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Syntheses and binding studies of oligonucleotides containing *N*-hydroxycarbamate linkages: potential DNA cleaving antisense oligomers

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Abstract

An efficient synthesis of *N*-hydroxycarbamate containing thymidine dimer **9** was accomplished and it was incorporated into automatic DNA syntheses to make thymidine 16mers **T*-1**, **T*-2** and **T*-3**. Binding abilities of **T*-1**, **T*-2** and **T*-3** with poly (dA)-16mer were assayed by melting denaturation (Tm) studies of the corresponding duplexes. Iron binding ability of a thymidine oligomer with *N*-hydroxycarbamate linkages was studied by MALDI-MS. Nuclease stability assays showed that the modified oligonucleotides have enhanced resistance toward nuclease S1 (endonuclease) compared to natural oligonucleotides. © 2000 Elsevier Science Ltd. All rights reserved.

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The use of oligonucleotide analogs as rationally designed drugs continues to attract attention in medicinal chemistry. In principle, antisense oligonucleotides target, in a sequence specific manner, a particular gene, or mRNA to inhibit the expression of that gene.[1–5] In recent years, chemical modifications of antisense oligonucleotides have resulted in improved solubility, stability towards nucleases, cellular uptake, and appropriate hybridization to target genes or mRNAs.[6,7] In the antisense approach, oligonucleotides have been designed to bind to a given mRNA to inhibit the translation process via duplex formation and/or RNase H-induced degradation of the message. We proposed a novel backbone replacement for antisense oligonucleotides with *N*-hydroxycarbamates as linkages (Fig. 1).[8,9] Hydroxamic acids can effectively chelate Fe(III), which can undergo Fenton chemistry to generate hydroxyl radicals.[10] Therefore, in addition to potential RNase-H cleavage processes, antisense oligonucleosides with *N*-hydroxycarbamate linkages might cleave the target mRNA through radical reactions. Moreover, the Fe(III) binding ability of the antisense oligonucleoside would have the potential to assist in its cell permeability

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through iron uptake systems.[11] In order for the proposed Fenton chemistry to work, the oligonucleotides with *N*-hydroxycarbamate linkages are required to bind both iron and target DNA or mRNA strands with reasonable affinity. Herein, we report the syntheses of thymidine oligomers with *N*-hydroxycarbamate linkages and their binding ability toward poly(dA) and Fe(III).

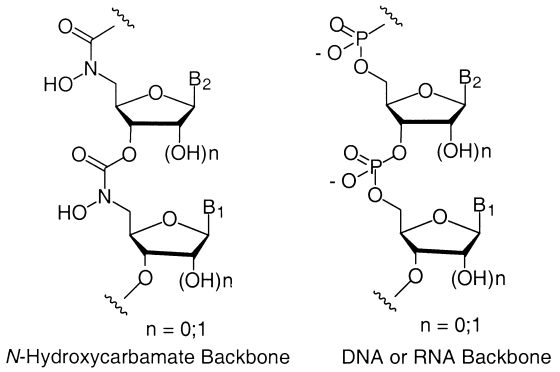
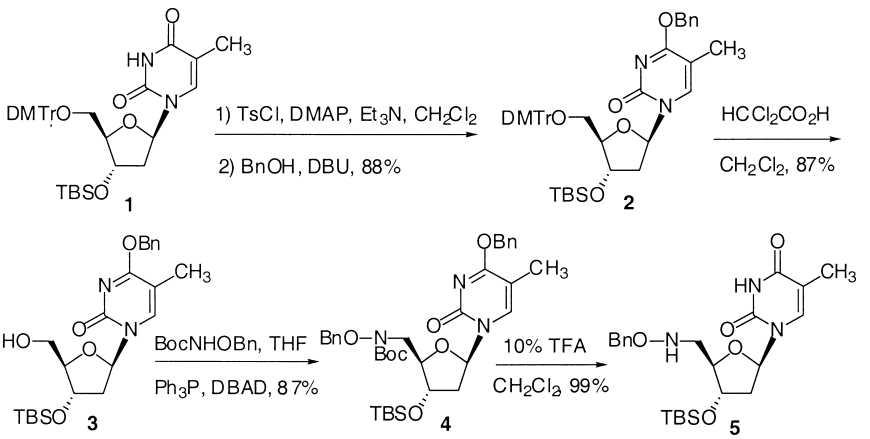


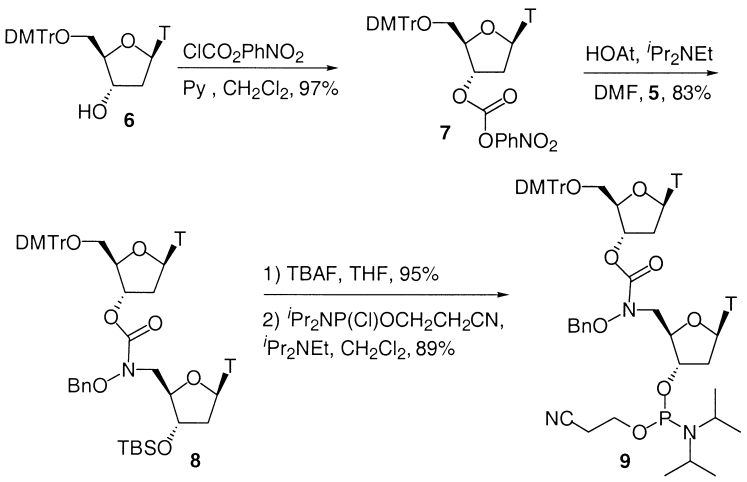
Figure 1.

In a previous report,[8] we described the syntheses of the building blocks of oligonucleotides with *N*-hydroxycarbamate linkages. The conversion of the 5′-hydroxyl group of a nucleoside into the corresponding 5′-*N*-hydroxylamino substituted derivatives can be achieved by Mitsunobu reactions of *N*-(*tert*-butoxycarbonyl)-*O*-(benzyloxycarbonyl)hydroxylamine (BocNHOCbz) with nucleosides. Scheme 1 shows the application of this methodology to thymidine for the syntheses of thymidine oligomers with *N*-hydroxycarbamate linkages. In order for the Mitsunobu reaction to work, proper protecting groups were introduced to thymidine. Starting from 3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-dimethoxytritylthymidine **1**, a benzyl group was introduced to the 4-O-position of the thymidine in two steps by base-aided condensation with tosyl chloride in dichloromethane, followed by the reaction of the resulting sulfonate with benzyl alcohol, to give **2** in 88% overall yield (Scheme 1). Removal of the dimethoxytrityl group in a dichloroacetic acid in dichloromethane



Scheme 1.

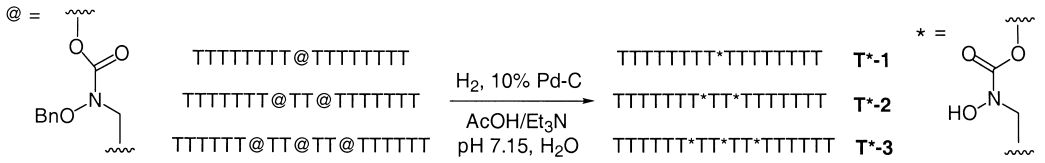
solution afforded an 87% yield of desired compound **3**. The Mitsunobu reaction of **3** with *N*-(*tert*-butoxycarbonyl)-*O*-(benzyl)hydroxylamine (BocNHOBn) gave desired hydroxyamino derivative **4** in good yield (Scheme 1). When **4** was exposed to 10% trifluoroacetic acid in dichloromethane, both the Boc and 4-O-Bn groups were removed to give **5** in 99% yield. Use of **5** in a coupling reaction provided the corresponding thymidine dimer with the desired *N*-hydroxycarbamate linkage as described below (Scheme 2).



Scheme 2.

The *p*-nitrophenyl (PNP) carbonate **7** was obtained in 97% yield by reaction between 5′-*O*-dimethoxytritylthymidine **6** and *p*-nitrophenyl chloroformate. Since direct reaction of **5** with **7** was sluggish, 1-hydroxy-7-azabenzotriazole (HOAt) was used to form a more active carbonate reaction intermediate for the coupling and generated dimer **8** in 83% yield. Compound **8** was then treated with *n*-tetrabutylammonium fluoride (TBAF) to remove the silyl group in 95% yield. The resulting 3′-hydroxy intermediate was reacted with chlorophosphoramidite to afford **9** in 89% yield (Scheme 2).

Dimer **9** was then incorporated into thymidine 16mers using phosphoramidite chemistry on solid support according to a standard 1 μmole synthesis cycle.[12] Oligonucleotides with *O*-benzyl-*N*-hydroxycarbamate (@) internucleoside linkages (up to three) were deprotected and removed from the solid support by treatment with concentrated aqueous ammonia solution at rt for 1 h. Benzyl groups were then removed by hydrogenolysis in acetic acid and triethylamine buffer solution (pH 7.15) to give **T*-1**, **T*-2** and **T*-3** (Scheme 3).



Scheme 3.

The binding of thymidine oligomers with *N*-hydroxycarbamate linkages to Fe(III) was studied by the synthesis of a model heptathymidylate analog using the same methodology mentioned above. When the heptamer T*TT*TT*TT was treated with iron(III)-acetylacetonate, $Fe(acac)_3$, MALDI-MS showed distinct Fe complex formation, indicating effective binding ability of *N*-hydroxycarbamate linkages toward Fe(III).

The stability of duplexes of the thymidine 16mer analogs with poly (dA) was investigated by thermal denaturation experiments (Tm).[13] Incorporation of one, two and three modifications in the middle of the sequence led to a progressive decrease in the melting temperature of the duplex compared to the natural DNA duplex. The degree of the destabilization depended on the steric factors of the linkages. While a more pronounced destabilization was observed with the *O*-benzyl-*N*-hydroxycarbamate linked compounds (ΔTm/mod$\sim$3.7°C), the affinity of the deprotected *N*-hydroxycarbamate linked oligonucleotides for poly (dA) was only slightly decreased (ΔTm/mod$\sim$1.4°C). It was also observed that **T*-3** did not further destabilize the duplex compared to **T*-2** (Table 1).

Table 1
Thermal stability (Tm)[a] of duplexes formed between oligothymidylate analogs and poly (dA) targets and half-lives of oligothymidylate analogs towards nuclease S1

	versus poly (dA)		
Compounds	**Tm (°C)**	**ΔTm/mod (°C)**	**Nuclease S1 $t_{1/2}$[(b)]**
$d(T)_{16}$	38.3		25.3 min
TTTTTTTT@TTTTTTTT	34.9	- 3.4	
TTTTTT@TT@TTTTTT	30.4	- 3.95	
TTTTT@TT@TT@TTTTT	27.1	- 3.7	
TTTTTTTT*TTTTTTTT **T*-1**	36.1	- 2.2	29.1 min
TTTTTTT*TT*TTTTTTT **T*-2**	34.2	- 2.05	32.7 min
TTTTT*TT*TT*TTTTT **T*-3**	34.1	- 1.4	44.4 min

[(a)]Melting temperature (Tm) measured at 10 μM oligomer concentration in 10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA buffer solution (pH 7.0). [(b)]50 mM sodium acetate buffer (pH 4.5) containing 300 mM sodium chloride and 100 mM zinc acetate, 37 °C.

Nuclease resistance of *N*-hydroxycarbamate linked oligonucleotides towards nuclease S1 (endonuclease) was investigated in comparison with the resistance of unmodified $d(T)_{16}$.[14] Due to the modified linkage, a stabilizing effect was observed. The half-life of oligonucleotides increased when more *N*-hydroxycarbamate linkages were incorporated into the sequence (Table 1).

N-Hydroxycarbamate linked oligonucleotides represent a new class of backbone modified DNA analogs. Thymidine 16mer analogs with alternating phosphodiester and *N*-hydroxycarbamate linkages were synthesized with one to three modifications and their biophysical properties were studied. Replacement of negatively charged phosphodiester linkages by *N*-hydroxycarbamate linkages only slightly weaken duplex stability of the oligonucleotide analogs when hybridized with poly (dA). The resistance of *N*-hydroxycarbamate linked oligonucleotides towards endonuclease is improved. Binding studies indicated a modified oligonucleotide with three *N*-hydroxycarbamate linkages does bind with Fe(III) and also binds to the target poly (dA). Work is currently in progress to study the ability of **T*-3** to perform Fenton chemistry and cleave the DNA sense strand sequence-specifically.

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References

1. *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*; Wickstrom, E., Ed.; Wiley-Liss: New York, 1991.
2. *Antisense Research and Applications*; Crooke, S. T.; Lebleu, B., Ed.; CRC Press: Boca Raton, FL, 1993.
3. *Antisense Therapeutics*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1996.
4. *Nucleosides and Nucleotides as Antitumor and Antiviral Agents*; Chu, C. K.; Baker, D. C., Eds.; Plenum Press: New York, 1993.
5. *Carbohydrate Modifications in Antisense Research*; Sanghvi, Y. S.; Cook, P. D., Ed.; ACS Symposium Series 580, 1994.
6. Uhlmann, E.; Peyman, A. *Chemical Reviews* **1990**, *90*, 544–584.
7. Hunziker, J.; Leumann, C. *Modern Synthetic Methods* **1995**, 331–417.
8. Li, H.; Miller, M. J. *J. Org. Chem.* **1999**, *64*, 9289–9293.
9. Li, H.; Miller, M. J. *Design and Synthesis of Thymidine Oligomer with Hydroxamate Linkages—A Novel Antisense Oligonucleoside*, 36th National Organic Chemistry Symposium, June 13–17, 1999, Madison, WI.
10. Ghosh, M.; Lambert, L. J.; Huber, P. W.; Miller, M. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2337–2340.
11. Roosenberg II, J. M.; Lin, Y.-M.; Lu, Y.; Miller, M. J. *Curr. Med. Chem.* **2000**, *7*, 2, 159–197.
12. *Oligonucleotides and Analogues: A Practical Approach*; Eckstein, F., Ed.; IRL Press: Oxford, New York, 1991.
13. Freier, S. M.; Albergo, D. D.; Turner, D. H. *Biopolymers* **1982**, *22*, 1107.
14. Peyrottes, S.; Vasseur, J. J.; Imbach, J. L.; Rayner, B. *Nucleosides Nucleotides* **1994**, *13*, 2135–2149.