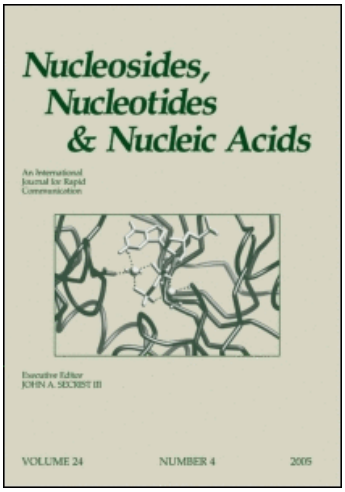


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A Convenient Synthesis of A Novel Nucleoside Analogue: 4-(δ-Diformyl-methyl)-1-(β-D-ribofuranosyl)-2-pyrimidinone

Kui Gao[a]; Leslie E. Orgel[a]
[a] The Salk Institute for Biological Studies, La Jolla, California, USA

To cite this Article Gao, Kui and Orgel, Leslie E.(2000) 'A Convenient Synthesis of A Novel Nucleoside Analogue: 4-(δ-Diformyl-methyl)-1-(β-D-ribofuranosyl)-2-pyrimidinone', Nucleosides, Nucleotides and Nucleic Acids, 19: 5, 935 — 940
To link to this Article: DOI: 10.1080/15257770008033033
URL: http://dx.doi.org/10.1080/15257770008033033

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(5&6), 935–940 (2000)

A CONVENIENT SYNTHESIS OF A NOVEL NUCLEOSIDE ANALOGUE: 4-(α-DIFORMYL-METHYL)-1-(β-D-RIBOFURANOSYL)-2-PYRIMIDINONE

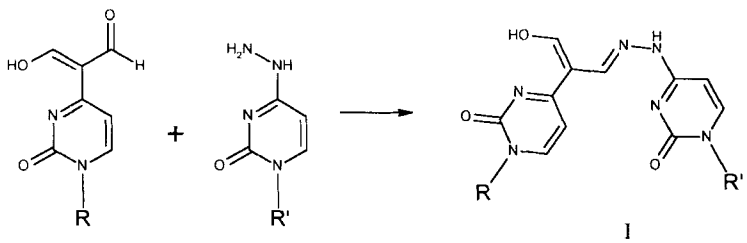
Kui Gao and Leslie E. Orgel*

The Salk Institute for Biological Studies,
10010 North Torrey Pines Road, La Jolla, California 92037, USA

ABSTRACT: The nucleoside analogue 4-(α-diformyl-methyl)-1-(β-D-ribofuranosyl)-2-pyrimidinone (**5**) was prepared from the corresponding 4-methyl pyrimidinone nucleoside by means of the Vilsmeier reaction. The unprotected nucleoside can be phosphorylated directly with phosphorus oxychloride in triethyl phosphate.

INTRODUCTION

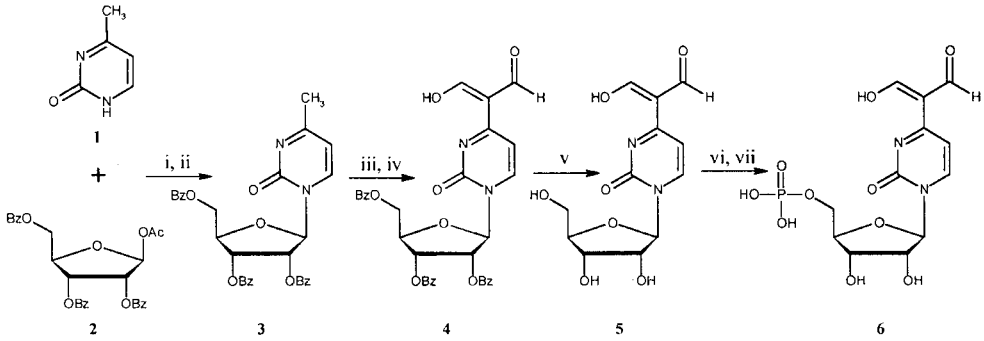
4-(α-Diformyl-methyl)-1-(β-D-ribofuranosyl)-2-pyrimidinone (**5**) is a nucleoside analogue in which the base carries a malonaldehyde group. This group reacts readily with hydrazines, hydroxylamines etc, so **5** is readily converted to a variety of potentially interesting nucleoside analogues. We have used **5** as the precursor of a covalent base pair (structure I), which we designed *de novo* by molecular modeling techniques[1].



I

RESULTS AND DISCUSSION

Condensation of 4-methyl-2-pyrimidinone with tri-benzoyl-D-ribofuranosyl chloride using the mercuric cyanide-nitromethane procedure to give the protected nucleoside **3** has been reported[2-5]. Here we describe a simpler one-pot procedure[6] for the preparation of **3**. Compound **1** was silylated in situ using hexamethyldisilazane (HMDS) and trimethylchlorosilane (TCS). The silylated base condensed directly with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **2** in presence of $SnCl_4$ to give 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-4-methyl-2-pyrimidinone **3** in 73% yield. The NMR spectrum of our material was identical to that reported for **3** in earlier publications[2, 5].



i, $SnCl_4$/HMDS/TCS. ii, $NaCO_3$. iii, $POCl_3$/DMF. iv. H_2O. v, MeONa/MeOH. vi, $POCl_3$. vii, H_2O

It is known that 4-methyl pyrimidines react with the Vilsmeier reagent[7, 8]. This reaction permits the 4-methyl group of **3** to be converted directly to a malonaldehyde derivative. The Vilsmeier reagent was prepared from $POCl_3$ and dimethylformamide (DMF), and was used directly to convert **3** to **4** (yield 86%). When compared with the NMR spectrum of compound **3**, the NMR spectrum of **4** has a new signal at ~9.6 ppm and has lost the signal of the 4-methyl group at 2.3 ppm. After debenzoylation with sodium methoxide, the yield of **5** was 63%. ^{1}H-NMR revealed a peak at 9.6ppm (2H), corresponding to the two aldehyde hydrogens. The shift of UV absorption from 309nm to 365nm (pH3) and the greatly increased extinction coefficient

indicate the formation of a conjugation aromatic system involving the malonaldehyde group. The composition of **5** was determined by high-resolution MS. As far as we are aware, this is the first application of Vilsmeier reaction to the direct synthesis of a nucleoside derivative containing a malonaldehyde group. Compound **5** is a potential precursor to be converted to a number of useful nucleoside analogues by treatment with hydrazines, hydroxylamines, etc. The nucleoside **5** can be converted to its 5'-phosphate by treatment with $POCl_3$ in triethyl phosphate, without protection of the dialdehyde function.

EXPERIMENTAL

All reagents were obtained from commercial sources. Analytical thin layer chromatography was performed on silica gel 60 F_{254} (Merck) in the following solvent systems: A. methanol-chloroform (1:19 v/v); B. methanol-chloroform (1:9 v/v); C. butanol-acetic acid-water (5:2:3 v/v). The compounds were visualized directly by UV absorption or by spraying with 0.5% 2,4-dinitrophenylhydrazine in 2N hydrochloride. Flash column chromatography was carried out on silica gel ('Baker' Silica gel 40 μm flash chromatography packing from J. T. Baker) eluting with a linear gradient of 0-10% (v/v) methanol in chloroform. High performance liquid chromatography was performed on a C_{18} column and eluting with a gradient of 0-50% acetonitrile in water in 35 minutes. UV spectra were recorded with a Beckman DU 640 spectrophotometer. The nuclear magnetic resonance spectrum of final product was measured on a Bruker 500MHz spectrometer. Chemical shifts are reported in parts per million (δ) and signals are described as S (singlet), D (doublet), or m (complex mutiplet). A JNM-PMX60SI NMR spectrometer was used in routine analysis. Mass spectra were determined in the Peptide Biology Laboratory at The Salk Institute. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter.

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-4-methyl-2-pyrimidinone (**3**)

700 μL $SnCl_4$ was added slowly under argon with vigorous stirring to a suspension of 0.45 g **1** and 1.52 g of **2** in 42 mL of anhydrous CH_3CN. 510 μL TCS and 550 μL HMDS were added to

the resulting clear solution, with continuous stirring. The reaction mixture was incubated at room temperature for 4 hours with careful exclusion of moisture. Analysis using TLC (system A) revealed a yield of **3** of >80%. The reaction solution was evaporated *in vacuo* and the residual syrup taken up in 30 mL dichloromethane. A saturated solution of sodium bicarbonate was dropped into this solution with stirring, until evolution of CO_2 ceased. After filtration through Celite, the aqueous layer was separated and discarded. The dichloromethane solution was washed with 2×10 mL of a saturated solution of sodium chloride and 2×10 mL of water, dried over magnesium sulfate and evaporated to dryness. The residue was purified by flash chromatography, affording 1.2 g (73%) of **3**. ^{1}H-NMR (60 MHz, $CDCl_3$) δ 2.3 (s, 3H), 4.8 (m, 3H), 6.0 (m, 1H), 6.2 (d, J=3, 1H), 6.5 (d, J=8, 1H), 7.2~7.7 (m, 9H), 7.8~8.3 (m, 7H).

4-(α-Diformyl-methyl)-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-pyrimidinone (**4**)

Phosphorus oxychloride (0.3 mL) was gradually added to 1 mL of anhydrous DMF with vigorous stirring, while maintaining the temperature at about 15°C. Next, 0.55 g of **3** dissolved in 1 mL DMF was added dropwise while holding the temperature at 15°C. The mixture was warmed to 25°C and stirred for 30 min and then incubated at 60°C for another 30 min. After cooling, the reaction mixture was poured into 3 g ice water and stirred until it reached room temperature. The product was precipitated by neutralization with sodium bicarbonate. The precipitate was washed with water several times, dried in a stream of air, and dissolved in 5 mL of chloroform. The resulting solution was washed with 2 mL water, dried over magnesium sulfate, and evaporated under vacuum. After flash chromatography, we obtained **4** (TLC pure in system B) as a syrup (521mg, 86%). ^{1}H-NMR (60 MHz, $CDCl_3$) δ 4.8 (m, 3H), 6.0 (m, 1H), 6.4 (d, J=5, 1H), 7.2~8.3 (m, 16H), 9.5(s, 1H), 9.6 (s, 1H).

4-(α-Diformyl-methyl)-1-(β-D-ribofuranosyl)-2-pyrimidinone (**5**)

The syrup obtained above was dissolved in 15 mL benzene-methanol (1:1 v/v), and 1.6 mL of a 0.5M alcoholic solution of sodium methoxide was added. The reaction mixture was stirred at

room temperature for 20 hours. The debenzoylated product was precipitated as the sodium salt by dilution of the reaction solution with 15 mL benzene. The precipitate was dissolved in 5 mL water, neutralized with Dowex® 50WX-100, filtered, and lyophilized. The product **5** was obtained as a light yellow powder (182 mg; 61% from **3**). Purity is >99% (analysis by HPLC on a C_{18} column): UV max (H_2O) λ_{max}^{pH3} 365 (ε 30,000), 247 (ε 14,000); $\lambda_{max}^{pH7.4}$ 363 (ε 25,000), 247 (ε 13,000); λ_{max}^{pH12} 338 (ε 20,000), 294(wide) (ε 12,000), 262 (ε 14,000); Optical rotation $[\alpha]_D^{20}$ +83° (0.01M, water).^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.6 (s, 2H), 8.5 (s, 1H), 7.5 (d, J=7Hz, 1H), 5.8 (d, J=3, 1H), 5.6 (s, 1H), 5.2 (s, 1H), 5.1 (s, 1H), 4.1 (s, 1H), 4.0 (s, 1H), 3.9 (m, 1H), 3.6 (d, J=12Hz, 1H), 3.6 (d, J=12Hz, 1H); Mass spectrum (MALDI-FTMS) $C_{12}H_{14}N_2O_7Na_2^+$ calculated 343.0518, found 343.0515.

4-(α-Diformyl-methyl)-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-pyrimidinone-5'-phosphate (**6**)

100 mg of **5** was dissolved in 1 mL triethyl phosphate, and cooled on ice. Then 360 μL of $POCl_3$ was added with stirring. After stirring for 3 hours on ice, the reaction mixture was poured into 20 g ice-water-pyridine (1:1:1 w/w). After the ice had melted, the resulting solution was concentrated to a small volume, diluted with 25 mL water, and adjusted to pH10 with 4M lithium hydroxide. The precipitate was filtered off and the filtrate evaporated to dryness, and then washed with acetone-ethanol (1:1 v/v) until the no chloride ion could be detected. The product was purified by ion-exchange chromatography (Sephadex A-25, elution with a gradient of triethylamine bicarbonate 0-0.5M). The final product **6** (TLC pure in system C) was obtained as a powder after lyophilization (107 mg, 83%). Mass spectra (FAB+) $C_{12}H_{14}N_2Na_2O_{10}P^+$ calculated 423.02, found 423.

ACKNOWLEDGMENTS

This work was supported by the National Aeronautics and Space Administration grant NAGW-1660 and National Aeronautics and Space Administration Specialized Center of Research and Training/Exobiology grant NAGW-2881. We thank Aubrey R. Hill Jr. for technical

assistance and Bernice Walker for manuscript preparation. We also thank Guoqiang Shi, Bin Zhou, Guangcheng Jiang, and Shaokai Jiang for helpful discussions.

REFERENCES

1) Gao, K. and Orgel, L. E. *Proc. Nat. Acad. Sci. USA,* **1999** *96,* 14837.

2) Klein, R. S.; Wempen, I.; Watanabe, K. A. and Fox, J. J. *J. Org. Chem.,* **1970** *35,* 2330.

3) Watanabe, K. A. and Fox, J. J. *J. Hererocycl. Chem.,* **1969** *6,* 106.

4) Yamaoka, N.; Aso, K. and Matsuda, K. *J. Org. Chem.,* **1965** *30,* 149.

5) Holy, A.; Ludzisa, A.; Vortube, I.; Sediva, K. and Pischel, H. *Collection Czechoslovak Chem. Commun.,* **1985** *50,* 393-417.

6) Vorbruggen, H. and Bennua, B. *Tetrahedron Letters,* **1978** *15,* 1339.

7) Bredereck, H. and Simchen, G. *Angew. Chem.,* **1963** *75,* 1102.

8) Bergstrom, D. E.; Inoue, I. and Leonard, N. J. *J. Org. Chem.,* **1972** *37,* 3902.

Received 9/24/99
Accepted 2/9/00