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## Nucleosides, Nucleotides and Nucleic Acids

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### CHIMERIC RNA WITH MODIFIED BACKBONES: ALTERNATING METHYLENE(METHYLIMINO) LINKED PHOSPHODIESTER BACKBONE OLIGONUCLEOTIDES WITH 2'-OH AND 2'-OMe GROUPS

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**CHIMERIC RNA WITH MODIFIED BACKBONES:  
ALTERNATING METHYLENE(METHYLIMINO)  
LINKED PHOSPHODIESTER BACKBONE  
OLIGONUCLEOTIDES WITH 2'-OH  
AND 2'-OMe GROUPS**

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**ABSTRACT**

Synthesis of a *novel* ribo-MMI dimer with 2'-OH and 2'-OMe in 5'- and 3'-nucleosides, respectively is presented. The synthesis was accomplished by reductive coupling of 3'-deoxy-3'-C-formyluridine and 2'-O-methyl-5'-O-methylaminouridine *via* a thioacetal as the key intermediate for the top part of the dimer. Incorporation of ribo-MMI dimers into oligonucleotides increased binding affinity for target RNA.

The MMI modification, a methylene group replacing the C-3' oxygen atom and the phosphodiester group replaced by a N-methylhydroxylamine (methylene-(methyl)imino, 3'-CH<sub>2</sub>-N(Me)-O-CH<sub>2</sub>-4'), is one of the most interesting backbone modifications of antisense oligonucleotides (1). The synthesis of MMI-linked dimeric nucleosides has been studied extensively due to the favorable properties of the oligonucleotides containing MMI and phosphodiester linkages (2). Analogs including 2'-deoxy-, 2'-deoxy-2'-fluoro- and 2'-O-methyl ribo derivatives have been synthesized and characterized (3) (Fig. 1). Here we report the synthesis of

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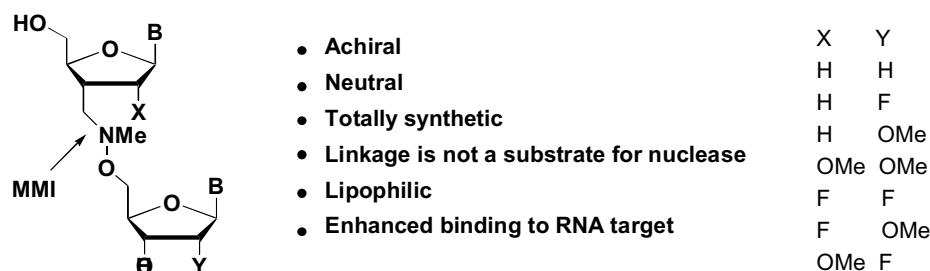
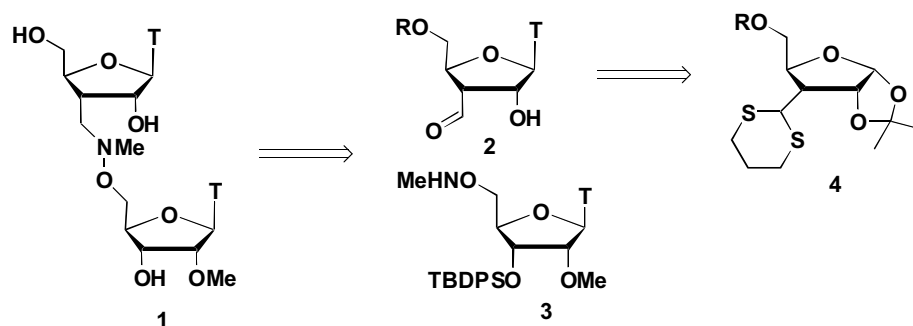
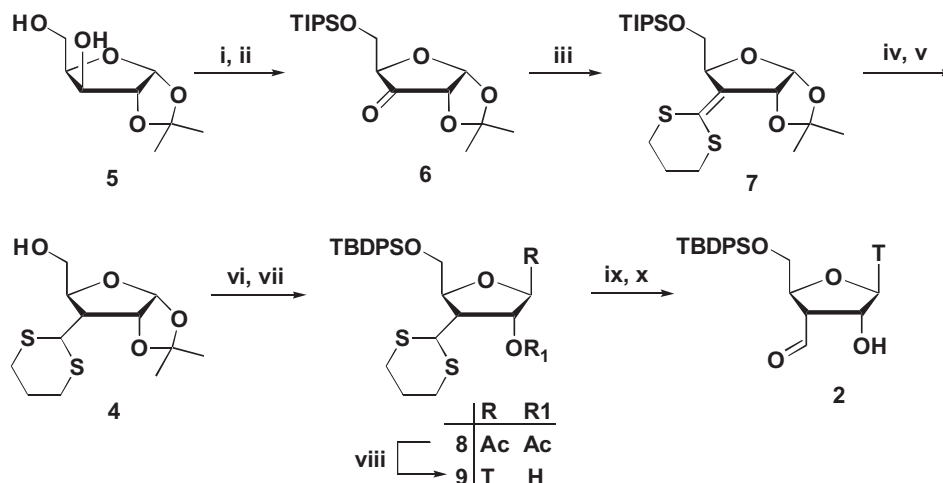


Figure 1. MMI backbone.



Scheme 1. Retrosynthetic analysis of Ribo-MMI-dimer.



Scheme 2. Synthesis of 3'-deoxy-3'-C-formyl-5-methyluridine. Reagents and conditions: i. TIPSO, Et<sub>3</sub>N, DMAP, DMF, rt, overnight, 100%; ii. DMSO, AC<sub>2</sub>O, rt, overnight, 78%; iii. 2-TMS-1, 3-dithiane, n-BuLi, THF, -78°C to 0°C, overnight, 60%; iv. TBAF, THF, 0°C, 0.5 h, 100%; v. LiAlH<sub>4</sub>, THF, 55°C, 6 h, 60%; vi. TBDPSCl, Imidazole, DMF, rt, 2 h, 100%; vii. Ac<sub>2</sub>O, AcOH, CSA, 70°C, 10 min; 80%; viii. (TMS)<sub>2</sub>T, TMSOTf, (CH<sub>2</sub>Cl)<sub>2</sub>, Δ, 0.5 h; ix. 0.1 N NaOH, MeOH, overnight, 95%; x. HgO (9 equiv), HgCl<sub>2</sub> (3 equiv), 90% aq. Me<sub>2</sub>CO, Δ, 1d, 94%.



a ribonucleoside (Fig. 1: X = OH, Y = OMe) MMI dimer. We wanted to examine the influence of 2'-OH on the solubility, pharmacokinetics and hybridization properties of the oligonucleotides. The 2'-OH substitution may also act as a starting point for further modifications (*e.g.* alkylation).

Retrosynthetic analysis (Scheme 1) of the desired dimer **1** indicated that dithiane **4** could serve as the key intermediate for the top part since thioacetals are known as masked aldehydes (4,5).

The synthesis started from commercially available 1,2-*O*-isopropylidene-D-xylose (**5**) (Scheme 2) which was upon 5-*O* silylation converted into 3-ketosugar **6**. Wittig condensation of **6** with 2-(trimethylsilyl)-1,3-dithiane followed by hydrogenation of ketene dithioacetal **7** with LiAlH<sub>4</sub> yielded dithiane **4**. Further acetolysis (6) of 5-*O*-TBDPS derivative and Vorbrüggen coupling of diacetate **8** with bis(trimethylsilyl)thymine followed by cleavage of 2'-*O* acetyl protection afforded 3'-dithianyl *ribo*-thymidine **9** which was successfully hydrolyzed with HgO and HgCl<sub>2</sub> in aqueous acetone (7) into 3'-C-formyl nucleoside **2**. Compound **2** was used for the reductive coupling with 5'-*O*-methylaminouridine **3** (8) into *ribo*-MMI-dimer **1** which was converted into phosphoramidite and incorporated into standard Isis sequences. Melting temperatures were measured against unmodified RNA compliments. In all cases a substantial increase (nearly +3°C/modification) was observed in comparison to unmodified oligodeoxy-nucleotides.

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