Procedure to obtain the 3´,5´-di-*O*-acetyl-5-formyl-2´-deoxyuridine

Marquiza Sablón-Carrazana, Guillermo Jiménez-Alemán,* Rafaela Pérez Perera, Hermán Vélez-Castro* and Chryslaine Rodríguez-Tanty.

Molecular Biology Department, Neurosciences Center, 25 Avenue No. 15202, Postal Code 12100, Havana City, P.O. Box 6414, Cuba. *Chemistry Deparment, Center of Pharmaceutical Chemistry, 200 Street and 21 Avenue, Atabey. E-mail: marquiza@cneuro.edu.cu

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RESUMEN Numerosos tipos de daños al ADN han sido identificados en células expuestas a radiación ionizante y a agentes oxidantes. La 5-formil-2´-desoxiuridina es un compuesto potencialmente mutagénico que ha sido identificado en células expuestas a radiaciones ionizantes y a agentes oxidantes. Este compuesto se emplea para la síntesis de varios derivados de la 2´-desoxiuridina sustituidos en la posición 5. En este trabajo se presenta un método práctico para la conversión de la 2´-desoxitimidina a 3´,5´-di-*O*-acetil-5-formil-2´-desoxiuridina (**4**), a través de la halogenación, hidrólisis y oxidación de la 3´,5´-di-*O*-acetil-2´-desoxitimidina (**1**). Se realizaron estudios para seleccionar los agentes adecuados para las etapas de bromación, hidrólisis y oxidación. El bromo molecular y la *N*-bromosuccinimida (NBS) fueron probados en la etapa de halogenación y la NBS permitió obtener el producto deseado, 5-(bromometil)-3´,5´-di-*O*-acetil-2´-deoxiuridina (**2**) y trazas del compuesto dibromado. Algunos procedimientos de hidrólisis fueron evaluados para obtener la hidroximetil-3´,5´-di-*O*-acetil-2´-deoxiuridina (**3**) a partir de **2** y se seleccionó para esta etapa un procedimiento neutro con agua. En la etapa de oxidación, se llevó a cabo un análisis comparativo entre el óxido de manganeso activo y el reactivo de Jones para establecer las condiciones óptimas para obtener **4**. Se utilizó, con buenos resultados, el reactivo de Jones para oxidar el grupo alcohol alílico, del nucleósido, a grupo formilo a través de un método más práctico, fácil y reproducible.

ABSTRACT Many types of DNA damage have been identified in cells exposed to ionizing radiation and oxidizing agents. 5-Formyl-2´-deoxyuridine has been identified in cells exposed to ionizing radiations and oxidizing agents and was recognized as a potentially mutagenic compound. Also, it can be an important and versatile intermediate for the synthesis of various 5-substituted 2´-deoxyuridine. In this work, a convenient method is presented for the conversion of 2´-deoxythymidine to 3´,5´-di-*O*-acetyl-5-formyl-2´-deoxyuridine (**4**) via halogenation, hydrolysis and oxidation of 3´,5´-di-*O*-acetyl-2´-deoxythymidine (**1**) intermediate. Studies to select the suitable brominating, hydrolyzing and oxidizing agents were performed. Thus, molecular bromine and *N*-bromosuccinimide (NBS) were essayed in the halogenation step, and NBS permitted to obtain the desired product, 5-bromomethyl)-3´,5´-di-*O*-acetyl-2´-deoxyuridine (**2**), and only trace of dibromide compound. Several hydrolyse procedures were evaluated to afford the hydroxymethyl 3´,5´-di-*O*-acetyl-2´-deoxyuridine (**3**) from **2** and a neutral procedure with water was selected for this step. In the oxidation step, a comparative analysis between the active manganese dioxide and the Jones reagent was carried out for establishing the optimal conditions to give **4.** The Jones reagent was successfully used to oxidize the allylic alcohol group of the nucleoside to formyl group through a method herein more practical since it is easier and more reproducible.

INTRODUCTION

Many types of DNA damage have been identified in cells exposed to ionizing radiation and oxidizing agents. 5-Formyl-2´-deoxyuridine (fdU) is the major product when 2´-deoxythymidine is treated with Fenton-type reagents, when DNA is irradiated *in vitro* and also, when the cultured human cells are exposed to hydrogen peroxide.[1-4]

Masaoka *et al*.[4] have previously shown that fdU is a potentially mutagenic lesion due to its elevated frequency to mispair with guanine. Therefore, fdU can exist in DNA as a correctly paired fdU:A form or an incorrectly paired fdU:G form.[5]

Several methods have been developed for introducing the 5-formyl group in 2´-deoxyuridine or 2´-deoxycytidine as intermediates, in order to synthesize other 5-substituted nucleosides derivatives. Mertes *et al*.[6] reported a condensation of the *bis*(trimethylsilyl) derivative of 5-formyluracil with 3,5-di-*O*-*p*-toluyl-2-deoxy-D-ribofuranosyl chloride and they obtained α- and β-

Correspondence:
Marquiza Sablón-Carrazana
Molecular Biology Department, Neurosciences Center, 25 Avenue No. 15202, Postal Code 12100, Havana City, P.O. Box 6414, Cuba. Email: marquiza@cneuro.edu.cu

nucleosides. Also, 5-formyluracil was synthesized by Ressner *et al*.,[7] via oxidation of 5-hydroxymethyluracil with ceric ammonium nitrate.

A direct procedure for introducing the formyl group at the 5-position of 2´-deoxythymidine was developed by Barwolff and Langen[8] using 5-dibromomethyl-2´-deoxyuridine as a key compound. Alkaline hydrolyses method with pyridine-water was employed in order to obtain fdU. Itahara *et al*.[9] also observed that when the 2´-deoxythymidine solution is treated with sodium peroxodisulfate, nucleoside oxidation occurred and 5-hydroxymethyl-2´-deoxyuridine as the main product was obtained together with fdU.

In this work the synthesis of **4** in reasonable yield from the 5-methyl-2´-deoxyuridine allylic alcohol (**3**) using Jones reagent as oxidizing agent is reported. The general procedure involves halogenation, hydrolyses and oxidation of the 2´-deoxythymidine 5-methyl group. The brominated nucleoside intermediate (**2**) was obtained and nucleophilic displacement of bromine under neutral conditions was easily performed to give the 5-methylhydroxy derivative (**3**) of uridine.

MATERIALS AND METHODS

All reagents were obtained from commercial suppliers and were pure for synthesis. Oxidizing reagents used were the Jones reagent and the active manganese dioxide, both prepared according to literature.[10,11] Thin layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel 60 F_{254} (Merck), and the solvent systems used were: EtOAc (A) and $CHCl_3$:MeOH 10:3 (B). Column chromatography was performed on Merck silica gel (35-70 mesh).

IR spectra were recorded on a ATI Mattson Genesis Series FTIR spectrophotometer.

Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Bruker Model AC 250F spectrometer with TMS as internal reference. The multiplicities for ^{1}H NMR were described using the following abbreviations; s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet and br: broad signal. The assignment was based on DEPT and COSY experiments.

Melting points (uncorrected) were determined on an Electrothermal (model 9100) apparatus.

3´,5´-Di-*O*-acetyl-2´-deoxythymidine (1)

Anhydride acetic (Ac_2O) (4.4 mL, 42.3 mmol), triethylamine (Et_3N) (5.5 mL, 39.6 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (0.1 g) were added to a solution of 2´-deoxythymidine (5 g, 20.6 mmol) in anhydrous $CHCl_3$ (70 mL). The reaction mixture was refluxed for 2 h, and then 2 mL of MeOH was added. The mixture was poured into a cold solution of $NaHCO_3$ (1 %, 50 mL) and extracted with $CHCl_3$ (3 X 15 mL). The organic phase was washed with water (3 X 10 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to dryness. The oily syrup crystallized from EtOH. Yield pure: 90 % (6.04 g).

Mp 127.5-128.5 °C (lit.[12] 125 °C); R_f = 0.55 (A); R_f = 0.75 (B).

IR (KBr): 3 000, 1 748.7, 1 684.6, 1 420, 1 243.5, 1 225.7 cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.9 (s, 3H, CH_3), 2.1 (s, 6H, 2 $COCH_3$), 2.19 (m, 1H, H-2´´), 2.48 (ddd, *J* = 14 Hz, 1H, H-2´), 4.26 (m, 1H, H-4´), 4.37 (dd, *J* = 12 Hz, *J* = 4.3 Hz, *J* = 2.1 Hz, 2H, H-5´), 5.25 (dt, *J* = 6.1 Hz, *J* = 2.1Hz, 1H, H-3´), 6.34 (dd, *J* = 8.2 Hz, *J* = 5.5 Hz, 1H H-1´), 7.25 (br s, 1H, H-6), 9.3 (s, 1H, NH).

5-Bromomethyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (2)

Method A. A suspension of **1** (1 g, 3.06 mmol) in anhydrous CCl_4 (300 mL) was stirred and heated at reflux in a three-necked flask (500 mL) fitted with an effective reflux condenser, a dry tube and a special device for bromine introduction, as described.[8] When the dissolution is complete a stream of dry nitrogen blows bromine (4.59 or 6.12 mmol) in anhydrous CCl_4 (5 mL) from the introduction device into the solution and a catalytic amount of 2,2´-azo*bis*(isobutyronitrile) (AIBN) was added to the solution. The mixture was refluxed and stirred for 5 or 6 h (Table 1), and then nitrogen was bubbled through the solution for 30 min to remove the hydrogen bromide. Dry ion exchange resin Dowex 50-W (10 g, 48 mequiv, Na^+ form) was added to eliminate trace amounts of HBr. The reaction mixture was shaken for two h and then the resin was filtered and washed with anhyd acetone. Finally, solvents were evaporated under reduced pressure and the crude syrup was used without further purification. R_f = 0.64 (A); R_f = 0.62 (B).

Method B. *N*-bromosuccinimide (NBS) (4.59 or 6.12 mmol, Table 1) and a catalytic amount of AIBN were added to a solution of **1** (1g, 3.06 mmol) in anhydrous CCl_4 (300 mL), under an inert atmosphere. The reaction mixture was refluxed for 2 or 5 h (Table 2) and then dry nitrogen was bubbled through the solution for 30 min to remove the hydrogen bromide. In a similar way to Method A, trace amounts of hydrogen bromide were eliminated with dry ion exchange resin Dowex 50-W (Na^+ form). R_f = 0.64 (A).

5-Hydroxymethyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (3)

Method I. the crude syrup **2**, obtained by bromination Method B, was treated with $NaHCO_3$, according to literature.[8]

Method II. the crude syrup **2**, obtained by bromination Method B, was stirred with a mixture of pyridine:water (3:1; 60 mL) at room temperature for 1 h . The solvent was evaporated under reduced pressure

Table 1. Reaction conditions assayed for obtaining 5-bromomethyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (**2**) from 3´,5´-di-*O*-acetyl-2´-deoxythymidine (**1**) with molecular bromine or NBS as bromine radical sources with AIBN as catalyst, in CCl_4 at reflux temperature.

Method	Brominating agents (BA)	Molar ratio 1 : BA[1]	Reaction time (h)	Conversion (%)[a]	Compound
A	Br_2	1 : 1.5	5	~ 70	2[b]
		1 : 2	6	100	[c]
B	NBS	1 : 1.5	2	~ 70	2[b]
		1 : 2	2	100	2[b]
		1 : 4	5	100	[c]

[1] 1 : BA nucleoside-brominating agents ratio. [a] Qualitatively determinated by TLC. [b] **2** was the major product obtained but with 5-dibromomethyl-3´,5´-di-*O*-acetyl–2´-deoxyuridine as by-product. [c] The product obtained was 5-dibromomethyl-3´, 5´-di-*O*-acetyl –2´-deoxyuridine.

Table 2. Hydrolyses reaction conditions assayed for obtaining 5-hydroxymethyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (**3**) from 5-bromomethyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (**2**), at room temperature.

Method	Hydrolytic agents	Reaction time (h)	Conversion[1] (%)	Compound
I	$NaHCO_3$ (5 %)	2	80	3[a]
II	Pyridine - H_2O (3 : 1)	< 0.10	100	[b]
III	H_2O	1 - 2	100	3

[1]Qualitatively determinated by TLC. [a]The formation of 5-hydroxymethyl-2´-deoxyuridine was observed, as by-product. [b]Many polar by-products were observed.

and then, the residue was purified by chromatography over silica gel (100 : 0 to 90 : 10, $CHCl_3$-MeOH, v/v).

Method III. Acetone:water (1 : 1, 40 mL) was added to the crude syrup **2**, obtained by bromination Method B. The reaction mixture was shaken for 1-2 h. The solvent was evaporated under reduced pressure and the crude syrup was purified by chromatography over silica gel (100 : 0 to 98 : 2, $CHCl_3$-MeOH, v/v). Yield pure (from **1**): 28.6 - 38.8 % (0.29- 0.41 g).

Mp 145 °C (sealed capillary); R_f = 0.33 (A).

IR (KBr): 3 400, 3 000, 1 739.7, 1 705.4, 1 685.8, 1 435.6, 1 235.6, 1 200.3, 1 052.3 cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.1 (s, 6H, 2 CH_3CO), 2.35 (m, 1H, H-2´´), 2.5 (m, 1H, H-2´), 3.8(br s, 1H, OH), 4.3 (m, 1H, H-4´), 4.4 (m, 4H, H-5´, H-5), 5.25 (br s, 1H, H-3´), 6.35 (dd, *J* = 8.2 Hz, *J* = 5.9 Hz, 1H, H-1´), 7.65 (br s, 1H, H-6), 10.3 (s, 1H, NH).

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 19.83, 19.92, 36.59, 56.98, 62.89, 73.26, 81.35, 84.17, 113.67, 135.44, 149.33, 162.60, 169.51, 169.70.

5-Formyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (4)

Method 1. Active dioxide manganese (68.8 mg, 789 µmol) was added to **3** (30 mg, 87.72 µmol) dissolved in toluene (2 mL). The reaction mixture was refluxed for 1 h . This mixture was filtered and the filtrate was concentrated under reduced pressure to dryness. R_f = 0.52 (A).

Method 2. Jones reagent (61.4 µmol) was added dropwise to a cold solution (0-5 °C) of **2** (30 mg, 87.72 µmol) in anhydrous acetone (2 mL). The reaction mixture was stirred at 25 °C for 24 h . MeOH (2 mL) was added to remove the excess of oxidant reagent. The reaction mixture was concentrated under reduced pressure to dryness. The residue was dissolved in $CHCl_3$ (5 mL) and washed first with $NaHCO_3$ (0.5 %, 3 X 2 mL) and later with water (3 X 2 mL). The organic phase was dried over anhyd Na_2SO_4 and concentrated under reduced pressure to dryness. The residue was purified by column chromatography over silica gel ($CHCl_3$). Yield pure: 45.3 % (13.5 mg). R_f = 0.52 (A), R_f = 0.9 (B).

IR (KBr): 3 003, 2 830, 2 720.9, 1 738.2, 1 679.3, 1 418, 1 239.3, 1 223.1, 1 050.1 cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.11 (s, 3H, CH_3CO), 2.19 (s, 3H, CH_3CO), 2.27 (m, 1H, H-2´´), 2.58 (ddd, *J* = 14 Hz, 1H, H-2´), 4.2 (br s, 1H, H-4´), 4.32 (dd, *J* = 13.9 Hz, 2H, H-5´), 5.24 (dt, *J* = 5.8 Hz, *J* = 2.1 Hz, 1H, H-3´,), 6.31 (dt, *J* = 7.6 Hz, *J* = 6.2 Hz, 1H, H–1´), 8.48 (br s, 1H, H-6), 9.89 (br s, 1H, NH), 9.99 (br s, 1H, CHO).

RESULTS AND DISCUSSION

The formyl group was introduced at 5-position of the 2´-deoxythymidine through a procedure developed by us. This method had five steps and two of them (bromination and hydrolyses) were done in one pot (Fig. 1).

The protection of the 3´- and 5´-hydroxyl groups of 2´-deoxythy-

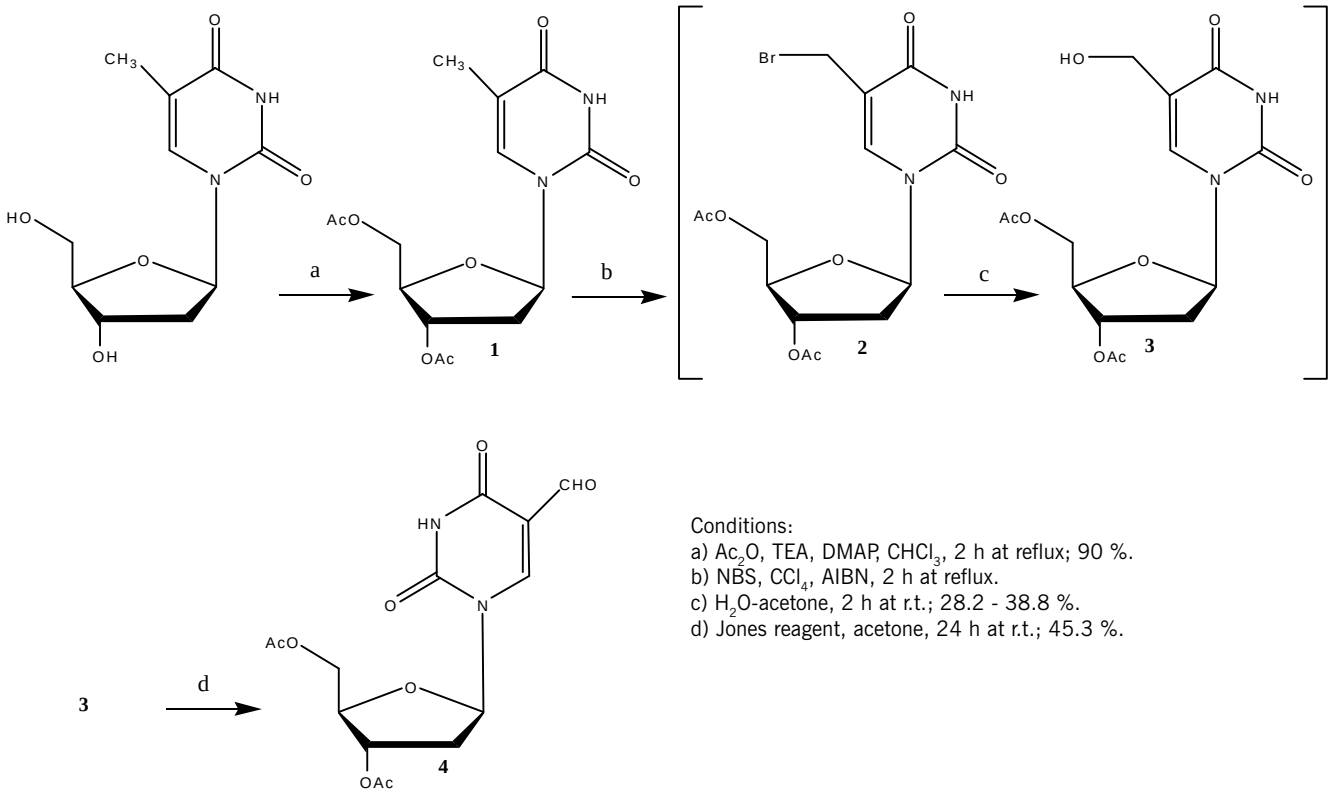

Fig. 1 *General procedure for obtaining 3´,5´-di-O-acetyl-5-formyl-2´-deoxyuridine (**4**).*

midine for the bromination reaction was performed using the acetyl group as protecting group due to the good solubility it provides, its lack of aromatic moieties, and its ease of addition and removal.

The synthesis of 3´,5´-di-*O*-acetyl-2´-deoxythymidine (**1**) was carried out eliminating the use of pyridine as solvent, since the trace of this solvent inhibit the subsequent radical bromination reaction. The 2´-deoxythymidine was acetylated using acetic anhydride, triethylamine, as base, and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) as catalyst, in dry chloroform at room temperature for 2 h. According to results, the product was obtained in 90 % yield and high purity. The structure of **1** was confirmed by IR and ^{1}H NMR spectra.

Barwolff and Langen[8] reported the 2´-deoxythymidine bromination with molecular bromine in carbon tetrachloride and this procedure was used for obtaining 2´-deoxythymidine derivates.[13-15] Furthermore, Basnak *et al*.[16] used *N*-bromosuccinimide (NBS) as source of bromine radical in the bromination of the 5-ethyl 2´-deoxy-4´-thiouridine for obtaining 5-(2-bromovinyl)-2´-deoxy-4´-thiouridine. Also, the photochemical bromination of the 3´,5´-di-*O*-butyldiphenylsilyl-2´-deoxythymidine using NBS to obtain the 5-monobromomethyl uridine derivative was reported by Matulic *et al*.[17] This intermediate compound has been used to synthesize 5-fluorothymidine and 5-difluorothymidine.

A comparative study of the efficiency of molecular bromine and NBS, as brominating agents, to obtain 5-bromomethyl 3´,5´-di-*O*-acetyl-2´-deoxyuridine (**2**) (Table 1) is reported. These agents were used at various molar ratios with regards to nucleoside, and with 2,2´-azo-*bis*(isobutyronitrile) (AIBN) as radical initiator.

According to results (Table 1), when the reaction was performed using molecular bromine (1.5 equiv) **2** was obtained at moderate yield. Also, the formation of the dibromide undesirable by-product was unavoidable and a small amount of starting material remained in the reaction mixture. Likewise, 5-dibromomethyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine as the main product of the reaction was found, when the bromine equivalents were increased.

Otherwise, when 2 equiv of NBS were used the desired product was successfully obtained, after 2 h of reaction (Table 1).The dibromide compound was only observed by TLC as trace. In order to remove the major quantity of hydrogen bromide (HBr) for avoiding the nucleoside's decomposition, it had to blow dry nitrogen into the reaction mixture. Finally, the HBr trace were totally eliminated by using ion exchange resin Dowex 50-W (Na^+ form) and **2** without further purification was used.

The introduction of the hydroxymethyl group at the 5-carbon of uracil was previously developed by Cline *et al*.[18] through an acid or base-catalyzed procedure. Likewise, the synthesis of 5-hydroxymethyl-2´-deoxyuridine from 2´-deoxyuridine under acidic conditions was reported by these authors and the base-catalyzed hydroxymethylation was described by Baker *et al*.[19] Although many researchers had extensively used these methods, none of them were suitable for a preparative scale due to the low yields and long reaction times. On the contrary, Barwolff *et al*.[8] described a more direct and effective method for obtaining 5-hydroxymethyl-2´-deoxyuridine via hydrolyses of **2**, than those mentioned previously.

The intermediate **2** is extremely labile to the moisture and the protic solvents. Therefore, its hydrolyses can be easily done in soft conditions where the removal of acetyl protecting groups is prevented. Several hydrolyses procedures to afford **3** were evaluated (Table 2).

Many polar by-products were observed.

The procedure described by Barwolff and Langen[8] was assayed (*Method I*). The 5-hydroxymethyl derivative **3** was obtained at low yield after the purification, and the undesired elimination of the diacetyl group was observed by TLC. On the other hand, the pyridine solution as basic hydrolytic agent (*Method II*) was evaluated. Many polar by-products were thus obtained but its isolation and purification by column chromatography were done without success.

In order to overcome the above disadvantages, a neutral hydrolytic agent was used. Thus, **3** was obtained by means of treatment of syrup **2**, dissolved in dioxane or acetone, with water. The product was purified by column chromatography. Then, **3** was afforded in 40 % overall yield from **1**.

The ^{1}H NMR spectrum of **3** indicated that the hydrolyses of **2** occurred as it was expected since the protons signal of the 5-CH_3 group disappeared. A multiplet at 4.4 ppm and a broad signal at 3.8 ppm appeared, corresponding to the 5'-CH_2 and 5-CH_2 group protons and hydroxyl group proton, respectively.

The unequivocal confirmation was carried out by a ^{1}H-^{1}H-COSY experiment. Thus, the 6-H resonance signal (7.65 ppm) correlated with the 5-CH_2 at 4.4 ppm and the 4´-H resonance signal at 4.3 ppm with the 5´-CH_2 at 4.4 ppm. Likewise, DEPT spectrum analysis allowed the 5-CH_2OH carbon signal assignation at 56.98 ppm.

The introduction of a formyl group has been studied through the oxidation reaction of the hydroxyl group, at the 5- position, of the uracil, thymine and cytosine derivatives and their nucleosidic derivatives.[6,17,18] For instance, the active manganese dioxide, the ceric ammonium nitrate and the platinum and oxygen mixture have been used as oxidizing agents for obtaining the formyl group. However, the partial overoxidation of hydroxyl group to carboxylic acid occurred when these oxidizing agents were employed.

A wide variety of chromium (VI) reagents are available in literature for the oxidation of alcohol to carbonyl compounds.[19] Jones reagent is the most known of these Cr (VI) reagents for obtaining ketones from secondary alcohols. Special conditions (removal of aldehyde as it is formed) are necessary for the primary alcohol oxidization to aldehyde. Jones reagent can produce satisfactory results when the primary alcohols are allylic or benzylic.[20]

In the present work, a comparative study between the active manganese dioxide and the Jones reagent was carried out for establishing the optimal conditions to give **4** (Table 3).

According to results, the 5-hydroxymethyl derivative **3** was completely oxidized to the 5-formyluracil nucleoside **4** with 9 equiv. of active manganese dioxide. However, when the MnO_2 amount was greater than 9 equiv., the oxidation of toluene could be observed. The main disadvantage of this method is the large amount of expensive active MnO_2 that it requires.

On the other hand, the whole oxidation of **3** occurred with a 0.7 molar ratio of the Jones reagent. This product was exhaustively oxidized when large amounts of oxidizing agent were used (Table 3). The by-products

were not characterized. However, the formation of hydrates, hemiacetals and their oxidizations to carboxylic acids and esters derivatives, respectively, was described in the Jones oxidation reaction.[19,21]

Structure of **4** was confirmed by ^{1}H NMR and ^{13}C NMR spectra. Proton chemical shifts of this nucleoside showed at downfield a broad signal at 9.99 ppm for the CHO group proton. The structure was corroborated by ^{13}C NMR spectrum which exhibited a signal for the 5-CHO group carbon at δ 185.83 ppm and also, showed the disappearance of the CH_2OH group carbon signal at 56.98 ppm. The DEPT spectrum confirmed the structure. As shown NMR data, the electron-withdrawing 5-formyl group in **4** causes the pyrimidine ring and deoxyribose C-1´ electron deficient relative to parent **3** (ΔδIV-III C-6´ = 8.94 ppm and Δδ IV-III C-1´ = 4.64 ppm).

CONCLUSION

The intermediate **4**, which is synthesized by discussed method, may be useful as a versatile product for the obtainment of a variety of nucleoside derivatives of 2´-deoxythymidine.

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Table 3. Reaction conditions assayed in the synthesis of 5-formyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (**4**) through the oxidation of 5-hydroxymethyl-3´,5´-di-*O*-acetyl-2´-deoxyuridine (**3**).

Method	Oxidizing agents (OA)	Molar ratio[1]	Solvents	Temperature (°C)	Reaction time (h)	Conversion[2] (%)	Compound
1	MnO_2	2	Acetone	Reflux	24	0	–
		2	Toluene	Reflux	2	0	–
		6	Acetone	Reflux	4	~ 20	4
		6	Toluene	Reflux	4	~ 50	4
		9	Toluene	Reflux	1	100	4
		≥10	Toluene	Reflux	1	100	4[a]
2	Jones reagent (H_2CrO_4)	0.7	Acetone	0 - 5	0.5 - 3	~ 50	4
		1.5	Acetone	0 - 5	1	~ 70	4[b]
		1.5	Acetone	0 - 5	6	100	[c]
		0.7	Acetone	25	5	~ 70	4
		0.7	Acetone	25	24	100	4[d]

[1] OA : **3** (oxidizing agent-nucleoside ratio). [2] Qualitatively determined by TLC. [a] **4** was obtained as major product but the oxidation of toluene was observed. [b] The presence of polar by-products was observed with R_f = 0 (TLC in A and B) and R_f = 0.21 (TLC in A). [c] **4** was completely transformed to polar by-products as [b]. [d] Complete conversion from **3** to **4** and the presence of traces of unknown by-product (R_f = 0.21, TLC in A) were observed.

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