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Synthesis of 1-(2'-*O*-Methyl-ß-D-ribofuranosyl)-1*H*-imidazo[4,5-*d*]pyridazine-4,7(5*H*,6*H*)-dione: An Attractive Building Block for Antisense and Triple-helical Applications ‡

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Abstract: Synthesis of the title compound,1-(2'-O-methyl-ß-D-ribofuranosyl)-1H-imidazo-[4,5-d]pyridazine-4,7(5H,6H)-dione (**1**), is reported. It was synthesized in four steps, starting from methyl 1-(ß-D-ribofuranosyl)imidazo-4,5-dicarboxylate (**2**). The 3',5'-hydroxyl groups of **2** was protected with a bis-silylating agent to form **3**, which was then methylated to form the corresponding 2'-O-methyl derivative **5**. The silyl deprotection of the latter (to form **6**), followed by treatment with hydrazine afforded the target nucleoside **1**. The reported nucleoside has potentially beneficial applications in biomedicine based on antisense and triple-helical nucleic acid technologies.

Keywords: Synthesis, Nucleoside Analogue, Imidazo[4,5-*d*]pyridazine, 2'-*O*-Methyl Nucleoside Introduction

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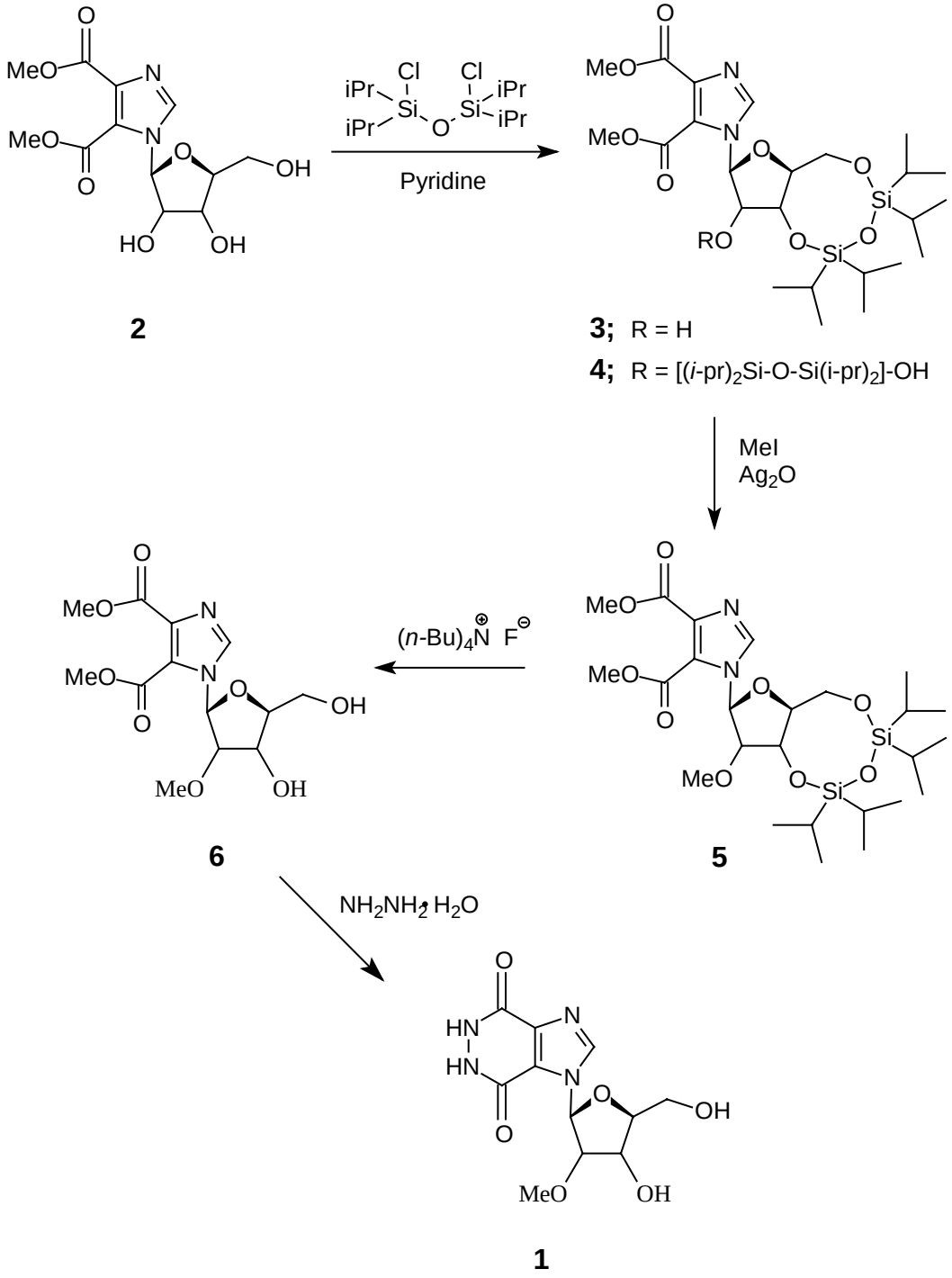
Introduction

2'-*O*-Methyl oligoribonucleotides are gaining wide attention in recent years because of their newly discovered, diverse biomedical applications in viral and cancer therapies [1,2]. Oligo-2'-*O*-methyl-ribonucleotides (2'-*O*-methyl RNA) were recently found to strongly inhibit restriction endonuclease *via* formation of triple-helices with oligoribonucleotides (RNA) and genomic sequences containing the recognition site for the class II-S restriction enzyme, Ksp632-I [1]. Synthetic 2'-*O*-methyl-modified hammerhead ribozymes, designed to be complementary to the RNA component of human telomerase, was shown to exhibit dose-dependent inhibition of human telomerase activity in tissue culture systems with a 0.4 micromolar IC_{50} value [2]. It has also been demonstrated that specific 2'-*O*-methyl-oligoribonucleotides, but not the corresponding 2'-deoxyribonucleotides, bind to E.*coli* tRNA (Cys), and inhibit aminoacylation of the latter by cysteine tRNA synthetase [3]. Furthermore, because of the significantly enhanced stability of their hybrids with complementary RNA as compared to that of the corresponding DNA**.**RNA duplexes, 2'-*O*-methyl-oligoribonucleotides are an attractive class of compounds for antisense-based therapeutic applications [4,5]. We report here the synthesis of a nucleoside analogue containing a methoxy functionality at the 2'-position, namely,1-(2'-*O*-methyl-*ß*-D-ribofuranosyl)-1*H*-imidazo[4,5-d]pyridazine-4,7(5*H*,6*H*)-dione (**1**), which can be considered an analogue of purine nucleoside in which a pyridazine moiety replaces a pyrimidine in fusion to an imidazole ring. Our molecular modeling studies suggest that nucleoside **1** has some unique structural features that would make it an attractive building block for oligonucleotides for potential antisense and triple-helical applications. These features include, but are not limited to, its potential capability to base-pair with cytidine, like guanosine, forming three H-bonds, but with a significantly shortened sugar-sugar (C-1' to C-1') distance, which in turn might lead to the decreased interstrand reach and consequently result in a somewhat compressed double-helix. The study of such an effect on the double-helical or triple-helical conformations, interactions, and stability would be interesting and rewarding.

Results and Discussion

Synthesis of the target compound commenced with methyl 1-(*ß*-D-ribofuranosyl)imidazole-4,5-dicarboxylate (**2**) [6] (**Scheme I**), which was selectively protected at the 3',5'-position using the Markiewicz reagent [7] 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane to form the corresponding TIPDS derivative **3** as the major product, along with a small amount of the completely silyl-protected derivative **4** [8]. Methylation of **3** with methyl iodide in the presence of silver oxide afforded the corresponding 2'-*O*-methyl derivative **5**. The silyl deprotection of the latter with tetra-*n*-butylammonium fluoride gave the free nucleoside **6**. The ring-closure of **6** to form the target **1** was accomplished by treatment with hydrazine. The synthesis of the parent ribofuranoside of **1**, containing a 2'-OH group in place of 2'-OMe, has long been reported in the literature, both by imidazole ring-closure [6f] as well as by glycosylation of the parent heterocyclic base, imidazo[4,5-*d*]pyridazine-4,7(5*H*,6*H*)-dione.

SCHEME I



2

3; R = H

4; R = [(*i*-pr)$_2$Si-O-Si(i-pr)$_2$]-OH

5

6

1

Acknowledgments

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Experimental

General

^{1}H-NMR spectra were recorded on a General Electric QE-300 (300 MHz) instrument. The spectral data are reported in the following format: chemical shift (all relative to Me_4Si as an internal reference standard unless otherwise indicated), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, b = broad), integration, coupling constants, exchangeability after D_2O addition, and assignment of resonances. Elemental Microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. The mass spectra were recorded at the Mass Spectral Facility, Department of Biochemistry, Michigan State University. Thin layer chromatography was performed on Merck Kieselgel 60 GF254 plates (0.2 mm thickness). Melting points were determined on a Thomas-Hoover capillary melting point apparatus, and are uncorrected.

Methyl 1-[(2'-O-methyl-3',5'-O-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl))-ß-D-erythropentofuranosyl]–4,5-imidazoledicarboxylate (5). A mixture of methyl 1-[(3',5'-*O*-(1,1,3,3,-tetraisopropyl-disiloxan-1,3-diyl))-*ß*-D-ribofuranosyl]-4,5- imidazoledicarboxylate (**3**) [8] (560 mg, 1 mmol), Ag_2O (1.85 g, 8 mmol) and MeI (10 mL) was refluxed for 5 h. The mixture was diluted with Et_2O, and was filtered over Celite.^{TM} The filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography, eluting with chloroform, to give **5** as a colorless oily product in quantitative yield, R_f 0.35 (hexane/ethyl acetate, 3:1); ^{1}H-NMR ($CDCl_3$) 8.20 (s, 1H, imidazole), 6.02(s, 1H, 1'-H), 4.42 (dd, 1H, *J*=4.2 and 9.6 Hz, 3'-H), 4.28 (d, 1H, J_{gem}=13.8 Hz, 5'-H), 4.16 (dd, 1H, *J*=9.6 and 2.4 Hz, 4'-H), 4.00 (dd, 1H, J_{gem}=13.8 Hz, *J5',4'*=2.4 Hz, 5'-H), 3.94 (s, 3H, $COOCH_3$), 3.93 (s, 3H, $COOCH_3$), 3.78 (d, 1H, *J*=4.2 Hz, 2'-H), 3.69 (s, 3H, OCH_3), 1.05 (m, 28H, isopropyl groups); ^{13}C-NMR ($CDCl_3$, 75.48 MHz) 12.53 (CHSi), 12.90 (CHSi), 12.96 (CHSi), 13.46 (CHSi), 16.87 (CCH_3), 17.01 (CCH_3), 17.01 (CCH_3), 17.15 (CCH_3), 17.30 (CCH_3), 17.30 (CCH_3), 17.38 (CCH_3), 17.49 (CCH_3), 52.45 ($COOCH_3$), 52.62 ($COOCH_3$), 59.46 (OCH_3), 59.96 (C-5'), 68.66 (C-3'), 81.59 (C-4'), 84.99 (C-2'), 90.76 (C-1'), 122.84 (C-4 or 5), 137.55 (C-2), 138.57 (C-5 or 4), 160.23 (C=O), 163.06 (C=O). *Anal*. Calcd. for $C_{25}H_{44}N_2O_9Si_2$ (MW 572.80): C, 52.42; H, 7.74; N, 4.89. Found: C, 52.46; H, 7.85; N, 4.63.

Methyl 1-(2'-O-methyl-ß-D-erythropentofuranosyl)-4,5-imidazoledicarboxylate (6). A 1*M* solution of tetra-*n*-butylammonium fluoride in THF (2 mL, 2 mmol) was added to an ice-cooled solution of methyl 1-[(2'-*O*-methyl-3',5'-*O*-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl))-*ß*-D-erythropentofuranosyl]-4,5-imidazoledicarboxylate (**5**) (573 mg, 1 mmol) in 10 mL of dry THF. The reaction mixture was stirred for 45 min at 0 ^{o}C. The solvent was evaporated *in vacuo* and the pure product was obtained as a foam after silica gel column chromatography, eluting with a mixture of chloroform-methanol (20:1), 85 % yield, R_f 0.29 (chloroform/methanol, 10:1); ^{1}H-NMR ($CDCl_3$) 8.67 (s, 1H, imidazole), 6.17 (d, 1H, *J*=2.1Hz, 1'-H), 5.03 (brs, 1H, 3'-OH, exchangeable with D_2O), 4.47 (t, 1H, 5'-OH, exchangeable with D_2O), 4.12 (m, 2H, 2',3'-H), 3.93 (s, 3H, $COOCH_3$), 3.91 (s, 3H, $COOCH_3$), 3.91 (m, 2H, 4',5'-H), 3.60 (s, 3H, OCH_3), 3.32 (m, 1H, 5'-H); ^{13}C-NMR ($CDCl_3$, 75.48 MHz) 52.31 ($COOCH_3$), 52.74 ($COOCH_3$), 59.10

(OCH_3), 60.03 (C-5'), 68.15 (C-3'), 84.85 (C-4'), 85.58 (C-2'), 88.83 (C-1'), 123.96 (C-4 or 5), 136.43 (C-5 or 4), 138.37 (C-2), 160.52 (C=O), 162.26 (C=O). *Anal.* Calcd. for $C_{13}H_{18}N_2O_8$ (MW 330.29): C, 47.27; H, 5.49; N, 8.48. Found: C, 47.54; H, 5.64; N, 8.28.

1-(2'-O-Methyl-ß-D-erythropentofuranosyl)-1H-imidazo[4,5-d]pyridazine-4,7(5H,6H)-dione (1). A solution of methyl 1-(2'-*O*-methyl-β-D-erythropentofuranosyl)-4,5-imidazoledicarboxylate (**6**) (165 mg, 0.5 mmol) and 99% hydrazine was refluxed for 6 hours. The excess hydrazine was removed by distillation *in vacuo* and the residue was coevaporated several times with water. The residue was dissolved in water and acidified with 2N HCl to pH 3. The solution was evaporated to dryness *in vacuo*. The residue was purified by preparative TLC on silica gel to afford a colorless solid 97 mg (65%). mp. >250 °C; R_f, 0.35 (chloroform/methanol/30% ammonium hydroxide, 2:2:1); ^{1}H NMR (DMSO-d_6): δ 8.73 (s,1H, imidazole), 6.50 (d, 1H, J=4.5 Hz, 1'-H), 4.27 (t, 1H, J= 4.8 Hz, 3'-H), 4.08 (t, 1H, J=4.5 Hz, 2'-H), 3.93 (m, 1H, 4'-H), 3.70 (dd, 1H, J=12.6 and 2.4 Hz, 5'-H), 3.58 (dd, 1H, J=12.6 and 3.0 Hz, 5'-H), 3.35 (s, 3H, OCH_3). ms: (FAB) m/z 299 (MH^+). HRMS Calcd. for $C_{11}H_{15}N_4O_6$: *m/z* 299.0992. Found (FAB): *m/z* 299.0990**.**

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Sample Availability: Available from authors