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SYNTHESIS OF TRITIUM-LABELLED 9-DEAZAINOSINE

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SUMMARY

The synthesis of labelled 9-deazainosine (1a,b) from the fully blocked 9-deazainosine (2) is achieved in six steps by selective detritylation, oxidation of the C-5' hydroxyl group, followed by purification via its N,N'-diphenylimidazolidine derivative, deprotection to obtain the 5'-aldehyde, borotritide reduction, and deisopropylidenation to give the labelled nucleoside. The sequence is of general utility in labelling nucleosides at the C-5' position for biochemical studies.

Key words: 9-Deazainosine, tritiated nucleoside, C-nucleoside, borotritide reduction.

INTRODUCTION

9-Deazainosine (1) [1, 7-(β-D-ribofuranosyl)-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine, "9-DINO"] has shown promising growth-inhibitory activities against Leishmania (2,3), Trypanosoma (3,4) and Pneumocystis carinii (5), while exhibiting low toxicity against mammalian cell lines (2). Metabolic studies of 9-DINO in African trypanosomes (4) indicated that it is first phosphorylated to the corresponding analogue of inosine monophosphate and then, through amination and further phosphorylation, converted to the corresponding analogues of adenosine mono, di, and triphosphates. Related studies have also shown that Leishmania donovani metabolizes 9-DINO to its adenosine and guanosine triphosphate analogues (2). In contrast, mammalian mouse L-cells convert 9-

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DINO to its inosine monophosphate analogue only to a small degree (2). This differential metabolism between protozoal and mammalian cell lines might explain the observed antiprotozoal chemotherapeutic properties of 9-DINO in animal models (5,6). For further studies of these metabolic pathways, we needed a radiolabelled isotopomer of 9-DINO, labelled at a specific position. We report herein a mild and efficient process for introducing a high specific activity tritium label at C-5'. The method has general applicability to the labelling of nucleosides at that position.

RESULTS AND DISCUSSION

Fully blocked 9-Deazainosine (2) synthesized earlier in our laboratories (1) was used as a convenient synthetic precursor (Figure 1). It was detritylated selectively by treatment with two equivalents of p-toluenesulfonic acid monohydrate ($TsOH.H_2O$) in dichloromethane at 0°C to give 3 in near quantitative yields. The acetonide protecting group stayed intact under these mild acidic conditions due to its relatively low reactivity (7). The molecular sieve-assisted pyridinium dichromate oxidation reaction (8) which has been successfully used in oxidizing the 5'-OH of AZT (9) (3'-azido-3'-deoxythymidine) was inapplicable to 3 due to its insolubility in dichloromethane. However, 3 was easily oxidized by treatment with dimethylsulfoxide (DMSO) and 1,3-dicyclohexyl carbodiimide (DCC) in the presence of dichloroacetic acid (10). Because of the difficulty in isolating aldehyde 4 directly from the reaction mixture in pure form, it was found more convenient to convert it first to its crystalline 1,3-diphenylimidazolidine derivative 5, which was isolated in 48% yield (10,11). Treatment of this derivative with Biorad AG 50W-X8 (H^+) resin in aqueous tetrahydrofuran at 22°C regenerated the aldehyde as its stable hydrate 6. Further purification of 6 by

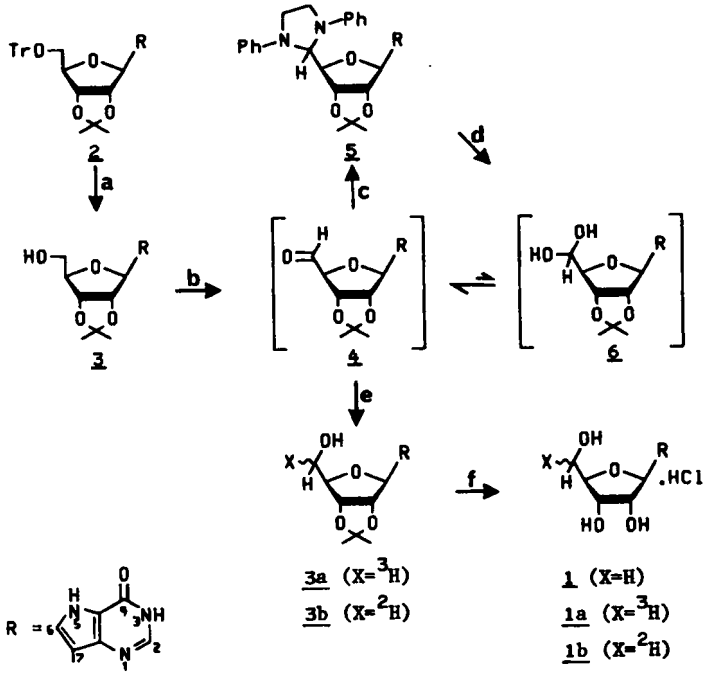

Figure 1. Synthesis of labelled 9-Deazainosine.
Reagents: (a) TSOH.H_2O, CH_2Cl_2, 0°C; (b) DCC, DMSO, $CHCl_2COOH$, 5° -> 22°C; (c) N,N'-Diphenylethylenediamine, MeOH, 22°C; (d) AG 50W-X8 (H^+), THF-water, 22°C; (e) [^{3}H]-$NaBH_4$ and/or NaB^2H_4, EtOH, 22°C; (f) 6% HCl/MeOH, 22°C.

preparative TLC was necessary in order to remove side products which interfered with the reduction step. Initial attempts to generate free (anhydrous) aldehyde 4 by azeotropic distillation with benzene in a Dean-Stark apparatus, as described for the dehydration of analogous nucleosidic aldehyde hydrates (10), led to partial decomposition and formation of polar side-products. However, multiple evaporations in vacuo of a suspension of 6 in dry benzene on a rotary evaporator gave the free aldehyde 4 in 52% yield. Preliminary reduction experiments with NaB^2H_4 showed that both aldehyde 4 and alcohol 3b had similar mobilities on normal and reverse phase TLC in various solvent systems (the charring behavior, however, was different; 4 charred to a blue

coloration, while 3b gave a brown spot). This chromatographic behavior precluded the separation of alcohol 3b from unreacted aldehyde 4 and, hence the use of the more conventional procedure where the unreacted aldehyde is separated from the product by chromatography. Therefore, after the reduction of 4 to 3a by [^{3}H]-$NaBH_4$ (54 mCi, specific activity 20 Ci/mmol) in ethanol at 22°C, it was necessary to add excess NaB^2H_4 (or $NaBH_4$) in order to reduce all of the unreacted aldehyde into alcohol. Specific radioactivity of 3(a,b) could thus be controlled by varying the ratio of aldehyde to borotritide. After purification by preparative TLC, 3(a,b) was treated with 6% methanolic hydrogen chloride at 22°C, which removed the isopropylidene group and afforded the labelled 9-deazainosine 1(a,b) as a white crystalline hydrochloride salt in 59% chemical yield (from 4), having a specific activity of 0.6 Ci/mmol. The ^{1}H-NMR (300 MHz) of 1(a,b) in D_2O exhibited two broad doublets (due to the presence of two 5'-epimers) in the region of H-5' which integrated for one proton only. The rest of the spectrum was similar to that of 1. In contrast to the spectrum of 1(a,b), that of 1 displayed a multiplet for H-5' which integrated for two protons.

EXPERIMENTAL

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Freshly opened bottles of anhydrous dimethyl sulfoxide (Aldrich) and absolute ethanol (Florida Distillers Company) were flushed with nitrogen and stored over molecular sieves (3Å) prior to use. Biorad AG 50W-X8 (H^+) resin was washed and air-dried before use. [^{3}H]-Sodium borohydride (20 Ci/mmol) was purchased from Research Products International Corporation (IL). Sodium borodeuteride (isotopic purity >98 Atom % ^{2}H) was purchased from MSD Isotopes (St. Louis, MO). Thin-layer chromatography was performed on 250 μm

silica gel GF plates (Analtech, Inc.), and the substances were visualized with a Mineralight lamp (short wave, 254 nm) followed by staining with I_2 vapors and/or by spraying with 10% ethanolic sulfuric acid and charring. Preparative TLC was performed on 1,000 μm, 20 x 20 silica gel plates (Uniplates by Analtech, Inc.). Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh ASTM). The ^{1}H-NMR spectra were recorded on a JEOL FX-90Q (90 MHz) and/or Nicolet 300 MHz NMR spectrometer and chemical shifts are reported in ppm downfield from TMS which was used as the internal standard. Microanalyses were performed by M.H.W. Laboratories, Phoenix, AZ. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Radioactive samples were counted in Fisher Scintiverse II fluid on an LKB Rackbeta Universal 1218 instrument operating at 50-60% efficiency for ^{3}H. All values were quench-corrected automatically by the external standard ratio method.

7-(2,3-O-Isopropylidene-β-D-ribofuranosyl)-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine 3.

To a stirred suspension of 2 (3.00 g, 5.46 mmol) in dichloromethane (100 mL) was added p-toluenesulfonic acid monohydrate (2.07 g, 10.92 mmol) at 0°C. Clarification of the reaction mixture occurred within 45 minutes and was immediately followed by precipitation of the p-toluenesulfonate salt of 3. Aqueous $NaHCO_3$ solution (1.83 g, 100 mL) was then added and the reaction mixture stirred vigorously at room temperature, until the solid had dissolved. The two layers were separated and the aqueous phase was extracted exhaustively with MeOH-CH_2Cl_2 (1:5, 20 x 75 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to ca. 50 mL. The solution was adsorbed on silica gel which was then dried by evaporation. Flash chromatography using MeOH-CH_2Cl_2 (1:9, 500 mL) followed by MeOH-CH_2Cl_2 (1:5, 1,000 mL) as successive eluents afforded 3 as a white crystalline solid (1.67 g,

near quantitative), mp 250-252°C. ^{1}H NMR (CD_3CN): δ 1.31 (s, 3H, CH_3), 1.56 (s, 3H, CH_3) 3.66 (m, 2H, H-5'), 4.20 (m, 1H, H-4'), 4.77 - 4.97 (m, 3H, H-1', H-2', H-3'), 5.17 (br. s., 1H, OH, D_2O exch.), 7.32 (d, 1H, H-6, $J_{H-6,NH}$ = 3.3 Hz, s after D_2O exchange), 7.78 (s, 1H, H-2), 10.05, 11.65 (2 br. s., 2NHs, D_2O exch.).

Anal. Calcd for $C_{14}H_{17}N_3O_5$: C, 54.72; H, 5.53; N, 13.68. Found: C, 54.58; H, 5.50; N, 13.53.

7-[5-Deoxy-2,3-O-isopropylidene-5,5-(N,N'-diphenylethylene-diamino)-β-D-ribofuranosyl]-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine 5.

Dichloroacetic acid (0.34 mL, 41.5 mmol) was added dropwise to a stirred solution of 3 (1.70 g, 5.53 mmol) and 1,3 dicyclohexyl-carbodiimide (3.63 g, 22.12 mmol) in anhydrous DMSO (10 mL) with ice cooling. The mixture was then stirred at 22°C for 90 minutes. After completion of the reaction, a solution of oxalic acid dihydrate (1.40 g, 11.06 mmol) in methanol (6 mL) was slowly added. After 20 minutes of stirring, the mixture was filtered and the crystalline 1,3-dicyclohexylurea was washed with cold methanol.

To the combined filtrates was added N,N'-diphenylethylene diamine (1.41 g, 6.64 mmol), and the mixture was stirred for 1 hour at 22°C. The reaction mixture was concentrated and partitioned between water and dichloromethane. The organic phase containing 5 was washed with brine, dried (Na_2SO_4) and evaporated. The crude product thus obtained was triturated with chloroform and some insoluble material was removed by filtration. Flash chromatography of the filtrate, using MeOH-CH_2Cl_2 (2:98) as the eluent, afforded 5 (1.34 g, 48%) as an off-white solid. An analytical sample was obtained by recrystallization of 5 from hot ethanol, mp 201-203° (dec.) ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.45-4.06 (m, 4H, 2NCH_2s), 4.44 (m, 1H, H-4'), 4.81 (m, 2H, H-2', H-3'), 5.14 (d, 1H, H-5', $J_{4',5'}$ = 3.5 Hz), 5.66 (s, 1H, H-1'), 6.74-7.25 (m, 11H, 2C_6H_5s and H-6), 8.13 (br. s., 1H, H-2), 10.78, 11.88 (2 br.s., 2H, 2NHs, D_2O exch.).

Anal. Calcd for $C_{28}H_{29}N_5O_4$: C, 67.33; H, 5.81; N, 14.02. Found: C, 67.21; H, 5.86; N, 13.85.

"Cold" Conversion of 5 to 3b.

To a stirred solution of 5 (40 mg, 0.08 mmol) in tetrahydrofuran:water (1:1 v/v, 8 mL) was added cation exchange resin Biorad AG 50W-X8 (H^+) (80 mg). After 1 h, the resin was removed by filtration and washed with THF (4 x 1 mL). The combined filtrates were concentrated and then co-evaporated with methanol (2 x 20 mL). The residue was subjected to preparative TLC (developed twice) using MeOH-CH_2Cl_2 (1:9) as the eluent. The desired band was extracted with CH_3OH-CH_2Cl_2 (1:3), filtered and concentrated in vacuo to give aldehyde hydrate 6. It was suspended in dry benzene and evaporated in vacuo (5 x 20 mL) at 42°C to give free aldehyde 4 (13 mg, 52%) which was chromatographically homogeneous as judged by TLC (R_f 0.56 in 10% MeOH-CH_2Cl_2; blue coloration upon charring).

To a solution of aldehyde 4 in ethanol (2 mL) was added excess NaB^2H_4 (2.2 mg, 0.05 mmol). The reaction was complete in 30 min as shown by TLC (R_f 0.54 in 10% MeOH-CH_2Cl_2, brown coloration upon charring). Acetone (1 mL) was added and the stirring was continued for another 15 min. Aqueous acetic acid (0.5 M, 250 µL) was then added and the reaction mixture was evaporated to dryness. The residue was dissolved in a minimum amount of MeOH-CH_2Cl_2 (1:9) and subjected to preparative TLC (developed twice) using MeOH-CH_2Cl_2 (1:9) as eluent. The desired band was extracted with MeOH-CH_2Cl_2 (1:3), filtered, and the filtrate evaporated in vacuo to afford 3b (9 mg, 66%) as a white crystalline solid. Its UV and chromatographic properties were identical to those of 3.

Conversion of 3b to 1b.

A suspension of 3b (9 mg, 0.029 mmol) in 6% methanolic hydrogen chloride (2 mL) was stirred at 22°C for 1 h. The reaction mixture was then concentrated to dryness in vacuo. The residue was

suspended in CH_2Cl_2 and evaporated in vacuo (3 x 10 mL), which removed residual HCl and afforded 1b (8 mg, 90%) as a white crystalline solid. Its UV and chromatographic properties were identical to those of an authentic sample of 9-deazainosine 1.

Tritiation of Aldehyde 4.

A solution of 4 (30.2 mg, 0.099 mmol) (obtained as described above in the conversion of 5 to 3b) in dry EtOH (4 mL) was added to an ampoule containing a stirring bar and 54.05 mCi of [^{3}H]-$NaBH_4$ (specific activity 20.0 Ci/mmol). After stirring for 1 h, excess $NaBD_4$ (2.8 mg, 0.067 mmol) was added to reduce any unreacted aldehyde. Acetone (2 mL) was then added, and stirring was continued for another 15 min. Aqueous acetic acid (0.5 M, 500 µL) was added and the reaction mixture was transferred to a 50 mL round bottom flask. The ampoule was rinsed several times with EtOH and the washings were added to the reaction mixture. Solvents were removed in vacuo and the residue was dissolved in a minimum amount of MeOH - CH_2Cl_2 (1:9) and subjected to preparative TLC (two plates, developed twice) using MeOH - CH_2Cl_2 (1:9) as a developing agent. Working inside a glove-bag, the desired bands were scraped off and transferred into a flask containing MeOH - CH_2Cl_2 (1:3, 75 mL). (Using the glove-bag allowed effective containment of silica gel dust. The bag was deflated by bubbling the air out through water.) The slurry was stirred for 0.5 h and then allowed to filter through well-packed glass wool in a wide-bore tapered glass tube. The filtrate was concentrated in vacuo to afford 3(a,b) (20.2 mg, 66%) as a white crystalline solid.

Conversion of labelled 3(a,b) to 1(a,b).

A suspension of 3(a,b) (20.2 mg, 0.06 mmol) in 6% methanolic hydrogen chloride (4 mL) was stirred at 22°C and the reaction was followed by TLC (MeOH - CH_2Cl_2, 1:4). After complete deisopropylidenation (≃ 1 h), volatiles were removed in vacuo. The

residue was suspended in CH_2Cl_2 and evaporated in vacuo (5 x 20 mL), which removed residual HCl and afforded 1(a,b) (17.8 mg, 59% from 4, specific activity 0.6 Ci/mmol) as a white crystalline solid. It was stored in a 1:1 mixture of ethanol and degassed water at 4°C.

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