Published in final edited form as:

Bioorg Med Chem Lett. 2008 May 1; 18(9): 2957–2958. doi:10.1016/j.bmcl.2008.03.063.

Synthesis of gemcitabine triphosphate (dFdCTP) as a tris (triethylammonium) salt

Prabhakar A. Risbood^{a,*}, Charles T. Kane Jr.^b, Md. Tafazzal Hossain^b, Sudhakar Rao Vadapalli^b, and Satish K. Chadda^b

^a*Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*

^b*Starks Associates Inc., 1280 Niagara Street, Buffalo, New York 14213*

Abstract

First synthesis of gemcitabine triphosphate (dFdCTP) as a tris(triethylammonium) salt is reported.

Keywords

gemcitabine triphosphate (dFdCTP)

Gemcitabine (2', 2'-difluoro-2'-deoxycytidine) is registered as a clinical anti-cancer agent for the treatment of a number of solid tumor types including pancreatic, non-small cell lung (NSCL), ovary, bladder, and breast cancer.¹ Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleoside metabolites.¹ The NCI Cancer Imaging Program needed gemcitabine triphosphate (dFdCTP), to be used as a standard in imaging studies using a radiolabeled probe for assessing the uptake and retention of gemcitabine in tumors and potentially identifying tumors sensitive to the drug. To facilitate such biochemical, pharmacological and other detailed studies of the metabolism of gemcitabine, a steady supply of authentic gemcitabine triphosphate (dFdCTP) was essential.²

Here we wish to report the first synthesis of gemcitabine triphosphate (dFdCTP), as shown in Scheme 1. Although, several methods have been reported for the general synthesis of nucleoside triphosphates,³ many of these approaches did not produce the desired triphosphate (dFdCTP) in our hands. Our synthesis incorporates the method described by Bogachev,⁴ using a modified purification procedure to give gemcitabine triphosphate as the tris (triethylammonium) salt.

Gemcitabine hydrochloride was phosphorylated by portion wise addition to a mixture of phosphorus oxychloride (POCl₃) in trimethyl phosphate at 5°C.⁵ The reaction was warmed to room temperature and stirred for two hours. The progress of the reaction was followed by HPLC (small aliquots were worked up by treatment with aq NaHCO₃). Work up of the reaction

*Corresponding author. Tel.: +1 301 435 9159; fax: +1 301 480 4817; e-mail risboodp@mail.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary data Detailed experimental procedures for the synthesis of compounds 2, 3, 4 and 5 are included in the Supporting Information section of this report. Supplementary data associated with this article can be found, in the online version, at ---.

afforded the monophosphate **2** (dFdCMP), as a white solid (105%). This material was used in the next step without further purification. ^1H NMR (500 MHz, CD_3OD): δ 7.95-7.93 (d, 1H); 6.25-6.22 (m, 1H); 6.01 (m, 1H); 4.39-4.32 (m, 1H); 4.25-4.23 (m, 1H); 4.14-4.10 (m, 1H); 4.00-3.98 (m, 1H). An impurity related to trimethyl phosphate is seen at δ 3.57 (s) and 3.55 (s). MS: Electrospray (negative), Calc'd for $\text{C}_9\text{H}_{12}\text{F}_2\text{N}_3\text{O}_7\text{P}$ = 343; Found: $(\text{M}-\text{H})^-$ 342 (100%). HPLC: Luna C-18 column (4.6×150 mm, 5μ), Isocratic 10% MeOH/90% 20mM NH_4OAc , Flow rate: 1 mL/min at 20°C , Detection: UV at 254 nm, Results: The compound elutes at 1.837 min with 92.77% area.

The monophosphate **2** (dFdCMP) was treated with trifluoroacetic anhydride in the presence of triethylamine and N, N-dimethylaniline in acetonitrile at -5°C . The reaction mixture was warmed to room temperature and stirred for 20 minutes until the formation of a clear solution indicating the completion of the reaction. This reaction mixture was then evaporated in vacuo to complete dryness (TFA free) to yield crude **3**, which was used in the next step without further purification. Crude **3** was dissolved in acetonitrile containing 1-methylimidazole and triethylamine. The mixture was stirred at 0°C for 5 minutes, when TLC analysis of an aliquot showed no presence of **3**. The reaction mixture containing **4** was allowed to warm to 20°C and then was treated with a solution of tris(tetrabutylammonium) hydrogen pyrophosphate in acetonitrile. After stirring for 7–10 minutes at room temperature, the reaction was quenched by the addition of water. Following an aqueous workup, the crude gemcitabine triphosphate (dFdCTP) was purified by chromatography on DEAE Sephadex A25 (1.6×20 cm bed volume) eluted with deionized water (one column volume), followed by elution with a linear gradient from deionized water (500 mL) to 0.4 M triethylammonium bicarbonate (pH 7.5, 500 mL). Fractions containing pure dFdCTP as a tris(triethylammonium) salt were combined and concentrated in vacuo to give the desired triphosphate **5** (dFdCTP) (140 mg, 17%). The reproducibility of the above procedure was confirmed by repeating the reaction sequence several times. ^1H NMR (500 MHz, D_2O): δ 8.17-8.15 (d, 1H); 6.39-6.38 (d, 1H); 6.28-6.26 (m, 1H); 4.66-4.57 (m, 1H); 4.44-4.42 (m, 1H); 4.32-4.25 (m, 2H); 3.23-3.19 (q, 18H); 1.30-1.27 (t, 27H); ^{31}P NMR (202.456 MHz, D_2O): δ -10.35 - -10.54 (m, 1P); -10.94 - -11.03 (d, 1P); -22.79 (m, 1P). HRMS: TOF Electrospray (negative): Calc'd for $\text{C}_9\text{H}_{13}\text{F}_2\text{N}_3\text{O}_{13}\text{P}_3$ = 501.9629, Found: m/z (relative intensity) 501.9611 (100%), $(\text{M}-\text{H})^-$. HPLC: Bio Basic column and gradient elution using ammonium acetate buffer and acetonitrile eluted **5** with a retention time of 4.17 min showing 97.03% purity.

Milligram quantities of this material (dFdCTP, NSC 746306) for the use as a standard can be obtained from the NCI (<http://dtp.nci.nih.gov>).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Linda J. Whited for her assistance with the HPLC analysis. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, under Contract No. N02-CM-52209.

References and notes

1. Norenberg JPUS. Patent Application 20070025910, 2007. Chem. Abstr 2007;146:200935.
2. From an assay of dFdCTP described by S. A. Veltkamp and coworkers, the source of the dFdCTP is unknown Veltkamp SA, Hillebrand MJX, Rosing H, Jansen RS, Wickremsinha ER, Perkins EJ, Schellens JHM, Beijnen JH. J. Mass Spectrometry 2006;41:1633.
3. Burgess K, Cook D. Chem. Rev 2000;100:2047. [PubMed: 11749283]

4. Bogachev VS. Russ. J. Bioorg. Chem 1996;22:599.(English Translation)
5. Bonjouklian R, Grindey GB, Hertel LW. European Patent Application 0376518, 1990. Chem. Abstr 1991;114:43489.

Scheme 1.

Reagents and conditions: (a) POCl_3 , $(\text{MeO})_3\text{PO}$; (b) H_2O ; (c) $(\text{CF}_3\text{CO})_2\text{O}$; (d) 1-methylimidazole; (e) $(\text{nBu}_4\text{N})_3\text{HP}_2\text{O}_7$; (f) DEAE Sephadex A25 $\text{Et}_3\text{NHHCO}_3$ (pH 7.5).