2002 Vol. 4, No. 24 4209–4212

ORGANIC LETTERS

Synthesis, Incorporation into Triplex-Forming Oligonucleotide, and Binding Properties of a Novel 2'-Deoxy-*C*-Nucleoside Featuring a 6-(ThiazolyI-5)benzimidazole Nucleobase

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Received July 25, 2002

ABSTRACT



6-(Thiazolyl-5)benzimidazole (B¹) was designed as a novel nucleobase for the specific recognition of an inverted A·T base pair in a triple helix motif. It was successfully incorporated into an 18-mer triplex-forming oligonucleotide (TFO) using the 2'-deoxy-C-nucleoside phosphoramidite 16. The triple helix binding properties of the modified TFO were examined by means of thermal denaturation experiments targeting an oligopyrimidine-oligopurine 26-mer DNA duplex containing an A·T base pair inversion.

Pyrimidine oligonucleotides can interact, in a parallel orientation, with the oligopurine strand of an oligopyrimidine• oligopurine DNA duplex through the formation of T•AxT and C•GxC⁺ triplets.¹ This recognition process occurs in the major groove of the double stranded DNA (ds-DNA) and involves specific Hoogsteen hydrogen bonds between pyrimidines of the triplex forming-oligonucleotide (TFO) and the Watson–Crick purine bases of its cognate ds-DNA duplex.² Accordingly, TFOs have attracted considerable interest because of their potential applications in gene expression modulation and gene targeting technologies.³ Unfortunately, when a single A•T or G•C base pair interrupts an oligopyrimidine•oligopurine ds-DNA target, one observes a strong triplex destabilization.⁴ Hence, to date, the recognition of mixed purine/pyrimidine sequences remains a challenge. In the past few years, many TFO chemical modifi-

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⁽¹⁾ The symbols \cdot and x indicate Watson–Crick and Hoogsteen hydrogen bonds, respectively.

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cations were reported to overcome this sequence limitation.⁵ The most promising approach consists of designing new modified bases able to form hydrogen bond contacts on both bases of the A·T or G·C Watson–Crick inverted base pairs in the major groove.⁶ We have recently reported on the selective recognition of A·T base pair in ds-DNA by pyrimidine-motif TFO containing an unnatural nucleoside **S**.⁷ This new base analogue consists of two unfused aromatic rings (3-aminophenyl-thiazole) linked to a 2-deoxyribose unit by an acetamide motif. This interesting result led us to design a nucleobase analogue featuring a thiazolyl-benzimidazole system (**B**^t), to form selective hydrogen bonds with an inverted A·T base pair, as shown in Figure 1.



Figure 1. The proposed interactions between nucleobase B^t and the inverted $A \cdot T$ base pair.

 \mathbf{B}^{t} was designed on the basis of molecular modeling studies suggesting that a more rigid modified base would better stabilize triplex formation. Here we report on the synthesis of the new nucleobase \mathbf{B}^{t} together with its incorporation into a TFO whose recognition properties have been thoroughly examined. To incorporate \mathbf{B}^{t} into a TFO we needed to synthesize, as suggested by modelization, a 2'-deoxy-*C*nucleoside derivative of \mathbf{B}^{t} in which the deoxyribose unit is connected to the C2-position of the thiazolyl-benzimidazole aglycone \mathbf{B}^{t} (Figure 1). A 2'-deoxy-C-nucleoside, in which the aglycone moiety is reduced to a single benzimidazole ring \mathbf{B} (Figure 1), was also synthesized as a control.

A large number of synthetic approaches to a variety of C-nucleosides have been reported.⁸ In our case, the use of

an aldol-type condensation between the 2-lithiated derivative of **5** and **6** with a suitably protected 2-deoxyribose appeared to be the most appropriate and straightforward procedure toward the preparation of **B**- and **B**^t-derived 2'-deoxy-*C*nucleosides. The key steps in this synthesis consisted of a Stille-type coupling, for the biaryl aglycone **B**^t formation (Scheme 1), and an aryl-aldol type condensation followed



^{*a*} Reagents and conditions: (i) Boc₂O, pyridine (100%); (ii) K_2CO_3 , MeI, DMF (95%); (iii) LDA, THF, -78 °C then Bu₃SnCl (>95%); (iv) NaH, DMF, ClSO₂NMe₂ (80%); (v) H₂, Pd/C, MeOH/ THF (1/1) (>95%); (vi) TMSI, NaNO₂, TEBAC, CH₃CN (73%); (vii) Pd(PPh₃)₄, DMF, 60 °C (95%). *Compound **6** was obtained from benzimidazole following conditions (iv).

by a regioselective isopropylidene cleavage-ring closure (Schemes 2 and 3).

In this study, the *N*,*N*-dimethylsulfamoyl and *tert*-butoxycarbonyl protecting groups were used for the respective protection of the benzimidazole and aminothiazole rings of \mathbf{B}^{t} , and they were found to be compatible with oligonucleotide solid-phase synthesis.

First, the biaryl nucleobase \mathbf{B}^{t} was obtained, according to our previously described procedure,⁹ by a palladiumcatalyzed Stille cross-coupling reaction between derivative **2** and 5(6)-iodobenzimidazole **4** in 95% yield (Scheme 1).¹⁰ Compound **2** was obtained from 2-aminothiazole **1** in three steps (>90% overall yield), which consisted of (i) standard

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treatment with Boc₂O, (ii) methylation of the resulting carbamate, and (iii) stanylation at the thiazole C-5 position using LDA in THF, then trapping of the intermediate with tributyltin chloride. The 5(6)-iodo regioisomers 4^{11} derived from 5(6)-nitro-benzimidazole **3**: (i) benzimidazole protection (ClSO₂NMe₂/NaH/DMF), (ii) catalytic hydrogenolysis of the nitro function, and finally (iii) transformation of the obtained amine to the target iodo-derivatives **4** (NaNO₂/TMSI)¹² in 73% yield (Scheme 1).

Next, and as shown in Scheme 2, the protected B^t and benzimidazole **B** were lithiated by LDA in THF at -50 °C



^{*a*} Reagents and conditions: (i) LDA, THF, -78 °C then **7** (59–61%); (ii) MsCl, Et₃N, DMAP (88–90%).

to give the corresponding 2-lithio derivatives in quantitative yield.¹³ These 2-lithiated heterocycles were allowed to react with the known deoxyribose precursor 7^{14} to afford the corresponding pentitol derivatives **8** and **9** in 60% yield as a mixture of *R/S* diastereoisomers in 7/3 ratio, respectively, as determined by ¹H NMR. The diastereoisomers of each compounds **8** and **9** were then easily separated by silica gel flash chromatography.¹⁵ Mesylation of **8** and **9** with methanesulfonyl chloride afforded the 1-*O*-mesyl derivatives **10** (88%) and **11** (90%), respectively. To keep the required *N*,*N*-dimethylsulfamoyl and *tert*-butoxycarbonyl protecting groups on the aglycone moiety for oligonucleotide solid-phase synthesis, we needed to use mild conditions to eliminate the isopropylidene group of compounds **10** and **11**. Interestingly,

SnCl₂ treatment of 1'-O-mesyl derivatives **10**S and **11**S in methylene chloride at room temperature resulted in a clean conversion to the cyclized product **12** and **13**, respectively, (60% yield) following a cleavage-ring closure process (Scheme 3).



^{*a*} Reagents and conditions: (i) $SnCl_2 \cdot 2H_2O$, $CH_2Cl_2 (58-62\%)$; (ii) Bu_4NF , THF (85-87%); (iii) DMTCl, Et_3N , DMF (70-88%); (iv) $ClP(OCH_2CH_2CN)(P_2)$, Hunig's base, $CH_2Cl_2 (63-73\%)$.

Under these mild conditions, the S-isomers of 10 and 11 led to the cyclized products $12(\beta)$ and $13(\beta)$, respectively, whereas the *R*-isomers afforded the corresponding α -anomers $(12(\alpha) \text{ and } 13(\alpha), \text{ respectively, see Supporting Information}).$ In view of these data, it appears that pentitols 10 and 11 cyclize, under SnCl₂ treatment, according to an SN₂ process.¹⁶ The β -anometric configuration of **12** and **13** was assigned by 2D-NOESY experiments that showed, as expected for a β -configuration, a clear NOE correlation between H1' and H4'.¹⁷ The 5'-TBDPS group cleavage leading to the target B- and B^t-derived 2'-deoxy-C-nucleosides 14 and 15 was achieved by treatment of 12 and 13 with Bu₄NF in THF. Finally, the preparation of the phosphoramidite building blocks 16 and 17, which were used for TFO synthesis, was accomplished by successive 5'-dimethoxytritylation and 3'phosphitylation of nucleosides 14 and 15, respectively.

The phosphoramidites **16** and **17** were then incorporated into 18-mer TFOs (III) at the internal position Z (Figure 2) using automated oligonucleotide synthesis. The protected oligonucleotides thus obtained were purified by reverse phase HPLC, and the protecting DMT, SO₂NMe₂ and Boc groups

⁽¹¹⁾ The iodo derivative **4** was obtained as a mixture of 5- and 6-regioisomers in nearly 40/60 ratio, respectively. Both regioisomers will give the same oligonucleotide after cleavage of the sulfamoyl protecting group. To facilitate the interpretation of the ¹H and ¹³C NMR spectra, we only used the 6-isomer in the next steps of our synthesis.

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⁽¹⁵⁾ The R and S configurations of compounds 8 and 9 were determined on the basis of the anomeric stereochemistry of their cyclized products 12 and 13, respectively (NOESY experiments).

⁽¹⁶⁾ Under more drastic conditions (TFA, H₂O, 60 °C),¹⁴ the cyclization occurred but with (i) concomitant cleavage of all the protecting TBDPS, SO₂NMe₂ and Boc groups and (ii) epimerization of the C-1' stereocenter, which suggested a partial SN₂-type mechanism for the outcome of this reaction.

⁽¹⁷⁾ In the same way, we observed for the other anomers (12(α) and 13(α) obtained from 10*R* and 11*R*, respectively) clear NOE H1'-H3' and H1'-H5' correlations, in accordance with an α configuration.



Figure 2. Sequence of the studied triplexes I·IIxIII (X·Y = A·T and Z = G, B^t, or B) and melting temperature curves. Conditions: I·II (1.2 μ M), III (1.5 μ M) in 10 mM cacodylate buffer (pH 6) containing 100 mM NaCl, 10 mM MgCl₂, and 0.5 mM spermine.

were cleanly cleaved by treatment of the oligonucleotides with aqueous TFA solution (10%). To our knowledge, this is the first example of oligonucleotides synthesis in which N,N-dimethylsulfamoyl-protected nucleobases were involved.

The triple helix binding properties of these TFOs were examined by means of thermal denaturation experiments with the oligopyrimidine oligopurine 26-mer ds-DNA (I-II) containing an A·T interruption ($X \cdot Y = A \cdot T$) (Figure 2).

We observed that the T_m value obtained for the triplex containing an A·TxB^t triplet ($T_m = 43$ °C) is very close to that obtained with **G**, which is known to form the most stable natural triplet (A·TxG, $T_m = 45$ °C). However, as it is wellknown that **G** involves one hydrogen bond with **T** in A·TxG triplet (NH₂(G)-O4 (T)),^{5c} our B^t nucleobase most likely does not involve three hydrogen bonds as illustrated in Figure 1. Interestingly, the replacement of B^t by the benzimidazole nucleobase **B** caused a dramatic destabilization of the triplex ($\Delta T_m = -9$ °C from A·TxB^t to A·TxB) (Figure 2). This result shows clearly the implication of the methylaminothiazole moiety of B^t in the recognition process, which has not been yet elucidated.

To determine the specificity of the nucleobase \mathbf{B}^{t} , the 18mer TFO (III, $Z = \mathbf{B}^{t}$) was also screened against duplexes containing the three other possible base pairs (X·Y = T·A, C·G and G·C) (Table 1). It was observed that (i) the

Table 1.	Melting Temperature of All Combinations of Base
Triplets at	the X•YxZ Site ($T_{\rm m} \pm 1$ °C)

	Z			
Х•Ү	Т	С	G	Bt
T·A	51	31	31	37
C∙G	40	50	31	38
A·T	33	33	45	43
G·C	38	35	35	41

nucleobase \mathbf{B}^t can moderately discriminate a Pu·Py from a Py·Pu base pair (A·Tx \mathbf{B}^t and G·Cx \mathbf{B}^t vs T·Ax \mathbf{B}^t and C·Gx \mathbf{B}^t) and (ii) that \mathbf{B}^t provides a +3 °C triplex stabilization in the case of a G·C interruption compared to the best base triplet made of the natural bases G·CxT. The moderate discrimination of nucleobase \mathbf{B}^t for all four base pairs argues for a non-sequence-specific interaction, i.e, third strand base stacking/intercalation.

In conclusion, we have synthesized a novel 2'-deoxy-Cnucleoside analogue featuring a highly functionnalized aglycone moiety (**B**') and shown the *N*,*N*-dimethylsulfamoyl protecting group to be compatible with oligonucleotide synthesis. The new nucleobase was successfully incorporated into a TFO using automated oligonucleotide synthesis. The triplexes featuring an $A \cdot TxB^t$ triplet or the most stable natural triplet $A \cdot TxG$ were shown to be of comparable stability. In view of these results, **B**^t represents an interesting starting point for the synthesis of other nucleobases, for structureaffinity relationship studies which is currently in progress.

Acknowledgment. The authors thank M. Thomas for oligonucleotides synthesis, Dr. J.-C. Blais for characterization of oligonucleotides, and CNRS for a doctoral fellowship (BDI) to D.G.

Supporting Information Available: Experimental procedures and spectroscopic characterization (¹H, ¹³C, ³¹P (for phosphoramidites) NMR and MS) of all new compounds. Also automated oligonucleotides synthesis, melting temperature experiments, and MALDI-TOF/MS characterization of oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

OL026609H