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A CONVENIENT ONE-POT SYNTHESIS OF 2,5-ANHYDRO-3,4,6-TRI-O-BENZOYL-D-[1-^{14}C]ALLONONITRILE, AND ITS CONVERSION TO METHYL 2-β-D-RIBOFURANOSYL-4-[2-^{14}C]SELENAZOLECARBOXYLATE

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SUMMARY

Reaction of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (3) with [^{14}C]cyanotrimethylsilane, generated *in situ* by reaction of potassium [^{14}C]cyanide and chlorotrimethylsilane in the presence of sodium iodide and 2,6-lutidine, gave 2,5-anhydro-3,4,6-tri-O-benzoyl-D-[1-^{14}C]allononitrile (4). Sequential reaction of the latter with hydrogen selenide, ethyl bromopyruvate, and methanolic sodium methoxide gave methyl β-D-ribofuranosyl-4-[2-^{14}C]selenazolcarboxylate (7), penultimate precursor to [2-^{14}C]CI-935, or 2-β-D-ribofuranosyl-4-[2-^{14}C]selenazolecarboxamide (8).

Key Words: [^{14}C]cyanotrimethylsilane, 2,5-anhydro-3,4,6-tri-O-benzoyl-D-[1-^{14}C] allononitrile, methyl 2-β-D-ribofuranosyl-4-[2-^{14}C]selenazolecarboxylate, 2-β-D-ribofuranosyl-4-[2-^{14}C]selenazolecarboxamide, anticancer, antiviral.

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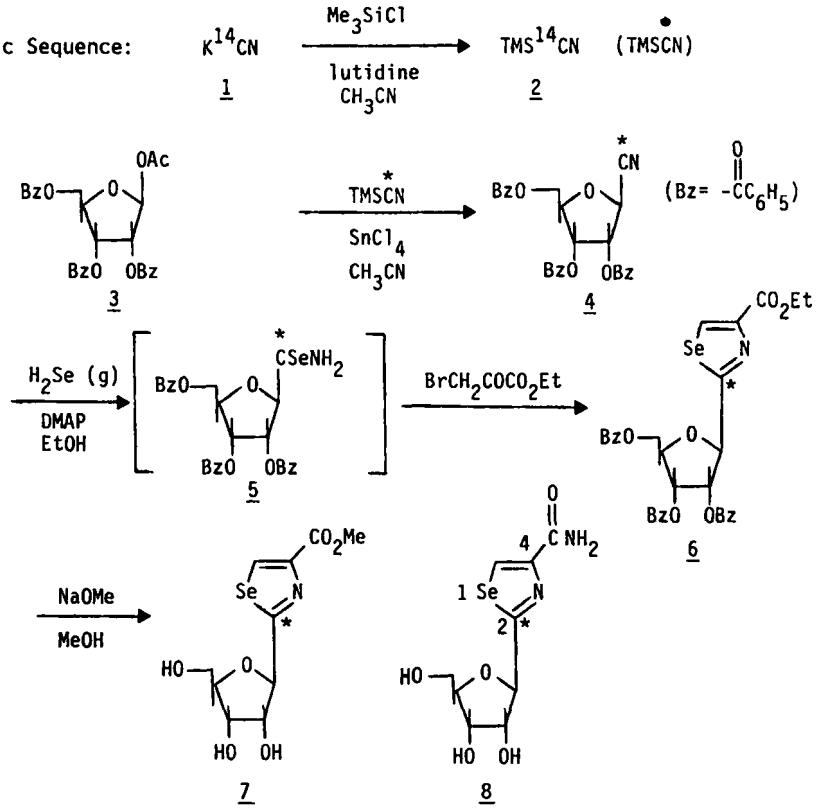
INTRODUCTION

CI-935, or 2-β-D-ribofuranosyl-4-selenazolecarboxamide (8),[1] has shown pronounced antitumor activity in animals and broad spectrum antiviral activity *in vivo*. Five- to ten-fold more potent in antitumor activity than its sulfur analog tiazofurin,[2] CI-935 presumably acts in an analogous mode by the inhibition of inosine monophosphate dehydrogenase (IMPD), via the intracellular metabolite 2-β-D-ribofuranosyl-4-selenazolecarboxamide adenosine dinucleotide.[3]

In the evaluation of CI-935 as an antitumor agent, a carbon-14 labelled form of the drug was required for pharmacokinetics and metabolism studies. This paper describes the synthesis of methyl 2-β-D-ribofuranosyl-4-[2-^{14}C]selenazole-carboxylate (7), the penultimate precursor to [2-^{14}C]CI-935 (8).[4]

Synthetic Sequence:



RESULTS AND DISCUSSION

Crucial to the synthesis was a novel, convenient one-pot conversion of the carbon-14 label from potassium [^{14}C]cyanide to the D-anhydroallononitrile (4), which we developed as an adaptation of the O-trimethylsilylcyanohydrin synthesis of Duboudin, et al.[5] The synthesis of [^{14}C]cyanotrimethylsilane in pure form from potassium [^{14}C]cyanide,[6] silver [^{14}C]cyanide,[7,8] or hydrogen [^{14}C]cyanide[7,9] by adaptation of conventional procedures would have been laborious or proceeded in low yield.

In unlabelled model runs, cyanotrimethylsilane was generated by the reaction of equimolar amounts of chlorotrimethylsilane and potassium cyanide in acetonitrile in the presence of a tertiary amine base and catalytic amount of sodium iodide. The rate of reaction, as monitored by nmr spectroscopy, was slow and base-dependent in approximate decreasing order of 2,6-lutidine, pyridine, 4-dimethylaminopyridine, and triethylamine, the half-time using pyridine being approximately 20 hours; however, all reactions eventually proceeded to completion after a few days. Since attempted isolation by distillation gave only trace amount of contaminated product, the cyanotrimethylsilane was used *in situ* to react with 1-O-acetyl-2,3,5-tri-O-benzoyl- β-D-ribofuranose in the presence of stannic chloride, giving 2,5-anhydro-3,4,6-tri-O-benzoyl-D-allononitrile (4) in 41% overall yield after chromatography. The procedure was similar to that using pure cyanotrimethylsilane as reported by Cook and McNamara,[4] except that a longer reaction time was found necessary.

The method was then utilized in the labelled synthesis giving the labelled nitrile 4 in 38% overall yield from potassium [^{14}C]cyanide. The [^{14}C]cyanosugar was then allowed to react with a measured amount of hydrogen selenide in a calibrated vacuum manifold, with careful tlc monitoring, since excess hydrogen selenide or reaction time would both be detrimental. However, approximately 1.6 equivalents of hydrogen selenide was found necessary; the use of 1.0 equivalent, recommended for large-scale synthesis,[4] resulted in incomplete reaction. The reactive and unstable intermediate 5 was promptly treated with ethyl bromopyruvate at the proper moment to give crude 6. The latter was then subjected to saponification and ester exchange with methanolic sodium methoxide to give 7. After chromatography and

fractional crystallization to remove a major uv-transparent radioactive contaminant, purified 7 was obtained in overall chemical yield of 9.7% and radiochemical yield of 9.4%, based on the potassium [^{14}C]cyanide used.

EXPERIMENTAL

Potassium [^{14}C]cyanide was purchased from New England Nuclear. Radioactivity was determined in Packard Tri-Carb 4530 liquid scintillation spectrometer, using Beckman Ready-Solv MP as the counting medium. Tlc plates, E. Merck silica gel 60 F-254, were scanned on a Berthold LB2832 Automatic Tlc Linear Analyzer. Column chromatography was performed with E. Merck silica gel, 230-400 mesh.

2,5-Anhydro-3,4,6-tri-O-benzoyl-D-[1-^{14}C]allononitrile (4)

A mixture of 329 mg of 96% potassium [^{14}C]cyanide (246 mci, 4.85 mmol, dried at 110°C at 2 mm Hg for 20 hrs), 127 mg (0.85 mmol) of sodium iodide (dried at 125°C *in vacuo* for 5 hrs), 550 mg (5.0 mmol) of 98% chlorotrimethylsilane, 137 mg (1.27 mmol) of 2,6-lutidine, and 2.1 g of acetonitrile was stirred at room temperature. Progress of the reaction was determined by nmr on a few drops of the solution in deuteriochloroform (chlorotrimethylsilane at δ 0.43, cyanotrimethylsilane at δ 0.38). Analysis of a similar run using unlabelled potassium cyanide showed that the reaction was approximately 60% complete after 19 hrs and complete after four days. The reaction mixture was then centrifuged, and the separated solid was washed twice with 0.6-ml portions of acetonitrile. The combined supernatant and washings were added to a mixture of 2.39 g (4.74 mmol) of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in 5.0 ml of acetonitrile. While the resulting mixture was stirred in a water bath at 20°C, 0.45 ml of stannic chloride was added. After 3.5 hrs the mixture was added with stirring to 33 ml of saturated aqueous sodium bicarbonate, and the aqueous mixture was extracted successively with 50-, 20-, and 20-ml portions of methylene chloride. The extracts were dried with anhydrous magnesium sulfate, filtered and evaporated to a residue. The residue, dissolved in hexane-chloroform (1:1), was chromatographed over 42 g of silica gel packed in hexane, and the effluent fractions were analyzed by tlc (1% methanol in chloroform, or two developments in chloroform). Elution with chloroform-hexane (2:1) and evaporation of the appropriate fractions gave 668 mg (29%) of product 4,

followed by 543 mg of a mixture of 4 and starting material 3. The latter was rechromatographed over 10.5 g of silica gel to give an additional 210 mg (9%) of product 4.

Methyl 2-β-D-Ribofuranosyl-4-[2-^{14}C]selenazolecarboxylate (7)

A 15-ml two-necked flask, fitted with a septum at the side-neck, was connected at the center-neck via a stopcock to a vacuum manifold which had been calibrated as containing 1.0 mmol of ideal gas per 11.58 mm Hg of pressure. A suspension of 303 mg (0.643 mmol) of 4, 7.5 mg (0.0613 mmol) of 4-dimethylaminopyridine, and 3.2 ml of ethanol in the flask was cooled in liquid nitrogen, and the system was evacuated. Hydrogen selenide, 9.15 ml (0.79 mmol) was introduced to the manifold at room temp, then transferred into the reaction flask by temperature difference.[10] With the stopcock closed, the flask was warmed up slightly with a bath of water at 25°C until the content could be stirred magnetically. The bath was then removed, and stirring was continued for 40 min. Tlc analysis using 1% methanol in chloroform (during which the reaction was stopped by freezing in liquid nitrogen) showed the presence of product 5 (Rf 0.33), 80%, and starting material 4 (Rf 0.71), 20%, as the two main radioactive components. In the same manner, another 4.0 ml (0.35 mmol) of hydrogen selenide was introduced and stirred for 30 min, during which a black liquid was gradually dispersed to give a light green solution, containing, by tlc analysis as above, 95% product and 3% starting material. A solution of 280 mg (1.29 mmol) of 90% ethyl bromopyruvate in a little ethanol was added, causing a red solid to precipitate. The mixture was stirred at room temperature to give 6.

In a second run, 575 mg (1.22 mmol) of 4 was converted to 6 in the same manner with hydrogen selenide, except that the latter (24.7 ml, 2.13 mmol) was added in one portion, with similar results.

The reaction mixtures from the two runs above, containing 6, were filtered through Celite. The filtrate and ethanol washing were adjusted to pH 7.0 with 2.5 ml of saturated aqueous sodium bicarbonate, then evaporated *in vacuo* to a residue. An extract of the residue in chloroform was washed with small portions of water, then dried with anhydrous magnesium sulfate and evaporated *in vacuo* to a residue, which was further dried by evaporation from benzene and evacuated at

0.025 mm Hg to a constant weight of 1.42 g. A partial solution of the residue in 5.7 ml of methanol was treated with 150 mg of sodium methoxide for 27 hrs. The resulting dark brown solution was neutralized by stirring for one hr with a strong cation exchange resin (Dowex 1x8, hydrogen form, washed with methanol and dried, 1.2 g), then filtered. The filtrate and methanol washing were evaporated in vacuo and further evacuated in high vacuum to 800 mg. The residue, dissolved by repeated trituration with fresh portions of 10% tetrahydrofuran in chloroform containing 1-2% of methanol, totaling 30 ml, was adsorbed onto a column of 5.7 g of silica gel. The product was eluted with 4% methanol in chloroform, together with a uv-transparent contaminant having the same Rf value (radiochemical purity, 93-96% by tlc, but only 60-69% by hplc). Further purification by fractional recrystallizations (in general by adding about five times the sample weight of ethyl acetate to a syrup containing about half the sample weight of methanol) gave 152 mg of light tan crystals, 23 mci at 49 mci/mmol, identical to an authentic unlabelled sample of 7 by tlc, hplc, and ^{1}H nmr (β-anomeric proton doublet at δ 4.82, $J_{1',2'}$ = 4.8 Hz, Me_2SO-d_6 + D_2O).[4,11] The purity by hplc (Alltech C-18, 10μ, 4.6 mm x 25 cm, methanol:1% acetic acid--25:75, 2 ml/min, retention time 4.4 min, contaminant at 2.0 min) was 98.9% by uv (256 nm) and 96% by radioactivity. The radiochemical purity in three tlc solvent systems was 97.4 to 98.3% ($CHCl_3$:THF:MeOH--75:15:10, Rf 0.28; EtAc:THF:MeOH--11:7:2, Rf 0.64; $CHCl_3$:MeOH--9:1, Rf 0.21).

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10. The hydrogen selenide should be removed from the manifold as soon as possible after the experiment to avoid formation of a red deposit which forms on standing. Hydrogen selenide is highly toxic and may be disposed in form of the insoluble copper selenide by treatment with a copper sulfate solution.

11. An upfield-shift of 0.33 ppm would have been expected from H-1' of the α-anomer;[1,2] such absorption was absent in the spectra.