A Facile Synthesis of 4'-[^{2}H]-Uridine and Its Derivatives

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SUMMARY

A facile method for the introduction of deuterium at the $C_{4'}$ position of uridine and its derivatives involves the reaction of appropriately protected 4',5'-unsaturated pyrimidine nucleosides with B_2D_6, followed by hydrogen peroxide oxidation under alkaline conditions. The method described herein is also adaptable to the introduction of tritium at $C_{4'}$ position of pyrimidine nucleosides for use in mechanistic studies of DNA-degrading drugs.

Key words: Hydroboration-oxidation, Deuterium labeling

INTRODUCTION

Recent studies from our laboratory and others have demonstrated that the antitumor antibiotic bleomycin degrades DNA in the presence of metal ions, such as Fe^{+2}, and oxygen yielding the four nucleic acid bases and base propenals[2-6]. Furthermore, our experiments using poly(dA-dU), specifically tritiated at the 4'-position of the deoxyribose of the 2'-deoxyuridine have established that the rate determining step in the formation of base and base propenal is the radical abstraction of the hydrogen atom from the 4'-carbon[4,5]. The poly [dA-(4'-^{3}H)dU] was synthesized from [4'-^{3}H]-2'-deoxyuridine-5'-triphosphate and the synthesis of the latter required a number of enzymatic and chemical steps[4]. In order to further elucidate the mechanism of bleomycin as well as other DNA-interacting molecules which may generate

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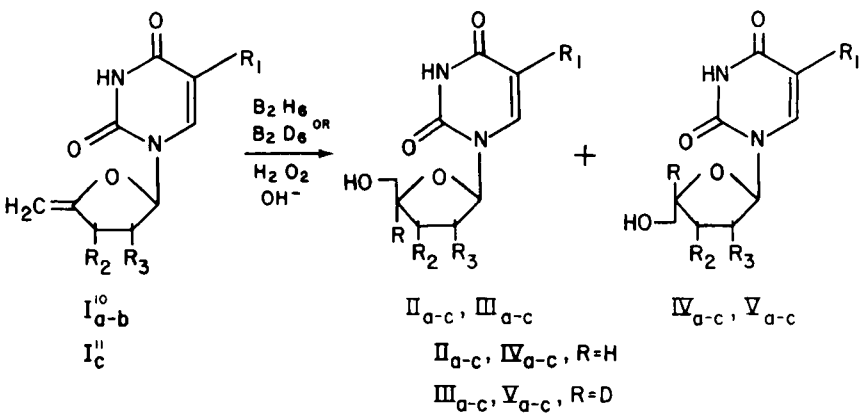
radical species at $C_{4'}$ position, we have investigated approaches to the chemical synthesis of $C_{4'}$ -labeled nucleosides. The present communication describes the synthesis of 4'-deuterium labeled uridine and its derivatives.

RESULTS AND DISCUSSION

Based upon the work of Moffatt and co-workers,[7] a versatile method for the introduction of a substituent at the $C_{4'}$ position of a nucleoside involves an olefinic addition reaction to the appropriately protected 4',5'-unsaturated nucleoside. Moreover, the applicability of hydroboration-oxidation in carbohydrate chemistry[8,9] suggested a method for labeling the $C_{4'}$ position of pyrimidine nucleoside. Accordingly, we have investigated the hydroboration-oxidation reaction of the 4',5'-unsaturated pyrimidine nucleosides[11,12] under a variety of conditions. In most cases, this procedure results in the formation of a mixture of two isomeric 4'-deuterated pyrimidine nucleosides in ~70% yield. Gradual addition of a 1M solution of B_2D_6 in tetrahydrofuran at -40°C, followed by alkaline hydrogen peroxide oxidation at room temperature led to the formation of a β-D-ribofuranosyl and an α-L-lyxofuranosyl isomer of 4'-deuterated pyrimidine nucleosides in a 1:3 ratio. The two isomers were separated by preparative thin layer chromatography. The NMR spectra of the fast moving components showed an absence of $C_{4'}$ proton in the region δ 3.9-4.2 ppm and the disappearance of $J_{3',4'}$ and $J_{4',5'}$ coupling. The spectra were identical, in all other respects, with that of the corresponding 4'(protio) pyrimidine nucleosides. These observations have led us to identify III_{a-c} as the β-D-ribofuranosyl isomer of 4'-deuterated pyrimidine nucleosides. The yield of III_{a-c} was ~20%. An analysis of the NMR spectra and mass spectra indicated 98% deuterium labeling at $C_{4'}$ position.

The NMR spectra of the slow moving components also showed the absence of $C_{4'}$ proton in the region δ 4.37-4.56 ppm and the disappearance of $J_{3',4'}$ and $J_{4',5'}$ coupling. In order to confirm V_{a-c} as the α-L-Lyxofuranosyl isomer of 4'-deuterated pyrimidine nucleosides, we synthesized 4'(protio)-α-L-

SCHEME



I^{10}_{a-b}

I^{11}_{c}

II_{a-c}, III_{a-c}

II_{a-c}, IV_{a-c}, R=H

III_{a-c}, V_{a-c}, R=D

IV_{a-c}, V_{a-c}

I_a-V_a, R_1=CH_3, R_2=OAc, R_3=H

I_b-V_b, R_1=H, R_2R_3=$\overset{O}{O}\!>\!CMe_2$

I_c-V_c, R_1=F, R_2R_3=$\overset{O}{O}\!>\!CMe_2$

Table. ^{1}H N.M.R. Data of II-V_{a-c}. Chemical Shifts δ (ppm) and Coupling Constant J (Hz)[a,b].

Compound	C_1'H ($J_{1',2'}$)	C_2'H ($J_{2',3'}$)	C_3'H ($J_{3',4'}$)	C_4'H ($J_{4',5'}$)	C_5'H ($J_{5',5''}$)	C_5H ($J_{5,6}$)	C_6H	Others
IIa	6.36(t) (6.4)	2.1(m) (4.2)	4.34(d) (<0.5)	3.91(m) (5.4)	3.75(m) (10)	(1.2)	7.8(brs)	1.9(s,3,C_5Me), 2.1(s,3,3'-OAc)
IIIa	6.33(t) (6.5)	2.1(m) (4.1)	4.33(d)		3.76(m) (9)	(1.2)	7.7(brs)	1.9(s,3,C_5Me), 2.1(s,3,3'-OAc)
IVa	6.30(t) (6.4)	2.1(m) (4.5)	4.33(d) (2)	4.37(m) (3)	3.76(m) b		7.4(s)	1.88(s,3,C_5Me), 2.12(s,3,3'-OAc)
Va	6.28(t) (6.5)	2.1(m) (5)	4.45(m)		3.75(m) (11)		7.4(s)	1.9(s,3,C_5Me), 2.1(s,3,3'-OAc)
IIb	5.9(d) (2.5)	4.9(dd) (7)	4.85(d) (<0.5)	4.18(m) (4.1)	3.78(m) (12)	5.6(d) (8)	7.82(d)	1.38 and 1.55 (s,3,CMe_2)
IIIb	5.9(d) (2.5)	4.9(dd) (7)	4.85(d)		3.77(m) (12)	5.6(d) (8)	7.82(d)	1.38 and 1.55 (s,3,CMe_2)
IVb	5.5(brs) (2)	5.23(dd) (7)	5.0(d) (2.5)	4.48(m) (6)	3.5(m) (13)	5.6(d) (8)	7.65(d)	1.31 and 1.58 (s,3,CMe_2)
Vb	5.5(brs) (2)	5.25(dd) (7)	5.0(d)		3.5(m) (12)	5.6(d)	7.65(d)	1.30 and 1.5 (s,3,CMe_2)
IIc	5.72(d) (2.4)	4.92(m) (6.3)	4.92(m) (<0.5)	4.34(m) (3.8)	4.0(m) (11)	(8)	7.68(d)	1.38 and 1.56 (s,3,CMe_2)
IIIc	5.7(d) (2)	4.9(m) (6.3)	4.85(m)		4.02(m) (11)	(8)	7.6(d)	1.37 and 1.56 (s,3,CMe_2)
IVc	5.4(brs) (2)	5.26(dd) (6)	5.05(d) (2)	4.56(m) (4.7)	3.92(m) (14)	(7.5)	8.02(d)	1.36 and 1.65 (s,3,CMe_2)
Vc	5.4(brs) (2)	5.2(dd) (6)	5.0(d)		3.9(m) (14)	(7.5)	8.02(d)	1.36 and 1.65 (s,3,CMe_2)

[a]Solvent: Acetone-d_6; [b]Unresolved.

lyxofuranosyl pyrimidine nucleosides by treating 4',5'-unsaturated pyrimidine[11,12] nucleosides with 1M solution of B_2H_6 in tetrahydrofuran, followed by alkaline hydrogen peroxide oxidation, and usual workup. An examination of the table shows that the $C_{4'}$, proton in the β-D-ribofuranosyl isomers (II_{a-c}) is at a higher field than in the corresponding lyxofuranosyl isomers (IV_{a-c}). Further, in β-D-ribofuranosyl isomers, the $J_{3',4'}$ coupling is less than 0.5 Hz while in the α-L-lyxofuranosyl isomers, it is 2-2.5 Hz. These observations are in agreement with the reported values for α-L-rhamnoside derivatives,[7] and thus, lead us to assign V_{a-c} as α-L-lyxofuranosyl isomers (yield ~50%). Thus, the hydroboration-oxidation reaction of an unsaturated nucleoside allows quantative introduction of deuterium labeling at $C_{4'}$ position of the uridine and its derivatives. The technique should be adaptable for tritium labeling at the $C_{4'}$ position of the pyrimidine nucleosides for the elucidation of the mechanism of bleomycin as well as other DNA-interacting molecules.

MATERIALS AND METHODS

Thin layer chromatography was performed on Kieselgel F 60_{254} (Merck) in chloroform:methanol (9:1). Proton magnetic resonance spectra were obtained using either a Brüker WM 400 or Brüker 500 MHz spectrometer. Sodium borodeuteride (99 atom % D) was obtained from Merck, Sharp and Dohme. The elemental analysis (C,H,N) were within ± 0.4% of the theoretical values. The purity of the compounds was checked by HPLC using 10% methanol in water.

GENERAL PROCEDURE

To a stirred solution of 4',5'-unsaturated pyrimidine[10,11] nucleoside (1_{a-c}) (5 mmol) in anhydrous tetrahydrofuran (15 ml) at -40°C in an atmosphere of dry nitrogen was added 1 M solution of B_2D_6 in tetrahydrofuran[10] (4 ml) dropwise over 30 min. The solution was stirred at this temperature for 1 hr and the mixture was allowed to warm at room temperature. The excess of B_2D_6

was decomposed by dropwise addition of water until the moderate effervescence subsided. To the mixture, 2M sodium hydroxide (4 ml) was added, followed by dropwise addition of 30% hydrogen peroxide (3 ml). The mixture was allowed to stir at room temperature for 2 hrs and the solvents were evaporated to dryness. The residue was partitioned between saturated sodium chloride (25 ml) and ethyl acetate (50 ml). The aqueous residue was extracted with ethyl acetate (5 X 50 ml). The combined ethyl acetate extraction was washed with water, dried over sodium sulfate, and evaporated to dryness. Preparative layer chromatography (chloroform:methanol; 9:1) of the residue afforded 4'-deuterated pyrimidine nucleosides (III_{a-c}) in ~20% yield and (V_{a-c}) in ~50% yield.

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