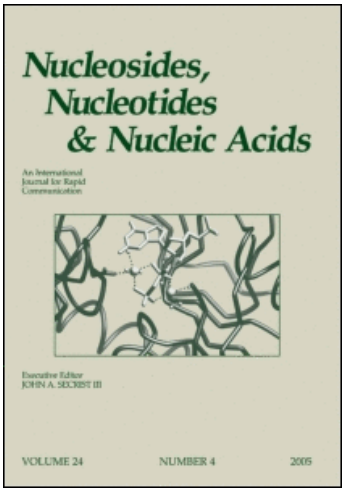

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 31 March 2001

To cite this Article Prhavc, Marija , Bhat, Balkrishen , Just, George , Cook, P. Dan and Manoharan, Muthiah(2001) 'CHIMERIC RNA WITH MODIFIED BACKBONES: ALTERNATING METHYLENE(METHYLIMINO) LINKED PHOSPHODIESTER BACKBONE OLIGONUCLEOTIDES WITH 2'-OH AND 2'-OMe GROUPS', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 995 — 997
To link to this Article: DOI: 10.1081/NCN-100002476
URL: http://dx.doi.org/10.1081/NCN-100002476

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 995–997 (2001)

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_2-N(Me)-O-CH_2-4′), 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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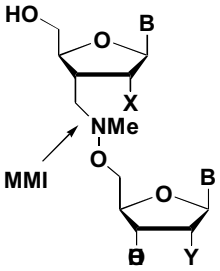
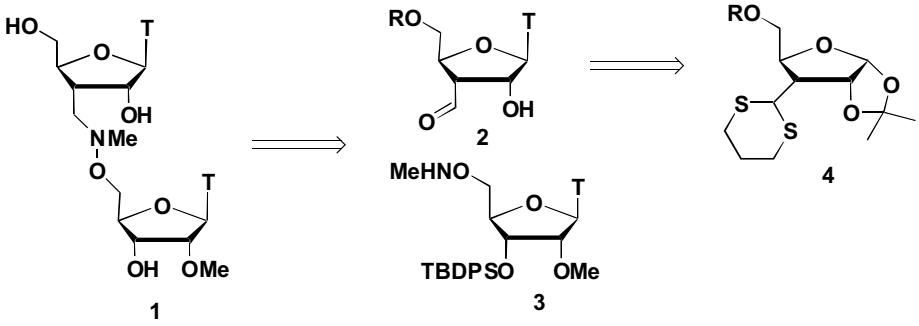


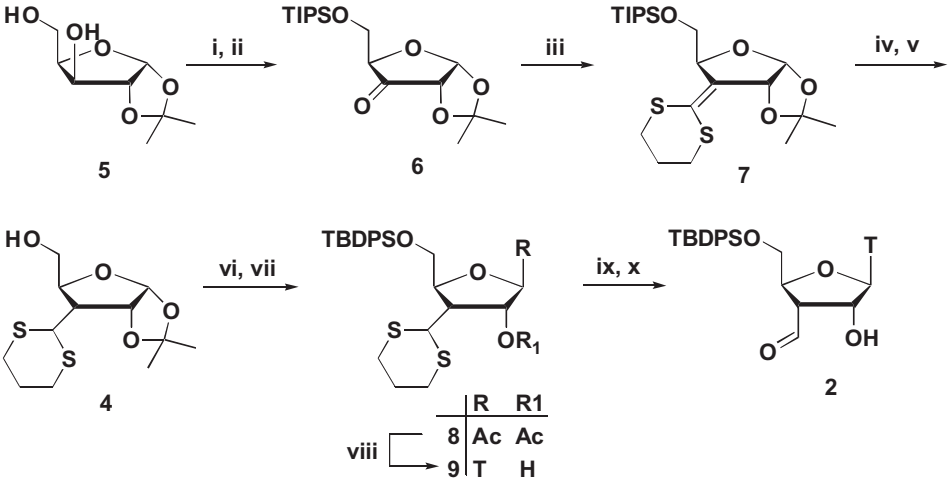
- **Achiral**
- **Neutral**
- **Totally synthetic**
- **Linkage is not a substrate for nuclease**
- **Lipophilic**
- **Enhanced binding to RNA target**

X	Y
H	H
H	F
H	OMe
OMe	OMe
F	F
F	OMe
OMe	F

Figure 1. MMI backbone.



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



Scheme 2. Synthesis of 3′-deoxy-3′-*C*-formyl-5-methyluridine. *Reagents and conditions*: **i.** TIPSCI, Et_3N, DMAP, DMF, rt, overnight, 100%; **ii.** DMSO, AC_2O, 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.** $LiAlH_4$, THF, 55°C, 6 h, 60%; **vi.** TBDPSCl, Imidazole, DMF, rt, 2 h, 100%; **vii.** Ac_2O, AcOH, CSA, 70°C, 10 min; 80%; **viii.** $(TMS)_2T$, TMSOTf, $(CH_2Cl)_2$, Δ, 0.5 h; **ix** 0.1 N NaOH, MeOH, overnight, 95%; **x**. HgO (9 equiv), $HgCl_2$ (3 equiv), 90% aq. Me_2CO, Δ, 1d, 94%.

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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-(trimethylsily)-1,3-dithiane followed by hydrogenation of ketene dithioacetal **7** with $LiAlH_4$ 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 $HgCl_2$ 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.

ACKNOWLEDGMENT

M.P. was a postdoctoral fellow at McGill University on leave from National Institute of Chemistry, Ljubljana, Slovenia, and wishes to acknowledge the financial support from Slovenian Ministry of Science and Technology and the National Science and Engineering Council of Canada.

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