Fourth International Electronic Conference on Synthetic Organic Chemistry (ECSOC-4), www.mdpi.org/ecsoc-4.htm, September 1-30, 2000

[C0032]

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

Huan-Ming Chen and Ramachandra S. Hosmane*

Laboratory for Drug Design and Synthesis
Department of Chemistry & Biochemistry
University of Maryland, Baltimore County
1000 Hilltop Circle, Baltimore, Maryland 21250, USA

* Phone: 410-455-2520 Fax: 410-455-1148 E-Mail: hosmane@umbc.edu

Received: 14 August 2000 / Uploaded: 17 August

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

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.*

INTRODUCTION

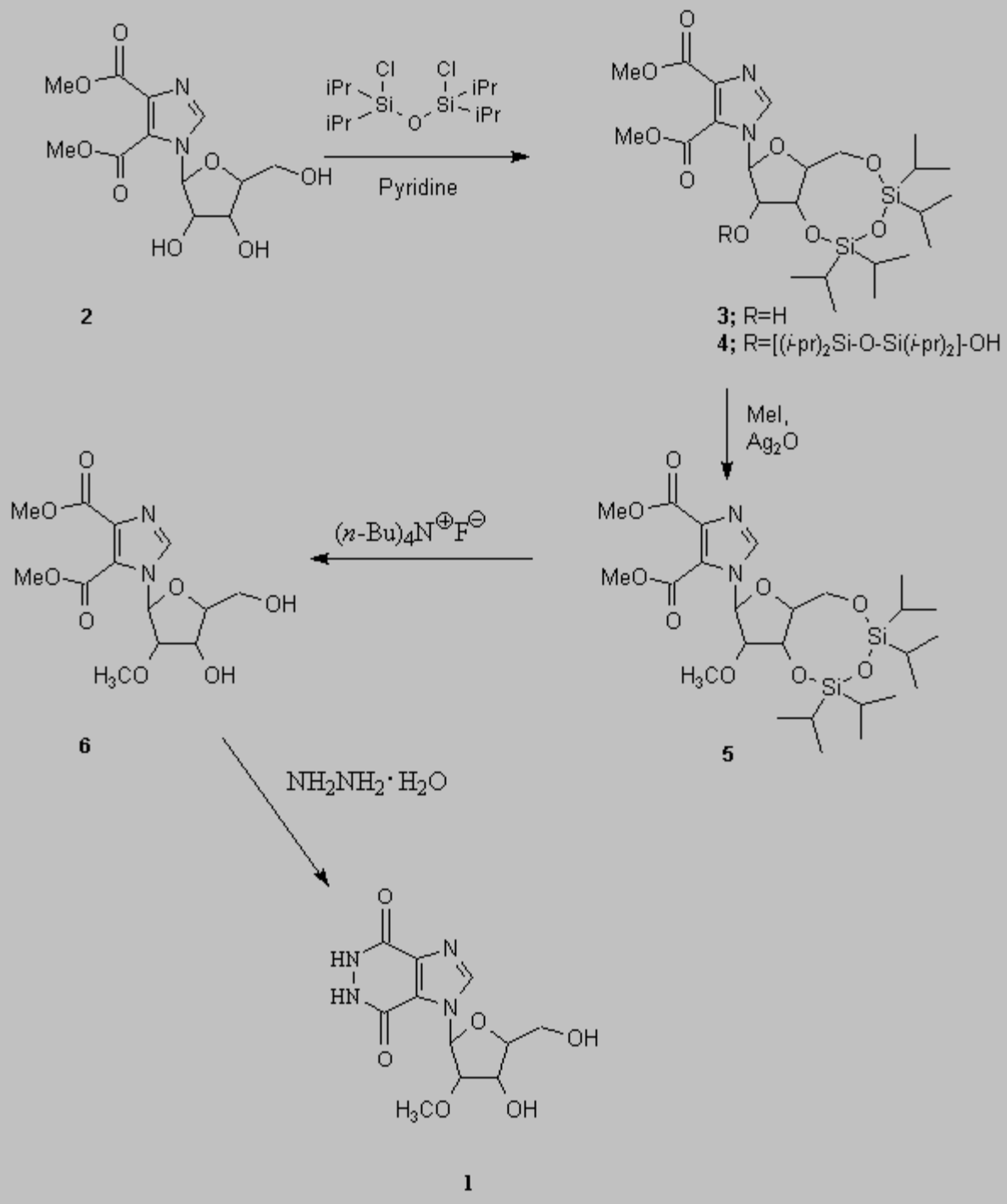
2'-*O*-Methyl ribonucleotides are gaining wide attention in recent years because of their newly discovered, diverse biomedical applications in viral and cancer therapies.[1,2] Oligo-2'-*O*-methyl-ribonucleotides (2'-*O*-methyl RNA) were recently found to strongly inhibit restriction endonuclease via 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'-deoxyribo nucleotides, 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**). The latter 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 somewhat compressed double-helix. The study of effect of such a compression on the double-helical or triple-helical conformations, interactions, and stability would be interesting and rewarding.

RESULTS & 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**. 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 reported here, as well as by glycosylation of the parent heterocyclic base, imidazo[4,5-*d*]pyridazine-4,7(5*H*,6*H*)-dione.[6b]

SCHEME I



EXPERIMENTAL SECTION

1H 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 GF_{254} plates (0.2 mm thickness). Melting points were determined on a Thomas-Hoover capillary melting point apparatus, and are uncorrected.

Methyl 1-[(3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl))-ß-D-ribofuranosyl]-4,5-imidazoledicarboxylate (3) and Methyl1-[((2'-O-(3-hydroxy-1,1,3,3,-tetraisopropyldisiloxyl)-3',5'-O-(1,1,3,3, -tetraisopropyldisiloxan-1,3-diyl))-ß-D-ribofuranosyl]-4,5-imidazoledicarboxylate (4)

To a solution of dry methyl 1-*ß*-D-ribofuranosyl-4,5-imidazoledicarbo xylate[6] (**2**) (500 mg, 1.58 mmol) in dry pyridine (10 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane[7] (550 mg, 1.75 mmol), and the mixture was stirred for 4 h at ambient temperature with protection from moisture. Volatile materials were evaporated *in vacuo*, and the residue was dissolved in chloroform. The chloroform solution was washed twice with cold water and dried over anhydrous sodium sulfate. The residue after evaporation was purified by silica gel flash chromatography, eluting with chloroform to give **3** and **4** as a colorless liquids, whose yield, spectral and analytical data are given below.

Compound **3**: Yield 800 mg (91%), Rf 0.38 (chloroform/ methanol, 30:1); ^{1}H-NMR ($CDCl_3$) 8.10 (s, 1H, imidazole), 6.11 (s, 1H, 1'-H), 4.44 (dd, 1H, *J*=4.2 and 9.0 Hz, 3'-H), 4.26 (d, 1H, J_{gem}=13.5 Hz, 5'-H), 4.18 (d, 1H, *J*=4.2 Hz, 2'-H), 4.16 (dd, 1H, *J*=9.0 and 2.4 Hz, 4'-H), 4.03 (dd, 1H, J_{gem}=13.5 Hz, *J5',4'*=2.4 Hz, 5'-H), 3.95 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 2.89 (brs, 1H, 2'-OH, exchangeable with D_2O), 1.05 (m, 28H, isopropyl groups); ^{13}C-NMR ($CDCl_3$, 75.48 MHz) 12.45 (CHSi), 12.82 (CHSi), 12.90 (CHSi), 13.35 (CHSi), 16.81 (CCH_3), 16.87 (CCH_3), 16.97 (CCH_3), 16.97 (CCH_3), 17.26 (CCH_3), 17.26 (CCH_3), 17.32 (CCH_3), 17.42 (CCH_3), 52.50 (OCH_3), 52.78 (OCH_3), 59.91 (C-5'), 68.43 (C-3'), 76.82 (C-2'), 81.87 (C-4'), 91.54 (C-1'), 122.83 (C-4 or 5), 137.26 (C-2), 138.57 (C-5 or 4), 160.26 (C=O), 162.91 (C=O).

Anal. Calcd. for $C_{24}H_{42}N_2O_9Si_2$ (MW 558.78): C, 51.59; H, 7.58; N, 5.01. Found: C, 51.50; H, 7.57; N, 5.04.

Compound **4**: Yield 120 mg (9%), Rf 0.45 (chloroform/ methanol, 30:1); ^{1}H-NMR ($CDCl_3$) 8.37 (s, 1H, imidazole), 6.06 (s, 1H, 1'-H), 4.48 (d, 1H, *J*=3.3 Hz, 2'-H), 4.35 (dd, 1H, *J*=3.3 and 9.6 Hz, 3'-H), 4.31 (d, 1H, J_{gem}=13.8 Hz, 5'-H), 4.27 (dd, 1H, *J*=9.6 and 2.4 Hz, 4'-H), 4.02 (dd, 1H, *Jgem*=13.8 Hz, $J_{5',4'}$=2.4 Hz, 5'-H), 3.93 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 1.03 (m, 56H, isopropyl groups); ^{13}C-NMR ($CDCl_3$, 75.48 MHz) 12.51 (CHSi), 12.91 (CHSi), 13.05 (CHSi), 13.05 (CHSi), 13.24 (CHSi), 13.35 (CHSi), 13.39 (CHSi), 13.48 (CHSi), 16.59 (CCH_3), 16.78 (CCH_3), 16.81 (CCH_3), 16.81 (CCH_3), 17.06 (CCH_3), 17.06 (CCH_3), 17.06 (CCH_3), 17.23 (CCH_3), 17.26 (CCH_3), 17.26 (CCH_3), 17.30 (CCH_3), 17.37 (CCH_3), 17.42 (CCH_3), 17.51 (CCH_3), 17.55 (CCH_3), 17.65 (CCH_3), 52.56 (OCH_3), 53.17 (OCH_3), 59.56 (C-5'), 68.47 (C-3'), 77.95 (C-2'), 81.44 (C-4'), 92.15 (C-1'), 121.16 (C-4 or 5), 138.64 (C-2), 139.48 (C-5 or 4), 161.49 (C=O), 163.11 (C=O).

Anal. Calcd. for $C_{36}H_{70}N_2O_{11}Si_4$ (MW 819.30): C, 52.78; H, 8.61; N, 3.42. Found: C, 52.45; H, 8.89; N, 2.93.

Methyl 1-[(2'-O-Methyl-3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl))-ß-D-ribofuranosyl] -4,5-

imidazoledicarboxylate (5)

A mixture of methyl 1-[(3',5'-O-(1,1,3,3,-tetraisopropyldisiloxan-1,3-diyl))-ß-D-ribofuranosyl]-4,5-imidazoledicarboxylate (**3**) (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, Rf 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-ribofuranosyl)-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,-tetraisopropyl disiloxan-1,3-diyl))--D-ribo furanosyl]-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, elting with a mixture of chloroform-methanol (20:1), 85 % yield, Rf 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-ribofuranosyl)-1H-imidazo[4,5-d]pyridazine-4,7(5H,6H)-dione (1)

ACKNOWLEDGMENT

This research was supported by a grant from the National Institutes of Health (#RO1 CA 71079). The Michigan State University Mass Spectrometry Facility was supported in part from a grant (#

P41RR00480-0053) from the National Institutes of Health

REFERENCES

1. Ushijima, K.; Ishibashi, T.; Yamakawa, H.; Tsukahara, S.; Takai, K.; Maruyama, T.; Takaku, H. *Biochemistry* **1999**, *38*, 6570.

2. Wan, M. S.; Fell, P. L.; Akhtar, S. *Antisense Nucleic Acid Drug Dev.* **1998**, *8*, 309.

3. Hou, Y. M.; Gamper, H. B. *Biochemistry* **1996**, *35*, 15340.

4. Lubini, P.; Zurcher, W.; Egli, M. *Chem. Biol.***1994**, *1*, 39.

5. Lesnik, E. A.; Guinosso, C. J.; Kawasaki, A. M.; Sasmor, H.; Zounes, M.; Cummins, L. L.; Ecker, D. J.; Cook, P. D.; Freier, S. M. *Biochemistry***1993**, *32*, 7832.

6. (a) Wyss, P. C.; Fischer, U. *Helv. Chim. Acta***1978**, *61*, 3149. (b) Cook, P. D.; Dea, P.; Robins, R. K. *J. Heterocyclic Chem.***1978**, *15*, 1. (c) Cook, P. D.; Robins, R. K. in *Nucleic Acid Chemistry, Part 1*, Townsend, L. B and Tipson, R. S., Ed.; John Wiley, Inc., New York, **1978**, p 211. (d) Fischer, U.; Wyss, P. C. *Ger. Offen. 2735458*, **1978**; *Chem. Abstr.* 89:24726e, **1978**. (e) Fischer, U.; Wyss, P. C. *Belg. 857512*, **1978**; *Chem. Abstr.* 89:44128q, **1978**. (f) Tapiero, C.; Imbach, J. L.; Panzicka, R. P.; Townsend, L. B. *J. Carbohyd. Nucleosides Nucleotides***1976**, *3*, 191.

7. Markiewicz, Wojciech T. *J. Chem. Res. (S)* **1979**, *1*, 24.
