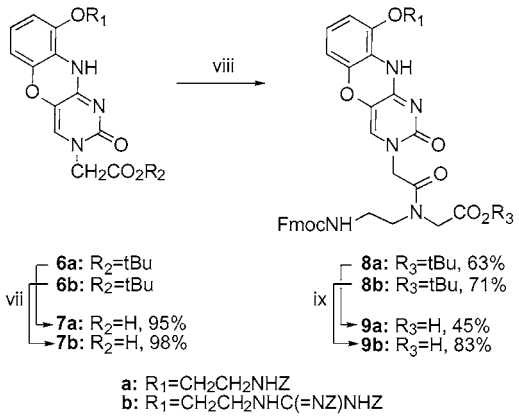
[a] Reaction conditions: (v) KF/EtOH, reflux, 20 h; (vi) R_1-OH, DEAD, $Ph_2PC_6H_4$-polymer/CH_2Cl_2, rt, 4 h.

guanidine. The former is commercially available and the latter was prepared by reaction of *N*,*N*′-bis-Z-*S*-methylisothiourea[13] and ethanolamine following literature procedures.[14] The alkylation reaction was carried out using polymer-bound triphenylphosphine[15] because it allows an easy separation of **6a** and **6b** from triphenylphosphine oxide.

In the synthesis of the amino-G-clamp nucleoside,[4b] these last two steps were reversed, that is the alkylation was followed by the cyclization. We have also performed the transformation of **4** into **6** in this order but slightly lower overall reaction yields were obtained (25% instead of 30%). When Mitsunobu reaction was performed first with **4**, it furnished undesirable dialkylated products lowering the overall yields. More importantly in our scheme we utilize a common intermediate **5** for the synthesis of both the amino- and the guanidino-G-clamp PNA monomers.

The remaining steps of the synthesis scheme are straightforward, but laborious (Scheme 3). Acid treatment of **6a**,**b** deprotected the carboxylic acid to give **7a**,**b**. EDC-mediated coupling of **7a**,**b** to *tert*-butyl *N*-Fmoc-aminoethylglycinate[16] afforded **8a**,**b**. Finally, anhydrous acid deprotection of the *tert*-butyl esters allowed the formation of the desired PNA monomer synthons **9a** and **9b**.

Scheme 3[a]



[a] Reaction conditions: (vii) 4 N HCl (g)/dioxane, rt, 16 h; (viii) $FmocNHCH_2CH_2NHCH_2CO_2tBu$, EDC/DMF, 0 °C to room temperature; (ix) TFA/CH_2Cl_2, 0 °C, 30 min, and room temperature, 30−60 min.

In summary, the protected amino-G-clamp and guanidino-G-clamp PNA monomers **9a** and **9b** have been obtained from 5-bromouracil in 5% and 10% overall yields, respectively. Work is in progress to incorporate these two cytosine analogues into PNA oligomers in order to exploit their remarkable capacity of recognition of guanine in the PNA series, and to enlarge their field of applications. It is also expected that the solubility of these modified PNAs will benefit from the charged amino- and guanidino-arms and improve with respect to that of the cytosine-containing oligomers.

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Supporting Information Available: Procedures for the preparation of compounds **2** to **9a**,**b** and their relevant spectroscopic (^{1}H and ^{13}C NMR, MS) data. This material is available free of charge via the Internet at http://pubs.acs.org.

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