AN IMPROVED METHOD FOR [5′-^{2}H]-LABELLING 3′-*O*-ACETYLTHYMIDINE

John Orban* and Brian R. Reid
Department of Chemistry
University of Washington
Seattle, Washington 98195

SUMMARY

3′-*O*-Acetylthymidine (1) was converted to the β-acetoxy aldehyde (3) using a modified Swern oxidation procedure. The crude product was reduced with sodium borodeuteride to give, after chromatography, [5′-^{2}H]-3′-*O*-acetylthymidine (4) in 68% overall yield from 1. The extent of deuterium incorporation was >95% as judged by ^{1}H NMR spectroscopy.

Key Words: [5′-^{2}H]-3′-*O*-Acetylthymidine, deuterium labelling, DNA.

INTRODUCTION

We are interested in the solid-state deuterium NMR analysis of specifically ^{2}H-labelled DNA sequences. Valuable information about the time-scale of internal motion in defined regions of biopolymers can be obtained from the deuterium quadrupolar coupling constant, lineshape analysis and simulation (1). In order to study DNA backbone motion an efficient method was needed to label the C5′-position of nucleosides. Using the solid-phase phosphite triester method (2), [5′-^{2}H]-labelled nucleosides can be incorporated into DNA sequences of interest *via* the corresponding dimethoxytrityl phosphoramidites (3). Earlier work in our laboratories (4) using a Moffatt-Pfitzner oxidation (5) of 3′-*O*-acetylthymidine (1) followed by sodium borodeuteride reduction provided only a 31% overall yield of [5′-^{2}H]-thymidine (5) (6). A higher yielding procedure was required to obtain sufficient quantities of [5′-^{2}H]-labelled phosphoramidite for dodecadeoxynucleotide synthesis at the 20 micromole level. This paper describes the synthesis of [5′-^{2}H]-3′-*O*-acetylthymidine (4) in 68% overall yield from unlabelled 1 using a modified Swern oxidation (7) followed by sodium borodeuteride reduction.

RESULTS AND DISCUSSION

3′-*O*-Acetylthymidine (1) was oxidized to the aldehyde (3) using DMSO activated with oxalyl chloride at -78°C followed by treatment of the intermediate alkoxysulfonium

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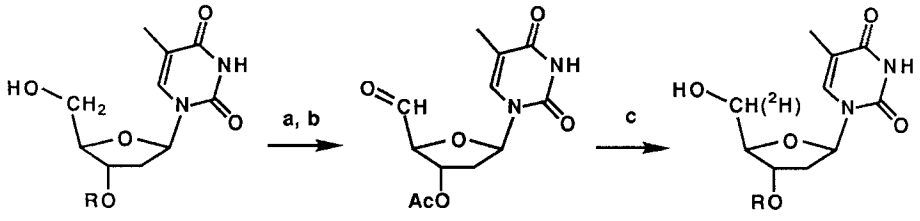
salt with triethylamine (7). After oxidation was complete, trifluoroacetic acid was added to the reaction mixture at -78°C to ensure that no significant base-promoted elimination of the acetate group [*cf.* (8)] from the β-acetoxy aldehyde (3) would occur upon warming to room temperature. Sodium borodeuteride reduction of the crude residue followed by chromatography on silica gel gave the [5′-^{2}H]-labelled product 4 in 68% overall yield from 1. The ^{1}H NMR spectrum of 4 showed a loss of intensity for the overlapped H5′ and H5″ signals compared with 1, corresponding to >95% isotopic purity.



1 R=Ac
2 R=H

3

4 R=Ac
5 R=H

a. DMSO, $(COCl)_2$, NEt_3, -78°C b. CF_3CO_2H, -78°C c. NaB^2H_4, 25°C

The Swern oxidation is faster than the Moffatt-Pfitzner approach and requires much smaller quantities of DMSO so the crude β-acetoxy aldehyde (3) is not subjected to any prolonged heating under vacuum. It is interesting to note the absence of any acetate hydrolysis during the reduction step. This contrasts with earlier observations (5) where borohydride reduction of 3 in water gave almost complete conversion to thymidine (2). The triethylammonium trifluoroacetate formed during the oxidation step may serve to buffer the reduction medium [*cf.* (9)] and prevent acetate hydrolysis. The acetate (4) is more soluble in organic solvents than [5′-^{2}H]-thymidine (5) and is therefore more readily isolated and purified.

This procedure more than doubles the isolated yield of pure [5′-^{2}H]-labelled material compared with the Moffatt-Pfitzner oxidation/ borodeuteride reduction method (4). Incorporation of 4 into a number of defined DNA sequences is in progress.

EXPERIMENTAL

Oxalyl chloride, triethylamine, and trifluoroacetic acid were all purchased from Aldrich Chemical Company. Dichloromethane and DMSO were refluxed over calcium hydride for 2-4 hours then distilled under argon and stored over activated 4A molecular sieves. Reagent grade ethyl acetate (Baker) was used. 3′-*O*-Acetylthymidine was obtained from Sigma Chemical Company. Sodium borodeuteride (98 atom % D) was purchased from Cambridge Isotope Laboratories. Column chromatography was carried out using Kieselgel 60 (230-400 mesh) obtained from E.M. Science. Glass-backed silica gel plates (Merck) were

used for TLC. NMR spectra were recorded on a Bruker WM-500 spectrometer at 37°C. Chemical shifts are referenced to the internal methyl signal of d_4-methanol at 3.35 ppm.

3'-*O*-Acetyl-5'-deoxy-5'-oxothymidine (3). A solution of DMSO (0.461 mL, 6.5 mmol, 2.0 eq.) in dichloromethane (1.6 mL) was added to a stirred solution of oxalyl chloride (0.312 mL, 3.58 mmol, 1.1 eq.) in dichloromethane (8.1 mL) at -78°C (dry ice/ acetone) under nitrogen. After 3 minutes, a solution of 3'-*O*-acetylthymidine (1) (0.924 g, 3.25 mmol) in DMSO/ dichloromethane (1.2 mL/ 3.7 mL) was added over a 5 minute period. Stirring was continued for 15 minutes then triethylamine (2.26 mL, 16.25 mmol, 5.0 eq.) was added and the reaction mixture was stirred for another 5 minutes. Trifluoroacetic acid (1.0 mL, 13.0 mmol, 4.0 eq.) was added and the mixture was allowed to warm to 25°C. Evaporation *in vacuo* afforded a mixture of the aldehyde (3) and triethylammonium trifluoroacetate as a pale yellow solid plus some residual DMSO. This material was placed under high vacuum for 18 hours at 25°C. Analysis by TLC (dichloromethane/ methanol, 9/1, v/v) showed the aldehyde (3) (R_f 0.49) as the major UV-active component with no residual alcohol (1) (R_f 0.44). ^{1}H NMR (500 MHz, d_4-methanol) δ 9.31 (s, aldehyde H). The mixture was not purified but used directly in the reduction step.

[5'-^{2}H]-3'-*O*-Acetylthymidine (4). The crude residue from the oxidation procedure was suspended in water (30 mL) and cooled in an ice bath under nitrogen. Sodium borodeuteride (0.480 g, 11.5 mmol) was added in portions over a 5 minute period and the mixture was allowed to warm to 25°C with stirring. TLC analysis after 45 minutes showed total consumption of the aldehyde (3). Acetone (2 mL) was added and, after 3 minutes, the solution was adjusted to pH~6 with 1M HCl. The mixture was frozen and placed under high vacuum for 24 hours. The sample was then partitioned between ethyl acetate (~200 mL) and water (~7 mL) and extracted. The ethyl acetate layer was dried (Na_2SO_4), filtered, and concentrated to give a pale yellow oil. Chromatography on a silica gel column (3 cm diameter x 22 cm length) using ethyl acetate gave 0.481 g of pure [5'-^{2}H]-3'-*O*-acetylthymidine (4) and 0.260 g of a mixture of 4 and an unidentified minor by-product. Rechromatography of the mixture using dichloromethane/methanol (92/8, v/v) provided a further 0.150 g of pure 4 (total 0.631 g) for a 68% overall yield from 1. TLC analysis (dichloromethane/ methanol, 9/1, v/v) showed that the deuterium labelled product (4) and unlabelled starting material (1) had identical R_f values (0.44) and that 4 was homogeneous. ^{1}H NMR (500 MHz, d_4-methanol) δ 7.86 (br s, 1H, $W_{\frac{1}{2}}$~4 Hz, H6), 6.31 (dd, 1H, J=6.1 and 7.3 Hz, H1'), 5.35 (m, 1H, $W_{\frac{1}{2}}$~10 Hz, H3'), 4.11(br s, 1H, $W_{\frac{1}{2}}$~7 Hz, H4'), 3.83 (br s, 1H, $W_{\frac{1}{2}}$~8 Hz, H5' and H5''), 2.38 (m, 2H, H2' and H2''), 2.12 (s, 3H, OAc), 1.93 (s, 3H, CH_3).

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