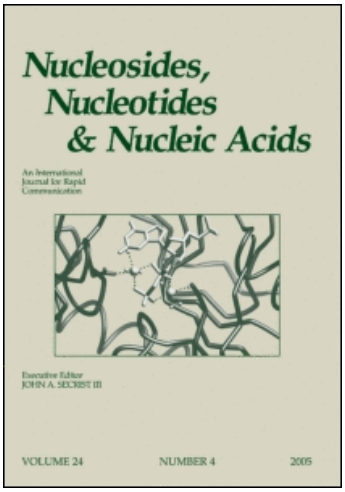


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The Synthesis of 2'-Deoxy-L-Cytidine-3'-Phosphate

Igor A. Kozlov[a]; Leslie E. Orgel[a]
[a] The Salk Institute for Biological Studies, San Diego, CA

To cite this Article Kozlov, Igor A. and Orgel, Leslie E.(1998) 'The Synthesis of 2'-Deoxy-L-Cytidine-3'-Phosphate', Nucleosides, Nucleotides and Nucleic Acids, 17: 12, 2249 — 2254
To link to this Article: DOI: 10.1080/07328319808004314
URL: http://dx.doi.org/10.1080/07328319808004314

NUCLEOSIDES & NUCLEOTIDES, 17(12), 2249-2254 (1998)

THE SYNTHESIS OF 2'-DEOXY-L-CYTIDINE-3'-PHOSPHATE

Igor A. Kozlov and Leslie E. Orgel*

The Salk Institute for Biological Studies
San Diego, CA 92138

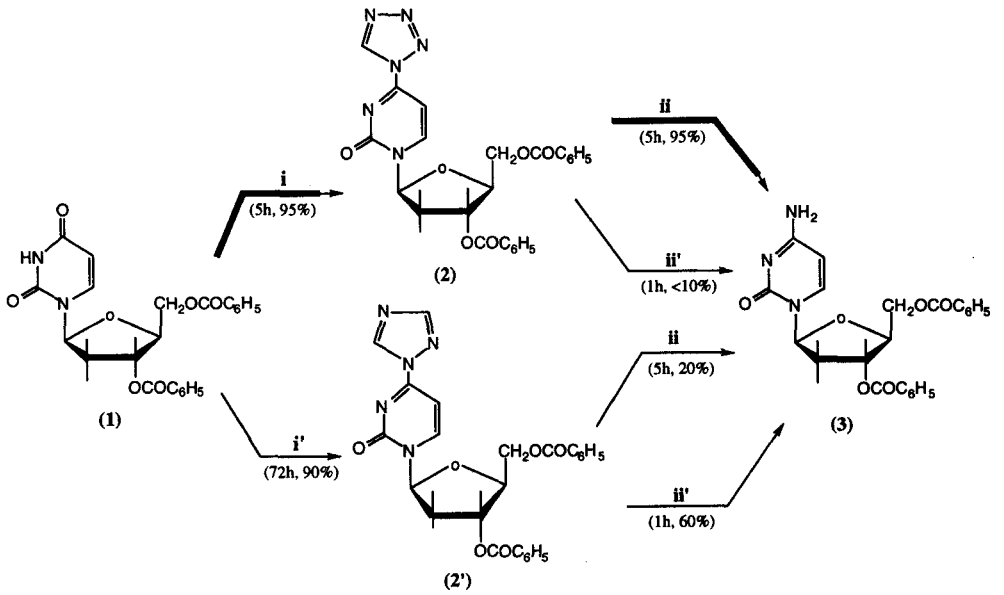
ABSTRACT: Improvements in the synthesis of 2'-deoxy-L-cytidine-3'-phosphate from L-arabinose are described.

INTRODUCTION

In the course of work on chimeras of DNA and peptide nucleic acid (PNA) we needed to synthesize 2'-deoxy-L-cytidine-3'-phosphate (L-dCp). This led us to modify the standard synthesis of 2'-deoxy-L-cytidine (L-dC) developed by Holy[1] using recent chemistry[2, 3]. The improvements that we were able to make may prove of use in the synthesis of other pyrimidine nucleoside derivatives. We also used recently developed chemistry[4] to phosphorylate the 3'-OH group of L-dC in place of the earlier procedures[5-7].

RESULTS AND DISCUSSION

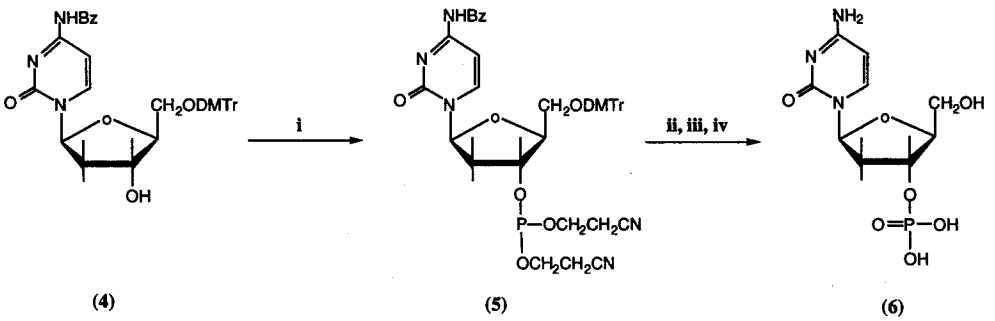
The standard method for the synthesis of L-dC[1] involves a well-developed five-step synthesis of 3',5'-di-benzoyl-2'-deoxy-L-uridine **(1)** from L-arabinose followed by its conversion to L-dC using phosphorus pentasulfide and methanolic ammonia. The second stage of the synthesis is not easy and does not give very good yields (50-60%). We improved the synthesis using modifications of more recent chemistry[2, 3].



Reagents and comments: i: 4-chlorophenyl dichlorophosphate, 1H-tetrazole, pyridine; **i':** 4-chlorophenyl dichlorophosphate, 1,2,4 -triazole, pyridine; **ii:** anhydrous NH_3, dioxane; **ii':** aqueous NH_3, dioxane. All reactions were run at room temperature. Product yields did not increase significantly if incubation times were increased beyond those indicated.

SCHEME 1

We explored two closely related routes via the 1H-tetrazole derivative **(2)** and 1,2,4-triazole derivative **(2')** (Scheme 1). The first route has been used for the synthesis of 5-fluoro-2'-deoxycytidine[3] and the second for the synthesis 5-methyl-2'-deoxycytidine[2]. We found that the formation of either intermediate proceeds with almost quantitative yield but is about 15 times faster with 1H-tetrazole then 1,2,4-triazole. In the next step, treatment of the intermediate **(2)** with aqueous ammonia in dioxane[2,3] leads to hydrolysis to **(1)** and gives less than 10% yield of **(3)**. The triazole intermediate **(2')** under these conditions gives **(3)** as main product and **(1)** as a side product. Clearly, the presence of fluoride in C5 position[3] makes the intermediate more stable to water hydrolysis relative to aminolysis. The reason for this effect is unclear. When we replaced



(4) (5) (6)

Reagents and comments: **i:** bis-cyanoethyl-N,N-diisopropyl phosphoramidite, 1H-tetrazole, acetonitrile; **ii:** I_2, pyridine, tetrahydrofuran, water; **iii:** trichloroacetic acid, dichloromethane; **iv:** aqueous NH_3. All reactions were run at room temperature.

SCHEME 2

aqueous ammonia by anhydrous ammonia we obtained compound **(3)** from **(2)** rapidly and in almost quantitative yield. The corresponding conversion of **(2')** to **(3)** was slow. The conversion of **(1)** to **(3)** is therefore best carried out via the 1H-tetrazole intermediate as indicated by bold arrows in Scheme 1.

We synthesized L-dCp from L-dC in two steps. We treated 5'-O-(Dimethoxytrityl)-4-N-benzoyl-2'-deoxy-L-cytidine **(4)** with bis-cyanoethyl-N,N-diisopropyl phosphoramidite in acetonitrile in the presence of 1H-tetrazole to give **(5)** (Scheme 2). The intermediate **(5)** was then converted into L-dCp **(6)** by oxidation followed by removal of the protecting groups.

EXPERIMENTAL

3',5'-Di-benzoyl-2'-deoxy-L-uridine was synthesized from L-arabinose (Sigma) by a published procedure[1] with an overall yield of 25%. 5'-O-(Dimethoxytrityl)-4-N-benzoyl-2'-deoxy-L-cytidine was synthesized from L-dC as previously reported[8] with a yield of 60%. Bis-cyanoethyl-N,N-diisopropyl phosphoramidite was synthesized by reaction of 2-cyanoethanol with diisopropylaminophosphodichloride in the presence of diisopropylethylamine[4].

^{1}H-NMR spectra were usually obtained on JEOL JNM-PMX60si spectrometer using DMSO-d_6 as a solvent with 1% tetramethylsilane as an internal standard (Aldrich). ^{1}H-NMR spectra of L-dCp and D-dCp were obtained on a Bruker AMX-400 spectrometer operating at 400 MHz using D_2O (Aldrich) as a solvent. The molecular mass of L-dCp was determined using a Bruker Esquire-LC electrospray ionization mass spectrometer. Silica gel column chromatography was performed on Merck, Kiesel gel 60 (200-400 mesh) using chloroform with a gradient of methanol (0-10%). Thin-layer chromatography (TLC) was performed on Merck TLC aluminium sheets silica gel 60 F_{254} (0.2 mm) in the following solvent systems: A - *n*-butanol saturated with water, B - *n*-butanol-acetic acid-water (5:2:3).

2'-Deoxy-L-cytidine

A 0.9 g (2 mmol) sample of **(1)** was dissolved in 30 ml of anhydrous pyridine and the solution was chilled in an ice bath. 4-Chlorophenyl dichlorophosphate (510 ml, 3 mmol) was added dropwise in about 5 min with stirring. After a further 5 min of stirring, 1H-tetrazole (0.42 g, 6 mmol) was added. The reaction mixture was removed from the ice bath, stirred for 5 h at room temperature and then evaporated at 35°C to give a glass. The glass was dissolved in 35 ml of chloroform, and the solution was washed twice with 20 ml of saturated sodium bicarbonate solution, washed twice with 20 ml of water and then dried using Aldrich molecular sieves (4A, 8-12 mesh). Finally the solution was concentrated and the product purified by silica gel column chromatography. Combined fractions were evaporated at 35°C to give about 1 g of **(2)**.

A 1 g sample of **(2)** was dissolved in 30 ml of a 0.5 M ammonia solution in dioxane(Sigma). The reaction mixture was stirred for 5 h at room temperature, and then evaporated to dryness. The residue was dissolved in 3 ml of chloroform and applied to a silica gel column. Combined fractions were evaporated to give 0.88 g of **(3)**. This product was dissolved in 1 ml of dioxane, and benzoyl protecting groups were removed by treating the solution with 10 ml of concentrated aqueous ammonia at 55°C for 12 h. The resulting solution was then evaporated and the residue dissolved in 20 ml of water and extracted 5 times with 15 ml of ether. The water fraction was evaporated to give a dry residue of L-dC. To obtain crystals, the product is dissolved in 3 ml of hot ethanol,

diluted with 40 ml of acetonitrile and allowed to stand in a refrigerator. The overall yield of chromatographically pure (TLC, solvent system A) L-dC from **(1)** was 85% (0.4 g). ^{1}H-NMR spectrum, molecular mass and chromatographic mobility for L-dC were identical to those for the natural D-dC.

2'-deoxy-L-cytidine-3'-phosphate

A 0.25 g (0.4 mmol) sample of **(4)** was dissolved in 1 ml dry acetonitrile. 1.5 ml of 1M bis-cyanoethyl-N,N-diisopropyl phosphoramidite solution in acetonitrile and 3 ml 0.5M 1H-tetrazole in acetonitrile were added under argon. After 15 min of stirring at room temperature the reaction mixture was filtered from a white precipitate. The solution was evaporated at 30°C. The residue was dissolved in 20 ml of 0.1 M iodine solution in tetrahydrofuran-2,6-lutidine-water mixture (40:10:1) and after 1 min at room temperature 4 ml of 1M sodium thiosulfate solution were added. The reaction mixture was evaporated at 30°C and then co-evaporated three times with 15 ml of toluene. Next, 20 ml of trichloroacetic acid solution (3% wt/vol) in dichloromethane (Applied Biosystems) was added to the residue and the reaction mixture was stirred for 30 min at room temperature. The solution was separated from precipitate and evaporated and the residue was co-evaporated two times with 15 ml of dichloromethane. The residue was dissolved in 1 ml of dioxane and transferred to a tube with 20 ml of concentrated aqueous ammonia and stored at 55°C for 12 h. The solution was next evaporated to dryness and the resulting solid was dissolved in 20 ml of water and extracted three times with 15 ml of ether. The water fraction was concentrated and applied on a column containing Dowex-1 anion exchange raisin (200-400 mesh, formate form). Yield of chromatographically pure (TLC, solvent system C) L-dCp was 58% (70 mg). $[\alpha]_D^{22}$ for L-dCp is - 45.6° (c 0.5, water); for D-dCp $[\alpha]_D^{22}$ is + 45.5° (c 0.5, water). All other analytical data for L-dCp were identical to those for the natural D-dCp.

ACKNOWLEDGMENTS

This work was supported by NASA Grant No. NAG5-4118 and NASA NSCORT/EXOBIOLOGY Grant No. NAG5-4546. We thank Aubrey R. Hill, Jr. for

technical assistance, Anthony G. Craig for mass spectrometry, Terry L. Sheppard for NMR measurements on dCp and Bernice Walker for manuscript preparation.

REFERENCES

1) Holy, A. *Coll.Czech. Chem. Comm.*, **1972** *37*, 4072-4087.

2) Sung, W. L. *J. Chem. Soc. Chem. Comm.*, **1981** *11*, 1089.

3) Schmidt, S.; Pein, C. D.; Fritz, H. J.; Cech, D. *Nucleic Acid Res.*, **1992** *20*, 2421-2426.

4) Uhlman, E.; Engels, J. *Tetrahedron Lett.*, **1986** *27*, 1023-1026.

5) Schaller, H.; Lerch, W. B.; Khorana, H. G. *J. Am. Chem. Soc.*, **1963** *85*, 3821-3827.

6) Hayakawa, Y.; Wakabayashi, S.; Nobori, T.; Noyori, R. *Tetrahedron Lett.*, **1987** *28*, 2259-2262.

7) Sekine, M.; Hamaoki, K.; Tsujiaki, H. *J. Org. Chem.*, **1979** *44*, 2325-2326.

8) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.*, **1982** *104*, 1316-1319.

Received 3/2/98
Accepted 4/28/98