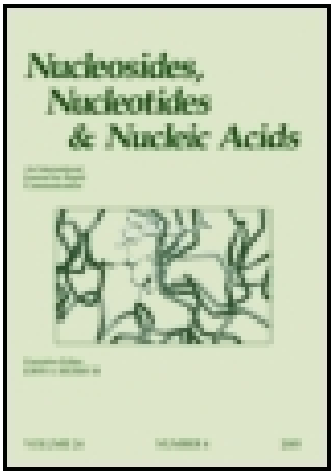

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Synthesis and Properties of Oligoribonucleotide Analogues Having Amide (3″-CH_2-CO-NH-5′) Internucleoside Linkages

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SYNTHESIS AND PROPERTIES OF OLIGORIBONUCLEOTIDE ANALOGUES HAVING AMIDE (3'-CH_2-CO-NH-5') INTERNUCLEOSIDE LINKAGES

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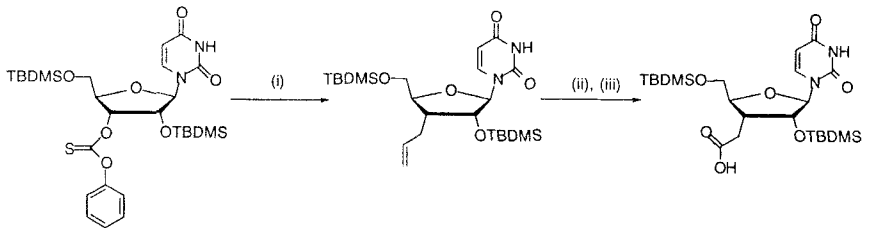
Abstract: Amide linked ribonucleoside dimer **5** was prepared and converted into selectively protected H-phosphonate building block **9**. Oligoribonucleotide duplexes having amide linkages at selected sites were synthesized and their stability was studied by UV melting experiments.

Oligonucleotides having the phosphodiesters replaced with dephosphono linkages have been proposed as potential second generation antisense compounds[1]. Most of these studies have been done on oligodeoxynucleotides and their analogues. Because the RNA-RNA duplexes are generally more stable than the DNA-RNA hybrids, oligoribonucleotide analogues having chemically and enzymatically stable dephosphono linkages could be promising as second generation antisense compounds. Recently we found that substitution of phosphodiester with formacetal linkage slightly improved the stability of oligoribonucleotide duplexes[2]. Herein we report preliminary results on synthesis and stability of RNA-RNA duplexes having amide (3'-CH_2-CO-NH-5') linkages at selected positions.

Acid **3** was prepared (Scheme 1) from 2',5'-O-di-TBDMS-3'-O-phenoxythiocarbonyl uridine[3] **1** according to procedures successfully used in the deoxy series: radical allylation[4] gave **2**, cleavage of the double bond[5] with OsO_4 and $NaIO_4$ and oxidation[6] of the aldehyde with $NaClO_2$ gave **3**. Correct configuration at C3' was confirmed by NOESY spectra of the intermediate aldehyde. Informative NOEs were observed between H1' and 3'-CH_2 and between H3' and H6. Amine **4** was prepared from 5'-azido-5'-deoxyuridine[7] *via* acylation with acetyl chloride in presence of pyridine and radical reduction with tributylstannane[8] (61%, two steps). Coupling of **3** and **4** (Scheme 2) in presence of DCC (1 eq.) and 1-hydroxybenzotriazole (1 eq.) gave dimer **5**. Selective



1 2 3

(i) allyltributyltin, AIBN (75%); (ii) OsO_4, $NaIO_4$ (82%); $NaClO_2$ (82%).

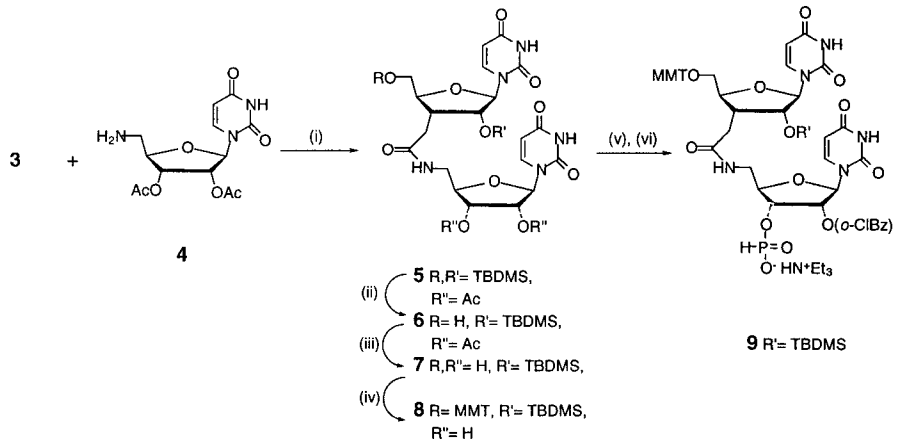
Scheme 1.

removal of the 5'-O-TBDMS group (limited acetic acid hydrolysis), cleavage of the terminal 2' and 3' acetyl groups and protection of the 5'-OH as monomethoxytrityl ether gave dimer **8**. Synthesis of the dimeric H-phosphonate building block **9** was achieved *via* one pot selective 2'-O-acylation (1.1 eq. of *o*-chlorobenzoyl chloride) followed by 3'-O-phosphonylation[2].

Correct structures of all compounds were confirmed using ^{1}H and ^{13}C NMR spectroscopy. The signal of H3' in the 3' uridine residue of **9** showed the coupling pattern expected (pentet, 1:2:2:2:1) from spin-spin interactions with H2' and H4' (J= ca 5 Hz) and with P (J= ca 10 Hz) thus confirming the correct structure of **9** as the 3'-O-hydrogenphosphonate.

Three model oligoribonucleotides (Table) having two, three and six internucleoside amide linkages (**x**) were synthesized using the automated solid phase H-phosphonate method for RNA synthesis[9] with 2'-O-*o*-chlorobenzoyl protection[10]. Dimer **9** was used under standard coupling conditions. Unmodified sequences and the complementary oligoribonucleotides were also synthesized and the stability of the corresponding duplexes were characterized by UV melting experiments (Table). Correct nucleoside residue composition of all oligonucleotides were confirmed by enzymatic degradation (SVPD and AP) followed by RP HPLC analysis.

Substitution of selected phosphodiester linkages with amides (3'-CH_2-CO-NH-5') did not significantly affect the stability of RNA-RNA duplexes. However, the data reveal some sequence dependence which makes it difficult to rationalize the effect of this substitution.



(i) DCC, HOBT (68%); (ii) 80% AcOH , 50°C, 3h; (iii) NH_3/EtOH, 1:1 6h; (iv) 4-Monomethoxytrityl chloride (43%, 3 steps); (v) o-Chlorobenzoyl chloride, 1.1 eq, -78°C; (vi) PCl_3, Imidazole, NEt_3, -78°C (69%).

Scheme 2.

Table. Oligonucleotide sequences and affinity towards complementary RNA (t_m)[a].

Entry	Oligonucleotides[b]	unmodified ribo	amide modified[c]
1	AAGCGAU**x**UU**x**UGACACU	56.7	56.7 (0)
2	ACAU**x**UCGU**x**UGU**x**UCAGA	53.4	52.5 (-0.3)
3	(U**x**U)$_6$U	13.8	17.6 (+0.6)

[a] Absorbance vs temperature profiles were measured at 260 nm in buffer containing 100 mM NaCl, 10 mM sodium phosphate (pH 7.2), 0.1 mM EDTA and 2 μM oligonucleotides [b] **x** denotes position of the amide linkage. [c] Δt_m per modification are given in parenthesis.

RNA-RNA duplexes are generally more stable than the corresponding DNA-RNA complexes. Although there are many different sides to developing antisense compounds, favourable duplex formation is likely to be of major importance. Oligoribonucleotide analogues can from this sense be considered as having even greater potential than their deoxy counterparts. The duplex with the complementary RNA for the full deoxy version of the 16mer (Table, Entry 1) had a t_m= 46.9°C, i.e. 9.8°C lower than for the RNA or amide modified oligomers. Other advantages are ready access to monomeric synthons (e.g. can often be readily prepared from modified sugars and heterocyclic bases) and less costly staring materials. Uniformly modified amide-linked oligoribonucleotides might be interesting candidates for use as second generation antisense compounds.

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