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THE SYNTHESIS OF [^{3}H]KW-2189, A NOVEL ACTIVE ANTITUMOR ANTIBIOTIC

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Summary

The synthesis of [^{3}H]KW-2189, **2**, a novel active antitumor antibiotic, is described. The key intermediate **6** in the synthesis, was synthesized in four steps from duocarmycin B2 (**1**). Treatment of **6** with [^{3}H]methyl iodide in the presence of $NaHCO_3$ in MeOH-Me_2CO (1:1) afforded the [^{3}H]KW-2189 with highly specific activity of 86.4 Ci/mmol.

Key Words: [^{3}H]KW-2189, duocarmycin, antitumor antibiotic

Introduction

Duocarmycins produced by *Streptomyces sp.*, possess exceptionally potent cytotoxicity (1). Duocarmycins and CC-1065 (2) derive their biological effects through the reversible, sequence-selective alkylation of DNA (3). Subsequent to their disclosure, extensive efforts have been devoted to define and exploit their properties (4). We have synthesized duocarmycin analogs with the aim of enhancing and broadening the spectrum of the antitumor activity, and improving the solubility (5). Recently, KW-2189, **2**, a novel derivative of duocarmycin B2, **1**, was synthesized and demonstrated excellent *in vivo* antitumor activity, aqueous solubility greater than 10 mg/mL (6). It is designed as a prodrug which requires enzymatic hydrolysis followed by regeneration of an active metabolite (7). KW-2189, **2**, is currently under phase I clinical trial. For evaluation of this

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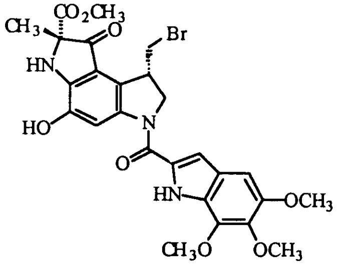
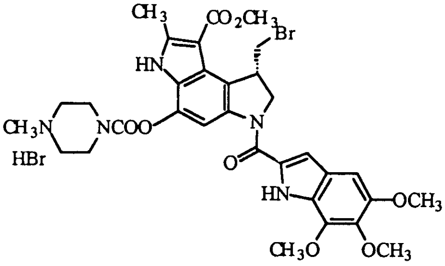
new duocarmycin derivative, drug metabolism and pharmacokinetic studies seemed to be very important. In such studies, isotopically labelled compounds with tritium or carbon-14 have been used in a general way. The synthesis of [^{3}H]KW-2189 is described herein.



duocarmycin B2 (1)

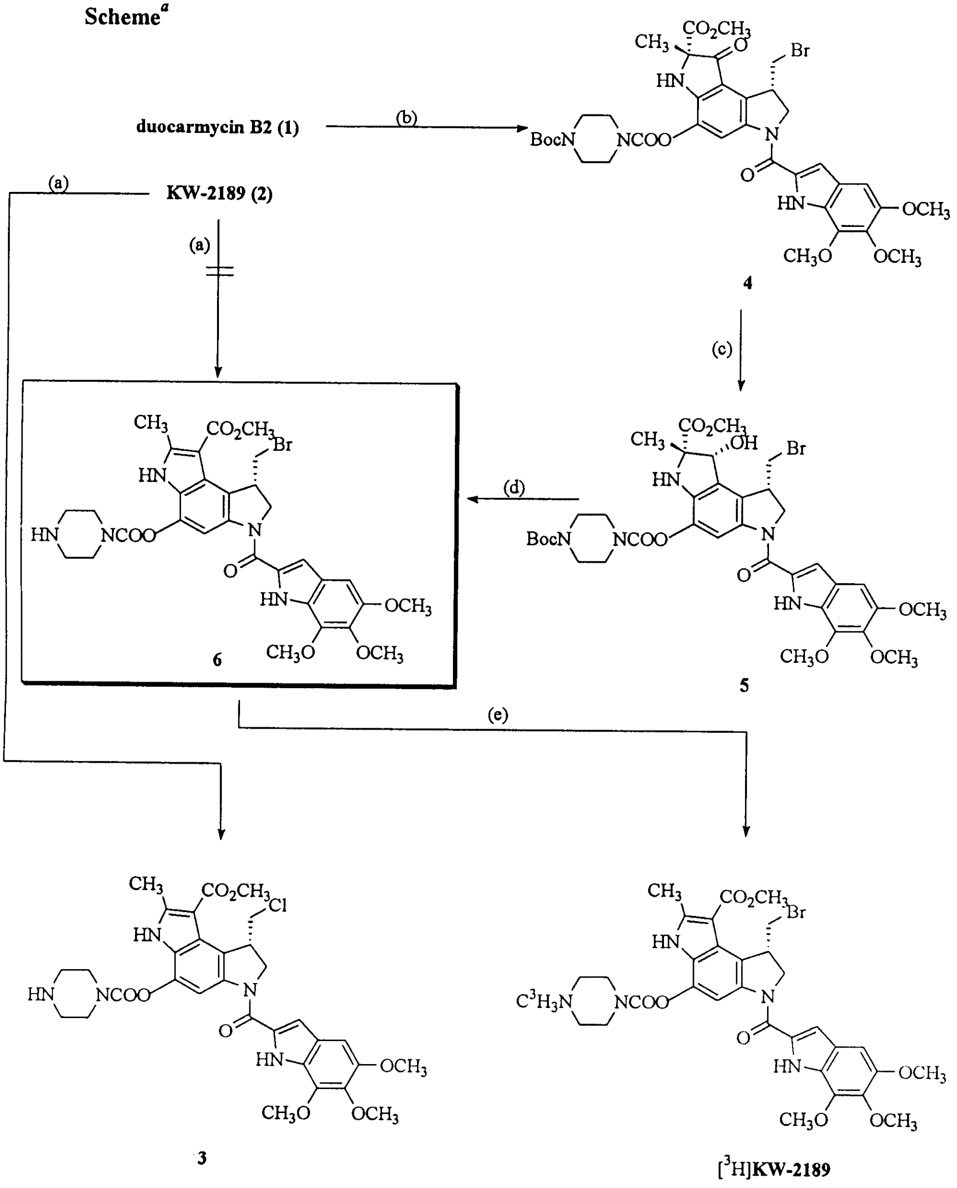


KW-2189 (2)

Results and Discussion

N-Methylation with [^{3}H]methyl iodide represents a convenient route for obtaining tritiated compounds with high specific activity. The 8-*O*-(piperaziny carbonyl)-A-ring pyrrole-duocarmycin B2, **6**, was selected as a suitable precursor for [^{3}H]KW-2189. We have already developed a convenient large scale preparation process of KW-2189, **2**. This synthetic route provided KW-2189, **2**, in three steps and 55 % overall yield from duocarmycin B2 (8). Therefore, we examined the direct conversion from the free base of **2** to the key intermediate **6** as reported by Olofson *et al.* (9). The free base of **2** was treated with 1-chloroethyl chloroformate in the presence of 1,8-bis(dimethylamino)naphthalene (Proton Sponge) in 1,2-dichloroethane, followed by warming in EtOH to give the demethylated product nearly quantitatively. However, since the bromo compound **6** is highly reactive, the undesirable compound **3** was formed by the concomitance of HCl during the reaction (6, 7). Therefore, conversion of duocarmycin B2 into the key intermediate **6** is shown in Scheme 1. Duocarmycin B2, **1**, was treated with 4-nitrophenyl chloroformate in methylene chloride in the presence of triethylamine to afford a carbonate as an intermediate, followed by addition of *N*-Boc-piperazine to give compound **4** in 79 % yield. Compound **4** was reduced with sodium borohydride in allyl alcohol at 0 °C to give **5** as the main compound in 61 % yield. The rearrangement reaction of **5** and its deprotection into **6** was achieved by treatment with camphorsulfonic acid in toluene (10). This one-pot conversion was acceptable enough to obtain the desired product **6** in 90 % yield. Compound **2** was prepared by the reaction with methyl iodide in the presence of a base.

Scheme^a



^{a} (a) $CH_3CHClOCOCl$, Proton Sponge, $ClCH_2CH_2Cl$, r.t., 24 h, then EtOH, reflux, 24 h; (b) 4-Nitrophenyl chloroformate, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, then *N*-Boc-piperazine, CH_2Cl_2, 0 °C, 2 h; (c) $NaBH_4$, Allyl alcohol, 0 °C, 1 h; (d) Camphorsulfonic acid, Toluene, 50 °C, 3 h; (e) [^{3}H]Methyl iodide, $NaHCO_3$, MeOH-Acetone, 35 °C, 10 h.

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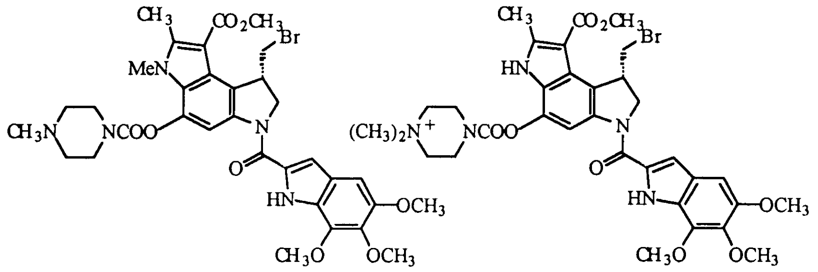
Optimal conditions for the reaction from **6** to **2** are characterized in Table 1. We found that the methylation was best carried out in MeOH-acetone (1:1) as reaction solvent in the presence of sodium hydrogencarbonate to give **2** as the main compound in 59 % yield. When [^{3}H]methyl iodide was used in the place of methyl iodide under identical condition of run 5, the desired [^{3}H]KW-2189 was obtained.

Table 1. Methylation of **6**

run[a]	solvent	base	HPLC area (%)[b]			
			6	**2**	N-1 Me	ammonium salt
1	DMF	$NaHCO_3$	63	23	0	0
2	DMF	K_2CO_3	26	56	14	0
3	MeOH	$NaHCO_3$	59	38	0	1
4	MeOH	K_2CO_3	40	35	14	0
5	MeOH-Acetone (1:1)	$NaHCO_3$	34	59	0	3
6	CH_2Cl_2	Et_3N	53	43	0	0

[a] Reaction was performed at room temperature. A base was 1.5 eq. against starting material **6**.
[b] HPLC analysis was performed after 24 h.



N-1 Me **ammonium salt**

In conclusion, starting from [^{3}H]methyl iodide, [^{3}H]KW-2189 was obtained at a specific activity of 86.4 Ci/mmol, and a radiochemical purity of 90.2 % after HPLC purification. Studies of phamacokinetics and mechanism of action of KW-2189, **2**, using this labelled compound are currently underway.

Experimental

^{1}H NMR spectra were measured on a JEOL JNM-GX270 spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane. Mass spectra were measured with a Hitachi B-80. All chemicals and solvents used were purchased commercially and used without any further purification. Thin layer chromatography was done with Merck Kieselgel

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60F (Art 5719). All compounds had identical Rf to that of unlabelled standards. A GL Science 5C18-250A column, 4.6 x 250 mm was used for analytical HPLC and Inertsil ODS-2 column, 6.0 x 150 mm was used for radiochemical HPLC of the tritiated product. Radioactivity was measured on a Berthold LB 2848 and a LB 507A Automatic Liner Analyzer.

8-*O*-(*N*-Boc-piperaziny carbonyl)-duocarmycin B2 (4):

p-Nitrophenyl chloroformate (103 mg, 0.51 mmol) and triethylamine (71 μL, 0.51 mmol) was added to a solution of duocarmycin B2 (**1**; 100 mg, 0.17 mmol) in dry CH_2Cl_2 (5 mL), and the mixture was stirred at 0 °C for 0.5 h. *N*-Boc-piperazine (111 mg, 0.60 mmol) was added to the solution, and the mixture was stirred at 0 °C for 2 h. The mixture was diluted with $CHCl_3$, and washed with 0.01 M phosphate buffer (pH 7) and brine. The organic extracts were concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$-CH_3OH (50:1) as an eluent to give 108 mg (79 %) of **4**. ^1H NMR (270 MHz, $CDCl_3$) δ 9.33 (1 H, br s), 8.45 (1 H, s), 6.96 (1 H, d, *J*= 2.4 Hz), 6.87 (1 H, s), 5.47 (1 H, s), 4.61 (2 H, m), 4.25 (1 H, m), 4.08 (3 H, s), 4.04 (1 H, dd, *J*= 10.0, 3.0 Hz), 3.94 (3 H, s), 3.91 (3 H, s), 3.79 (3 H, s), 3.69 (2 H, br s), 3.55 (7 H, br s), 1.69 (3 H, s), 1.50 (9 H, s). FABMS *m/z* 801 799 $(M + H)^+$, 234.

8-*O*-(*N*-Boc-piperaziny carbonyl)-3α-hydroxy-duocarmycin B2 (5):

$NaBH_4$ (14 mg, 0.37 mmol) was added to a solution of **4** (95 mg, 0.12 mmol) in allyl alcohol (4 mL), and the mixture was stirred at 0 °C for 1 h. Then, 1 N HCl was added, and the resulting mixture was extracted with EtOAc. The combined extracts were washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to column chromatography ($CHCl_3$-MeOH, 50:1) to give 58 mg (61 %) of **5**. ^1H NMR (270 MHz, $CDCl_3$) δ 9.36 (1 H, br s), 8.05 (1 H, s), 6.91 (1 H, d, *J*= 2.5 Hz), 6.87 (1 H, s), 5.37 (1 H, d, *J*= 9.9 Hz), 4.76 (1 H, m), 4.57 (2 H, m), 4.12 (1 H, dd, *J*= 10.4, 3.0 Hz), 4.07 (3 H, s), 3.94 (3 H, s), 3.91 (3 H, s), 3.75 (3 H, s), 3.65 (2 H, br s), 3.62 (7 H, br s) 2.73 (1 H, d, *J*= 9.9 Hz), 1.58 (3 H, s), 1.50 (9 H, s). FABMS *m/z* 803 801 $(M + H)^+$, 234.

8-*O*-(Piperaziny carbonyl)-A-ring pyrrole-duocarmycin B2 (6):

Camphorsulfonic acid (1.9 g, 8.2 mmol) was added to a solution of **5** (2.2 g, 2.7 mmol) in dry toluene (50 mL), and the reaction mixture was stirred for 3 h at 50 °C. Then, the mixture was poured into aqueous $NaHCO_3$ and the whole was extracted with $CHCl_3$. The extract was washed with brine. The organic layer was dried over Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel with CHCl$_3$-MeOH (5:1) to give 1.66 g (90 %) of **6**. ^{1}H NMR (270 MHz, CDCl$_3$) δ 9.39 (1 H, br s), 9.00 (1 H, br s), 8.14 (1 H, s), 7.00 (1 H, d, J= 1.4 Hz), 6.89 (1 H, s), 4.73 (1 H, br d, J= 10.3 Hz), 4.62 (1 H, dd, J= 8.3, 7.4 Hz), 4.53 (1 H, m), 4.08 (3 H, s), 3.96 (3 H, s), 3.95 (3 H, s), 3.92 (3 H, s), 3.81 (1 H, dd, J= 10.0, 2.0 Hz), 3.74 (2 H, br s), 3.59 (2 H, br s) 3.23 (1 H, dd, J= 10.0, 100 Hz), 2.97 (4 H, br s), 2.68 (3 H, s). FABMS *m/z* 686 684 (M + H)$^+$, 234.

8-*O*-(*N*-[^{3}H]Methyl-piperaziny carbonyl)-A-ring pyrrole-duocarmycin B2 ([^{3}H]KW-2189; 7): To a solution of **6** (500 mg, 0.73 mmol) and finely ground anhydrous sodium hydrogencarbonate (92 mg, 1.1 mmol) in MeOH-acetone (1:1; 50 mL) was distilled [^{3}H]methyl iodide (90 Ci, ca. 80 Ci/mmol, ca. 1.1 mmol) and reaction tube sealed. The reaction mixture was stirred at 35 °C for 10 h in the dark. The volatiles were removed by vacuum transfer. The residues were extracted into dichloromethane from brine. 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 50 mM ammonium acetate-acetonitrile (45:55) on a Inertsil ODS-2 (150 mm x 6.0 mm, 5 μm, detection: UV at 332 nm) yielding 8-*O*-(*N*-[^{3}H]Methyl-piperaziny carbonyl)-A-ring pyrrole-duocarmycin B2 [86.4 Ci/mmol, Radiochemical purity: 90.2 %].

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