

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

A Simple and Regioselective Synthesis of ^{13}C -Methyl-Labeled Thymidine

Mohammad Ahmadian^a; Donald E. Bergstrom^a

^a Department of Medicinal Chemistry, Molecular Pharmacology Purdue University, W. Lafayette, IN, USA

To cite this Article Ahmadian, Mohammad and Bergstrom, Donald E.(1998) 'A Simple and Regioselective Synthesis of ^{13}C -Methyl-Labeled Thymidine', *Nucleosides, Nucleotides and Nucleic Acids*, 17: 7, 1183 — 1190

To link to this Article: DOI: 10.1080/07328319808004231

URL: <http://dx.doi.org/10.1080/07328319808004231>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

**A SIMPLE AND REGIOSELECTIVE
SYNTHESIS OF ^{13}C -METHYL-LABELED
THYMIDINE**

Mohammad Ahmadian[†] and Donald E. Bergstrom^{†*}

[†]Department of Medicinal Chemistry and Molecular Pharmacology
Purdue University, W. Lafayette, IN 47907 and ^{*}Walther Cancer Institute,
Indianapolis, IN 46208, USA

ABSTRACT: A convenient and efficient procedure for the synthesis of ^{13}C methyl-labeled thymidine by way of lithiation of *t*-butyldimethylsilyl protected 2'-deoxyuridine is described.

The macroscopic properties of macromolecules depend on the structure, order and dynamics of the polymeric chains.¹ Among a variety of physicochemical techniques which has been applied to characterize these properties, nuclear magnetic resonance has proven particularly powerful and versatile. A very important advantage of nmr is its selectivity which could be achieved in a number of ways including selective labeling. This generally requires laborious chemical synthesis. Solid state nmr has been demonstrated to be useful in the study of DNA structure and dynamics.²

As part of our plan to carry out an extensive study on the correlation of the mobility of functional groups in the solid as determined by variable temperature X-ray crystallography and variable temperature solid state nmr spectroscopy, we needed to study the relaxation time of the methyl groups by ^{13}C nmr in a selected series of oligodeoxyribonucleotides each containing one thymidine residue. For the experiments to be selective and give high signal to noise ratio, it was essential that the methyl group of thymidine be isotopically enriched. Thus, a convenient method which would allow us to prepare gram quantities of ^{13}C -methyl-labeled thymidine seemed essential. The methodology described in this paper provides a convenient and efficient method for the preparation of thymidine.

Ideally, the synthetic preparation of labeled compound should require as few steps and physical manipulation as possible. Introduction of a methyl group at the C-5 position of uracil nucleosides by the way of acidic³, basic⁴⁻⁷ or neutral^{8,9} hydroxymethylation and subsequent catalytic hydrogenation of the resulting 5-hydroxymethyluracil nucleosides proved not to be the method of choice for isotope labeling because of low overall yields of the desired methyl pyrimidine derivatives. The three steps conversion of 2'-deoxyuridine by a procedure involving the intermediate Mannich methiodide into thymidine also proceeds in less than 20% overall yield.^{10,11} Furthermore, these methods generally require excess (up to 30 fold) formaldehyde. Alternative applicable methods of the preparation of isotopically labeled thymidine from 5-iodo- or 5-bromo-2'-deoxyuridine employing tetramethyltin¹² or trimethylaluminum¹³ are not favored since the isotopically enriched Me₄Sn or AlMe₃ are not commercially available.

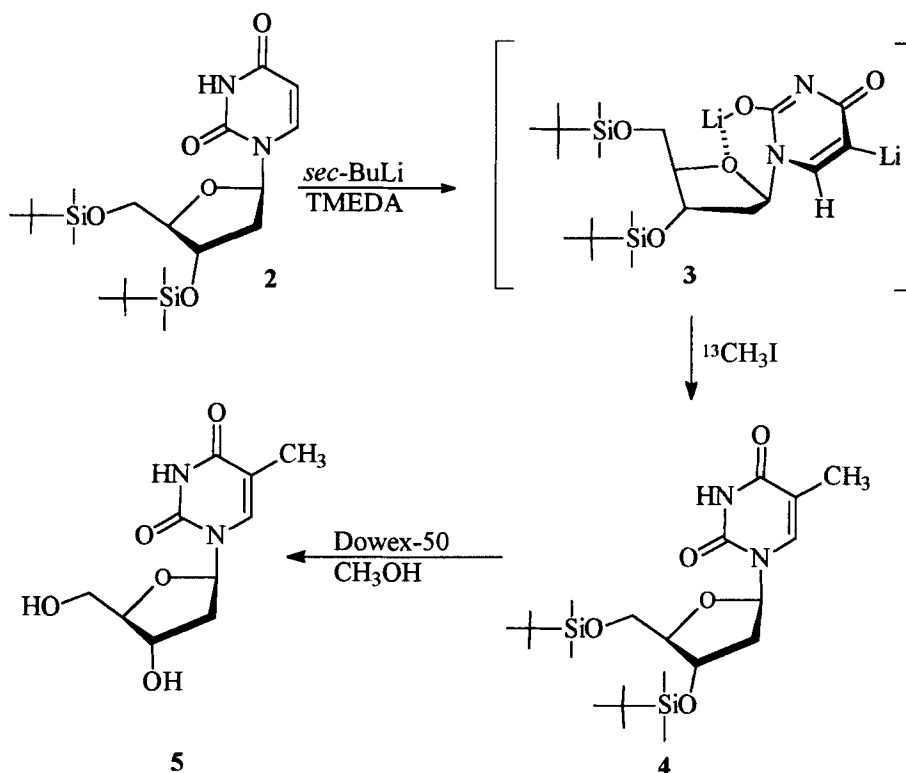
Lithiation of organic molecules has been studied extensively as an increasingly important tool for bond forming reactions with electrophiles. During the course of their studies, Miyasaka and co-workers observed that efficiency and regioselectivity (C-5 vs C-6) in the lithiation of uridine derivatives with lithium dialkylamides were dramatically affected by the protecting groups in the sugar moiety.¹⁴ That is, although lithiation of 2',3'-*O*-isopropylidene-5'-methoxymethyluridine with LDA occurs selectively at the C-6 position,¹⁵⁻¹⁷ neither H-6 nor H-5 is deprotonated when 2',3',5'-tris-*O*-(*t*-butyldimethylsilyl)uridine is treated with LDA. The above observations were rationalized by assuming that LDA was not basic enough to deprotonate the less acidic H-5¹⁸ and that the C-6 position in the substrate was sterically blocked by a free rotational C-2' substituent, the 2'-*O*-TBDMS group. In support of this assumption was the observation that lithium 2,2,6,6-tetramethylpiperidide (LTMP), which is a more basic lithiating agent than LDA, could metallate the C-5 position of 2',3',5'-tris-*O*-(*t*-butyldimethylsilyl)uridine, although the lithiation level (26%) was not satisfactory for practical use.¹⁴

In the quest for a general route to C-5 substituted uracil nucleosides, Miyasaka et al. considered the use of a lithiating agent which would act through a coordination mechanism. In fact, treatment of 2',3',5'-tris-*O*-(*t*-butyldimethylsilyl)uridine with *n*-

butyllithium (*n*-BuLi) in tetrahydrofuran (THF) followed by the addition of CD_3OD resulted in an increased C-5 deuteration level (72%). The highest C-5 lithiation level (86%) was accomplished when *sec*-butyllithium (*sec*-BuLi) was used in combination with equimolar amount of *N,N,N',N'*-tetramethylethylenediamine (TMEDA). This lithiation occurs with high regioselectivity and subsequent reactions of the resulting C-5 lithiated species with a variety of electrophiles provides a general entry to 5-substituted uridines.¹⁹ Consequently application of this methodology to 3',5'-bis-*O*-(*t*-butyldimethylsilyl)-2'-deoxyuridine (**2**) appeared to be an attractive route to ^{13}C -methyl-labeled thymidine.

The starting nucleoside 3',5'-bis-*O*-(*t*-butyldimethylsilyl)-2'-deoxyuridine (**2**) was obtained quantitatively from the commercially available 2'-deoxyuridine (**1**) using the procedures developed by Oglivie.²⁰ Treatment of **1** in the presence of imidazole with *t*-butyldimethylsilyl chloride (TBDMSCl) in *N,N*-dimethylformamide (DMF) resulted in **2**. Deprotonation of **2** in dry THF at -78°C with 3.2 equivalent of *sec*-BuLi under a stream of argon in the presence of 2.2 equivalent of freshly distilled dry TMEDA after 30 minutes afforded the bislithio intermediate **3**. To the resulting mixture, a solution of excess ^{13}C -methyl iodide in THF was added dropwise and the mixture stirred at -78°C for 20 minutes. The resulting pale yellow solution was allowed to warm up to room temperature and stirred for 30 more minutes to yield 72% isolated product **4**. We found that the optimum yield is obtained when measures were taken to ensure that the temperature of the reaction mixture remained below -70°C during the addition of the methyl iodide solution. This goal was accomplished by tailoring a plastic jacket filled with dry ice for the addition funnel to keep the solution below -20°C while it was being added to the reaction mixture.

The deprotection of the nucleoside employing trimethylsilyltriflate according to the method of Bou²¹ resulted in partial deprotection of **4**. However, the efficient deprotection of *t*-butyldimethylsilyl groups was best achieved using a cation exchange resin in acidic form (Dowex-50w, 8X). Thus, 3',5'-bis-*O*-(*t*-butyldimethylsilyl)-5-(^{13}C -methyl)-2'-deoxyuridine (**4**) was dissolved in methanol and the resin, Dowex-50w (8X) added, and the mixture stirred at room temperature for 10 hours. The residue, after removal of the resin and evaporation of the solvent, was recrystallized from methanol.



SCHEME 1

The product was characterized by the comparison of the ^1H and ^{13}C nmr spectra of **5** with those of thymidine. The ^1H nmr of **5**, along with all other expected signals, showed a characteristic doublet for the methyl group. The signal which appears at δ 1.75 is split by 129 Hz due to ^{13}C - ^1H couplings. In the proton decoupled ^{13}C spectrum of **5**, the C-5 signal appeared as a doublet (δ 112.35, $J_{\text{C-C}} = 48$ Hz) due to the homonuclear coupling to the adjacent methyl group. The spectrum also shows a signal at δ 14.1 with much higher intensity in comparison to other signals. The aforementioned spectral evidence confirm ^{13}C enrichment of the methyl group. Although it has been reported that under similar conditions a mixture of 5- and 6-substituted pyrimidine nucleosides were obtained,²² in our hands no detectable amounts of 6-methyl-2'-deoxyuridine formed. Furthermore, identical chromatographic (TLC) behavior ($R_f=0.61$) was observed when both **5** and an

authentic sample of thymidine were eluted with chloroform containing 20% methanol. The mass spectrum (CI) of the final compound also showed the $(\text{M}+\text{H})^+$ peak at m/z 244.

We have converted the ^{13}C -methyl-labeled thymidine into the corresponding 3'-phosphoramidite derivative after protection of the 5'-hydroxyl of **5** with 4,4'-dimethoxytrityl (DMTr) group and the nucleosides were successfully incorporated into the oligodeoxyribonucleotide sequence 5'-d(GGGTACCC)-3'.

EXPERIMENTAL:

General:

2'-Deoxyuridine was purchased from Crystal Chem Inc. (Chicago, IL) and used as received. *sec*-Butyllithium (1.3 M solution in cyclohexane) was purchased from Aldrich Chemical Co. (St. Louis MO). Nmr spectra were recorded using a Bruker ARX300 spectrometer. ^1H and ^{13}C signals were internally referenced to TMS unless otherwise stated. Chemical ionization mass spectra (CI-MS) were recorded by the mass spectrometry laboratory of the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University. Elemental analysis was performed by the microanalysis laboratory, Department of Chemistry, Purdue University. Analytical thin layer chromatography (TLC) was carried out on pre-coated Whatman 60F254 plates. Anhydrous solvents were freshly distilled from appropriate drying agents or purchased from Aldrich Chemical Co. All other chemicals were reagent grade or better quality and used as received.

3',5'-Bis-*O*-(*t*-butyldimethylsilyl)-2'-deoxyuridine (**2**):

In a 50 mL round bottom flask, 2'-deoxyuridine (5.7 g 25 mmol) and imidazole (8.5 g, 125 mmol) were dissolved in 30 mL dry DMF. To this solution, *t*-butyldimethylsilyl chloride (9.42 g 2.2 mmol) was added portionwise. The reaction mixture was stirred for 2 hours at 45-50 °C and then the resulting solution concentrated at reduced pressure. The residue was introduced on a column of silica-gel (230-400 mesh) and eluted with a mixture of CHCl_3 - CH_3OH (95:5). The title compound was obtained (R_f = 0.30) in quantitative yield. ^1NMR (CHCl_3 - d_7) δ 8.8 (s, 1H, $\text{N}^3\text{-H}$), 7.92 (d, J = 8.1 Hz, 1H, H-6), 6.30 (t, J =6 Hz, 1H, H-1'), 5.70 (d, J =8.1 Hz, 1H, H-5), 4.40 (m, 1H, H-4'), 3.92 (m,

1H, H-3') 3.75 (m, 2H, H-5'), 2.35 (m 1H, H-2'), 2.05 (m, 1H, H-2''), 0.92 (s, *t*-butyl, 9H), 0.89 (s, *t*-butyl, 9H), 0.10 (s, 2CH₃, 6H), 0.08 (s, CH₃, 3H), 0.075 (s, CH₃, 3H). IR (KBr) ν =3025, 2957.4, 2636, 2858, 1708, 1471, 1469, 1463, 1253, 837 cm⁻¹

3',5'-Bis-*O*-(*t*-butyldimethylsilyl)-2'-deoxy-5-(¹³C-methyl)uridine (4):

TMEDA (255 mg, 331 μ l, 2.2 mmol) was added to a solution of 3',5'-bis-*O*-(*t*-butyldimethylsilyl)-2'-deoxyuridine (2, 456 mg, 1 mmol) in 10 mL THF at -78 °C under argon followed by 2.5 mL of a 1.3 M solution of *sec*-BuLi in hexanes (3.2 mmol). After stirring for 30 minutes, a solution of ¹³CH₃I (250 μ l 4.0 mmol) in THF (3 mL) was added dropwise to the reaction mixture, using an addition funnel. In order to keep the temperature of the reaction mixture below -70°C, a plastic jacket was fitted onto the funnel and filled with dry ice during addition of the solution. The resulting pale yellow mixture was stirred for 20 minutes and then the reaction mixture allowed to warm up to room temperature and stirred for 30 more minutes. Then, the mixture was filtered and the solid residue washed with THF. The filtrates were combined and the solvent evaporated under reduced pressure. The residue was dissolved in dichloromethane containing 2% methanol and filtered through a 1 cm thick layer of silica-gel. The plug was washed with the same solvent and the combined filtrates evaporated under reduced pressure. The remaining oil was purified by column chromatography (silica gel) eluting with 2% methanol in dichloromethane to obtain 338 mg (72% yield) of 4. ¹H-NMR (CHCl₃-*d*₁) δ 8.31 (s, N³-H, 1H), 7.45 (s, H-6 1H), 6.31 (t, *J*=6.2 Hz, H-1', 1H), 4.38 (m, H-4', 1H), 3.91 (m, H-3', 1H), 3.85 (dd, *J*₁=22 Hz, *J*₂=9.3 Hz, H-5', 2H), 2.21 (m, H-2', 1H), 2.00 (m, H-2'' 1H), 1.93 (d, *J*_{C-H}=129 Hz, CH₃, 3H), 0.90 (s, *t*-butyl, 9H), 0.87 (s, *t*-butyl, 9H), 0.09 (s, 2CH₃, 6H), 0.05 (s, 2CH₃, 6H).

2'-Deoxy-5-(¹³C)methyluridine (5):

3',5'-Bis-*O*-(*t*-butyldimethylsilyl)-2'-deoxy-5-(¹³C-methyl)uridine (4, 235 mg 0.5 mmol) was dissolved in methanol and Dowex-50w-8X (H-form, 3 g) was added. The mixture was stirred overnight at room temperature. Then the reaction mixture was filtered, the cation-exchange resin washed with methanol, and the solvent evaporated. The residue was recrystallized from methanol to give 120 mg (98% yield) ¹³C-labeled thymidine. ¹H NMR

[$\text{H}_2\text{O}-d_2$ referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS)] δ 7.65 (s, H-6, 1H), 6.15 (t, H-1', $J=6.5$ Hz), 4.32 (m, H-3' 1H), 3.88 (m, H-4', 1H) 3.73-3.59 (m, H-5', 2H), 2.23 (t, $J=6.5$ Hz), 1.75 (d, CH_3 , $J_{\text{C-H}}=129$ Hz). Proton decoupled ^{13}C NMR, (D_2O , referenced to 1,4-dioxane at δ 67.5) 167.4, 152.6, 138.4, 112.35 (d, $J_{\text{C-C}}=48$ Hz, C-5), 87.5, 86.0, 71.4, 62.15, 39.5, 12.45 (very strong signal assigned to ^{13}C enriched methyl group). MS-CI m/z calculated for $\text{C}_9\text{H}_{14}\text{C}[^{13}]\text{N}_2\text{O}_5$ 243. Found 244 ($\text{M}+\text{H}$) $^+$ Anal. Calculated for $\text{C}_9\text{H}_{14}\text{C}[^{13}]\text{N}_2\text{O}_5$: C, 44.44; C[13], 5.35; H, 5.80; N, 11.52. Found: C (total), 49.42; H, 5.77; N, 11.15.

ACKNOWLEDGMENTS. The National Institutes of Health is gratefully acknowledged for support of this research through NIH Grants GM53155 and AI36601. We thank Purdue Research Foundation for a fellowship to M. A.

REFERENCES:

1. Spiess, H. W., *Chem. Rev.* (1991), **91**, 1321-1338.
2. Alam, T. M.; Drobný, G. P., *Chem. Rev.* (1991), **91**, 1545-1590.
3. Cline, R. E.; Fink, R. M.; Fink, K., *J. Am. Chem. Soc.* (1959), **81**, 2521-2527.
4. Scheit, K. H., *Chem. Ber.* (1966), **99**, 3884-3891.
5. Armstrong, V. W.; Eckstein, F., *Nucl. Acids. Res. Special Pub.* (1975), **1**, s97-s100.
6. Ikeda, K.; Tanaka, S.; Mizuno, Y., *Chem. Pharm. Bull.* (1975), **23**, 2958-2964.
7. Wang, B.; Kagel, J. R.; Mertes, M. P.; Bowman-James, K., *Bioorganic Chem.* (1994), **22**, 405-420.
8. Scheit, K. H., *Tetrahedron Lett.* (1965), 1031-1039.
9. Kahilainen, L. I.; Bergstrom, D. E.; Vilpo, J. A., *Acta Chem. Scand.* (1985), **B39**, 477.
10. Jones, S. S.; Reese, C. B.; Ubasawa, A., *Synthesis* (1987), 259.
11. Reese, C. B.; Sanghvi, Y. S., *J. Chem. Soc. Chem. Commun.* (1983), 877.
12. Herdewijn, P.; Kerremans, L.; Wegerinck, P.; Vandendriessche, F.; Van Aerschot, A., *Tetrahedron Lett.* (1991), **32**, 4397-4400.
13. Hirota, H.; Kitade, Y.; Kanbe, Y.; Isobe, Y.; Maki, Y., *Synthesis* (1993), 213-215.
14. Hayakawa, H.; Tanaka, H.; Maruyama, Y.; Miyasaka, T., *Chem. Lett.* (1985), 1401-1404.

15. Tanaka, H.; Nasu, I.; Miyasaka, T., *Tetrahedron Lett.* (1979), **20**, 4755-4758.
16. Tanaka, H.; Hayakawa, H.; Miyasaka, T., *Chem. Pharm. Bull* (1981), **29**, 3565-3572
17. Kagel, J. R.; Wang, B.; Mertes, M. P., *J. Org. Chem.* (1993), **58**, 2738-2746.
18. Rabi, J. A.; Fox, J. J., *J. Am. Chem. Soc.* (1973), **95**, 1628-1632.
19. Hayakawa, H.; Tanaka, H.; Obi, K.; Itoh, M.; Miyasaka, T., *Tetrahedron Lett.* (1987), **28**, 87-90.
20. Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L., *Can. J. Chem.* (1978), **56**, 2768-2780.
21. Bou, V.; Vilansa, J., *Tetrahedron Lett.* (1990), **31**, 567-568.
22. Armstrong, R. W.; Gupta, S.; Whelihan, F., *Tetrahedron Lett.* (1989), **30**, 2057-2060