Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 12, pp. 1553-1556, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00270-9

REPLACEMENT OF THE PHOSPHODIESTER LINKAGE IN OLIGONUCLEOTIDES BY HETEROCYCLES: THE EFFECT OF TRIAZOLE- AND IMIDAZOLE-MODIFIED BACKBONES ON DNA/RNA DUPLEX STABILITY

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Abstract: Thymidine dinucleotide analogs with imidazole-derived backbones (**III** and **IV**) were synthesized. These modified dimeric building blocks were incorporated into oligodeoxyribonucleotides. The hybridization affinity of the modified oligonucleotides for RNA complements was determined and compared to the corresponding olefinic modifications **I** and **II**. © 1997 Elsevier Science Ltd.

In the previous communication of this issue, we have described the replacement of the natural phosphodiester linkage in T-T dimers with a *cis* C=C double bond (**I**, Figure 1) or with a triazole heterocycle. *Cis* C=C double bonds **I** and **II**[1a] and aromatic heterocycles serve to mimic the overall geometry of an internucleosidic amide bond fixed in a *cis* conformation.[1] In order to further assess the contributions of polarity and/or basicity of the heterocycle to the RNA binding affinity of modified oligonucleotides, these studies were extended to imidazole-containing backbone modifications of type **III** and **IV**. In this communication, we report on the synthesis of modified dimeric building blocks corresponding to **III** and **IV** as well as on the thermodynamic properties of the duplexes formed between modified oligodeoxyribonucleotides containing these novel backbone modifications and their RNA complements.

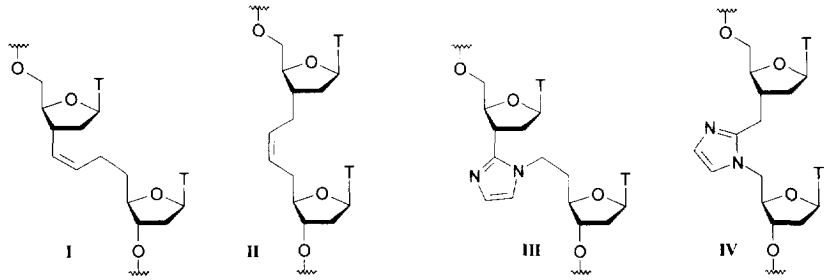
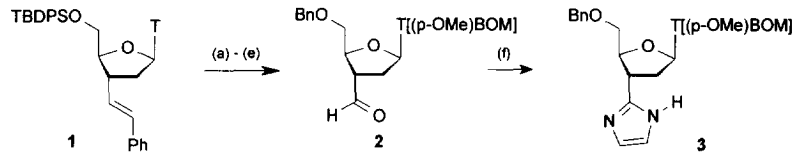


Figure 1.

A number of approaches to the imidazole-containing dinucleotide analogs of type **III** and **IV** were initially examined. The most viable strategy that emerged from these studies was based on the construction of the imidazole heterocycle from an aldehyde carbonyl destined to become C(2) of the imidazole and subsequent N(1)-alkylation. Several challenges associated with this route were to be met. Most notable among them were, first, the elaboration of conditions for imidazole formation compatible with the stability of the glycosidic bond and protecting groups of potential aldehyde precursors and, second, the identification of a suitable electrophile for N(1)-alkylation of the basic but only weakly nucleophilic imidazole anion. Schemes 1-3 summarize how these issues were addressed in the context of the synthesis of dinucleotide analog **10**. Construction of the imidazole moiety of **10** was achieved by reaction of protected aldehyde **2** (prepared in 5 steps

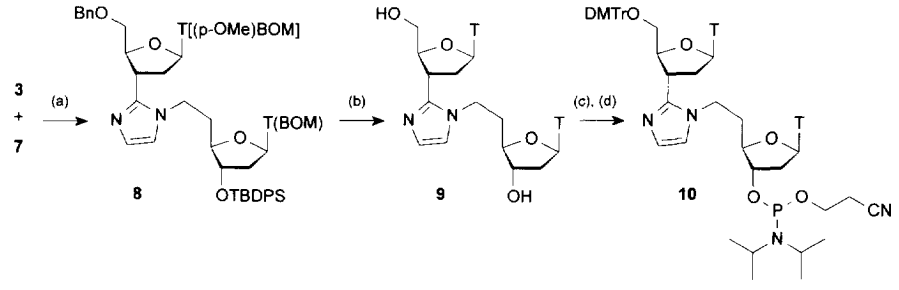


Scheme 1. (a) 1.8 equiv (*p*-OMe)BOM-Cl, 2.0 equiv DBU, MeCN, 0 °C, 1 h, 84%. (b) 1.2 equiv Bu_4NF, THF, 0 °C → rt, 30 min, quant. (c) 1.9 equiv BnBr, 1.9 equiv NaH, 0.05 equiv Bu_4NI, THF, 0 °C → rt, 36 h, 99%. (d) 0.03 equiv OsO_4, 1.0 equiv NMO, acetone/H_2O (4:1), rt, 0.5 h, 93%. (e) 1.1 equiv $NaIO_4$, dioxane/H_2O (3:1), 2 h, quant. (f) 1.0 equiv glyoxal (40% aq), 3.0 equiv NH_3 (25% aq), dioxane/H_2O (6:1), 80 °C, 4 h, 49%. TBDPS: *tert*-butyldiphenylsilyl; (*p*-OMe)BOM: *para*-methoxybenzyloxymethyl.

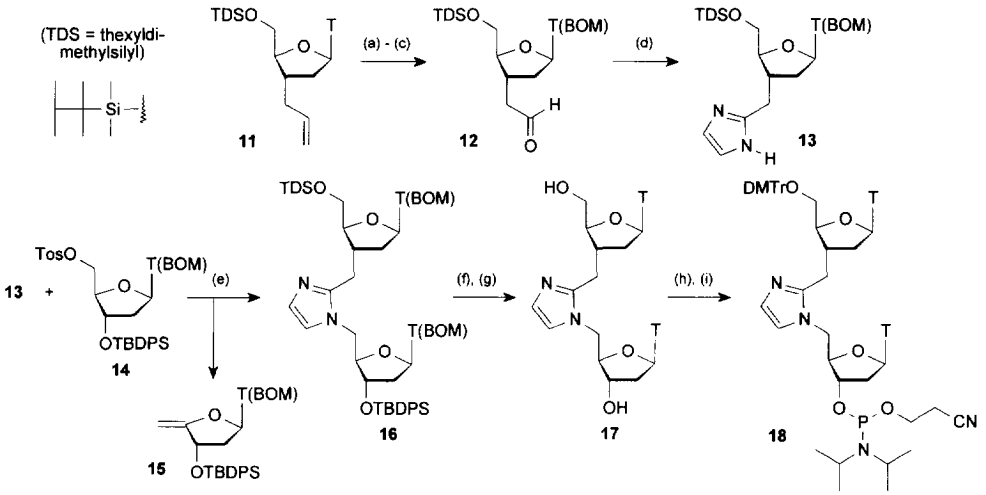
Scheme 2. (a) (1) 6.2 equiv oxalyl chloride, 12.2 equiv DMSO, 20.3 equiv NEt_3, CH_2Cl_2, -78 °C → -30 °C, 3 h; (2) 1.4 equiv methyltriphenylphosphonium bromide, 1.3 equiv potassium *tert*-butoxide, THF, rt → 50 °C, 5 h, 55%. (b) 1.5 equiv 9-BBN, THF, 0 °C, 24 h; NaOH, H_2O_2, 0 °C → rt, 98%. (c) 3.0 equiv Tos-Cl, 12 equiv pyridine, cat DMAP, $CHCl_3$, 0 °C → rt, 16 h, 93%. BOM: benzyloxymethyl.

and 77% overall yield from the known vinyl compound **1**[2]) with 1 eq of glyoxal and 3 eq of NH_3 in a 6:1 mixture of dioxane and water at 80 °C (Scheme 1).[3] Different electrophiles, derived from homologated thymidine analog **6**, were then evaluated for N(1)-alkylation of imidazole **3**. As illustrated in Scheme 2, **6** was prepared from protected thymidine derivative **4**[4] by Swern oxidation, immediate reaction of the resulting C(5') aldehyde with methylenetriphenylphosphorane and highly regioselective hydroboration of alkene **5** with 9-BBN. Imidazole N(1)-alkylation was best accomplished with tosylate **7**, since under the basic reaction conditions, the corresponding C(6') bromide or triflate exclusively led to olefin **5**. After careful optimization, coupling of the sodium salt of imidazole **3** and tosylate **7** afforded dimer **8** in 65% yield (Scheme 3),[5] even though substantial quantities of **5** were still formed by elimination of TsOH. In the next step, all four different protecting groups of **8** were removed simultaneously and in quantitative yield through hydrogenation over stoichiometric amounts of Pd-C in methanol containing 2% of concentrated aqueous HCl (→ **9**). Reaction of **9** with dimethoxytritylchloride (DMTr-Cl) and subsequent activation as the phosphoramidite afforded dinucleotide analog **10**.

The synthesis of the backbone-modified dimeric building block **18**, a positional isomer of **10**, is outlined in Scheme 4. Aldehyde **12**, which serves as substrate for the elaboration of imidazole **13**, was synthesized by a well-established sequence from the known C(3')-allylated thymidine derivative **11**.[6] Heating of aldehyde **12** in the presence of glyoxal and ammonia in dioxane/water afforded imidazole **13** in a rewarding 68% yield. Coupling of **13** to a C(5')-activated thymidine derivative such as **14** was seriously hampered by the exceedingly facile abstration of the C(4') hydrogen to form exocyclic alkene **15**. After testing several leaving



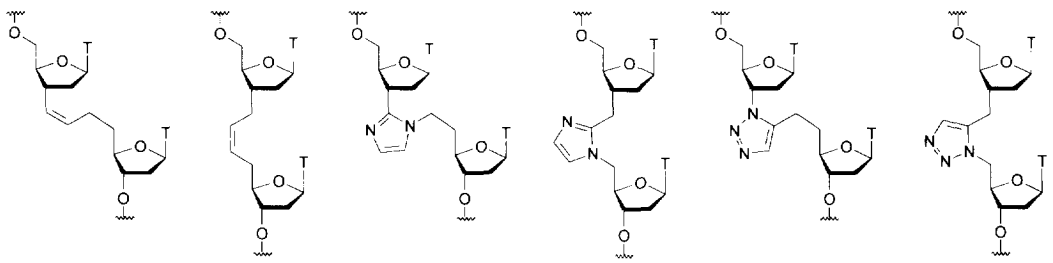
Scheme 3. (a) 1.0 equiv **3**, 1.4 equiv **7**, 1.1 equiv NaH, DMF, 75 °C, 16 h, 65%. (b) 1.0 equiv Pd-C (10%), H_2 (1 bar), MeOH/conc HCl (aq) 50:1, 50 °C, 24 h, quant. (c) 1.2 equiv DMTr-Cl, pyridine, rt, 16 h, 78%. (d) 3.0 equiv $((i\text{-}Pr)_2N)_2POCH_2CH_2CN$, 5.0 equiv diisopropylammonium tetrazolide, CH_2Cl_2, rt, 16 h, 91%.



Scheme 4. (a) 2.0 equiv BOM-Cl, 2.0 equiv DBU, MeCN, 0 °C - rt, 4 h, 79%. (b) 0.01 equiv OsO_4, 1.1 equiv NMO, acetone/H_2O (4 : 1), rt, 18 h, 83%. (c) 1.7 equiv $NaIO_4$, dioxane/H_2O (3:1), rt, 2 h, 93%. (d) 1.0 equiv glyoxal (40% aq), 3.0 equiv NH_3 (25% aq), dioxane/H_2O (6:1), 70 °C, 5 h, 68%. (e) 1.0 equiv **13**, 1.4 equiv **14**, 1.1 equiv NaH, DMF, 75 °C, 18 h, 29%. (f) 2.4 equiv Bu_4NF, THF, 0 °C, 8 h, 99%. (g) 1.5 equiv Pd-C (10%), $HCOONH_4$ (25% aq), THF, rt, 16 h; 6 equiv Na_2CO_3, MeOH, rt, 6 h, 94%. (h) 1.2 equiv DMTr-Cl, pyridine, 16 h, rt, 86%. (i) 3.0 equiv $((i\text{-}Pr)_2N)_2POCH_2CH_2CN$, 5.0 equiv diisopropylammonium tetrazolide, CH_2Cl_2, rt, 16 h, 98%.

groups on C(5') such as Br^-, I^-, OTf^- and $OTos^-$ and some nucleophilic imidazole derivatives (Li-, Na-, K-, and Cs-salts; Me_3Si and Bu_3Sn-imidazoles), it was again only the combination of the sodium salt of imidazole **13** with tosylate **14**[7] which afforded the desired coupling product **16** in 29% yield. While desilylation of **16**

Table 1: Hybridization properties of duplexes of oligodeoxyribonucleotides containing modified dimers **I-VI** with their RNA complements.[a]



sequence	wild-type[b]	modification					
		I	II[c]	III[d]	IV[e]	V	VI
5' TTT**txt**C TCT CTCTCT 3' (**A**)	53.0	-3.3	-1.1	-1.6	-3.4	-2.3	-
5' GCGTT**txt**TTTT**txt**GCG 3' (**B**)	48.0	-	-	-3.7	-4.6	-	-
5' GCG**txttxttxttxttxt**GCG 3' (**C**)	48.0	-2.2	-1.3	-	-	-6.1	-

[a] ΔT_m/mod values [°C]; ΔT_m/mod = [T_m(modified oligonucleotide) - T_m(wild-type oligonucleotide)]/(number of backbone modifications). The extinction *vs.* temperature profiles were measured for each strand (4 μM) in 10 mM phosphate (pH 7.0, Na^+ salts) with 100 mM total [Na^+] (supplemented as NaCl) and 0.1 mM EDTA. [b] T_m of duplex formed by unmodified DNA with complementary RNA. [c] Data published in ref 1a. [d] At pH 6: ΔT_m/mod = -1.8 (sequence **A**), wild-type: T_m = 53.0 °C. [e] At pH 6: ΔT_m/mod = - 4.1 (sequence **A**).

proceeded smoothly, the N(1)-BOM protecting groups were rather reluctant to undergo cleavage. Ultimately, they could be removed by resorting to a transfer hydrogenation protocol (→ **17**).[8] Dimethoxytritylation and subsequent phosphitylation led to the desired phosphoramidite **18**.

The phosphoramidites described in this and the preceding communication were incorporated into oligodeoxynucleotide sequences 5'-d(TTT**txt**CTCTCTCTCT)-3' (**A**), 5'-d(GCGTT**txt**TTTT**txt**GCG)-3' (**B**) and 5'-d(GCG**txttxttxttxttxt**GCG)-3' (**C**) (where **txt** signifies the modified dimer unit) using standard solid-phase protocols.[9] While dimers **I**, **II**,[1a] and **V** (see Table 1) could be incorporated with high yields,[10] the modifications **III** and **IV** were more difficult to oligomerize.[11] Attempts to synthesize oligonucleotides containing the triazole-derived dimer **VI** were completely unsuccessful.[12] The thermal melting temperatures (T_m) of the duplexes formed by the oligonucleotides containing backbone-modified building blocks **I** - **V** with complementary RNA strands are summarized in Table 1.

Inspection of the data compiled in Table 1 reveals that the backbone replacements evaluated lead to a destabilization of the duplexes formed by all modified sequences with their RNA complements. In the case of modifications **III** and **V**, substitution of a heterocycle for the *cis* C=C double bond leads to no significant improvement, while the substitution of the olefin moiety of **II** by an imidazole ring (→ **IV**) is clearly deleterious. In all instances, multiple incorporations of heterocyclic backbone modifications lead to an additional decrease of the thermal stability of the duplexes. In view of molecular mechanics results which did not reveal any steric interference of the heterocyclic backbone structures in a standard A-type DNA/RNA duplex, these findings were rather surprising. They may, however, be indicative of the fact that the increased polarity of the aromatic heterocycles relative to a C=C double bond did not lead to an improved solvation of the modified backbone structure as we had originally anticipated.

Acknowledgments: We thank Dr. F. Natt, Dr. D. Hüsken and Dr. S. M. Freier (ISIS Pharmaceuticals) for the synthesis, purification and characterization of the modified oligodeoxyribonucleotides.

References and Notes.

1. (a) Wendeborn, S.; Wolf, R. M.; De Mesmaeker, A. *Tetrahedron Lett.* **1995**, *36*, 6879. (b) De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 873. (c) Lebreton, J.; De Mesmaeker, A.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Tetrahedron Lett.* **1993**, *40*, 6383.
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3. *Cf.* (a) Rothenburg, A. S.; Dauplaise, D. L.; Panzer, H. P. *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 560. (b) Schaumann, E. Houben-Weyl, E8c, Georg Thieme Verlag, Stuttgart, New York, 1994; pp. 1-215.
4. **4** was synthesized from thymidine by: (i) reaction with BOM-Cl (DBU, CH_2Cl_2, 76%); (ii) reaction of 5' OH with DMTr-Cl (pyridine, 95%); (iii) silylation of 3' OH by reaction with TBDPS-Cl (imidazole, CH_2Cl_2, 92%); (iv) AcOH/H_2O, 84%.
5. Conditions were adapted from: Rotstein, D. M.; Walker, K. A. M. *Tetrahedron: Asymmetry* **1993**, *4*, 1521.
6. De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Synlett* **1993**, 733.
7. **14** was synthesized from 5' O-tritylated thymidine by: (i) reaction with BOM-Cl (DBU, CH_2Cl_2, 82%); (ii) silylation of 3' OH (TBDPS-Cl, imidazole, CH_2Cl_2, 88%); (iii) AcOH/H_2O (80%); (iv) Tos-Cl (pyridine, CH_2Cl_2, cat. DMAP, 94%).
8. For the use of a similar transfer hydrogenation protocol, see: (a) Kende, A. S.; Liu, K.; Jos Brands, K. M. *J. Am. Chem. Soc.* **1995**, *117*, 10597. (b) Bieg, T.; Szeja, W. *Synthesis*, **1985**, 76.
9. Gait, M. J. *Oligonucleotide Synthesis: A Practical Approach*; IRL: Oxford, 1984. For couplings involving modified building blocks, extended reaction times of 10 to 12 minutes were employed.
10. After completion of chain assemblage, the 5'-O-DMTr-protected product was released from controlled pore glass (CPG, 500 Å) with concentrated aqueous NH_3 for 16 h at 55 °C. The 5'-O-DMTr-protected oligonucleotide analogs were purified by RP-HPLC (Hyposil RP C-18), and the 5'-O-DMTr group was subsequently removed by treatment with 80% aqueous acetic acid at rt. According to capillary gel electrophoresis, the fully deprotected oligonucleotides were at least 95% pure. The expected molecular weights of the modified oligonucleotides were confirmed by MALDI-TOF MS (Pieles, U.; Zürcher, W.; Schär M.; Moser, H. *Nucleic Acids Res.* **1993**, *21*, 3191).
11. These difficulties prompted us to synthesize sequence **B** with only two incorporations of modified dimeric building blocks.
12. Despite surveying variations of the standard coupling protocol (ref 9), we were unable to pinpoint the reasons for these failed coupling reactions.

(*Received in USA* 18 *April* 1997; *accepted* 14 *May* 1997)