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A new approach for the synthesis of 3′-deoxy-3′-*C*-formyl-ribonucleosides and the synthesis of alternating methylene(methylimino) linked phosphodiester backbone oligonucleotides with 2′-OH and 2′-OMe groups

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Abstract

A stereoselective synthesis of 3′-deoxy-3′-*C*-formyl-5-methyluridine **2** is described via the dithiane **4** as the key intermediate. Compound **2** was coupled with **3** into a novel *ribo*-MMI dimer **1**. The dimer was then incorporated into antisense oligonucleotides which were found to have high binding affinity to the target RNA.

Keywords: dithianes; nucleoside; nucleic acid analogs; antisense; oligonucleotides.

The methylene(methylimino), (MMI, 3′–CH_2–N(Me)–O–CH_2–4′) modification, in which a methylene group replaces the C-3′ oxygen atom and a *N*-methylhydroxylamine replaces the phosphodiester group, is a promising modification of the backbone of antisense oligonucleotides.[1] The synthesis of MMI-linked dimeric nucleosides has been studied extensively due to the favorable properties of oligonucleotides containing alternating MMI and phosphodiester linkages,[2] and analogs including 2′-deoxy-, 2′-deoxy-2′-fluoro-, and 2′-*O*-methyl *ribo*-derivatives have been synthesized and characterized.[3] Here we report the synthesis of a novel ribonucleoside containing (2′-OH in the 5′-nucleoside end) MMI-dimer (**1**, Scheme 1) utilizing a unique synthetic approach to 3′-deoxy-3′-*C*-formyl *ribo*-nucleosides. The 2′-OH may influence solubility, pharmacokinetics, and hybridization properties of the oligonucleotides. Biologically, the native RNA structure with its 2′-OH group is crucial in determining many RNA secondary structures (zippers, U-turns and bulges) and also in many nucleic acid–protein interactions.[4] The 2′-OH may also act as a starting point for further modifications (e.g. alkylation).

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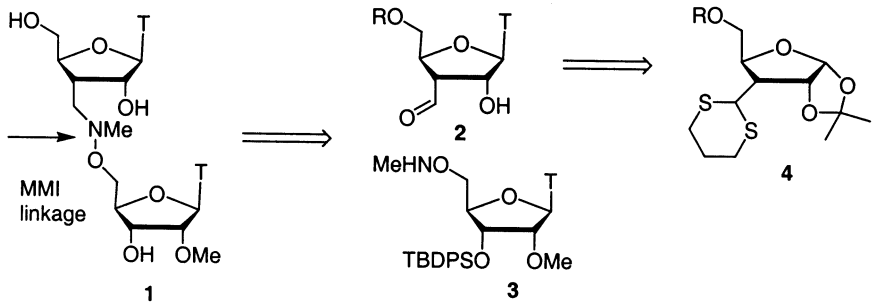
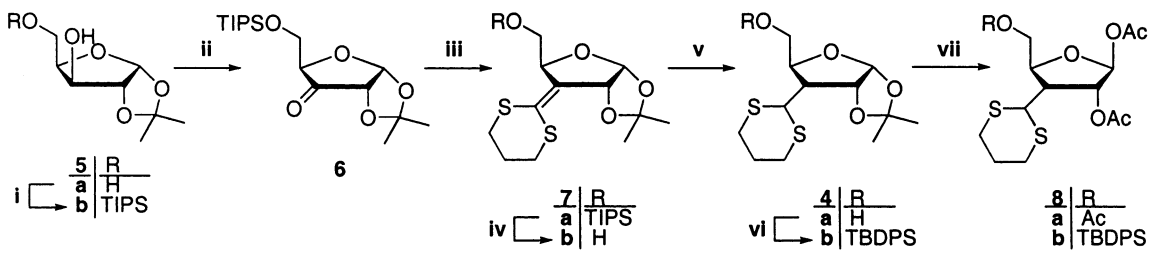


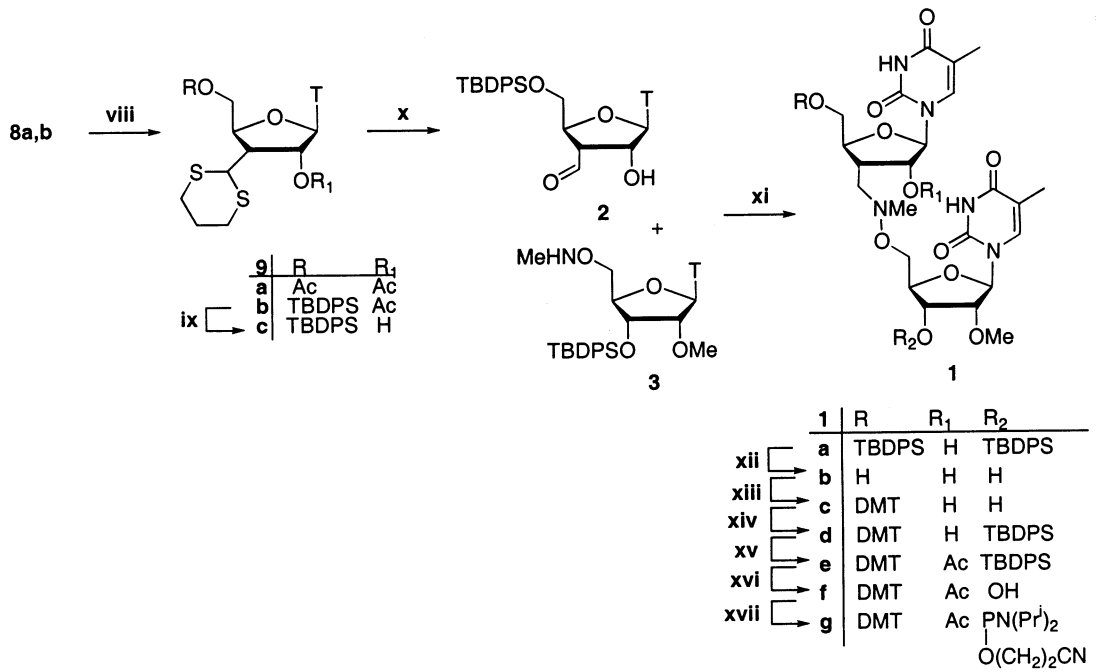
Scheme 1.

Recently, a mild procedure for the synthesis of *N*,*N*,*O*-trisubstituted hydroxylamines via reductive alkylation of *N*,*O*-disubstituted hydroxylamines with aldehydes has been developed and applied to the synthesis of MMI-linked dinucleosides,[5] with nucleoside aldehydes being prepared by *cis*-dihydroxylation/oxidation of the corresponding olefins.[5–7] In an analogous fashion the synthesis of *ribo*-MMI dimer **1** through the coupling of 3′-deoxy-3′-*C*-formyl-5-methyluridine (**2**) and 5′-*O*-methylaminouridine derivative **3** was envisaged (Scheme 1). Our search for a versatile intermediate for the 5′-portion of the molecule led to the dithiane **4**, since thioacetals are masked aldehydes.[8,9]

The commercially available 1,2-*O*-isopropylidene-D-xylose (**5a**) was quantitatively converted into the corresponding 5-*O*-silyl ether **5b** with triisopropylsilyl chloride (TIPSCl) (Scheme 2). A 20-fold excess of Ac_2O/DMSO was required for the Moffat reaction[10] to give the 3-ketosugar **6** in good yield (78%). Wittig condensation with 2-(trimethylsilyl)-1,3-dithiane[11] afforded ketene dithioacetal **7a** in 60% yield. Attempts to reduce the double bond by catalytic hydrogenation were unsuccessful. However, removal of the 5-*O*-TIPS protecting group (**7b**), followed by treatment with $LiAlH_4$ yielded the dithiane **4a** exclusively in the *ribo* configuration[12] in 80% yield.[13] The acetonide **4a** was treated with Ac_2O/AcOH/(±)-camphor-10-sulfonic acid (CSA) at 70°C for 10 min[14] to afford the triacetate **8a** in 60% yield. Vorbrüggen coupling of **8a** with bis(trimethylsilyl)thymine[15] gave the *ribo*-thymidine derivative **9a** in quantitative yield (Scheme 3). In an alternate route to improve the moderate yield (60%) during the acetolysis of **4a**, a



Scheme 2. Reagents and conditions: (i) TIPSCl, Et_3N, DMAP, DMF, rt, overnight, 100%; (ii) DMSO, Ac_2O, rt, overnight, 78%; (iii) 2-TMS-1,3-dithiane, *n*-BuLi, THF, −78 to 0°C, overnight, 60%; (iv) TBAF, THF, 0°C, 0.5 h, 100%; (v) $LiAlH_4$, THF, 55°C, 6 h, 80%; (vi) TBDPSCl, imidazole, DMF, rt, 2 h, 100%; (vii) Ac_2O, AcOH, CSA, 70°C, 10 min; 60% for **8a**, 80% for **8b**



Scheme 3. Reagents and conditions: (viii) $(TMS)_2T$, TMSOTf, $(CH_2Cl)_2$, Δ, 0.5 h, 100%; (ix) 0.1N NaOH, MeOH, rt, overnight, 95%; (x) HgO (9 equiv.), $HgCl_2$ (3 equiv.), 90% aq. Me_2CO, Δ, 1 day, 94%; (xi) 1 M PPTS (1 equiv.), MeOH, 0°C, 8 M Py·BH_3 (1 equiv.), 0°C–rt; 2 h, 60%; (xii) 1 M TBAF/THF, rt, 6 h, 100%; (xiii) DMTCl, Py, DMAP, rt, overnight, 80%; (xiv) TBDPSCl, imidazole, CH_2Cl_2, rt, 8 h, 93%; (xv) Ac_2O, Et_3N, DMAP, MeCN, rt, 2 h, 85%; (xvi) TBAF(SiO_2), THF, rt, 15 h, 82%; (xvii) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphsphordiamidite, diisopropylammonium tetrazolide, MeCN, rt, 22 h, 90%

5-*O*-TBDPS group was introduced to give **4b**, which eventually increased the yield of the reaction **4b**→**8b** to 80%. Vorbrüggen coupling of **8b** with bis(trimethylsilyl)thymine, followed by cleavage of 2′-*O*-acetyl protection of **9b** afforded the 3′-dithianyl *ribo*-thymidine **9c**. Compound **9c** was hydrolyzed in high yield (94%) to the 3′-*C*-formyl nucleoside **2**[16] after a one day treatment with HgO and $HgCl_2$ (9 and 3 equivalents, respectively) in boiling 90% aqueous acetone.[17] The crude compound **2** was used for the reductive coupling with 5′-*O*-methylamino-5-methyluridine (**3**).[5] The resulting *ribo*-MMI-dimer **1a**[18] was isolated in 60% yield after purification on silica column (Scheme 3). Deprotection with TBAF and dimethoxytritylation provided the crystalline product **1c**, which was further converted into MMI-phosphoramidite **1g**[19] in four steps.

The *ribo*-MMI-dimer was incorporated into standard Isis oligonucleotide sequences (Table 1). The oligonucleotides were synthesized with a 0.1 M CH_2Cl_2 solution of phosphoramidite **1g** following standard protocols with extended (3×15 min) couplings for all MMI dimers and using (1*S*)-(+)-10-(camphorsulfonyl)oxaziridine as the oxidizer.[20] The oligonucleotides were deprotected using standard conditions and purified by reverse phase HPLC. Electrospray mass spectral measurements (ESMS) and capillary gel electrophoresis measurements confirmed the integrity of the novel oligonucleotides. Helix to coil transition melting temperatures were measured against RNA complements (Table 1). In all cases, substantial increase in Tm was observed in comparison to unmodified oligodeoxyonucleotides.

Table 1
Properties of MMI-linked chimeric (2′-OH/2′-OMe) oligonucleotides

Isis No.	Sequence (5′→3′)	Anal. HPLC[a]	Mass		Tm (°C)	ΔTm/mod.[b]
		Time (min)	Calcd	Found		
17221	CTC GTA CCT*TTC CGG TCC	18.4	5387.3	5386.8	67.9	+2.26
17222	CTC GTA CCT*TT*T CGG TCC	19.9	5411.4	5411.1	67.9	+3.19
17223	GCG T*TT*TT*TT*T*T GCG	20.6	4921.3	4921.1	67.2	+3.83

* Chimeric *ribo*-MMI backbone modifications.
[a] C-18 Waters Delta Pak (15 μ; 3.8 × 300 mm) RP-column; solvents: (A) 50 mM triethylammonium acetate pH 7.0; (B) MeCN; Linear gradient: 5–60% B in 60 min.
[b] Compared to unmodified DNA.

In summary, we have described a novel synthesis of 3′-deoxy-3′-*C*-formyl-5-methyluridine (**2**) via the 3-deoxyribose-3-dithiane **4** as the key intermediate. This chemistry opens many avenues in nucleoside and oligonucleotide synthesis for diagnostic and therapeutic applications. It also allows the synthesis of a novel *ribo*-MMI dimer with 2′-OH and 2′-OMe in 5′- and 3′-nucleosides, respectively. The MMI-dimer was incorporated into antisense oligonucleotides, which have high binding affinity to the target RNA. It is already known that the MMI backbone provides extremely high nuclease stability to antisense oligonucleotides by providing stability to adjacent phosphate linkages. Protein binding and pharmacology of this new class of antisense compounds will have to be evaluated.

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12. Ribo configuration of **4a** was confirmed with ^{1}H NMR analysis: $J_{H2,H3}=3.9$ and $J_{H3,H4}=9.8$ Hz (in $CDCl_3$).
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16. Compound **2**: ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 9H, *t*-Bu), 1.46 (d, 3H, C-5-Me; $J_{Me,H-6}=0.8$ Hz), 1.9 (br, 1H, 2′-OH), 3.30 (dd, 1H, H-3′; $J_{3',4'}=9.4$, $J_{3',2'}=5.7$ Hz), 3.95 (dd, 1H, H-5′a; $J_{5'a,5'b}=12.2$, $J_{5'a,4'}=2.4$ Hz), 4.26 (dd, 1H, H-5′b; $J_{5'b,4'}=1.8$ Hz), 4.79 (apparent td, 1H, H-4′), 4.90 (d, 1H, H-2′), 5.75 (s, 1H, H-1′), 7.33–7.44 and 7.59–7.65 (2m, 10H, 2Ph), 7.67 (q, 1H, H-6), 9.83 (s, 1H, CHO), 10.24 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 12.00 (C5-Me), 19.45 (CMe$_3$), 27.03 (CMe$_3$), 52.63 (C-3′), 63.11 (C-5′), 77.31 (C-2′), 80.90 (C-4′), 93.26 (C-1′), 110.75 (C-5), 127.95, 128.04, 130.07, 130.17, 132.26, 133.04, 135.18, 135.44 (2Ph, C-6), 150.84 (C-4), 164.43 (C-2), 197.81 (CHO). FABMS (glycerol): *m*/*z* 509.2100 (MH$^+$); $C_{27}H_{33}N_2O_6Si$ requires 509.2108.
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18. Compound **1a**: ^{1}H NMR ($(CD_3)_2CO$) (U1 and U2 represent 3′- and 5′-substituted uridine moieties, respectively) δ 1.08 (s, 18H, 2 *t*-Bu), 1.45 (d, 3H, U1:C-5-Me; $J_{Me,H-6}=0.7$ Hz), 1.74 (d, 3H, U2:C-5-Me; $J_{Me,H-6}=1.0$ Hz), 2.52 (s, 3H, NMe), 2.54 (m, 1H, U1:H-3′), 2.80 (m, 1H, H-3″a), 3.07 (dd, 1H, H-3″b; $J_{3''a,3''b}=11.3$, $J_{3',3''b}=8.2$ Hz), 3.21 (s, 3H, OMe), 3.49 (t, 1H, U2:H-2′; $J_{1',2'}=4.1$ Hz), 3.67 (dd, 1H, U2:H-5′a; $J_{5'a,5'b}=11.2$, $J_{5'a,4'}=4.2$ Hz), 3.84 (m, 1H, U2:H-5′b), 3.89 (dd, 1H, U1:H-5′a; $J_{5'a,5'b}=11.7$, $J_{5'a,4'}=4.2$ Hz), 4.10–4.19 (3m, 3H, U1:H-5′b, U1:H-4′, U2:H-4′), 4.23 (t, 1H, U2:H-3′; $J_{3',4'}=J_{3',2'}=5.4$ Hz), 4.46 (m, 1H, U1:H-2′), 4.75 (d, 1H, 2′-OH; $J_{2',2'\text{-OH}}=2.7$ Hz), 5.82 (d, 1H, U1:H-1′; $J_{1',2'}=2.2$ Hz), 5.93 (d, 1H, U2:H-1′; $J_{1',2'}=3.7$ Hz), 7.38–7.48, 7.66–7.69 and 7.71–7.77 (3m, 22H, 4Ph, 2H-6), 9.98 (s, 2H, 2NH). ^{13}C NMR ($(CD_3)_2CO$) δ 12.01, 12.40 (2C5-Me), 19.21, 19.42 (2CMe$_3$), 26.81, 27.03 (2CMe$_3$), 38.89 (NMe), 44.95 (U1:C-3′), 56.00 (U1:C-3″), 57.73 (OMe), 63.51 (U1:C-5′), 70.36 (U2:C-5′, C-3′), 76.74 (U2:C-2′), 81.41 (U1:C-2′), 82.34, 83.38 (2C-4′), 88.77, 92.28 (2C-1′), 110.50, 110.56 (2C-5), 127.65, 127.79, 127.90, 127.94, 130.03, 130.08, 132.53, 132.77, 132.93, 133.01, 135.25, 135.42, 135.59, 135.73 (4Ph, 2C-6), 149.92, 150.71 (2C-4), 163.87, 164.09 (2C-2). ESMS: *m*/*z* 1032.7 (M$^+$); $C_{55}H_{69}N_5O_{11}Si_2$ requires 1032.5.
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