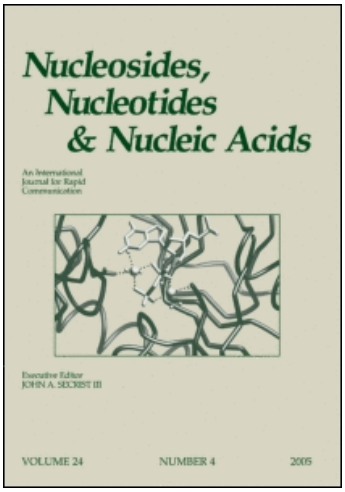

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OLIGONUCLEOTIDES CONTAINING ACYCLIC NUCLEOSIDE ANALOGUES WITH CARBAMATE INTERNUCLEOSIDE LINKAGES

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Abstract. Synthesis of 2'-deoxy-2',3'-secothymidine **t** and its dimer **t*t**, where the two 2'-deoxy-2',3'-secothymidine **t** units are connected *via* a carbamate, *=3'-NH-CO-O-5', internucleoside linkage has been achieved. These building blocks were protected in the 5'-position, converted into their phosphoramidites, or attached onto CPG, and then used for "chimeric oligonucleotide" synthesis.

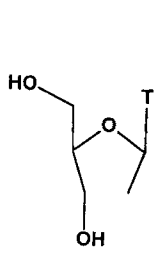
For "antisense" oligonucleotides, to modify gene expression, they must be taken up by cells, be resistant to nucleases, and bind to complementary nucleic acids targets effectively[1,2]. Recently, significant efforts have been made to develop modified oligonucleotides in order to improve these conditions.

Several acyclic analogues of naturally occurring deoxynucleosides have been synthesized[3] and have shown high potency as antiviral agents. In these molecules, the usual sugar deoxyribose or ribose has been replaced with a "flexible" acyclic structure such as glycerol. Oligonucleotide analogues in which these glyceronucleoside residues are substituted for the normal DNA or RNA sugars may demonstrate antiviral activity and may also be stable to nucleases[4] which would improve their bioavialability as antisense drugs.

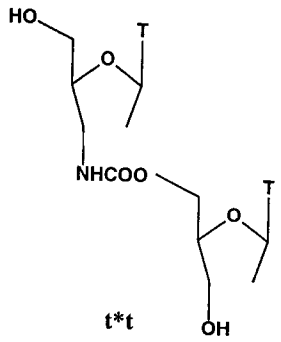
Early studies suggested that incorporation of acyclic nucleotide analogues into antisense oligonucleotides stabilizes the oligonucleotides against degradation by nucleases but prevents formation of duplexes with complementary natural oligonucleotides[5]. Recent reports[6], however, show that these acyclic oligonucleotide analogues are not only stable against enzymatic degradation, but also posses acceptable hybridization properties in double and triple helix formations with natural nucleic acids, and their use as universal nucleosides for the design of probes with ambiguous positions.

The reports on peptide nucleic acids (PNA), describing their ability to hybridize and form stable duplexes with the complementary natural oligonucleotides[7] have generated increased interest in the synthesis of acyclic oligonucleotides[8].

Continuing our recent work[9] on chimeric oligonucleotides, we incorporated 2'-deoxy-2',3'-secothymidine **t** and its dimer **t*t**, where the two 2'-deoxy-2',3'-secothymidine units are connected *via* a carbamate, * = 3'-NH-CO-O-5', internucleoside linkage into oligonucleotide analogues. Carbamate linkages have several beneficial characteristics, including planar geometry, non - ionic and achiral characters, and the



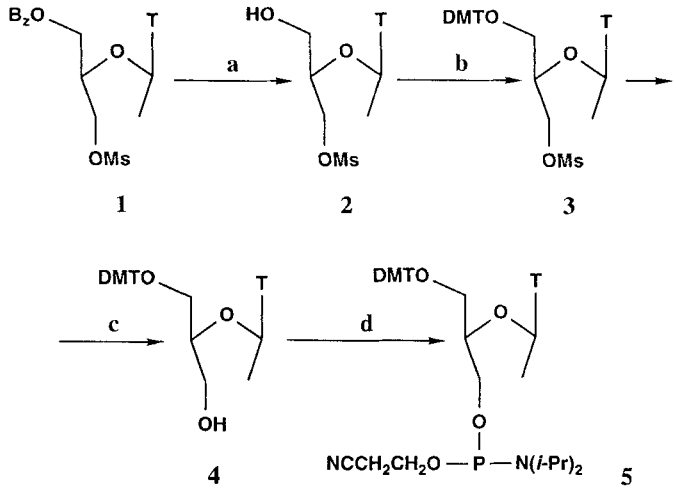
t



t*t

ability to provide increased nuclease stability[9] and cellular uptake[10] to chimeric oligonucleotides. We hoped that replacing the rigid cyclopentane unit of deoxyribose with a flexible acyclic sugar analogue, would reduce the restriction in rotation[11] of the carbamate linkage as the backbone builder. We also hoped that the flexibility of the acyclic sugar analogue would adjust for the shortage in distance[12] between two nucleoside units created by the carbamate internucleoside linkages increasing the thermal stability of duplexes formed with natural oligonucleotides.

5'-O-Benzoyl-2'-deoxy-3'-O-mesyl-2',3'-secothymidine **1**, was synthesized[13] in a multi-step process, and was selectively deprotected at the 5'-position with ammonia at room temperature to give **2**. After DMT protection at 5'-OH, the mesyl group at the 3'-position of the DMT-derivative **3** was hydrolyzed with ammonia at 55°C for 48 hours, (Scheme 1). The resulting 5'-O-DMT-2'-deoxy-2',3'-secothymidine **4** was converted[14] into the corresponding phosphoramidite **5**. 5'-O-DMT-2'-Deoxy-2',3'-secothymidine **4** was also attached onto a long chain alkylamidopropanoic acid controlled-pore glass (CPG) support[15] to give CPG-**t**-5'-O-DMT. The loading efficiency was 84.27 μmole/g CPG.



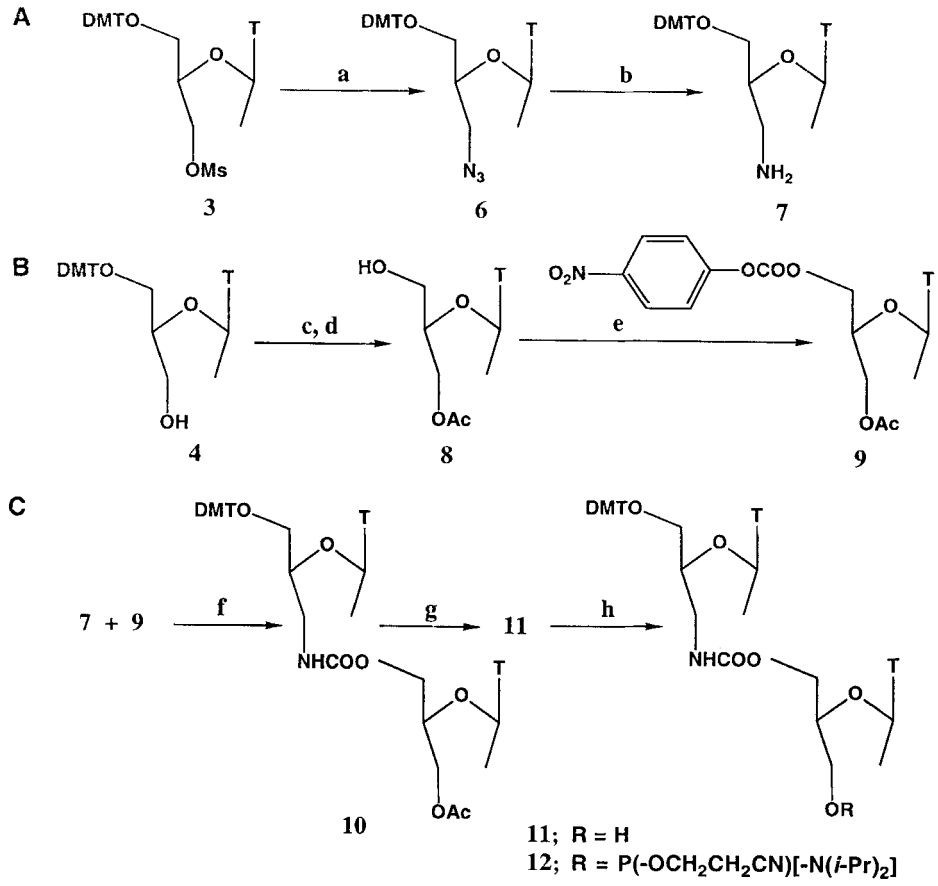
1 2 3 4 5

a. NH_4OH; b. DMTCl, Pyridine; c. NH_4OH, 55°C;
d. $(NCCH_2CH_2O\text{-})[(i\text{-}Pr)_2N\text{-}]PCl$, Et_3N, CH_2Cl_2.

Scheme 1

We synthesized the starting materials **7** and **9** in order to form the acyclic dimer **10.** To synthesize the 3'-amino compound **7**, we started from **3**, from which the mesyl group was displaced with sodium azide to give the 3'-azido compound **6**, (Scheme 2A). 3'-Azido **6** was further catalytically reduced using 10% Pd/C as a catalyst and hydrogen to give 3'-amino **7**. The 3'-OH position of **4** was acetylated with acetic anhydride in pyridine, followed by detritylation in 80% acetic acid at room temperature to give **8**. The 5'-OH in **8** was activated[9,12,16] by treatment with bis(*p*-nitrophenyl)carbonate to give **9**, (Scheme 2B). The acyclic dimer **10** was then obtained in the reaction of 5'-O-*p*-nitrophenyl carbonate **9** with 3'-amino **7** in pyridine[9,12,16], (Scheme 2C). The 3'-O-acetyl of this acyclic dimer was hydrolyzed in ammonia to give **11**, which was further converted[14] into the phosphoramidite **12**, (Scheme 2C).

The phosphoramidites **5** and **12** were precipitated in hexane at -78°C to give white powders and were used directly for oligonucleotide synthesis with ~90% coupling efficiency. Oligonucleotides, Table 1, were synthesized using the β-cyanoethyl phosphoramidite approach, on a 1 μmole scale, using the standard DNA and RNA coupling cycles for **5** and **12**, respectively. After ammonia treatment at 55°C for six hours, oligonucleotides at pre- and post-DMT removal stages were purified by reverse phase



a. NaN_3, DMF, 100°C; b. Pd/C-H_2; c. $(CH_3CO)_2O$, Pyridine; d. 80% CH_3COOH; e. bis(*p*-nitrophenyl)carbonate, Pyridine; f. Pyridine; g. NH_4OH; h. $[(i\text{-}Pr)_2N\text{-}](NCCH_2CH_2O\text{-})PCl$, Et_3N, CH_2Cl_2.

Scheme 2 A, B, C

HPLC and exhibited a single band on polyacrylamide gel-electrophoresis. Detritylation was carried out by treatment with 80% aqueous acetic acid at room temperature for 30 minutes.

The hybridization properties of the oligonucleotides were examined in the presence of their complementary DNA strands and the melting temperatures were determined by measuring absorbance vs temperature profiles, (Table 1). The results depicted in Table 1 indicate that introduction of one 2'-deoxy-2',3'-secothymidine **t** unit at the 3'-end of oligonucleotide Seq.#2 had little effect (ΔTm = -1.9°C) on the stability of the duplex

Table 1. Hybridization Data of Oligonucleotides[a]

Oligonucleotide	Sequence (5'-3')	Tm, (°C)	ΔTm, (°C)
Seq.#1	GCACTCATCT	41.8[b]	
Seq.#2	GCACTCATCt	39.9[b]	-1.9
Seq.#3	GCACTCATCG	43.0[b]	
Seq.#4	GCACtCATCG	25.8[b,d]	-17.2
Seq.#5	TTGTTCTTCTTGTTGTTCTTCTTTT	56.2[b]	
Seq.#6	T*TGT*TCT*TCT*TGT*TGT*TCT*TCT*T*T*T	16.9[b]	-39.3
Seq.#7	T_{15}	40.9[c]	
Seq.#8	t*tt*tt*tt*tt*tt*tt*tt	<0[c]	

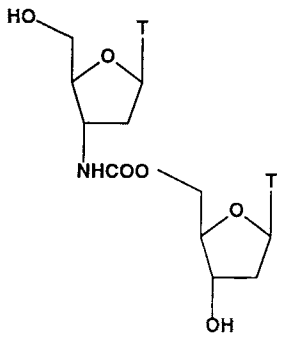
[a]Oligonucleotides Seq.#1-8 were hybridized with complementary DNA; phosphodiester, t = 2'-deoxy-2',3'-secothymidine; * = 3'-NH-CO-O-5'. Absorbance vs temperature profiles were measured at 0.2 A_{260} units of each strand in 1ml of [b]buffer I : 100mM Na^+, 10mM phosphate, pH 7.5; or [c]buffer II: 200mM Na^+, 20 mM phosphate, pH 7.5. [d]Similar duplex destabilization was created by a TC mismatch, Tm^b = 27.9°C, ΔTm = -15.1°C.

formed. In contrast, the introduction of the same unit at a more interior site in the oligonucleotide, Seq.#4, caused a significant reduction in binding (ΔTm = -17.2°C) which is similar to the duplex destabilization created by a TC mismatch, (ΔTm = -15.1°C; Table 1).

We compared the duplex stabilities of oligonucleotide analogues containing **t*t** and **T*T** dimers with carbamate internucleoside linkages (3'-NH-CO-O-5') with those of natural oligonucleotides. The oligonucleotides with 1-3 consecutive carbamate linkages at the 3'-end had very little or no effect[9] on the stability of the duplexes formed with their complementary nucleic acids. The presence of three **T*T** dimers with carbamate internucleoside linkages alternating with phosphodiester linkages at the 3'-end had a modest inhibitory effect on duplex stability, (ΔTm = -1.2°C)[9]. The presence of more than three **T*T** dimers Seq.#6 resulted in weak hybridization the oligonucleotide to its complementary sequence, (Table 1). However, the introduction of **t*t** containing carbamate internucleoside linkage into oligonucleotide Seq.#8 resulted in no binding affinity to natural nucleic acids.

We found similar results using mixed oligonucleotide analogues with an acyclic carbohydrate moiety and a N-cyanoguanidine functionality[17].

The presence of acyclic nucleoside analogues significantly reduced the ability of oligonucleotides to form stable duplexes with complementary nucleic acids. The loss in entropy caused by introducing the flexible structures into oligonucleotides is so significant that is impossible to compensate for it by introducing a carbamate linkage with restricted



T*T

rotation. Although oligonucleotides with acyclic nucleotide analogues exhibited better stability toward nucleases, their inability to hybridize to complementary natural nucleic acids precludes their use as antisense agents.

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References

1. (a) Agrawal, S.; Iyer, R.P. *Current Opinion in Biotechnology* **1995**, *6*, 12. (b) Agrawal, S. *Trends in Biotech.* **1992**, *10*, 152. (c) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.

2. (a) Gait, J.M.; Jones, A.S.; Walker, R.T. *J. Chem. Soc. Perkin I* **1974**, 1684. (b) Nyilas, A.; Glemarec, C.; Chattopadhyaya, J. *Tetrahedron* **1990**, *46*, 2149. (c) Huang, Z.; Schneider, K.C.; Benner, S.A. *J. Org. Chem.* **1991**, *56*, 3869. (d) Kawai, S.H.; Wang, D.; Giannaris, P.A.; Damha, M.J.; Just, G. *Nucleic Acids Res.* **1993**, *21*, 1473. (e) Mungall, W.S.; Greene, G.L.; Heavner, G.A.; Letsinger, R.L. *J. Org Chem.* **1975**, *40*, 1659. (f) Mag, M.; Engels, J.W. *Nucleic Acids Res.* **1989**, *17*, 5973. (g) Vandendriessche, F.; Van Aerschot, A.; Voortmans, M.; Janssen, G.; Busson, R.; Van Overbeke, A.; Van den Bossche, W.; Hoogmartens, J.; Herdewijn, P. *J. Chem. Soc. Perkin Trans. I* **1993**, 1567. (h) Vasseur, J.-J.; Debart, F.; Sanghvi, Y.S.; Dan Cook, P. *J. Am. Chem. Soc.* **1992**, *114*, 4006.

3. (a) Schaeffer, H.J. in *Nucleosides, Nucleotides and Their Biological Applications*, Rideout, J.L.; Henry, D.W.; Beachan, L.M., Eds.; Academic Press: New York, **1983**, pp.1-17. (b) Chu, C.K.; Cutler, S.J. *J. Heterocyclic Chem.* **1986**, *23*, 289.

4. Ogilvie, K.K.; Nguyen-ba, N.; Gillen, M.F.; Radatus, B.K.; Cheriyan, U.O.; Hanna, H.R.; Smith, K.O.; Galloway, K.S. *Can. J. Chem.* **1984**, *62*, 241.

5. (a) Usman, N.; Juby, C.D.; Ogilvie, K.K. *Tetrahedron Lett.* **1988**, *29*, 4831. (b) Schneider, K.C.; Benner S.A. *J. Am. Chem. Soc.* **1990**, *112*, 453. (c) Augustyns, K.; Van Aerschot, A.; Van Schepdael, A.; Urbanke, C.; Herdewijn, P. *Nucleic Acids Res.* **1991**, *19*, 2587. (d) Azymak,

M.; Chavis, C.; Lucas, M.; Morvan, F.; Imbach, J.-L. *Nucleosides & Nucleotides* **1992**, *11*, 1241.

6. (a) Vandendriessche, F.; Augustyns, K.; Van Aerschot, A.; Busson, R.; Hoogmartens, J.; Herdewijn, P. *Tetrahedron* **1993**, *49*, 7223. (b) Nielsen, P.; Kirpekar, F.; Wengel, J. *Nucleic Acids Res.* **1994**, *22*, 703.

7. (a) Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Science* **1991**, *254*, 1497. (b) Egholm, M.; Buchardt, O.; Nielsen, P.E.; Berg, R.H. *J. Am. Chem. Soc.* **1992**, *114*, 1895. (c) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S.; Driver, D.; Berg, R.; Kim, S.; Norden, B.; Nielsen, P. *Nature* **1993**, *365*, 566. (d) Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Anti-Cancer Drug Design* **1993**, *8*, 53.

8. (a) Weller, D.D.; Daly, D.T.; Olson, W.K.; Summerton, J.E.; *J. Org. Chem.* **1991**, *56*, 6000. (b) Huang, S.-B.; Nelson, J.S.; Weller, D.D. *J. Org. Chem.* **1991**, *56*, 6007. (c) Garner, P.; Yoo, J.U. *Tetrahedron Lett.* **1993**, *34*, 1275. (d) Lewis, J. *Tetrahedron Lett.* **1993**, *34*, 5697. (e) Pannecouque, C.; Schepers, G.; Rozenski, I.; Van Aerschot, A.; Cloes, P.; Herdewijn, P. *BioMed. Chem. Lett.* **1994**, *4*, 1203.

9. Habus, I.; Temsamani, J.; Agrawal, S. *BioMed. Chem. Lett.* **1994**, *4*, 1065.

10. According to our unpublished experimental results, the carbamate linkages have provided increased cellular uptake for modified oligonucleotides containing **T*T** dimers with carbamate internucleoside linkages (* = 3'-NH-CO-O-5') compare to those of natural oligonucleotides. For example, oligonucleotide phosphorothioate (PS) with three consecutive carbamate linkages contiguously at the 3'-end (Seq.#7, ref. 9) and fluorescein labeled at the 5'-end, exhibited 34% increase in cellular uptake, as monitored by flow cytometry using H9 cells, than the same PS oligonucleotide with no carbamate linkages at the 3'-end.

11. Waldner, A.; De Mesmaeker, A.; Lebreton, J. *BioMed. Chem. Lett.* **1994**, *4*, 405.

12. Stirchak, E.P.; Summerton, J.E.; Weller, D.D. *Nucleic Acids Res.* **1989**, *17*, 6129.

13. (a) Kumar, A.; Walker, R.T. *Tetrahedron* **1990**, *46*, 3101. (b) Maggio, A.-F.; Boyer, V.; Aubertin, A.-M.; Obert, G.; Kirn, A.; Imbach, J.-L. *Nucleosides & Nucleotides* **1991**, *10*, 1431.

14. (a) Schulhof, J.C.; Molko, D.; Teoule, R. *Nucleic Acids Res.* **1987**, *15*, 397. (b) Caruthers, M.H.; Barone, A.D.; Beaucage, S.L.; Dodds, D.R.; Fisher, E.F.; McBride, L.J.; Matteucci, M.; Stabinsky, Z.; Tang, J.-Y. *Methods in Enzymology* **1987**, *154*, 287.

15. Damha, M.J.; Giannaris, P.A.; Zabarylo, S.V. *Nucleic Acids Res.* **1990**, *18*, 3813.

16. (a) Mungall, W.S.; Kaiser, J.K. *J. Org. Chem.* **1977**, *42*, 703. (b) Stirchak, E.P.; Summerton, J.E.; Weller, D.D. *J. Org. Chem.* **1987**, *52*, 4202. (c) Coull, J.M.; Carlson, D.V.; Lee Weith, H. *Tetrahedron Lett.* **1987**, *28*, 745. (d) Wang, H.; Weller, D.D. *Tetrahedron Lett.* **1991**, *32*, 7385.

17. Pannecouque, C.; Schepers, G.; Rozenski, J.; Van Aerschot, A.; Claes, P.; Herdewijn, P. *BioMed. Chem. Lett.* **1994**, *4*, 1203.

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