Synthesis of 7-amino-9a-methoxy-10-[^{14}C]carbamoyl mitosane (10-[^{14}C]carbamoyl Mitomycin C)

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SUMMARY

The synthesis of the title compound (5) is described. Treatment of mitomycin C with sodium methoxide gave decarbamoyl mitomycin C (2). Reaction with acetic anhydride in methanol yielded N1a-acetyl-mitomycin C (3). Treatment with [^{14}C]phosgene in the presence of N,N-dimethylaniline yielded [^{14}C]chloroformate (4). Conversion of the chloroformate to the carbamate and removal of the protecting group with excess ammonia produced the title compound (5).

Key Words:

Anticancer, 7-amino-9a-methoxy-10-[^{14}C]carbamoyl mitosane, 10-[^{14}C]carbamoyl-Mitomycin C, [^{14}C]-MMC

INTRODUCTION

Radiolabelling of mitomycin C has been a long standing problem mainly because there is no easily accessible position in the molecule to be substituted with a radio nucleus. Apart from labelling by precursor feeding in the fermentation process[1a,b], there are few chemical pathways to generate labelled mitomycin C. There exists a need for labelled mitomycin C in pharmacokinetic studies of free mitomycin C on carrier-conjugated mitomycin C. This paper describes the synthesis of carbamoyl [^{14}C]mitomycin C. The C-10 carbamoyl group is a possible leaving group[2]. The primary reaction site with DNA, however, is believed to be the C-1 aziridine position, and therefore mitomycin C labelled at the carbamoyl carbon with ^{14}C is considered to have some utility.

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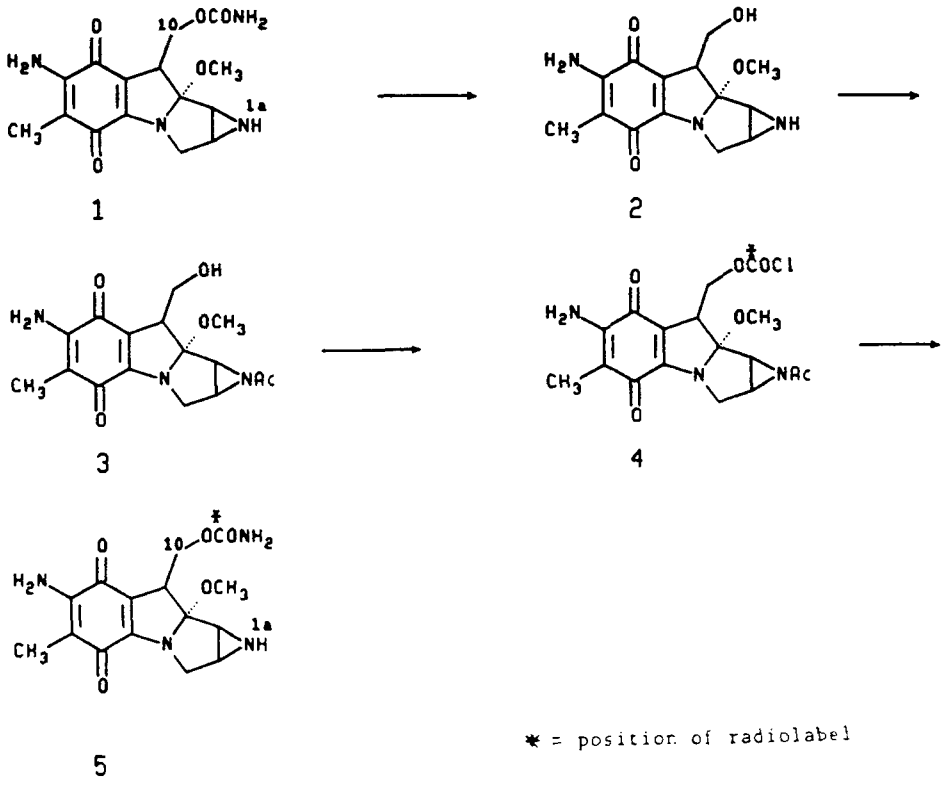
SYNTHETIC PATHWAY



EXPERIMENTAL

Materials

[^{14}C]Phosgene was purchased from NEN Research Corporation. All chemicals used in the synthesis were purchased commercially and used without any purification. All other solvents were either distilled or analytical reagent quality. Thin layer chromatography plates used were Analtech silica gel GF scored 10 x 20 cm and high pressure liquid chromatography was carried out on Waters Associates instrumentation. Radioactivity was measured by a Beckman LS 9000 liquid scintillation counter. Nuclear magnetic resonance spectra were measured on a Bruker 360. Weighings were carried out on a Sartorius 200 balance and a Mettler Microanalytical M5AS Balance.

Decarbamoyl mitomycin C (2)

Mitomycin C (1) (600 mg, 1.7 mmole) was dissolved in methanol (60 ml) and dry benzene (60 ml). Solid sodium methoxide (6.0 g) was added. The resulting mixture was stirred at room temperature and monitored by thin layer chromatography (10% methanol-methylene

chloride). After 15 hr, the reaction mixture was quenched by a slow addition of dry-ice and the precipitated sodium carbonate was removed by filtration and washed with ethyl acetate (150 ml). The combined filtrates were washed with brine (2 x 10 ml). The organic layer was dried over sodium sulfate and then concentrated under reduced pressure to give a purple foam. It was purified by chromatography on silica gel (2.5% methanol-methylene chloride to 5% methanol-methylene chloride) to give the product (300 mg, yield = 59%) as a purple foam.

N1a-Acetyl mitomycin C (3)

Decarbamoyl mitomycin (2) (290 mg, 1 mmole) was dissolved in methanol (25 ml) and cooled to 0°C. Acetic anhydride (225ul, 2.5 mmol) was added and the solution was stirred for 15 min at room temperature. Thin layer chromatography (10% methanol-methylene chloride) showed single spot at Rf=0.5. The solvent and excess reagent was removed under reduced pressure and the resulting purple oil dried *in vacuo* for 2 hr. This material was used as is for the following reaction.

[^{14}C]Mitomycin C (5)

N1a-acetyl mitomycin C (3) was dissolved in dry methylene chloride (25 ml), and cooled to 0°C. Dimethylaniline (488ul, 4 mmol) and [^{14}C]phosgene in toluene (100 mCi, 60 mCi/mmol) was added. The resulting reddish-purple solution was stirred at room temperature for 1 hr. This was cooled to 0°C and methanolic ammonia (8.4 ml, 2.2 M) was added. The reaction mixture was stirred for 15 hr at room temperature, filtered through celite and washed with methanol (50 ml). Non-labelled mitomycin C (100 mg) was dissolved in the filtrate and the solution concentrated to a purple foam. Purification by column chromatography on silica gel (5% methanol -methylene chloride) yielded the title compound (140 mg). This material had a radiochemical purity of 89.7% and a specific activity of 53.3 µCi/mg.

RESULTS AND DISCUSSION

Decarbamoyl mitomycin C was obtained by a sodium methoxide hydrolysis of mitomycin C[3]. It was then treated with acetic anhydride in methanol to effect a selective protection of the aziridine nitrogen yielding the N-acetyl derivative (3). This was reacted with [^{14}C]phosgene in the presence of N,N-dimethyl-aniline to give the [^{14}C]chloroformate (4)[4]. It was treated with excess ammonia to convert the [^{14}C]chloroformyl group to a [^{14}C]carbamate and at the same time remove the aziridine protecting group. This produced [^{14}C] mitomycin C (5) having a specific activity of 53.3 uCi/mg and radiochemical purity of

89.7%. The radiochemical purity was determined by high pressure liquid chromatography using Waters Assoc. instrumentation with a phenyl column and a 20 min. gradient of 25% to 75% methanol containing 0.02M ammonium acetate. The UV profile at 310 nm was identical to that of the unlabeled mitomycin C used to generate the decarbamoyl mitomycin C starting material. All experimental conditions were optimized using non-radiolabelled materials.

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