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Selective Alkylation of Pyrimidyl Dianions II: Synthesis, Characterization, and Comparative Reactivity of 3', 5'-O-Bis- Tetrahydropyranyl, Trimethylsilyl and *tert*-Butyldimethylsilyl Derivatives of 5-Bromo-2'-Deoxyuridine

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Abstract: Three compounds which can be used as precursors for thymidine synthesis via methylation at the 5 position, including 3', 5'-o-bis(tetrahydropyranyl)-5-bromo-2'-deoxyuridine **1a** , 3', 5'-o-bis-(trimethylsily)-5-bromo-2'-deoxyuridine **1b** , and 3', 5'-o-bis-(*t*-butyldimethylsilyl)-5-bromo-2'-deoxyuridine **1c**, were prepared, isolated and characterized by spectroscopic methods. Alkylation with methyl iodide using an organo-lithium reagent at low temperature produced 72%, 41% and 74% of thymidine **6** , respectively. Tetrahydropyranyl and *t*-butyldimethylsilyl ethers are found to be better precursors for introduction of a methyl group at the 5 position.

INTRODUCTION

Radiolabeled thymidine has been widely used in in vitro and in vivo models for assessment of cellular replication. With the advent of positron emission tomography, [methyl-^{11}C]thymidine has been considered as a potential compound for in vivo tumor imaging and liver regeneration studies using positron emission tomography (PET). Thymidine kinase is the enzyme which catalyses phosphate transfer from ATP to thymidine to form thymidine 5'-phosphate[1]. The activity of thymidine kinase in rapidly growing cells is known to be very high compared to the non-dividing adult tissue. The anabolic product of thymidine phosphorylation, thymidyllic acid, cannot transverse the cellular membrane and therefore remains trapped in the tumor cells[2]. Radiolabeled thymidine uptake in vivo has been correlated with DNA synthesis in cells[3]. Studies with [methyl-^{14}C]thymidine[4], [methyl-^{11}C]thymidine[5,6] and [methyl-^{3}H]thymidine[7,8] have demonstrated that tumor uptake and tumor/tissue ratios are sufficiently high to justify the use of [methyl-^{11}C]thymidine for PET imaging.

The earlier bio-synthesis of [methyl-^{11}C]thymidine using an enzymatic procedure produced only 4 mCi of radiotracer with low specific activity[5,6], insufficient for human studies. An alternative organic synthetic methodology was reported thereafter[9,10] using 3', 5'-o-tris-(trimethylsilyl)-5-bromo-2'-deoxyuridine as precursor of thymidine. This method suffered from low radiochemical yield, poor reproducibility and longer reaction times. Another synthesis of [methyl-^{11}C]thymidine was reported in which 3', 5'-o-bis-(tetrahydropyranyl)-5-bromo-2'-deoxyuridine was used as the precursor[11]. In this method 2.4-7.4 mCi of [methyl-^{11}C]thymidine was produced with 17-25% radiochemical yield. The low specific activity was due to the addition of carrier methyl iodide during the synthesis. More recently a third synthesis of [methyl-^{11}C]thymidine has been reported, which was a modification of an earlier method[12]. In this method radiochemical yield and specific activity have been reported to be high. However, an unknown radiolabeled by-product was observed in significant quantity.

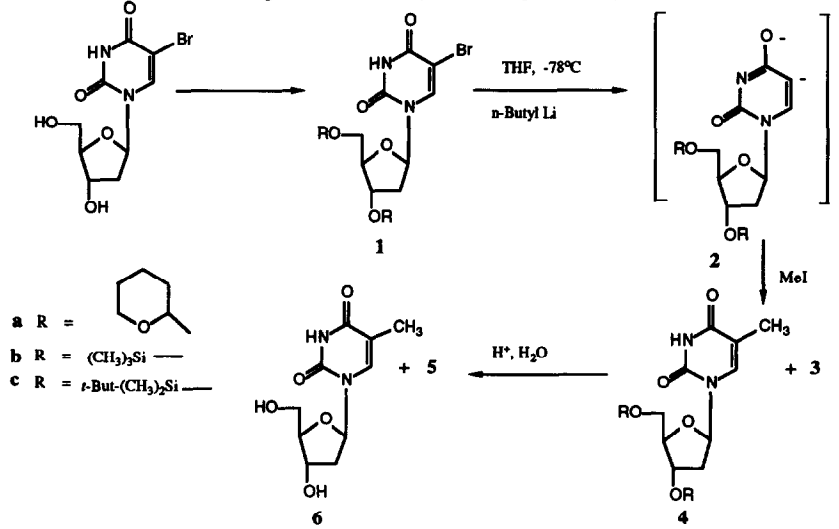
All the organic syntheses mentioned above are similar, in that they involve treatment of a bis- or tris-hydroxy-protected-5-bromo-2'-deoxyuridine with n-butyllithium at low temperature followed by reaction with [^{11}C]methyl iodide. To date, 3', 5'-o-bis-(tetrahydropyranyl)-5'-bromo-2'-deoxyuridine[11], 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine[12] and 2, 3', 5'-o-tris-

(trimethylsilyl)-5-bromo-2'-deoxyuridine[9,10] have been used for [methyl-^{11}C]thymidine synthesis. Only o-bis-(tetrahydropyranyl) ether of 5-bromo-2'-deoxyuridine has been isolated and successfully characterized by ^{1}HNMR and mass spectrometry[11]. Treatment of this compound with two equivalents of n-butyllithium has been shown to produce a dianion intermediate which can be selectively methylated at the 5 position[11]. The o-bis-(trimethylsilyl) ether was prepared in situ and used without isolation[12]. Analysis by mass spectrometry did not provide adequate information on this compound. The o-tris-(trimethylsilyl) ether derivative was identified only by IR. The intermediate hydroxy-protected compounds have not been isolated and characterized in any of the synthetic methods. The issues of stability of the o-bis-(trimethylsilyl) ether[11] and the reported difficulty in removal of the tetrahydropyranyl group[12] remain unresolved. Detailed investigations, including isolation of the precursors, their reactions with methyl iodide, and isolation of the hydroxy-protected intermediates, as well as their hydrolysis to the final product, thymidine, were conducted in an effort to help resolve these questions.

Therefore, based upon the previously established techniques for selective alkylation and, in order to establish an efficient, high yield method of [methyl-^{11}C]thymidine synthesis for studies using positron emission tomography, we have studied three different precursors of thymidine. We wish to report synthesis of a new compound, 3', 5'-o-bis-(*t*-butyldimethylsilyl)-5-bromo-2'-deoxyuridine **1c** and a comparative evaluation of its alkylation reactions with methyl iodide with the 3', 5'-o-bis-(tetrahydropyranyl) and 3', 5'-o-bis-(trimethylsilyl) derivatives. The intermediate products and their studies on hydrolysis are also reported. The techniques described are adaptable for radiolabeling with either C-14 or C-11 in the 5 position.

RESULTS AND DISCUSSION

In order to develop an efficient method of [methyl-^{11}C]thymidine synthesis we have evaluated the reaction of three different precursors, two of which were used earlier. A general method of synthesis is represented by the Scheme 1:



Scheme 1

We have improved the quantitative yield of 3', 5'-o-bis(tetrahydropyranyl)-5-bromo-2'-deoxyuridine **1a** in a shorter reaction time than previously published. Following the literature procedure[11] the reaction was not complete in 72h. However, by increasing the amount of 3, 4-dihydro-2*H* -pyran from 3 equivalent to 15 equivalent the reaction was complete in 2h with quantitative yield. The reaction is quite simple and straightforward. ^{1}HNMR spectrum of this compound was slightly different due

to the use of our high field (300 MHz) NMR spectrometer. In our spectrum four different singlets were observed for the C_6 protons of four diastereomers compared to one singlet previously reported.

Syntheses of 3', 5'-o-b-s(trimethylsilyl)-5-bromo-2'-deoxyuridine **1b** were reported earlier using bis-(trimethylsilyl)-acetamide-chlorotrimethylsilane mixture[13] or bis-(trimethylsilyl)-trifluoroacetamide[12]. This compound was characterized either by IR, or by mass spectrometry without isolation. We have synthesized this compound by reacting 5-bromo-2'-deoxyuridine with chlorotrimethylsilane in the presence of triethylamine in THF at room temperature. The reaction produced a high crude yield of 95%. However, after purification on basic alumina column the yield was lowered to 45% due to partial hydrolysis of the compound in the column. The melting point of the compound was 52-56°C, relatively a broad range due to the fact that it was used without recrystalization. However, ^{1}HNMR spectrum of the compound was very clean and explainable, and high resolution mass spectrometry also supported the identity of the compound.

Synthesis of the third precursor 3', 5'-o-bis-(tert-butyldimethylsilyl)-5-bromo-2'-deoxyuridine **1c** was difficult. Following a literature method[14] no desired product was obtained. The compound was synthesized in excess triethylamine by reaction of 5-bromo-2'-deoxyuridine and *t*-butyl-dimethylsilyl chloride in THF at 75°C for 40h. The isolated yield after chromatographic purification on silica gel column was 65%. Melting point of this compound was also broad 54-59°C due to the fact that it was used without recrystalization. The ^{1}HNMR spectrum of the compound was very clean and explainable. High resolution mass spectrometry also supported the structure of the compound.

Earlier alkylation reaction of bis-hydroxy-protected-5-bromo-2'-deoxyuridine involved various solvent systems such as THF[11,12], THF/HMPA[9] and two different temperatures, -78°C and -50°C[12,13]. In those reactions generally 2 compounds were observed by HPLC, thymidine **6** and 2'-deoxyuridine **5**. However, in the reaction of 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine an additional unidentified compound was observed, which was assumed to be isothymidine. The ratio of thymidine to 2'-deoxyuridine was about 25 : 75[12] and 40 : 60[13]. In all of our alkylation reactions the ratio of thymidine to 2'-deoxyuridine was much higher (Table 1).

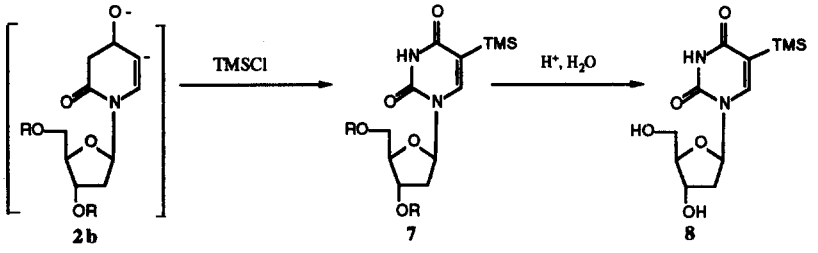
Precursors		Products	Product Ratio
THP ether	**1a**	Thymidine **6**, Deoxyuridine **5**	72 :28
TMS ether	**1b**	Thymidine **6**, Deoxyuridine **5**, 5-TMS-Deoxyuridine **8**	41 : 34 : 25
TBDMS ether	**1c**	Thymidine **6**, Deoxyuridine **5**	74 : 26

Table 1

The ratio of these compounds were obtained by ^{1}HNMR integration of the C_6 protons. In the reaction of 3', 5'-o-bis-(tetrahydropyranyl)-5-bromo-2'-deoxyuridine **1a** this ratio was 72 : 28, and in 3', 5'-o-bis-(*t*-butyldimethylsilyl)-5-bromo-2'-deoxyuridine **1c** it was 74 : 26, which are similar. However, the isolated yield of the intermediate bis-hydroxy-protected thymidine **4** was higher in the latter reaction (52%), compared to the former one (40%). The alkylation reaction of 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine **1b** gave 3 compounds, bis-hydroxy-protected thymidine **4b** , bis-hydroxy-protected-2'-deoxyuridine **3b** and a third compound characterized by ^{1}HNMR and mass spectrometry as 3', 5'-o-bis-(trimethylsilyl)-5-(trimethylsilyl)-2'-deoxyuridine **7** (scheme 2). The ratio of these three compounds in the mixture was about 41 : 34 : 25. In a separate experiment when the crude precursor 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine was used , the ratio of these compounds were slightly different. The isolated yield of the desired thymidine intermediate **4b** in this particular reaction was 12%,

whereas the uridine intermediate **3b** was 20% and the 5-trimethylsilyl uridine intermediate **7** was 5%. The crude products could not be purified on basic alumina column, since all the protecting groups were hydrolyzed in the column. However, they could be purified on silica gel column (pretreated with 1% triethylamine in hexane) in lower yields. During the reaction of 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine **1b**, formation of the 3', 5'-o-bis-(trimethylsilyl)-5-(trimethylsilyl)-2'-deoxyuridine **7** reveals that the precursor is unstable under the reaction condition. The labile Si-Oxygen bond presumably is cleaved by the attack of stronger nucleophilic organo-lithium reagent especially by the dianion intermediate **2b**. This type of reaction was not observed in other precursors.

The intermediate compound 3', 5'-o-bis(trimethylsilyl)-5-(trimethylsilyl)-2'-deoxyuridine **7** was hydrolyzed to 5-(trimethylsilyl)-2'-deoxyuridine **8** and analyzed by TLC using 10% methanol in dichloromethane as eluent, and thymidine as a reference compound. The Rf value of this compound was 0.41 and that of thymidine was 0.22 in the above solvent system. This compound could not be identified by HPLC through 30 minutes where as the retention time of thymidine was 12 minutes. Therefore, it is most likely that the previous workers did not see this compound by HPLC even though it may have been present in the reaction mixture. In order to verify the formation of this compound, it was prepared by treatment of the dianion **2b** with trimethylchlorosilane instead of methyl iodide (Scheme 2). The ^{1}HNMR spectrum of the product was identical as the isolated compound from the methylation reaction.



Scheme 2

The earlier reported unidentified compound (so-called isothymidine)[12] was observed by HPLC as less than 5%. The retention time of this compound was very close to that of 2'-deoxyuridine. A small amount of pure compound was isolated and analyzed by mass spectrometry. The mass spectrum of the compound demonstrated a molecular ion of 391, consistent with M+H with the base peak of 279. The loss of 112 mass unit from 391 to 279 indicates the loss of an uracil unit by rearrangement. This suggests that at least the uracil unit was not methylated, since it has 18 mass unit higher molecular weight than thymidine. Therefore, the reported unidentified radioactive peak is not likely to be isothymidine. Further characterization of the compound was not pursued due to the insignificant amount produced in the reaction.

In general, alkylation reactions proceeded rapidly when performed in THF at -78°C. In these reactions 2.5 equivalent of n-butyllthium were used instead of 2 equivalent, since frequently when 2 equivalents were used incomplete reaction was observed, probably secondary to partial hydrolysis in storage or during transfer. Hydrolysis of protecting groups went smoothly under acidic conditions, trimethylsilyl derivative hydrolyzed at room temperature within a minute. Tetrahydropyranyl and tert-butyldimethylsilyl derivatives required heating to 65°C for complete hydrolysis within 5 minutes.

Although it has been reported that synthesis of [methyl-^{11}C]thymidine using 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine **1b** produced high radiochemical yield and specific activity[12], this precursor produces an additional by-product, 5-(trimethylsilyl)-2-deoxyuridine **8** in a significant amount (15-25%) compared to other precursors, we have studied. Production of more by-products not only reduce the chemical yield but also make isolation of the pure desired product more difficult. Therefore,

based on our studies it appears that either tetrahydropyranyl or tert-butyldimethylsilyl protected precursors would be better choice for [methyl-^{14}C] or [methyl-^{11}C]thymidine synthesis. Although the protecting groups can be readily removed from both derivatives, the 3', 5'-o-bis-(tetrahydropyranyl)-5-bromo-2'-deoxyuridine has the advantage over the tert-butyldimethysilyl precursor, since it can be synthesized in quantitative yield compared to 65% yield of the latter case.

The synthesis of radiolabeled thymidine requires a precursor which is stable enough for routine storage and handling, and provides readily removable protecting groups. Our studies revealed that tetrahydropyranyl or tert-butyldimethylsilyl protected precursors have the advantage over the trimethylsilyl derivative. Preparation of thymidine from tetrahydropyranyl or tert-butyldimethylsilyl derivatives produces cleaner product in high yield. Considering the synthesis of the precursors, the tetrahydropyranyl derivative has the advantage over the tert-butyldimethylsilyl one, as it can be synthesized more easily and quantitatively compared to the latter which gave 65% yield. Therefore, we conclude that 3', 5'-o-bis-(tetrahydropyranyl)-5-bromo-2'-deoxyuridine is the best choice of the evaluated precursors in the preparation of [methyl-^{14}C] or [methyl-^{11}C]thymidine.

EXPERIMENTAL

Chemicals and Instrumentation:

Triethylamine, trimethylchlorosilane, *t*-butyldimethylsilyl chloride, p-toluenesulfonyl chloride and 2, 3-dihydropyrane were purchased from Aldrich Chemical company and were used without further purification. Flash chromatography were performed using silica gel 60 (E.M.Science) and basic Alumina (Aldrich), and HPLC grade solvents.

Thin layer chromatography (TLC) were performed on pre-coated Kisselgel 60 F254 (Merck) glass plates. Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}HNMR studies were performed on a Bruker AMX300 spectrometer in the Biophysics facility at The Johns Hopkins University using tetramethylsilane as internal reference, unless otherwise specified. Mass spectra were obtained on a Finnigan 4000 mass spectrometer at The University of Minnesota using ammonia chemical ionization technique, and m/z are reported only on the major peaks with relative intensity in the parenthesis. High performance liquid chromatography (HPLC) were performed on a Perkin-Elmer HPLC dual pump system using a LC-75 spectrophotometric detector at 254 nm, 024 recorder, Hewlet Packard integrator and an analytical reverse phase C18 column (Alltech). A solvent system of 90% water and 10% methanol was used as mobile phase in an isocratic system.

PREPARATION OF PRECURSORS

Preparation of 3', 5'-o-bis-(tetrahydropyranyl)-5-bromo-2'-deoxyuridine **1a**:

This compound was prepared following a literature method[11] with modification. Briefly, 5-bromo-2'-deoxyuridine (1.02 g, 3.25 mmol) was dissolved in dry THF (25 mL). p-Toluenesulfonic acid (60 mg, catalytic amount) was added to the reaction flask followed by addition of 3,4-dihydro-2*H* -pyran (4.45 mL, 48 mmol). The reaction mixture was stirred at room temperature for 2h, when TLC showed no remaining starting material. The reaction was quenched by adding 2 drops of triethylamine. Solvent was evaporated, and the crude product was purified by flash chromatography using silica gel column and 20% acetone in hexane as eluent. White solid, 1.57g of pure compound was obtained as a mixture of four diastereomers, yield 99.70%(quantitative). M.P. 58-75°C, Lit. 56-75°C (11). ^{1}HNMR ($CDCl_3$): 8.48 (b s, 1 H, NH), 8.26, 8.23, 8.21, 8.20 (4s, 1H, C_6H), 6.40-6.21 (m, 1H, 1'H), 4.69 (bs, 1H, 3'H), 3.62-3.60 (m, 7H, O-CH-O), 3.60-3.45 (m, 2H, CH_2-O), 2.70-2.44 (m, 1H, 2'H), 2.30-2.20 (m, 1H, 2'H), 1.98 -1.60 (m, 12H, CH_2). MS: 494 (M+NH_4, 2), 492 (M+NH4, 2), 477 (m+H, 11), 475 (M+H, 10), 410 (10), 408 (11), 332 (8), 330 (8), 118 (100), 102 (41), 85 (29).

Preparation of 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine **1b**:

5-Bromo-2'-deoxyuridine (0.50 g, 1.63 mmol) was dissolved in dry THF (18 mL). Triethylamine (5 mL, 35.8 mmol) was added followed by addition of trimethylchlorosilane (0.5 mL, 6.38 mmol). The reaction mixture was stirred at room temperature,

with continuous stirring for an additional 2.5 hours after the flask became warm. TLC showed no residual starting material at this time. The reaction mixture was diluted with 50% ethyl acetate in hexane (40 mL), washed with water (2x35 mL) and brine (1x15 mL). The aqueous phase was back extracted with 50% ethyl acetate in hexane (1x30 mL). The combined organic phase was dried (Na_2SO_4), and evaporated to give 890 mg of crude product. The crude product was chromatographed on a basic alumina column using 20% acetone in hexane as eluent. Pure compound, 300 mg was obtained as white solid, yield 45%. M.P. 52-56°C. ^{1}HNMR ($CDCl_3$): 8.26 (s, 1H, C_6H), 6.28 (t, 1H, 1'H, J = 6 Hz), 4.37-4.33 (m, 1H, 3'H), 3.98-3.96 (m, 1H, 4'H), 3.86 (dd, 1H, 5'H, J = 4 Hz, 2 Hz), 3.69 (dd, 1H, 5'H, J = 4 Hz, 2 Hz), 2.35-2.27 (m, 1H, 2'H), 2.10-2.01 (m, 1H, 2'H), 0.19 (s, 9H, TMS), 0.15 (s, 9H, TMS). MS: 470 (M+NH_4, 43), 468 (M+NH4, 44), 453 (M+H, 100), 451 (M+H, 98), 398 (2), 396 (2), 373 (37), 278 (29), 90 (35).

Preparation of 3', 5'-o-bis-(t-butyldimethylsilyl)-5-bromo-2'-deoxyuridine **1c**:

5-Bromo-2'-deoxyuridine (0.50 g, 1.63 mmol) was dissolved in dry THF (18 mL). Triethylamine (5 mL, 35.8 mmol) was added followed by addition of *t*-butyldimethylsilyl chloride (0.98 g, 6.51 mmol). The reaction mixture was heated at 75°C for 40h, when TLC showed no remaining starting material. The reaction mixture was diluted with 50% ethyl acetate in hexane (30 mL), washed with water (2x25 mL) and brine (1x15 mL). The aqueous phase was back extracted with 50% ethyl acetate in hexane (1x30 mL). The combined organic phase was dried (Na_2SO_4), and evaporated to give 1.00 g of crude product. After chromatography on silica gel column using 20% acetone in hexane as eluent, pure compound (570 mg) was obtained in 65% yield. M.P.: 54-59°C. ^{1}HNMR ($CDCl_3$): 8.07 (s, 1H, C_6H), 6.27 (t, 1H, 1'H, J = 6 Hz), 4.37-4.33 (m, 1H, 3'H), 3.98-3.96 (m, 1H, 4'H), 3.89 (dd, 1H, 5'H, J = 4 Hz, 2 Hz), 3.76 (dd, 1H, 5'H, J = 4 Hz, 2 Hz), 2.70-2.35 (m, 1H, 2'H), 2.10-1.90 (m, 1H, 2'H), 0.94 (s, 9H, t-butyl), 0.89 (s, 9H, *t*-butyl), 0.15 (s, 3H, Si-CH_3), 0.14 (s, 3H, Si-CH_3), 0.08 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3).
MS: 537 (M+H, 100), 535 (98), 457 (60), 419 (5), 399 (7).

ALKYLATION REACTIONS

Preparation of 3', 5'-o-bis-(tetrahydropyranyl)thymidine **4a**:

3', 5'-o-Bis-(tetrahydropyranyl)-5-bromo-2'-deoxyuridine (100 mg, 0.21 mmol) was dissolved in dry THF (1 mL) under argon and cooled to -78°C. n-Butyllithium (1.6M solution in hexane, 0.330 mL, 0.25 mmol) was injected to the cold solution by a microsyringe, and the reaction mixture was stirred for 30 seconds. Methyl iodide (0.020 mL, 0.31 mmol) was injected to the above reaction mixture, stirred for one minute, then the cold bath was removed. After 2 minutes TLC showed no remaining starting material. The reaction was quenched with 2 drops of saturated ammonium chloride solution and warmed to room temperature. Ethyl acetate (10 mL) was added to the reaction flask and transferred to a separatory funnel, washed with water (1x10 mL), and brine (1x8 mL). The aqueous phase was back extracted with ethyl acetate (1x10 mL), and the combined organic phase was dried (Na_2SO_4) and evaporated to give 81 mg of crude product. ^{1}HNMR of the crude product showed a mixture of 2 compounds in the ratio 72 : 28, as obtained by integration of the C_6 protons. The crude product was chromatographed on silica gel column using 20% acetone in hexane as eluent to produce 35 mg of pure compound as a mixture of 4 diastereomers, in 40% yield. M.P. : 62-78°C. ^{1}HNMR ($CDCl_3$): 8.75 (bs, 1H, NH), 7.68-7.52 (4s, 1H, C_6H), 6.48-6.32 (m, 1H, 1'H), 4.80-4.70 (m, 1H), 4.55-3.60 (m, 7H), 3.58 - 3.40 (m, 2H), 2.46-2.36 (m, 1H, 2'H), 2.21-2.00 (m, 1H, 2'H), 1.78, 1.77 (2s, 3H, CH_3), 1.82-1.44 (m, 12H, CH_2). MS: 411 (M+H, 13), 327 (76), 118 (100), 102 (62), 101 (49), 85 (87).

Preparation of 3', 5'-o-bis-(tetrahydropyranyl)-2'-deoxyuridine **3a**:

This compound was isolated as a by-product in the above alkylation reaction. ^{1}HNMR ($CDCl_3$): 8.75 (bs, 1H, NH), 7.90-8.10 (4d, 1H, C_6H, J = 7 Hz), 6.38-6.28 (m, 1H, 1'H), 5.70 (t, 1H, C_5H, J = 7 Hz), 4.71-4.60 (m, 1H), 3.60-3.30 (m, 7H), 3.59-3.48 (m, 2H), 2.60-2.40 (m, 1H, 2'H), 2.25-2.00 (m, 1H, 2'H), 1.80-1.40 (m, 12H). MS: 414 (M+NH_4, 20), 397 (M+H, 66), 330 (96), 313 (94), 118 (100), 101 (77), 85 (67).

Preparation of 3', 5'-o-bis(trimethylsilyl)-thymidine **4b**:

The general method of preparation has been described above. The crude product after workup gave 81 mg of materials which was a mixture of 3 compounds as observed by ^{1}HNMR. The crude mixture was separated on a silica gel column (pretreated with 1% triethylamine) using 20% acetone in hexane to produce 10 mg of pure desired compound, yield 12%. ^{1}HNMR ($CDCl_3$): 8.46 (bs, 1H, NH), 7.60 (s, 1H, C_6H), 6.33 (t, 1H, 1'H, J = 6 Hz), 4.36-4.33 (m, 1H, 3'H), 3.94-3.92 (m, 1H, 4'H), 3.82 (dd, 1H, 5'H, J = 11 Hz, 2.5 Hz), 3.71 (dd, 1H, 5'H, J = 11 Hz, 2.5 Hz), 2.25-2.22 (m, 1H, 2'H), 2.09-2.01 (m, 1H, 2'H), 1.92 (s, 3H, CH_3), 0.18 (s, 9H, TMS), 0.14 (s, 9H, TMS). MS: 383 (M+H, 33), 261 (32), 199 (14), 171 (40), 103 (100).

Preparation of 3', 5'-O-bis-(trimethylsilyl)-2'-deoxyuridine **3b**:

This compound was isolated from the above reaction as a by-product. ^{1}HNMR ($CDCl_3$): 8.56 (bs, 1H, NH), 7.96 (d, 1H, C_6H, J = 6 Hz), 6.29 (t, 1H, 1'H, J = 6 Hz), 5.67 (d, 1H, C_5H, J = 6 Hz), 4.37-4.34 (m, 1H, 3'H), 3.97-3.91 (m, 1H, 4'H), 3.85 - 3.82 (dd, 1H, 5'H, J = 11 Hz, 2.5 Hz), 3.72-3.68 (dd, 1H, 5'H, J = 11 Hz, 2.5 Hz), 2.36-2.32 (m, 1H, 2'H), 2.12-2.04 (m, 1H, 2'H), 0.15 (s, 9H, TMS), 0.13 (s, 9H, TMS). MS: 373 (M+H, 100), 261 (40), 185 (44), 103 (60).

Preparation of 3', 5'-O-bis(trimethylsilyl)-5-(trimethylsilyl)-2'-deoxyuridine **7**:

This compound was also isolated from the above reaction, and it was prepared by the reaction of 3', 5'-o-bis-(trimethylsilyl)-5-bromo-2'-deoxyuridine with trimethylchlorosilane through the organolithium intermediate **2b**. ^{1}HNMR ($CDCl_3$): 7.95 (bs, 1H, NH), 7.44 (s, 1H, C_6H), 6.27 (t, 1H, 1'H, J = 6 Hz), 4.36-4.33 (m, 1H, 3'H,), 3.94-3.92 (m, 1H, 4'H), 3.72 (d, 2H, 5'H, J = 4 Hz), 2.36-2.25 (m 1H, 2'H), 2.06-1.95 (m, 1H, 2'H), 0.24 (s, 9H, TMS), 0.14 (s, 9H, TMS), 0.138 (s, 9H, TMS). MS: 445 (M+H, 19), 339 (8), 261 (40), 171 (17), 169 (40), 103 (100), 73 (32).

Preparation of 3', 5'-o-bis-(t-butyldimethylsilyl)-thymidine **4**:

The method of preparation of this compound was same as described above. The crude product produced 81 mg of compounds, after chromatography on silica gel column using 20% acetone in hexane 46 mg of pure desire compound was obtained, yield 52%. M. P. 97-99°C. ^{1}HNMR ($CDCl_3$): 8.61 (bs, 1H, NH), 7.47 (s, 1H, C_6H), 6.33 (t, 1H, 1'H, J = 6 Hz), 4.43-4.35 (m, 1H, 3'H), 3.96-3.92 (m, 1H, 4'H), 3.86 (dd, 1H, 5'H, J = 11 Hz, 2.5 Hz), 3.75 (dd, 1H, 5'H, J = 11 Hz, 2.5Hz), 2.22-2.19 (m, 1H, 2'H), 2.06 - 1.94 (m, 1H, 2'H), 1.90 (s, 3H, CH_3), 0.93 (s, 9H, *t*-butyl), 0.89 (s, 9H, *t*-butyl), 0.11 (s, 6H, Si-CH_3), 0.07 (s, 6H, Si-CH_3). MS: 471 (M+H, 100), 413 (5), 391 (7), 362 (6).

Preparation of 3', 5'-O-bis-(t-butyldimethylsilyl)-2'-deoxyuridine **3c**:

This compound was isolated from the above reaction mixture as a by-product. ^{1}HNMR ($CDCl_3$): 8.35 (bs, 1H, NH), 7.91 (d, 1H, C_6H, J = 7 Hz), 6.26 (t, 1H, 1'H, J = 6 Hz), 5.58 (d, 1H, C_5H, J = 7 Hz), 4.43-4.35 (m, 1H, 3'H), 3.96-3.92 (m, 1H, 4'H,), 3.86 (dd, 1H, 5'H, J = 11 Hz, 2.5 Hz), 3.75 (dd, 1H, 5'H, J = 11 Hz, 2.5 Hz), 2.22-2.19 (m, 1H, 2'H), 2.06-1.94 (m, 1H, 2'H), 0.93 (s, 9H, *t*-butyl), 0.89 (s, 9H, *t*-butyl), 0.11 (s, 6H, Si-CH_3), 0.07 (s, 6H, Si-CH_3). MS: 457 (M+H, 100), 399 (35), 287 (69), 267 (92), 227 (48), 145 (51), 81 (61).

HYDROLYSIS OF PROTECTING GROUPS

Preparation of thymidine **6**:

All the alkylated intermediate products were hydrolyzed under acidic conditions. The trimethylsilyl derivative was hydrolyzed at room temperature, however, other compounds were hydrolyzed by heating them at 65°C for 5 minutes. Briefly, 10 mg of 3', 5'-o-bis(tetrahydropyranyl)thymidine was dissolved in methanol or THF (1 mL). The solution was acidified by adding 2 drops of 1N HCl acid and heated for 5 minutes at reflux, when TLC showed no starting material left. The reaction mixture was neutralized by adding 2 drops of sodium bicarbonate solution. After evaporating solvent the flask was washed with 50% ethyl acetate in hexane, and the washing was discarded. The residual solvent was pumped off on high vacuum line and product analyzed by

^{1}HNMR and HPLC. ^{1}HNMR (DMSO): 7.51 (s, 1H, C_6H), 6.17 (t, 1H, 1'H, J = 6 Hz), 5.21 (bs, 1H, OH), 5.04 (t, 1H, OH), 4.2-4.19 (m, 1H, 3'H), 3.70-3.68 (m, 1H, 4'H), 3.5-3.48 (m, 2H, 5'H), 2.14-1.98 (m, 2H, 2'H), 1.71 (s, 3H, CH_3).

Preparation of 2'-deoxyuridine **5**:

This compound was prepared by hydrolysis of the isolated intermediate 3', 5'-o-bis(tetrahydropyranyl)-2'-deoxyuridine **4b**, following the above procedure. ^{1}HNMR (DMSO): 7.57 (d, 1H, C_6H, J = 8 Hz), 6.18 (t, 1H, 1'H, J = 6 Hz), 5.41 (d, 1H, C_5H, J = 8 Hz), 5.21 (bs, 1H, OH), 5.04 (bs, 1H, OH), 4.21-4.17 (m, 1H, 3'H), 3.72-3.68 (m, 1H, 4'H), 3.55-3.44 (m, 2H, 5'H), 2.1 -1.98 (m, 2H, 2'H).

Preparation of 5-(trimethylsilyl)-2'-deoxyuridine **8**:

This compound was prepared from the intermediate 3', 5'-o-bis(trimethylsilyl)-5-(trimethylsilyl)-2'-deoxyuridine **7** by acidic hydrolysis. ^{1}HNMR ($CDCl_3$): 8.50-8.20 (bs, 1H, NH), 7.46 (s, 1H, C_6H), 6.19 (t, 1H, 1'H, J = 6 Hz), 4.66-4.62 (m, 1H, 3'H), 4.08-4.06 (m, 1H, 4'H), 3.90-3.81 (m, 2H, 5'H), 2.55-2.35 (m, 2H, 2'H), 1.60 (bs, 2H, OH), 0.27 (s, 9H, TMS). MS: 318 ($M+NH_4$, 3), 301 (M+H, 100), 287 (10), 185 (9).

HPLC ANALYSIS OF THYMIDINE AND 2'-DEOXYURIDINE

An isocratic HPLC system (Perkin Elmer) was used for analysis of the final products. A mobile phase of 90% water and 10% methanol was used in a C_{18} reverse phase analytical column with a flow of 1 mL per minute. Thymidine was eluted at 12 minutes (Lit. retention time = 12 minutes) and 2'-deoxyuridine was eluted at 6 minutes (Lit. retention time = 6 minutes)[11].

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