# SYNTHESIS OF [C6-$CH_3$-$^{14}C$] AND [C6-$CH_3$-$^3H_3$]MITOMYCIN C

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## SUMMARY

A facile synthesis of the title compounds **2** and **3** is described. The key intermediate in the synthesis, 6-demethyl-7,7-(ethylenedioxy)-6-(phenylselenenyl)mitosane (**4**) was synthesized in five steps from mitomycin A. Treatment of **4** with [$^{14}C$]methyl iodide in the presence of $K_2CO_3$ afforded the [$^{14}C$]-labelled mitosane (**5**). The removal of the phenylselenenyl group of **5** and subsequent treatment of the resultant mitosane **7** with ammonia led to the desired [$^{14}C$]mitomycin C (MMC) (**2**) with specific activity of 50 mCi/mmol. Similarly, [$^3H$]-labelled MMC (**3**) with highly specific activity of 78.4 Ci/mmol was obtained.

Key words: [C6-$CH_3$-$^{14}C$]mitomycin C, [C6-$CH_3$-$^3H_3$]mitomycin C

## INTRODUCTION

In studies of pharmacokinetics and metabolism of a drug, isotopically labelled drugs with tritium or carbon-14 at a metabolically stable position are necessary tools. Only one method for synthesis of mitomycins labelled at a metabolically stable position, *i.e.*, the biosynthetic method *(1)* is known.

In a previous paper, we first reported a synthesis of mitomycin C specifically mono-labelled with deuterium *(2)* or tritium *(3)* at the C6-methyl position. However, this procedure results in products with low specific activities *(3)*. As a follow-up study, we have developed methodology for replacement of the C6-methy

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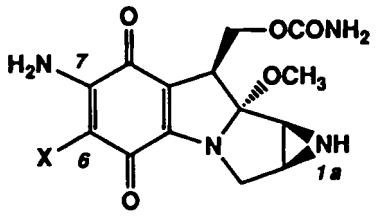
group of **1**, in which the methyl group was derived from easily available labelled methyl iodide *(4)*. Herein, we describe a facile synthesis of [C6-$CH_3$-$^{14}C$]-mitomycin C (**2**) and [C6-$CH_3$-$^3H_3$]mitomycin C (**3**).




**1**, Mitomycin C (X=$CH_3$)
**2**, [C6-$CH_3$-$^{14}C$]Mitomycin C (X=$^{14}CH_3$)
**3**, [C6-$CH_3$-$^3H_3$]Mitomycin C (X=$C^3H_3$)

## RESULTS AND DISCUSSION

The process shown in Scheme 1 consists of the treatment of the readily available 6-demethyl-6-(phenylselenenyl)mitosane (**4**), prepared in five steps from mitomycin A *(4)*, with [$^{14}C$]methyl iodide in the presence of $K_2CO_3$ followed by removal of the phenylselenenyl group by treatment with $nBu_3SnH$ and $Et_3B$. Amination at C7 and deacetylation of the 1a-aziridine nitrogen of the resultant product **7** with ammonia led to the desired [C6-$CH_3$-$^{14}C$]mitomycin C (**2**). Reverse phase HPLC analysis of **2** showed a radiochemical purity of 97 % and a specific activity of 50 mCi/mmol.

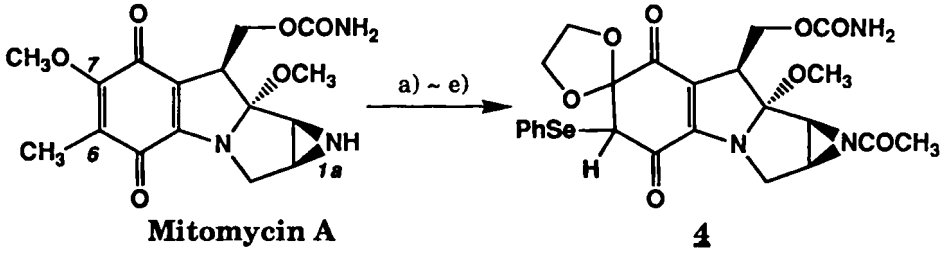
Similarly, starting from [$^3H$]methyl iodide, [C6-$CH_3$-$^3H_3$]mitomycin C (**3**) was obtained at a specific activity of 78.4 Ci/mmol and a radiochemical purity of 98 % after HPLC purification.

In conclusion, we have shown a facile route for synthesis of the radioactive mitomycins. Studies of pharmacokinetics and mechanism of action of **1** using these labelled compounds are currently underway.

## EXPERIMENTAL

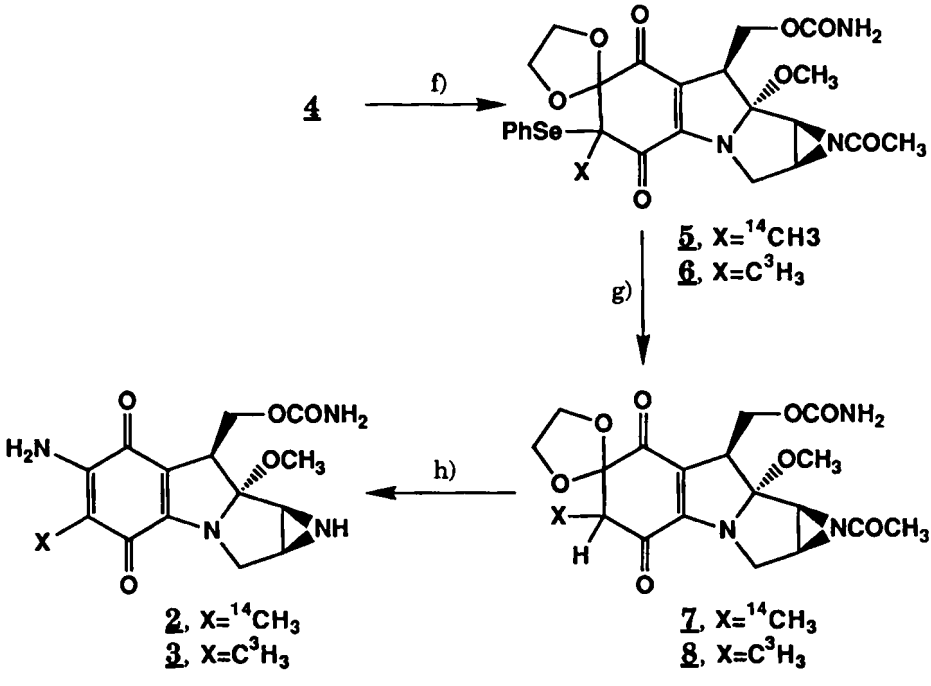
All chemicals and solvents used were purchased commercially and used without any further purification. Thin layer chromatography was done with

## Scheme 1. Synthetic Pathway of Labelled Mitomycin C (2) and (3)




**Mitomycin A** **4**

Reagents and conditions: a) ethylene glycol, KOH, tetrahydrofuran, room temp.; b) $Ac_2O$-pyridine, $CHCl_3$, room temp.; c) PhSeBr, $Et_3N$, MeCN, room temp.; d) *m*CPBA, $K_2CO_3$, $CHCl_3$, -40° to room temp. *(2)*; e) *N*-(phenylseleno)morpholine, $CHCl_3$, room temp. *(4)*.




Reagents and conditions: f) $^{14}CH_3I$ or $C^3H_3I$, $K_2CO_3$, acetone, room temp.; g) $nBu_3SnH$, $Et_3B$, $C_6H_6$, room temp.; h) $NH_3$, MeOH, room temp.

Merck Kieselgel 60F and Whatman LKC 18F ODS TLC plates. All compounds had identical Rf to that of authentic unlabelled standards. Radioactivity was measured on a Berthold LB 2848 and a LB 507A Automatic Linear Analyser.

[C6-$CH_3$-$^{14}$C]-1a-Acetyl-7-demethoxy-6,7-dihydro-7,7-(ethylenedioxy)-6-(phenylselenenyl)mitomycin A (**5**)

To a solution of 1a-acetyl-7-demethoxy-6-demethyl-6,7-dihydro-7,7-(ethylenedioxy)-6-(phenylselenenyl)mitomycin A (**4**) *(4)* (1968 mg, 3.5 mmol) and freshly ground anhydrous potassium carbonate (967 mg, 7.0 mmol) in dry acetone (20 mL) was distilled [$^{14}$C]methyl iodide (200 mCi, ca. 55 mCi/mmol, ca. 3.6 mmol). The reaction mixture was stirred at room temperature in the dark for 48 h and heated at 35 °C for 20 h. The volatiles of the flask were pumped into a trap cooled to -196 °C on a vacuum manifold, then the crude product was dissolved in chloroform (20 mL), filtered through a pad of celite and washed with chloroform (100 mL). The solution was concentrated and purified by silica gel chromatography using chloroform (150 mL), 2 % and 5 % methanol/chloroform (150 mL each) yielding a brown solid of **5** [675 mg, 56.3 mCi, radiochemical purity: ca. 97 %. by TLC (ODS plates, solvent: 50 % methanol/water)]

[C6-$CH_3$-$^{14}$C]-1a-Acetyl-7-demethoxy-6,7-dihydro-7,7-(ethylenedioxy)-mitomycin A (**7**).

To a solution of **5** (56.3 mCi, 1.02 mmol) in benzene (100 mL) under nitrogen was added tributyltin hydride (2.75 mL, 10.2 mmol) followed by triethylborane (1M in THF, 350 μL). After 5 min further triethylborane (350 μL) was added and stirred for 4 h. To the reaction mixture was added saturated sodium carbonate solution (20 mL) together with chloroform (30 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The crude product was purified by C-18 derivatized silica gel column (Sorbsil C60 silica gel RP18, 40~60 μ, May & Baker) chromatography using 40 %, 50 %, 60 % and 70 % methanol/water (50 mL each) to afford **7** [25.7 mCi, radiochemical purity: ca. 70 % by TLC (ODS plates, solvent: 50 % methanol/water)].

[C6-$CH_3$-$^{14}C$]Mitomycin C (**2**).

To a solution of **7** (25.7 mCi) in dry methanol (25 mL) under nitrogen was added methanolic ammonia (1.5 mL). The solution was stirred at room temperature overnight. The solvent was removed *in vacuo*. The crude product was purified by silica gel chromatography using chloroform (150 mL), 2 %, 5 % and 10 % methanol/chloroform (150 mL each) to afford **2** [radiochemical purity: ca. 90 % by TLC (silica gel plates, solvent: 20 % methanol/chloroform)]. This was further purified by HPLC using 5 % methanol/chloroform with a flow rate of 20 mL/min on a Dynamax silica column (250 mm x 21.4 mm i.d., detection: UV at 254 nm). The product fractions were combined and concentrated *in vacuo* giving **2** as a purple solid [77.8 mg, 50 mCi/mmol, 11.6 mCi, radiochemical purity: 97 % by HPLC (column: Ultrasphere ODS, 5 μ, 250 mm x 4.6 mm i.d., eluant: from 5 % acetonitrile/0.02 M pH 7.0 phosphate buffer to acetonitrile)]. **2** : CI-MS ($NH_3$) m/z 337 ($M^+$+H).

[C6-$CH_3$-$^3H_3$]Mitomycin C (**3**).

To a solution of **4** (160 mg, 0.285 mmol) and finely ground anhydrous potassium carbonate (78.9 mg, 0.57 mmol) in dry acetone (4 mL) was distilled [$^3$H]methyl iodide (50 Ci, ca. 80 Ci/mmol, ca. 0.6 mmol) and reaction tube sealed. The reaction mixture was stirred at 35 °C for 65 h in the dark. The volatiles were removed by vaccum transfer. The residues were extracted into dichloromethane (3 x 20 mL) from brine (20 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and then the solvent was removed *in vacuo*. The crude product was purified by HPLC using 40 % acetone/dichloromethane on a Dynamax silica column ( 350 mm x 22 mm i.d., detection: UV at 340 nm) yielding [C6-$CH_3$-$^3H_3$]-1a-acetyl-7-demethoxy-6,7-dihydro-7,7-(ethylenedioxy)-6-(phenylselenenyl)-mitomycin A (**6**) [10 Ci, radiochemical purity: ca. 40 % by HPLC (column: Ultrasphere $C_8$, 250 mm x 4.6 mm i.d., eluant: 35 % acetonitrile/water)].

To a solution of the above compound in dry benzene (6 mL) under argon was added tributyltin hydride (400 μL) and triethylborane (50 μL). After 5 min further triethylborane (50 μL) was added and stirred for 1h. The reaction mixture was extracted into dichloromethane (45 mL) from saturated aqueous sodium

bicarbonate (20 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. The crude product [7.7 Ci, radiochemical purity: ca. 30 % by HPLC (column: Ultrasphere $C_8$, 250 mm x 4.6 mm i.d., eluant: 35 % acetonitrile/water)] was purified twice by HPLC using 40 % methanol/water on an Ultrasphere $C_8$ column (250 mm x 10 mm i.d.) to afford [C6-$CH_3$-$^3H_3$]-1a-acetyl-7-demethoxy-6,7-dihydro-7,7-(ethylenedioxy)mitomycin A (**8**) (417 mCi). To the above compound (207 mCi) under argon was added saturated methanolic ammonia (250 µL). The reaction mixture was stirred at room temperature for 1h 40 min in the dark and blown down under a stream of argon. The crude product was purified twice by HPLC using 30 % methanol/water on an Ultrasphere $C_8$ column (250 mm x 10 mm i.d.) to afford [C6-$CH_3$-$^3H_3$]mitomycin C (**3**) [100 mCi, 78.4 Ci/mmol, radiochemical purity: 98 % by HPLC (column: Ultrasphere $C_8$, 250 mm x 4.6 mm i.d., eluant: 35 % methanol/water)]. **3** : CI-MS ($NH_3$) m/z 341 ($M^+$+H).

## ACKNOWLEDGEMENT

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## REFERENCES

1. (a) Hornemann U. and Cloyd J. C. - J. Chem. Soc., Chem. Commun. 301 (1971), (b) Hornemann U., Eggert J. H. and Honor D. P. - ibid., 11 (1980).
2. Kanda Y. and Kasai M. - J. Org. Chem. 55: 2515 (1990).
3. Kanda Y., Akinaga S. and Kasai M. - J. Labelled Compd. Radiopharm. 28: 1033 (1990).
4. Kasai M., Arai H. and Kanda Y. - J. Chem. Soc., Chem. Commun. 1991 (in press).