

# An Unusual Replacement of a Methylene Moiety by a Phenylseleno Group. Synthesis of Mitomycin C labelled at C-6 by $^{13}\text{CH}_3$ and $\text{C}^2\text{H}_3$

Masaji Kasai,\* Hitoshi Arai and Yutaka Kanda

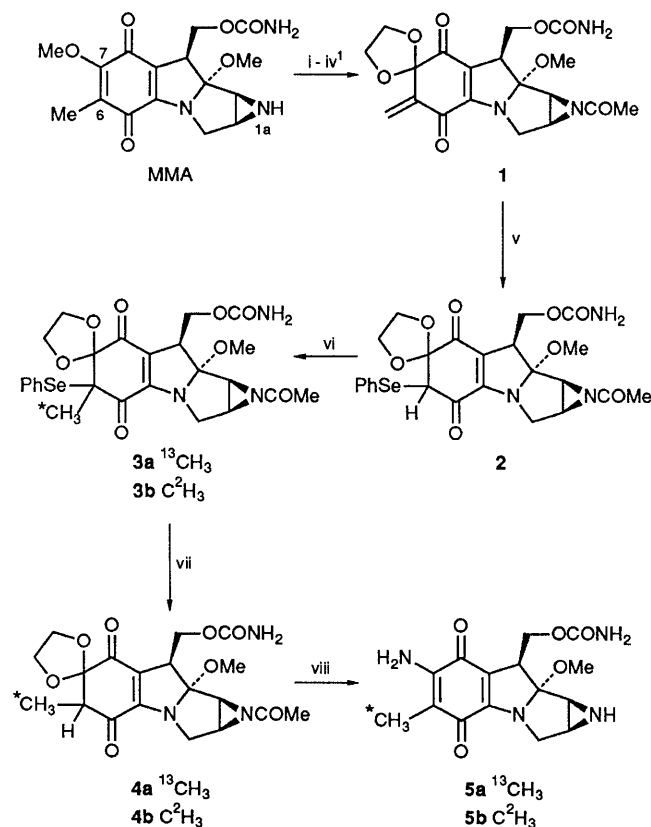
Kyowa Hakko Kogyo Co. Ltd., Pharmaceutical Research Laboratories, 1188 Shimotogari, Nagaizumi, Sunto, Shizuoka 411, Japan

A novel replacement of the C-6-methylene group of 7,7-ethylenedioxy-6-methylenemitosane by a phenylseleno group was employed to prepare a critical intermediate in the synthesis of mitomycin C labelled at C-6 by  $^{13}\text{CH}_3$  and  $\text{C}^2\text{H}_3$ .

Mitomycin C (MMC) has proved to be an effective drug against a variety of solid tumours. In order to obtain needed pharmacokinetic data on the behaviour of this drug and its derivatives it was necessary to synthesize metabolically stable, radiochemically labelled compounds. While there are procedures for preparing labelled mitomycins,<sup>1-3</sup> use of these to incorporate  $^3\text{H}$  or  $^{14}\text{C}$  results in products with low specific activities. We recently described<sup>1</sup> the preparation of a mono-deuterium or -tritium labelled MMC and mitomycin A from 7,7-ethylenedioxy-6-methylenemitosane **1**, and now describe the synthesis of MMC containing C-6- $^{13}\text{CH}_3$  and C-6- $\text{C}^2\text{H}_3$ , prototypes for the synthesis of the corresponding  $^{14}\text{C}$  and  $^3\text{H}$  analogue (Scheme 1).<sup>†</sup> The critical compound **2** in these syntheses was formed *via* a novel reaction from **1**. The intermediate was converted into MMC containing C-6- $^{13}\text{CH}_3$  and C-6- $\text{C}^2\text{H}_3$  groups.

Synthesis of 7,7-ethylenedioxy-6-methylenemitosane **1** and its conversion to a mono-deuterio derivative of MMC and MMA was recently described.<sup>1</sup> In order to prepare the  $^{14}\text{C}$  labelled compound or a  $^3\text{H}$  derivative of higher specific activity a new synthetic route was required. In the course of

reaction of 7,7-ethylenedioxy-6-phenylselenomitosane with *m*-chloroperbenzoic acid (*m*CPBA), followed by addition of secondary amines, we isolated 6-demethyl-6-phenylselenomitosane **2** in low yield. In considering how this useful product might have been formed, we noted the report by Hase and Kukkola<sup>4</sup> describing the conversion of 1-phenylbut-2-en-1-one into  $\alpha$ -phenylselenoacetophenone on treatment with lithium diisopropylamide (LDA) and benzene selenenyl bromide, *i.e.* an ethylidene group was eliminated and replaced by a phenylseleno group. Compound **2** might have been formed in an analogous reaction. This reaction was explained as occurring by two sequential reactions, where the amine would add to the initially generated enone **1** followed by the phenylseleno group to afford an intermediate adduct which would then lose iminium by retro-Mannich reaction to yield **2**. In searching for a more efficient way to effect this conversion, we found that the reaction of **1** with *N*-(phenylseleno)-morpholine<sup>5</sup> yielded **2** in 58% yield.



**Scheme 1** Reagents and conditions: i, ethylene glycol, KOH, tetrahydrofuran (THF), room temp.; ii,  $\text{Ac}_2\text{O}$ -pyridine,  $\text{CHCl}_3$ , room temp.; iii,  $\text{PhSeBr}$ ,  $\text{Et}_3\text{N}$ ,  $\text{MeCN}$ , room temp.; iv, *m*CPBA,  $\text{K}_2\text{CO}_3$ ,  $\text{CHCl}_3$ ,  $-40^\circ\text{C}$  to room temp.; v, *N*-(phenylseleno)morpholine,  $\text{CHCl}_3$ , room temp.; vi,  $^{13}\text{CH}_3\text{I}$  or  $\text{C}^2\text{H}_3\text{I}$ ,  $\text{K}_2\text{CO}_3$ , acetone, room temp.; vii,  $\text{Bu}^n_3\text{SnH}$ ,  $\text{Et}_3\text{B}$ ,  $\text{C}_6\text{H}_6$ , room temp.; viii,  $\text{NH}_3$ ,  $\text{MeOH}$ , room temp.

<sup>†</sup> All new compounds were characterized by  $^1\text{H}$  NMR and high-resolution mass spectrometry and/or combustion analysis: **2** (3.5:1 mixture of stereoisomers at C-6; data for minor isomer in square brackets):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.20 [2.10] (s, 3H, 1a-NCOMe), 3.21 [3.21] (s, 3H, 9a-OMe), 3.26 [3.23] (dd,  $J$  4.7 and 2.0 [4.4 and 2.0] Hz, 1H, 2-H), 3.41 [3.39] (dd,  $J$  13.0 and 2.0 [13.0 and 2.0] Hz, 1H, 3 $\alpha$ -H), 3.52 [3.48] (d,  $J$  4.7 [4.4] Hz, 1H, 1-H), 3.73 [3.67] (dd,  $J$  10.8 and 4.9 [10.8 and 4.7] Hz, 1H, 9-H), 3.84 [4.40] (d,  $J$  13.0 [13.0] Hz, 1H, 3 $\beta$ -H), 4.02 [4.17] (s, 1H, 6-H), 4.19 (t,  $J$  10.8 Hz, 1H, 10a-H), 4.01–4.20 (m, 3H, ethylenedioxy) [4.01–4.20 (m, 4H, 10a-H + ethylenedioxy)], 4.41 [4.31] (m, 1H, ethylenedioxy), 4.91 [4.89] (br s, 2H, 10-OCONH<sub>2</sub>), 4.95 [4.81] (dd,  $J$  11.1 and 4.9 [10.8 and 4.7] Hz, 10b-H), 7.28–7.38 [7.28–7.38] (m, 3H, 6-SePh), and 7.60 [7.61] (m, 2H, 6-SePh).  $^{13}\text{C}$  NMR (67.5 MHz,  $\text{CDCl}_3$ )  $\delta$  23.5 [23.5] (1a-NCOCH<sub>3</sub>), 39.3 [39.3] (C-2), 42.6 [42.2] (C-9), 43.1 [42.9] (C-1), 47.8 [47.3] (C-3), 49.9 [49.8] (9a-OCH<sub>3</sub>), 55.1 [58.0] (s + d,  $J$  [ $^{13}\text{C}^{77}\text{Se}$ ] 69.6 [73.9] Hz, C-6), 61.7 [61.3] (C-10), 66.2 [66.6] and 67.6 [67.4] (ethylenedioxy), 105.3 [105.7] (C-9a), 105.6 [105.7] (C-7), 119.1 [119.8] (C-8a), 127.6 [127.7] (SePh-1), 128.9 [129.1] (SePh-4), 129.3 [129.2] (SePh-3 + -5), 135.7 [135.5] (SePh-2 + -6), 155.2 [153.0] (C-4a), 156.4 [156.4] (10-OCONH<sub>2</sub>), 180.6 [180.7] (1a-NCOMe), 184.3 [184.5] (C-5), and 186.1 [185.9] (C-8). FAB-MS:  $m/z$  564 and 562 ( $\text{M}^+ + 1$ ). Satisfactory elemental analyses were obtained.

**5a** [differences in the spectrum of **5b** are in square brackets]:  $^1\text{H}$  NMR (400 MHz,  $\text{C}_5\text{D}_5\text{N}$ )  $\delta$  2.02 [absent] (d,  $J_{\text{CH}}$  127.2 Hz, 3H, 6- $^{13}\text{CH}_3$ ), 2.08 (br s, 1H, 1a-NH), 2.75 (br s, 1H, 2-H), 3.16 [3.15] (br s, 1H, 1-H), 3.22 (s, 3H, 9a-OMe), 3.60 (br d,  $J$  12.3 Hz, 1H, 3 $\alpha$ -H), 4.03 (dd,  $J$  11.2 and 4.2 Hz, 1H, 9-H), 4.55 (d,  $J$  12.7 Hz, 1H, 3 $\beta$ -H), 5.11 [5.12] (br t,  $J$  10.6 Hz, 1H, 10a-H), 5.44 (dd,  $J$  10.4 and 4.2 Hz, 1H, 10b-H) and 7.3–7.9 [7.3–8.0] (br s, 4H, 7-NH<sub>2</sub> + 10-OCONH<sub>2</sub>).  $^{13}\text{C}$  NMR (100 MHz,  $\text{C}_5\text{D}_5\text{N}$ )  $\delta$  8.77 (s, 6- $^{12}\text{C}$ - $^{13}\text{CH}_3$  and d,  $J_{\text{CC}}$  46.2 Hz, 6- $^{13}\text{C}$ - $^{13}\text{CH}_3$ ), [8.01 (sept.,  $J_{\text{CD}}$  19.4 Hz, 6- $\text{CD}_3$ )], 32.67 [32.66] (C-2), 36.77 (C-1), 44.38 (C-9), 49.61 [49.60] (9a-OCH<sub>3</sub>), 50.67 (C-3), 62.59 [62.60] (C-10), 104.38 [104.26] (d,  $J_{\text{CC}}$  46.2 Hz, [S], C-6), 106.88 [106.87] (C-9a), 110.88 [110.90] (C-8a), *ca.* 149.6 (C-7), 156.12 [156.11] (C-4a), 158.14 [158.12] (10-OCONH<sub>2</sub>), 176.78 [176.80] (d,  $J_{\text{CC}}$  2.5 Hz, [S], C-8) and 178.46 [178.52] (C-5).

Mass spectra: **5a**,  $m/z$  335 ( $\text{M}^+$ ); 303.1072 (calc. for  $\text{C}_{13}^{13}\text{CH}_4\text{N}_4\text{O}_4$  303.1048); **5b**,  $m/z$  337 ( $\text{M}^+$ ); 337.1465 (calc. for  $\text{C}_{15}^2\text{H}_3\text{H}_5\text{N}_4\text{O}_5$  337.1465).

This critical intermediate **2** was first methylated with  $^{13}\text{CH}_3\text{I}$  and  $\text{C}^2\text{H}_3\text{I}$  to establish a reaction sequence that later would be employed to prepare  $^{14}\text{C}$ - and  $^3\text{H}$ -labelled MMC. Methylation of **2** with  $^{13}\text{CH}_3\text{I}$  or  $\text{C}^2\text{H}_3\text{I}$  in the presence of  $\text{K}_2\text{CO}_3$  afforded the mitosanes **3a** and **3b** in 69 and 75% yields, respectively. The phenylseleno group in **3a** was removed employing  $\text{Bu}^n_3\text{SnH}$  and  $\text{Et}_3\text{B}$  in a radically initiated reaction<sup>6</sup> to afford the 7,7-ethylenedioxymitosane **4a** in 84% yield. The 7-amino group in MMC was introduced by reaction of **4a** with methanolic ammonia. Hydrolysis of the *N*-acetyl group of the 1a-aziridine unit also occurred under these reaction conditions to yield labelled MMC **5a** in 75% yield.

The 400 MHz  $^1\text{H}$  NMR spectrum of  $^{13}\text{C}$ -labelled compound **5a** showed clearly the resonance of the C-6-methyl protons at  $\delta$  2.02 ( $\text{C}_5\text{D}_5\text{N}$ ) as a doublet,  $J$  127.2 Hz. In the proton-decoupled  $^{13}\text{C}$  NMR spectrum of **5a**, the C-6-methyl resonance at  $\delta$  8.77 ( $\text{C}_5\text{D}_5\text{N}$ )<sup>7</sup> showed strong singlet intensity together with satellite signals arising from  $^1J_{\text{CC}}$  coupling ( $J$  46.2 Hz). The resonances of C-6 and C-8 were observed as doublets at  $\delta$  104.38 ( $^1J_{\text{CC}}$  46.2 Hz) and  $\delta$  176.78 ( $^3J_{\text{CC}}$  2.5 Hz), respectively. The incorporation of the isotope into the C-6-methyl position of **5a** was confirmed by mass spectrometry, which indicated over 99%  $^{13}\text{C}$  content, the same as  $^{13}\text{C}$ -labelled methyl iodide used as the C-6-methyl origin.

Similarly, MMC labelled with the deuterium, **5b** was obtained from **3b** in 70% yield. As expected, the resonance due to the C-6-methyl group of **5b** was absent in the  $^1\text{H}$  NMR spectrum of **5b**.

In summary the reaction sequence described offers an unusual and facile route for introducing metabolically stable groups of high specific activity in MMC, and should allow hitherto inaccessible C-6-substituted MMC derivatives to be prepared.

Received, 19th December 1990; Com. 0/05721E

## References

- 1 Y. Kanda and M. Kasai, *J. Org. Chem.*, 1990, **55**, 2515; Y. Kanda, S. Akinaga and M. Kasai, *J. Labelled Compd. Radiopharm.*, 1990, **28**, 1033.
- 2 U. Hornemann, J. H. Eggert and D. P. Honor, *J. Chem. Soc., Chem. Commun.*, 1980, 11.
- 3 M. G. Anderson, J. J. Kibby, R. W. Rickards and J. M. Rothschild, *J. Chem. Soc., Chem. Commun.*, 1980, 1277.
- 4 T. A. Hase and P. Kukkola, *Synth. Commun.*, 1980, **10**, 451.
- 5 P. Lerouge and C. Paulmier, *Bull. Soc. Chim. Fr.*, 1985, 1219.
- 6 K. Nozaki, K. Oshima and K. Utimoto, *J. Am. Chem. Soc.*, 1987, **109**, 2547.
- 7 P. J. Keller and U. Hornemann, *J. Natural Prod.*, 1983, **46**, 569.