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

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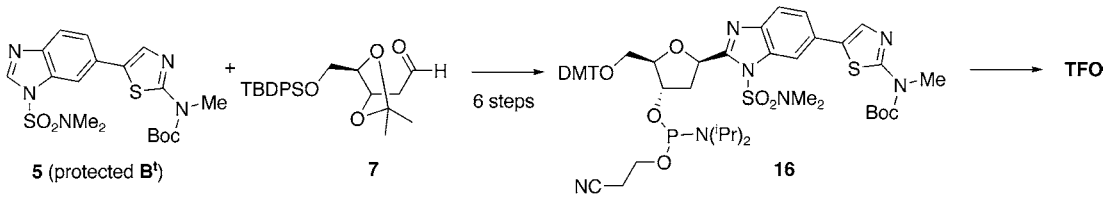
ABSTRACT



6-(Thiazolyl-5)benzimidazole (B[t]) 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 · 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.

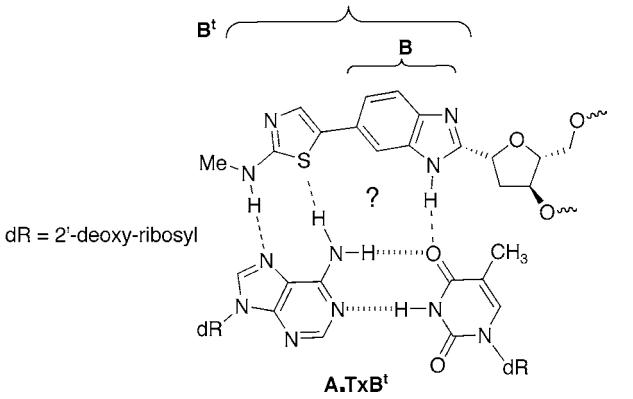
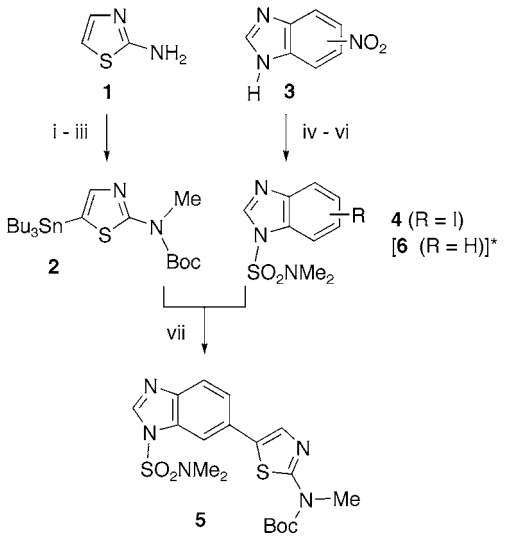


Figure 1. The proposed interactions between nucleobase **B**^{t} and the inverted **A·T** base pair.

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 **B**^{t} together with its incorporation into a TFO whose recognition properties have been thoroughly examined. To incorporate **B**^{t} into a TFO we needed to synthesize, as suggested by modelization, a 2′-deoxy-*C*-nucleoside derivative of **B**^{t} in which the deoxyribose unit is connected to the C2-position of the thiazolyl-benzimidazole aglycone **B**^{t} (Figure 1). A 2′-deoxy-C-nucleoside, in which the aglycone moiety is reduced to a single benzimidazole ring **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 by a regioselective isopropylidene cleavage-ring closure (Schemes 2 and 3).

Scheme 1[a]



[a] Reagents and conditions: (i) Boc_2O, pyridine (100%); (ii) K_2CO_3, MeI, DMF (95%); (iii) LDA, THF, −78 °C then Bu_3SnCl (>95%); (iv) NaH, DMF, $ClSO_2NMe_2$ (80%); (v) H_2, Pd/C, MeOH/THF (1/1) (>95%); (vi) TMSI, $NaNO_2$, TEBAC, CH_3CN (73%); (vii) $Pd(PPh_3)_4$, DMF, 60 °C (95%). *Compound **6** was obtained from benzimidazole following conditions (iv).

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 **B**^{t}, and they were found to be compatible with oligonucleotide solid-phase synthesis.

First, the biaryl nucleobase **B**^{t} was obtained, according to our previously described procedure,[9] by a palladium-catalyzed 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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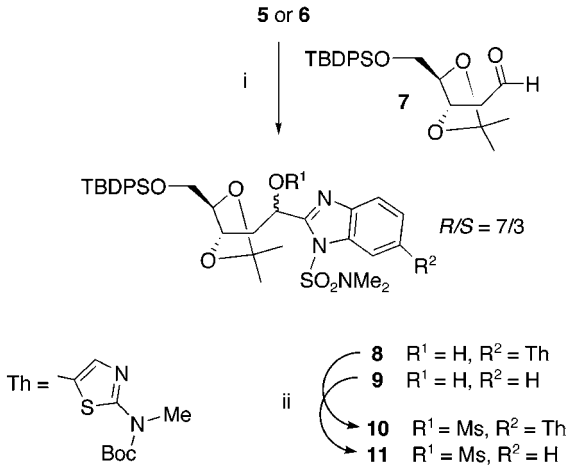
treatment with Boc_2O, (ii) methylation of the resulting carbamate, and (iii) stanylation at the thiazole C-5 position using LDA in THF, then trapping of the intermediate with tributyltin chloride. The 5(6)-iodo regioisomers **4**[11] derived from 5(6)-nitro-benzimidazole **3**: (i) benzimidazole protection ($ClSO_2NMe_2$/NaH/DMF), (ii) catalytic hydrogenolysis of the nitro function, and finally (iii) transformation of the obtained amine to the target iodo-derivatives **4** ($NaNO_2$/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

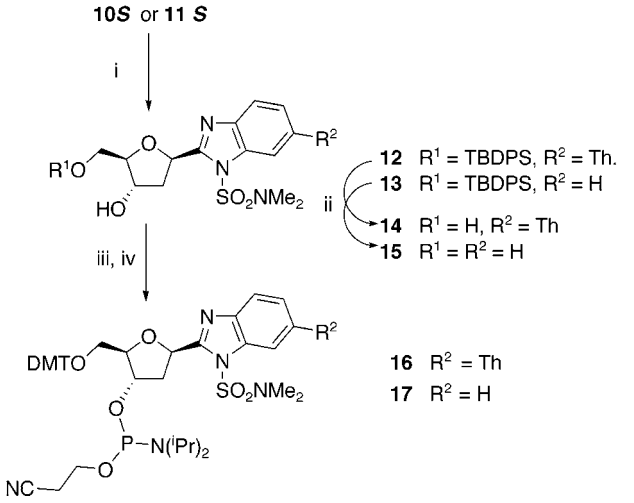
Scheme 2[a]



[a] Reagents and conditions: (i) LDA, THF, −78 °C then **7** (59−61%); (ii) MsCl, Et_3N, 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 ^{1}H 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, $SnCl_2$ 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).

Scheme 3[a]



[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) $ClP(OCH_2CH_2CN)(^iPr)_2$, 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**(*β*) and **13**(*β*), respectively, whereas the *R*-isomers afforded the corresponding α-anomers (**12**(α) and **13**(α), respectively, see Supporting Information). In view of these data, it appears that pentitols **10** and **11** cyclize, under $SnCl_2$ treatment, according to an SN_2 process.[16] The *β*-anomeric 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 Bu_4NF 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, SO_2NMe_2 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 ^{1}H and ^{13}C NMR spectra, we only used the 6-isomer in the next steps of our synthesis.

(12) Lee, J. G.; Cha, H. T. *Tetrahedron Lett.* **1992**, *33*, 3167−3168.

(13) We have verified that this lithiation occurred exclusively and quantitatively at the C-2 position of the benzimidazole moiety by trapping the lithio derivatives with hexachloroethane.

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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, H_2O, 60 °C),[14] the cyclization occurred but with (i) concomitant cleavage of all the protecting TBDPS, SO_2NMe_2 and Boc groups and (ii) epimerization of the C-1′ stereocenter, which suggested a partial SN_2-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.

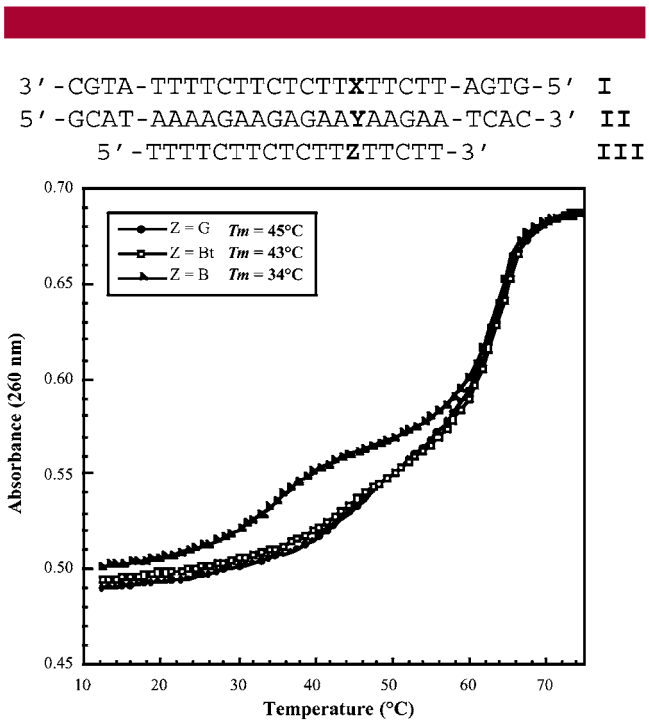

Figure 2. Sequence of the studied triplexes I·IIxIII (X·Y = A·T and Z = **G**, **B**^{t}, or **B**) and melting temperature curves. Conditions: I·II (1.2 μM), III (1.5 μM) in 10 mM cacodylate buffer (pH 6) containing 100 mM NaCl, 10 mM $MgCl_2$, and 0.5 mM spermine.

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·Y = A·T) (Figure 2).

We observed that the T_m value obtained for the triplex containing an A·Tx**B**^{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·Tx**G**, T_m = 45 °C). However, as it is well-known that **G** involves one hydrogen bond with **T** in A·Tx**G** triplet (NH_2(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 (ΔT_m = −9 °C from A·Tx**B**^{t} to A·Tx**B**) (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 **B**^{t}, the 18-mer TFO (III, Z = **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_m ± 1 °C)

	Z			
X·Y	T	C	G	**B**^{t}
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 **B**^{t} can moderately discriminate a Pu·Py from a Py·Pu base pair (A·Tx**B**^{t} and G·Cx**B**^{t} vs T·Ax**B**^{t} and C·Gx**B**^{t}) and (ii) that **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·Cx**T**. The moderate discrimination of nucleobase **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-C-nucleoside analogue featuring a highly functionnalized aglycone moiety (**B**^{t}) 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·Tx**B**^{t} triplet or the most stable natural triplet A·Tx**G** 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 structure-affinity relationship studies which is currently in progress.

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Supporting Information Available: Experimental procedures and spectroscopic characterization (^{1}H, ^{13}C, ^{31}P (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.

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