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Synthesis of dinucleotides containing nitrone, hydroxylamine and amidoxime linkages

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Abstract—Three new thymidine dinucleotides with nitrone, hydroxylamine and amidoxime backbone linkages, suitable for incorporation into oligonucleotide chains, were easily synthesized, by coupling of readily available thymidine monomers and applying short, simple and efficient procedures.
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The synthesis of oligonucleotides with modified backbone linkages remains a very active area of research.[1] Current efforts are focused on the complete replacement of the phosphorus atom in order to achieve higher affinity for the RNA target, enhanced nuclease resistance and improved permeability and cellular uptake.

From the plethora of modifications reported hitherto, oligonucleotides with the amide linkage **1** (Fig. 1) have shown very promising antisense properties by displaying higher binding affinity and very good resistance towards nucleases.[2] Oligomers with positively charged guanidine[3] and methylthiourea[4] linkers **2** and **3** are also promising leads in the development of new antisense therapeutics, since the attractive forces between them and the negatively charged DNA or RNA contribute significantly to the stability of heteroduplex and triplex structures formed between these species. In addition, they are stable to enzymatic hydrolysis due to the lack of a phosphodiester linkage. Interestingly, oligonucleotides containing the hydroxamate functionality[5] **4** display a binding affinity similar to the natural analogues and substantial resistance towards nucleases. Moreover, the hydroxamate functionality can effectively chelate with a ferric ion, which can undergo Fenton chemistry to generate hydroxy radicals. Thus, hydroxamate nucleic acids might cleave the target RNA through radical reactions.

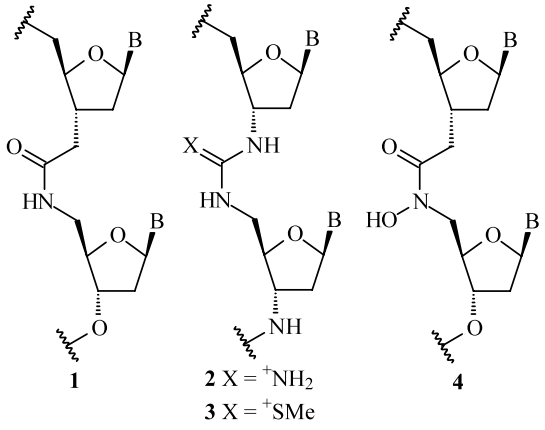

Figure 1.

We now report the synthesis of three new dinucleotides containing nitrone **5**, hydroxylamine **6** and amidoxime **7** functionalities as internucleotide backbone linkages (Fig. 2). In all these cases the existing 3′-CH_2 substituent might favor the $C_{3'}$-*endo* conformation, which is beneficial for an RNA binding behavior.[1,6] The nitrone and amidoxime functionalities in **5** and **7**, respectively, have a structural preorganization similar to that of the amide functionality in **1** and the hydroxamate in **4**. Due to their neutral character at physiological pH, all these linkages will contribute to the overall charge reduction and therefore might potentially be favorable for penetration through cellular membranes. Finally, the hydrophilicity of the nitrone, hydroxylamine and amidoxime backbones will increase the solubility of these molecules in biological fluids.

Keywords: antisense oligonucleotides; thymidine; nitrone; hydroxylamine; amidoxime.

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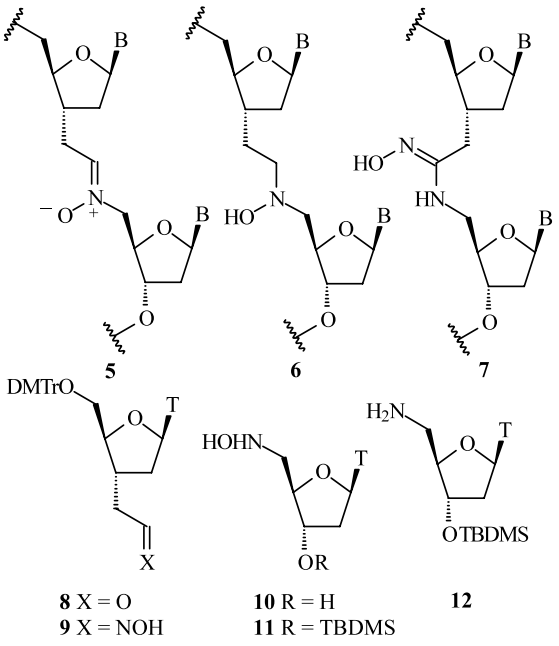
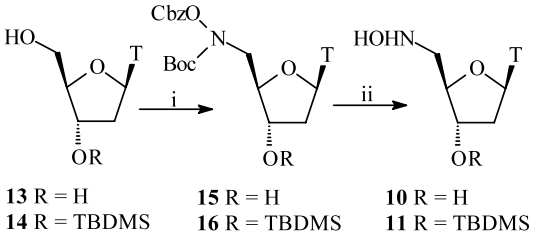
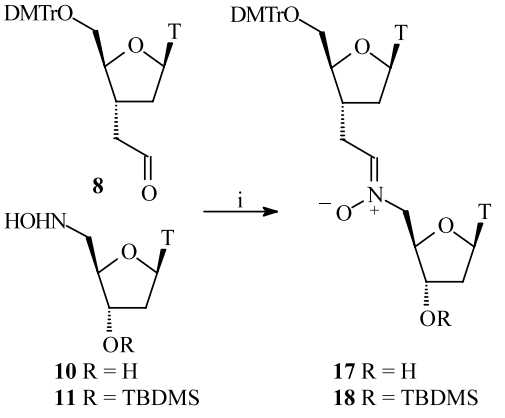


Figure 2.



Scheme 1. *Reagents and conditions*: (i) BocNHOCbz, PPh_3, DEAD, THF, 0→20°C, 12 h, 47% for **15** and 80% for **16**. (ii) Pd/C, H_2, MeOH, 20°C, 2 h; then 10% TFA, CH_2Cl_2, 20°C, 2 h, 94% for **10** and 81% for **11**.



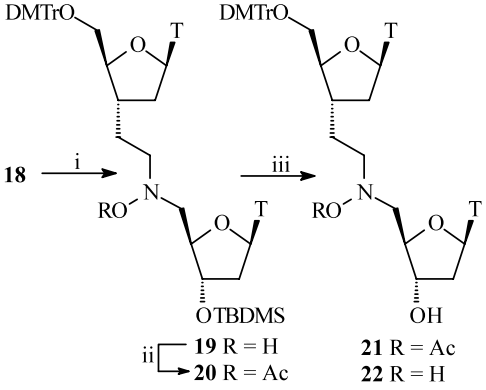
Scheme 2. *Reagents and conditions*: (i) Na_2CO_3, EtOH, 20°C, 15 min for **5** (95%) and 12 h for **17** (84%).

Thymidine dinucleotides **5**, **6** and **7** (B = thymine) were easily prepared by condensation of aldehyde **8** and its oxime **9** with the hydroxylamines **10** and **11** or the amine **12**. Compounds **8**, **9**, and **12** were prepared according to the literature methods,[7] while hydroxylamines **10** and **11** were accessible from thymidine, applying the method developed by Miller,[8] namely replacement of the 5′-OH by BocNHOCbz under Mitsunobu conditions, followed by deprotection (Scheme 1).

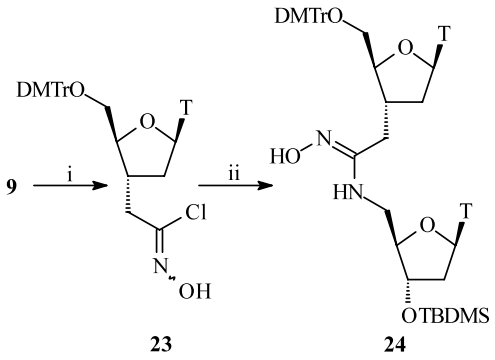
Thymidine hydroxylamines **10** and **11** were readily condensed with the thymidine aldehyde **8** to give the desired unprotected and protected dimers **17** and **18**,[9] respectively, isolated as white foams in high yield (Scheme 2). It is interesting that the 3′-OH of the hydroxylamine moiety needs no protection prior to the condensation. Although the existing data did not allow the unequivocal assignment of the nitrone geometry, it is most probable that this group adopts the thermodynamically more stable *Z*-form, as happens in all heavily substituted nitrones.[10]

Reduction of the nitrone group in **17** could apparently result in a dinucleotide with the hydroxylamine backbone **6** (Fig. 2). It was considered, however, that the presence of the free *N*-OH group could not be discerned from 3′-OH in the phosphoramidite formation reaction. For this reason, we decided to protect the *N*-OH group as *N*-OAc, as it could be regenerated after formation of the oligonucleotide chain. Dinucleotide **18** was reduced by $NaBH_4$ and the *N*-OH group thus formed was acetylated, in high yield (Scheme 3). Having protected the internucleotide hydroxylamine group, the 3′-OH was deprotected by treatment with TBAF to give the desired dimer **21**,[9] unfortunately accompanied by the doubly unprotected one **22**.

The amidoxime backbone linker in **7** (Fig. 2) could be prepared according to the most general method of making such molecules,[11] namely addition of the known primary amine **12** to the nitrile oxide generated from the oxime **9**. To this end, the latter was converted to chloroxime **23** (Scheme 4) by addition of pyridine to a solution of NCS in dry chloroform followed by careful addition of **9** to the resulting mixture. This procedure was necessary in order to avoid hydrolysis of the dimethoxytrityl ether. The formation of compound **23** was detected by TLC and without isolation, its



Scheme 3. *Reagents and conditions*: (i) $NaBH_4$, EtOH, 20°C, 12 h, 92%. (ii) Ac_2O, pyridine, 0→20°C, 12 h, 92%. (iii) TBAF, THF, 20°C, 10 min, 50% of **21** and 46% of **22**.



Scheme 4. *Reagents and conditions*: (i) NCS, pyridine, $CHCl_3$, 20°C, 30 min. (ii) **12**, Et_3N, $CHCl_3$, 20°C, 12 h, 57% overall.

chloroform solution was treated with the amine **12** and triethylamine to afford the desired dimer **24**,[9] in satisfactory overall yield, apparently with intermediate formation of the respective nitrile oxide.

In conclusion, the nitrone, hydroxylamine and amidoxime dithymidines reported here represent three new types of modification in the internucleotide backbone bridges with potential antisense properties. These dimers were easily prepared by coupling of known and readily available thymidine monomers, applying short, simple and efficient procedures. Our efforts directed towards the exploration of the compatibility of other bases with these procedures as well as in the incorporation of the prepared dimers into oligonucleotide chains and investigation of their biophysical properties are now in progress and results will be reported in due course.

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