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SYNTHESIS OF [^{14}C]ANTHRACYCLINE ANTICANCER AGENT 14-*O*-(β-ALANYL-*N*-HCL)-7-*O*-(2',6'-DIDEOXY-2'-FLUORO-α-L-TALOPYRANOSYL) ADRIAMYCINONE-14-^{14}C(DA-125-^{14}C)

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SUMMARY

Synthesis of the anthracycline anticancer agent, 14-*O*-(β-alanyl-*N*-HCl)-7-*O*-(2',6'-dideoxy-2'-fluoro-α-L-talopyranosyl) Adriamycinone (DA-125-^{14}C) labeled with carbon-14 regiospecifically for ADME (absorption, distribution, metabolism, and excretion) studies is described. Unlabeled 7-*O*-(2',6'-dideoxy-2'-fluoro-α-L-talopyranosyl) adriamycinone (Dong-A Pharm. Lot MI-8008) (**B-1**) was employed as the starting material in this nine-step radiosynthesis. The ^{14}C-labeled *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide (methyl-^{14}C), prepared in five steps from $^{14}CH_3I$, served as the source of the label. A total of 335 μCi of 14-^{14}C-DA-125 (specific activity 6.63 mCi/mmol, radiochemical purity of 98%, and overall isolated radiochemical yield >1 % based on N-methyl-^{14}C-N-nitroso-p-toluenesulfonamide,A-5) was prepared.

KEY WORDS: [^{14}C]-diazomethane, [^{14}C]-anthracycline, anticancer agent.

INTRODUCTION

The anthracycline antibiotic adriamycin (ADM) is one of the most widely used cancer chemotherapeutic agents, and is highly active against hemological tumors and a number of human solid tumors.[1,2] However, ADM has severe side effects, such as myelosuppression, alopecia, and cardiotoxicity which limit its clinical use. Recently, Research Lab., Dong-A Pharmaceutical Co. Ltd. (Yongin, South Korea) developed an ADM analogue DA-125 containing fluorine in the sugar moiety, and a β-alanine in 14-carbon position, as a water soluble prodrug. DA-125 was found to have higher *in vivo* and *in vitro* cytocidal activity,[3] and lower cardiotoxicity and hematoxicity than that of ADM.[4,5]

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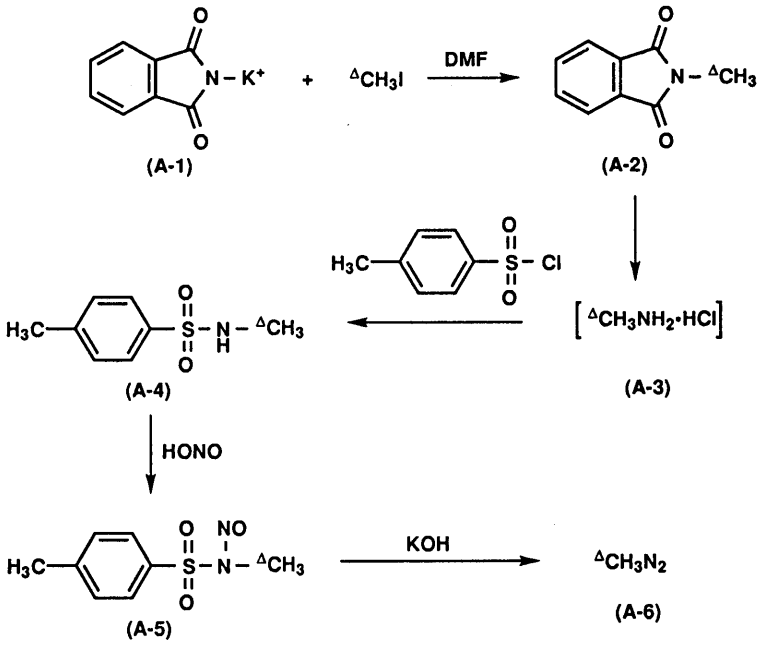
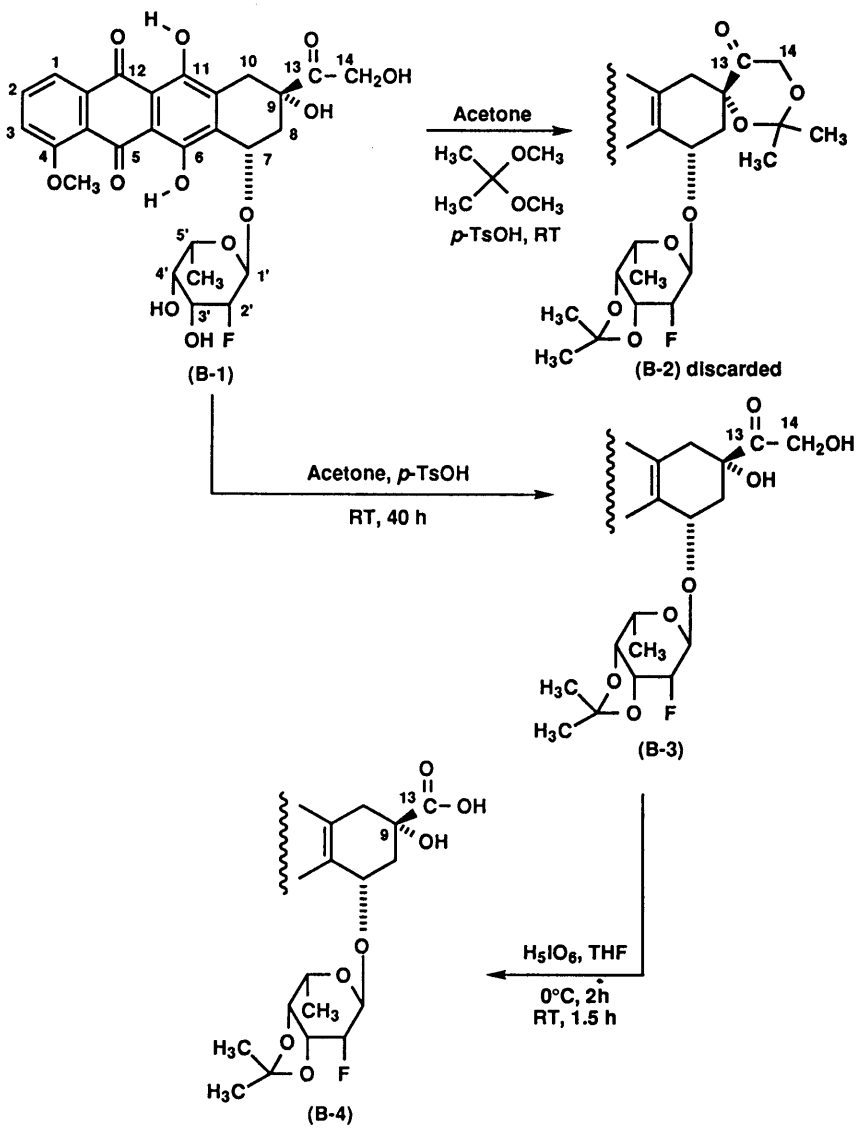
The preparation of DA-125 with a metabolically stable radioisotope (carbon-14) became necessary for ADME (absorption, distribution, metabolism, and excretion) and whole body radioautographic studies. ^{14}C-Labeled DA-125 was prepared according to radiosynthetic Schemes A and B.



Radiosynthesis Scheme A



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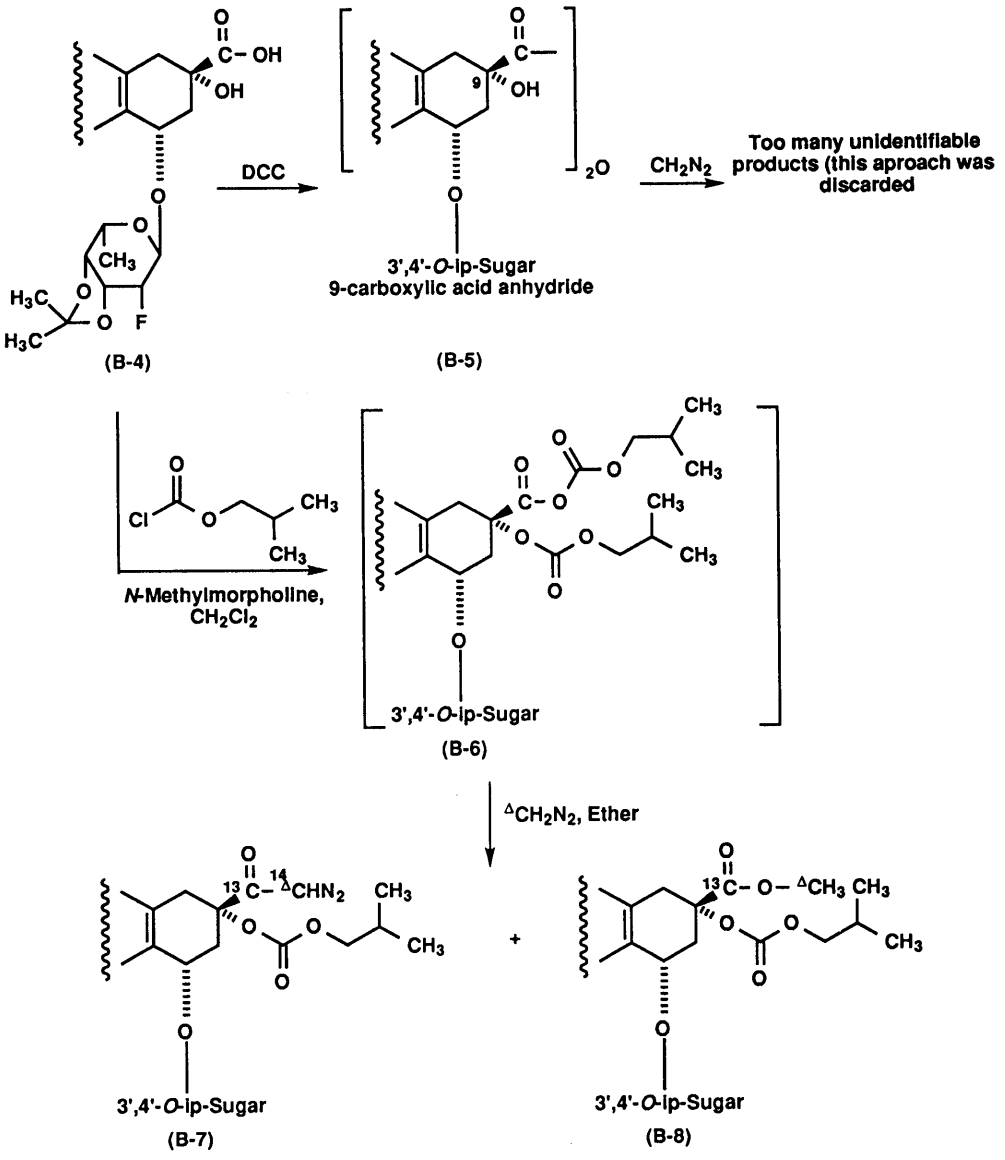
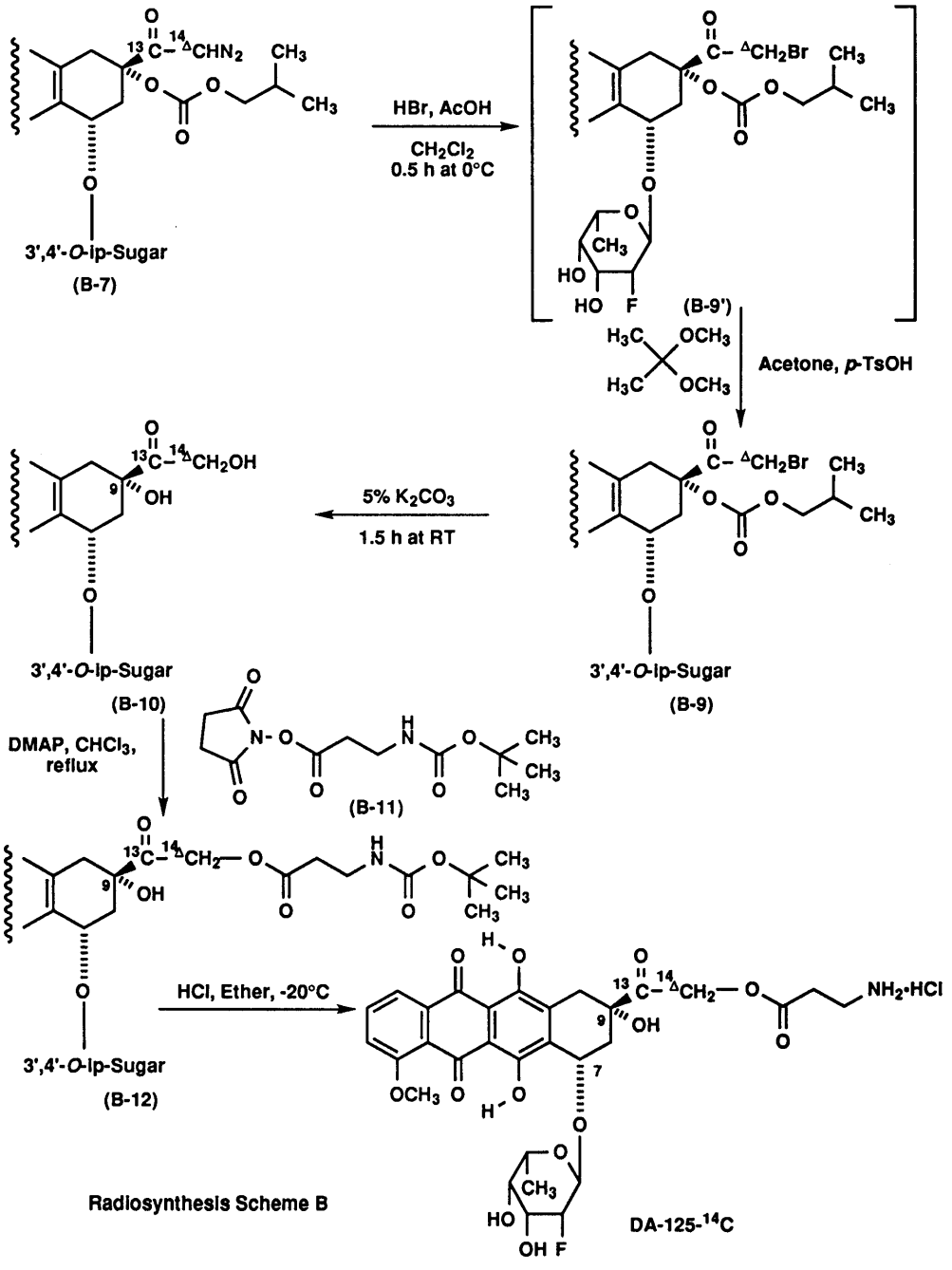


Radiosynthesis Scheme B



DISCUSSION AND RESULTS

Tritium-labeled anthracyclines (daunorubicin and doxorubicin) have previously been synthesized by the Wilzbach procedure.[6] The tritium-labeled anthracyclines, however, due to exchange of tritium in body fluid, are of limited use. Anthracyclines labeled with ^{14}C should not present the drawbacks of the tritium-labeled compound. For this reason, processes for the synthesis of [^{14}C] anthracyclines have been developed in different laboratories.[7-9] All three procedures involve carbon-14 labeling in the C_{14} position of the aglycone (i.e. tetracyclic portion without a sugar moiety), which can be manipulated using selective cleavage of C_{13} and C_{14} by controlled periodate oxidation, followed by treatment with ^{14}C-labeled diazomethane.

The first step is to protect the C_3'-C_4' bond in the fluoro sugar moiety during selective cleavage of the C_{13}-C_{14} bond of the aglycone. Employment of dimethoxypropane for isopropylidene formation gave mainly the product (**B-2**), in which the 9-OH and 14-OH groups formed the dioxolane even under the mild reaction conditions, in addition to the desired 3',4'-isopropylidene product (**B-3**). Stirring the hydroxymethyl ketone (**B-1**, FT-ADM) in dry acetone with a catalytic amount of *p*-TsOH for 40 h at room temperature gave **B-3** in quantitative yield.

Treatment of 3',4'-Ip-FT-ADM (**B-3**) in aqueous THF at 0°-25°C with 1.1 equivalent of periodic acid effected selective cleavage of the C_{13}-C_{14} bond of the hydroxymethyl ketone moiety with formation of the key 9-carboxylic acid intermediate (**B-4**) in high yield. The acid (**B-4**) was characterized by mass spectrometry (MS), infrared spectroscopy (IR), and nuclear magnetic resonance spectroscopy (NMR). The periodate cleavage was highly specific under our conditions; although the C_9-C_{13} bond is also potentially susceptible, we saw no evidence of attack at these positions (the C_3'-C_4' bond is protected by the isopropylidene group). Similar reactions were employed for the conversion of adriamycin to the corresponding acid.[7,8,10] However, interestingly, using sodium periodate instead of periodic acid gave a mixture of unidentifiable products. Reaction of the acid (**B-4**) with 2 equivalents of isobutyl chloroformate and 3 equivalents of *N*-methylmorpholine in dichloromethane (ice bath:1.5 h) afforded the unstable mixed anhydride (**B-6**). We observed that removal of *N*-methylmorpholine hydrochloride by filtration before reaction with diazomethane improved the yield of the diazoketone (**B-7**). We also attempted to prepare the acid anhydride intermediate (**B-5**) as a precursor to labeling, using dicyclohexylcarbodiimide (DCC) as condensing agent.[11] This procedure would circumvent the potential problems of deprotection, especially of the 9-*O*-isobutylcarbonate in the diazoketone (**B-7**). However, treatment of the acid (**B-4**) with DCC gave an unidentifiable mixture of products. Thus, we discontinued this rather attractive approach.

The mixed anhydride (**B-6**) in dichloromethane/ether was added to diazomethane(13 fold excess, generated from *N*--methyl-^{14}C-N-nitroso-*p*-toluenesulfonamide) in ether at 0°C. After the solution was allowed to stand overnight in the dark at room temperature, the excess diazomethane was removed under reduced pressure. Thin-layer chromatography (TLC system B) of the residue afforded diazoketone (**B-7**) in 11.5% chemical yield; IR ($CHCl_3$)

2114 cm^{-1} (N=N), NMR (300 MHz; $CDCl_3$) δ 5.5 (s, 1H, -C<u>H</u>N_2). A major by-product (~36%) in this reaction was identified as methyl ester (**B-8**), NMR δ 3.80 (s, 3H, $CO_2C\underline{H}_3$). Before converting the diazoketone (**B-7**) to the corresponding bromoketone (**B-9**), we attempted to remove the 9-*O*-isobutylcarbonate group, which will facilitate the esterification step by using sodium salt of *N-t*-Boc-β-alanine instead of *N*-hydroxysuccinimide. However, treatment of the diazoketone (**B-7**) with 5% K_2CO_3 solution under various reaction conditions gave a mixture of unidentifiable products. Therefore, we adopted an alternative approach that required bromination and subsequent hydrolysis to obtain the hydroxymethyl ketone (**B-10**) [essentially carbon-14-labeled starting material 3',4'-Ip-FT-ADM (**B-3**)].

Not surprisingly, bromination (HBr/HOAc/CH_2Cl_2, 0°C, 30 min) of the diazoketone (**B-7**) gave a mixture of the bromoketones (**B-9** and **B-9'**) with retention of the 3',4-*O*-isopropylidene group in sugar (**B-9**) and loss of the 3',4'-*O*-isopropylidene group (**B-9'**). Treatment of the mixture with dimethoxypropane/acetone and a catalytic amount of *p*-TsOH at room temperature gave the desired bromoketone (**B-9**) in quantitative yield. Hydrolysis of the bromoketone (**B-9**) with 5% K_2CO_3 solution afforded the desired hydroxymethyl ketone (**B-10**), at the same time removing 9-*O*-isobutylcarbonate group. Conversion of the 9-hydroxymethyl ketone (**B-10**) to the corresponding *N-t*-Boc-β-alanyl ester was accomplished by refluxing with the *N*-hydroxysuccinimide ester of *N-t*-Boc-β-alanine in chloroform (24 h). The ester (**B-12**) was purified by preparative TLC (solvent system C). We noted that the *p*-nitrophenyl ester of *N-t*-Boc-β-alanine (Aldrich) required longer reflux, resulting in poor yield. Also the water solubility of *N*-hydroxy succinimide, a by-product of this transesterification reaction, made work-up simpler, resulting in a purer product (**B-11**). We believe that this modification gives a significant improvement in the yield and in the reaction conditions. The protecting groups, *N-t*-Boc and 3',4'-*O*-isopropyliedene were removed by treating the ester (**B-11**) with ether/HCl (-20°C, 2 h). The resulting product was identical to an authentic sample of DA-125. By this method we synthesized 35.9 mg (355 μCi) of 14-^{14}C-DA-125 (specific activity 6.63 mCi/mmol, radiochemical purity >98%), which is sufficient for the ADME and radioautography studies.

EXPERIMENTAL DETAILS

Thin Layer Chromatography (TLC)

TLC analyses were performed using Whatman silica gel 60 (normal phase) or $KC_{18}F$ (reverse phase). The eluting solvent systems were as follows:

A: Benzene:Acetone = 4:1

B: EtOAc:Chloroform = 5:95

C: Acetone:Chloroform = 8:92

D: $CHCl_3$:MeOH:H_2O:HOAc = 80:20:1.5:3.5

E: Triethylammonium formate (0.25N, pH 3.0):CH_3CN:H_2O = 2:2:1

All reactions were performed under inert atmosphere, and solvents were purified before use. ^{1}H-NMR spectra for the cold intermediates were recorded on a Gemini 300-MHz or a Varian XL-400-MHz NMR spectrometer. IR spectra were obtained on a Perkin Elmer FTIR. MS spectra were obtained on Ribermag R10-10C GC/MS. Radiochemical purity of every labeled intermediate was determined by TLC radiochromatogram with BIOSCAN-OC-SCAN using 5 × 20 cm silica gel plate. Radiochemical counting was performed on a Beckman liquid scintillator system, Model LS-250 using ICN's Ecolite as cocktail, correcting for counting efficiency using an internal standard technique.

Preparation of *N*-methyl-^{14}C-*N*-nitroso-*p*-toluenesulfonamide (A-5)[12,13]

***N*-Methyl-^{14}C-phthalimide (A-2).** Methyl-^{14}C iodide (500 mCi, 59.1 mCi/mmol, 8.46 mmol, dried over P_2O_5, Wizard Laboratory, CA, USA) was vacuum-distilled, with liquid nitrogen cooling, into the flask containing 20 mL of dry DMF and 2.04 g (11.0 mmol) of potassium phthalimide, which had been recrystallized from acetone and dried by heating at 160°C overnight under vacuum. The reaction flask was degassed by two freeze-thaw cycles using liquid nitrogen cooling and then heated gradually to 60°C, reaction was then stirred overnight. The solution was concentrated under vacuum, and the solid residue was dissolved with 100 mL of $CHCl_3$ and washed with100 mL of 0.1 N NaOH to remove inorganic materials. The organic layers were washed with 100 mL of water and dried over anhyd. Na_2SO_4, leaving a yellow product after evaporation yielded 1.56 g (113%, based on weighing of the crude product).

***N*-Methyl-^{14}C-*N*-nitroso-*p*-toluenesulfonamide (A-4).** A mixture of 1.56 g of *N*-methyl-^{14}C-phthalimide (**A-2**) in 7 mL of 20% hydrochloric acid was sealed in a 10 mL ampoule and stirred for 16 h at 160°C in an oil bath. The product, methylamine-^{14}C-hydrochloride was dissolved in 50 mL of H_2O, 50 mL of CH_2Cl_2 was added, and the mixture was cooled with an ice-H_2O bath. After addition of 1.81 g of *p*-toluenesulfonylchloride the aqueous phase was carefully made basic to pH paper with 50% NaOH. After stirring at 0°C for 3 h, the reaction mixture was then made acidic with 20% hydrochloric acid. The reaction mixture was filtered, the precipitate extracted with another 50 mL of CH_2Cl_2. After separation of the layers the organic phases were washed with 50 mL of 0.5 M $NaHCO_3$ and dried over anhyd. Na_2SO_4. Concentration of the dried extracts gave the desired *N*-methyl-^{14}C-*p*-toluenesulfonamide (**A-4**); yield 1.615 g (90%).

***N*-Methyl-^{14}C-*N*-nitroso-*p*-toluenesulfonamide (A-5).** First, 831 mg (4.44 mmol) of *N*-methyl-^{14}C-*p*-toluenesulfonamide (**A-4**, 200 mCi, 95% radiochemical purity) in 9 mL of glacial acetic acid was cooled in an ice bath. Then, 1 mL of 5% $NaNO_2$ (7.8 mmol) was added dropwise by a syringe, through a septum. After the reaction mixture was stirred at 0°C for 20 min, it was diluted with 50 mL of H_2O, extracted with ether (3 × 30 mL) and the

combined ether extracts (90 mL) were washed with H_2O (2 × 30 mL) followed by neutralization with saturated $NaHCO_3$ (1 × 30 mL). The organic phases were concentrated and dried *in vacuo* to give *N*-methyl-^{14}C-*N*-nitroso-*p*-toluenesulfonamide (**A-5**) in almost quantitative yield; 894 mg (93%). The ^{14}C-labeled (**A-5**) (200 mCi, 50 mCi/mmol) was identified by codeveloping with an authentic unlabeled *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald, Aldrich) in TLC (hexane:EtOAc = 5:1), R_f = 0.25, and it was found to be about 98% radiochemically pure by radio TLC.

Preparation of DA-125-^{14}C (Scheme B)

7-*O*-(2',6'-dideoxy-2'-fluoro-3',4'-*O*-isopropylidene-α-L-talopyranosyl) Adriamycinone (B-3). To 1 g (1.7 mmol) of 7-*O*-(2',6'-dideoxy-2'-fluoro-a-L-talopyranosyl)adriamycinone (FT-ADM, Dong A Pharm. Lot. M1-008) (**B-1**) in 300 mL of dry acetone was added 43 mg (0.2 mmol) of *p*-toluenesulfonic acid. The reaction mixture was stirred at room temperature under an argon atmosphere in the dark. After the mixture was stirred for 40 h, the reddish clear solution was concentrated under reduced pressure. The residue was dissolved in 200 mL of chloroform, this solution was washed with 0.5 M aqueous sodium bicarbonate solution (1 × 100 mL), followed by water (1 × 100 mL), then dried over anhydrous sodium sulfate. Concentration of chloroform extracts gave 1.00 g (98%) of 3',4'-*O*-isopropylidene compound (**B-3**). The analytical sample was prepared by recrystallization from boiling acetone, TLC (System A) R_f = 0.21. IR ($CHCl_3$), 3440 (OH), 1720, 1620, 1580 cm^{-1} (C=O'S) MS ($M+Na^+$) 625; NMR ($CDCl_3$) δ 1.31 (d, 3H, *J* = 6.5 Hz, 6'-CH_3); 1.42 and 1.61 (each s, 6H, $(CH_3)_2C$); 2.20 (dd, 1H, *J* = 3.9 and 14.8 Hz, 8-H_{ax}); 2.57 (dd, 1H, *J* = 2.1 and 14.8 Hz, 8-H_{eq}); 3.09 (d, 1H, *J* = 19.0 Hz, 10-H_{ax}); 3.32 (dd, 1H, *J* = 1.7 abd 19.0 Hz, 10-H_{eq}); 3.83 (dq, 1H, *J* = 1.9 and 6.5 Hz, 5'-H); 4.08 (s, 3H, OCH_3); 4.22 (dd, *J* = 1.9 and 7.4 Hz, H, 4'-H); 4.48 (ddd, 1H, *J* = 3.0, 8.4 and 46.3 Hz, 2'-H); 4.66 (ddd, 1H, *J* = 3.0, 7.4 and 10.4 Hz, 3'-H); 4.80 (s, 2H, 14-CH_2); 5.46 (q, 1H, *J* = 1.7 Hz, 7-H); 5.61 (dd, 1H, *J* = 5.8 and 13.9 Hz, 1'-H); 7.42 (dd, 1H, *J* = 1.0 and 8.7 Hz, 3-H); 7.81 (dd, 1H, *J* = 7.6 and 8.7 Hz, 2-H); 8.06 (dd, 1H, *J* = 1.0 and 7.6 Hz, 1-H); 13.21 and 13.83 (each s, 1H, 6, 11-OH).

7-*O*-(2',6'-dideoxy-2'-fluoro-3',4'-*O*-isopropylidene-α-L-talopyranosyl)-9-carboxy Adriamycinone (B-4). A solution of 259.1 mg (1.13 mmol) of periodic acid in 5 mL of water was added to a solution of 636 mg (1.05 mmol) of 3',4'-*O*-isopropylidene compound (**B-3**) in 6.5 mL of dry tetrahydrofuran at 0°C. The mixture was stirred and slowly warmed to room temperature under argon. After 2 h at room temperature, the clear red solution was concentrated under reduced pressure; 75 mL of water, was added and the mixture was extracted with chloroform (3 × 60 mL). The combined chloroform extracts were washed with water (2 × 30 mL), dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give 575 mg (90%) of an oil-solid residue. The crude products were dissolved in dichloromethane (15 mL) and 50 mL of hexane added dropwise. The resulting precipitate was collected, washed with hexane (3 × 15 mL), and dried at room temperature to give 508

mg (82%) of the 9-carboxy adriamycinone derivative (**B-4**) as a red powder. TLC (System A) R_f = 0.01. IR ($CHCl_3$) 3425 (br, CO_2H, OH) 1736, 1619, 1580 cm^{-1} (C=O'S) MS ($M+Na^+$) 611; NMR ($CDCl_3$) δ 1.36 (d, 3H, J = 6.9 Hz, 6'-CH_3); 1.41 and 1.60 (each s, 3H, $(CH_3)_2C$); 2.47 (dd, 1H, J = 4.7 and 14.7 Hz, 8-H_{ax}); 2.69 (br dd, 1H, J = 1.6 and 14.7 Hz, 8-H_{eq}); 3.23 (d, 1H, J = 18.7 Hz, 10-H_{ax}); 3.46 (dd, 1H, J = 1.3 and 18.7 Hz, 10-H_{eq}); 4.03 (dq, 1H, J = 2.3 and 6.9 Hz, 5'-H); 4.11 (s, 3H, OCH_3); 4.17 (dd, 1H, J = 2.3 and 7.0 Hz, 4'-H); 4.54 (ddd, 1H, J = 3.7, 5.0 and 60.8 Hz, 2'-H); 4.59 (dd, 1H, J = 3.7 and 7.0 Hz, 3'-H); 5.41 (ddd, 1H, J = 1.3, 1.6 and 4.7 Hz, 7-H); 5.65 (dd, 1H, J = 5.0 and 13.2 Hz, 1'-H); 7.41 (d, 1H, J = 8.5 Hz, 3-H); 7.79 (dd, 1H, J = 7.7 and 8.5 Hz, 2-H); 8.06 (d, 1H, J = 7.7 Hz, 1-H); 13.5 and 14.5 (each s, 1H, 6, 11-OH).

14-Diazo-9-*O*-isobutylformyl-7-*O*-(2',6'-dideoxy-2'-fluoro-3',4'-*O*-isopropylidene-α-L-talopyranosyl) Adriamycinon14-^{14}C (B-7). A total of 1.74 g (8.0 mmol) of *N*-methyl-^{14}C-*N*-nitroso-*p*-toluenesulfonamide (100 mCi, 12.5 mCi/mmol) in 30 mL of dry ethylether was added dropwise to 3 g of potassium hydroxide in 6 mL water/6 mL 95% ethanol, at 65°C in a diazomethane generator. An additional 10 mL of ether was added until the distillate became clear.

To a stirred solution of 364 mg (0.60 mmol) of the acid (**B-4**) in 21 mL of dichloromethane, cooled in an ice bath, was added 248 μL (2.2 mmol, d = 0.92) of *N*-methylmorpholine followed by 188 μL (1.44 mmol, d = 1.05) of isobutylchloroformate. The solution was stirred for 1 h on the ice bath, followed by the addition of 10 mL of ether with vigorous stirring for another 1 h. The resulting *N*-methylmorpholine-hydrochloride salt was removed by filtration, and the filter cake was washed with 10 mL of ether. The red filtrate was added dropwise to a stirred solution of 50 mL of ether containing ^{14}C-labeled diazomethane at 0°C over a period of 30 min, then stirred in the dark for 16 h. The red solution was concentrated on a rotary evaporator (acetic acid in dry ice trays to react with excess diazomethane) and the residue dissolved in dichloromethane (50 mL) and washed with water (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give 178 mg of a red oil-solid. This residue showed two major spots on TLC (System B). These compounds were separated on three (20 × 20 × 0.1 cm) silica gel plates developing with same solvent system. The more polar compound on TLC [49.5 mg (11.5%)] was identified as the diazoketone (**B-7**). TLC (System A), R_f = 0.71, (System B), R_f = 0.21, IR ($CHCl_3$) 2114 (N=N), 1717, 1619, 1578 cm^{-1} (C=O'S) MS ($M+H^+$-N_2) (712 + 1 - 28 = 685) NMR ($CDCl_3$) δ 0.80 (d, 6H, J = 6.7 Hz, $CH(CH_3)_2$); 1.22 (d, 3H, J = 6.4 Hz, 6'-CH_3); 1.26 and 1.45 (each s, 3H, $(CH_3)_2C$); 1.82 (dq, 1H, J = 6.7 and 6.7 Hz, $CH(CH_3)_2$); 2.36 (dd, 1H, J = 5.0 and 18.4 Hz, 8-H_{ax}); 2.69 (d, 1H, J = 18.4 Hz, 8-H_{eq}); 3.11 (dd, 1H, J = 1.8 and 18.6 Hz, 10-H_{ax}); 3.39 (dd, 1H, J = 1.8 and 18.6 Hz, 10-H_{eq}); 3.62 and 3.91 (each dd, 1H, J = 6.7 and 10.3 Hz, O-$C\underline{H}_2$-$CH(CH_3)$); 3.78 (dq, 1H, J = 1.9 and 6.4 Hz, 5'-H); 3.99 (s, 3H, OCH_3); 4.06 (dd, 1H, J = 1.9 and 7.3 Hz, 4'-H); 4.25 (ddd, 1H, J = 3.1, 5.3 and 46.5 Hz, 2'-H); 4.46 (ddd, 1H, J = 3.1, 7.3 and 10.2 Hz, 3'-H); 5.20 (dd, 1H, J = 1.8 and 5.0 Hz, 7-H); 5.48 (s, 1H, CHN_2); 5.61 (dd, 1H, J = 5.3 and 13.6 Hz, 1'-H); 7.29 (d, 1H, J = 8.3 Hz, 3-H); 7.66 (dd, 1H, J = 7.9 and 8.3 Hz, 2-H); 7.88 (d, 1H, J = 8.3 Hz, 1-H); 13.0 and 14.0

(each s, 6, 11-OH). The less polar compound [55 mg (36%)] was identified as the methyl ester (**B-8**). TLC (System A) R_f = 0.78 (System B) R_f = 0.32. NMR ($CDCl_3$) δ 3.8 (s, 3H, CO_2CH_3), No $C\underline{H}N_2$ peak.

14-Bromo-9-*O*-isobutylformyl-7-*O*-(2',6'-dideoxy-2'-fluoro-3',4'-*O*-isopropylidene-α-L-talopyranosyl) Adriamycinone-14-^{14}C(B-9). To 49.5 mg (0.069 mmol) of 7-O-2',3'-dideoxy-2'-fluoro-3',4'-O-isopropylidene-α-L-talopyranosyl)-9-*O*-isobutoxycarbonyl-14-diazo-adriamycinone (**B-7**) in 10 mL of dichloromethane (ice bath, protected from moisture) was added 3.0 mL (0.11 mmol) of HBr/HOAc/CH_2Cl_2 (1 g of 30% HBr/HOAc diluted to 100 mL with CH_2Cl_2) and stirred for 0.5 h at 0°C. The reaction mixture was washed with 3 × 40 mL of cold 5% NaCl solution, the aqueous layer was back extracted with 2 × 20 mL CH_2Cl_2, and the combined CH_2Cl_2 layers were dried over Na_2SO_4, filtered, and evaporated to dryness to yield 32 mg of deprotected 14-bromo compound (**B-9'**). The residue was dissolved in 10 mL of acetone and 1 mL of dimethoxypropane plus 1 crystal of *p*-TsOH and the orange solution stirred for 3 h at room temperature then evaporated to dryness. The residue was dissolved in 20 mL of CH_2Cl_2 and washed with 20 mL of saturated NaCl solution. The aqueous layer was back extracted with 2 × 20 mL CH_2Cl_2 (some emulsion formed). The combined CH_2Cl_2 layers were dried over Na_2SO_4, filtered and evaporated to dryness to yield 41 mg (78%) of **B-9**. TLC (System A) R_f = 0.82 (System B) R_f = 0.27. IR ($CHCl_3$) 1739, 1618, 1581 cm^{-1} (C=O'S) MS ($M+NH_4^+$) 780, 782 NMR ($CDCl_3$) δ 0.85 (d, 6H, J = 6.6 Hz, $CH(CH_3)_2$); 1.23 (d, 3H, J = 6.5 Hz, 6'-CH_3); 1.31 and 1.51 (each s, 6H, $(CH_3)_2C$); 1.85 (dq, 1H, J = 6.6 and 6.8 Hz, $CH(CH_3)_2$); 2.41 (dd, 1H, J = 5.1 and 18.5 Hz, 8-H_{ax}); 2.75 (dd, 1H, J = 18.5 Hz, 8-H_{eq}); 3.02 (dd, 1H, J = 1.8 and 18.4 Hz, 10-H_{ax}); 3.55 (dd, 1H, J = 1.8 and 18.4 Hz, 10-H_{eq}); 3.64 and 3.92 (each dd, 2H, J = 6.8 and 10.0 Hz, $CH_2CH(CH_3)_2$); 3.70 (dq, 1H, J = 2.3 and 6.5 Hz, 5'-H); 4.01 (s, 3H, OCH_3); 4.05 (dd, 1H, J = 2.3 and 6.9 Hz, 4'-H); 4.10 and 4.24 (each d, 2H, J = 13.0 Hz, CH_2Br); 4.26 (ddd, 1H, J = 2.3, 5.4 and 46.2 Hz, 2'-H); 4.58 (ddd, 1H, J = 2.3, 6.9 and 10.4 Hz, 3'-H); 5.20 (dd, 1H, J = 1.8 and 5.1 Hz, 7-H); 5.49 (dd, 1H, J = 5.4 and 13.6 Hz, 1'-H); 7.38 (d, 1H, J = 8.6 Hz, 3-H); 7.77 (dd, 1H, J = 7.7 and 8.6 Hz, 2-H); 8.02 (d, 1H, J = 7.7 Hz, 1-H).

14-Hydroxy-7-*O*-(2',6'-dideoxy-2'-fluoro-3',4'-*O*-isopropylidene-α-L-talopyranosyl)-9-*O*-isobutoxycarbonyl Adriamycinone-14-^{14}C (B-10). 14-Bromo compound (**B-9**) was dissolved in 20 mL of THF and added to 20 mL of 5% K_2CO_3/H_2O. The mixture was stirred for 1.5 h at which time a red spot began to form at the origin of TLC. The purple mixture was neutralized with solid tartaric acid to give a red mixture which was extracted with EtOAc (3 × 60 mL). The EtOAc extracts were washed with 50 mL of cold 5% NaCl solution, dried over Na_2SO_4, filtered and evaporated to dryness to give 14-hydroxy adriamycinone (**B-10**), in 19 mg (60%). The product has identical IR, MS, and NMR spectral results as 3',4'-IP-FT-ADM (**B-3**).

***N*-Hydroxysuccinimide Ester[14] of *N*-*t*-Boc-β-alanine (B-11).** To 5 g (26.4 mmol) of *N*-*t*-Boc-β-alanine, 3.1 g (26.9 mmol) of *N*-hydroxysuccinimide in 100 mL of dry ethyl acetate with stirring was added a solution of 5.5 g (26.6 mmol) of dicyclohexylcarbodiimide (DCC) in 10 mL of ethyl acetate. The mixture (precipitates are formed almost immediately) was stored overnight at room temperature. The mixture was then filtered to remove dicyclohexylurea and the filter cake washed with ethyl acetate (3 × 25 mL). The combined filtrates were concentrated, and the residue was recrystallized from isopropanol to give **(B-11)**, 7.2 g (95%); NMR ($CDCl_3$) δ 1.47 (s, 9H, -C(CH_3)$_3$), 3.50 (q, 2H, C$\underline{H_2}$-NH), 2.87 (t, 2H, O=CC$\underline{H_2}$-), 2.89 (s, 4H, OCC$\underline{H_2}$C$\underline{H_2}$CO), 5.16 (t, 1H, NH).

14-*O*-(3-*N*-*t*-butoxycarbonyl-β-alanyl)-7-*O*-(2',6'-dideoxy-2'-fluoro-3'4'-isopropylidene-α-L-talopyranosyl) Adriamycinone-14-^{14}C (B-12). First, 39 mg [0.065 mmol diluted with 20 mg of unlabeled **(B-10)**] of 7-*O*-(2',6'-dideoxy-2'-fluoro-3',4'-isopropylidene-α-L-talopyranosyl) C-14 adriamycinone, 35 mg (0.12 mmol) of *N*-hydroxy-succinimide ester of β-alanyl-*N*-*t*-Boc (**B-11**)and 13.3 mg (0.1 mmol) of 4-dimethylamino-pyridine in 100 mL of chloroform were refluxed for 24 h. The orange solution was evaporated to dryness and the residue purified by a preparative TLC (20 × 20 × 0.2, eluant 8% acetone/$CHCl_3$, System C). The nonpolar band was eluted with 10% MeOH/$CHCl_3$ to give 44 mg (88%) *N*-*t*-Boc-β-alanyl ester (**B-12**). TLC (System A) R_f = 0.5. NMR ($CDCl_3$) δ 1.38 (d, 3H, *J* = 6.6 Hz, 6'-CH_3); 1.44 and 1.64 (each s, 6H, ($C\underline{H_3}$)$_2$C); 1.49 (s, 9H, C(CH_3)$_3$); 2.12 (dd, 1H, *J* = 3.8 and 14.8 Hz, 8-H_{ax}); 2.62 (brdt, 1H, *J* = 2.0 and 14.8 Hz, 8-H_{eq}); 2.67 (t, 2H, *J* = 6.7 Hz, C$\underline{H_2}$C=O); 3.20 (d, 1H, *J* = 19.0 Hz, 10-H_{ax}); 3.30 (dd, 1H, *J* = 2.0 and 19.0 Hz, 10-H_{eq}); 3.49 (dd, 2H, *J* = 5.8 and 6.0 Hz, NC$\underline{H_2}$-); 3.82 (dq, 1H, *J* = 2.3 and 6.6 Hz, 5'-H); 4.12 (s, 3H, OCH_3); 4.23 (dd, 1H, *J* = 2.3 and 7.5 Hz, 4'-H); 4.49 (ddd, 1H, *J* = 2.8, 5.6 and 46.4 Hz, 2'-H); 4.70 (ddd, 1H, *J* = 2.8, 7.5 and 10.6 Hz, 3'-H); 5.15 and 5.36 (each d, 1H, *J* = 18.1 Hz, 14-CH_2); 5.48 (dd, 1H, *J* = 2.0 and 3.8 Hz, 7-H); 5.63 (dd, 1H, *J* = 5.6 and 14.0 Hz, 1'-H); 7.43 (d, 1H, *J* = 8.6 Hz, 3-H); 7.82 (dd, 1H, *J* = 7.7 and 8.6 Hz, 2-H); 8.07 (d, 3H, *J* = 7.7 Hz, 1-H).

14-*O*-β-alanyl-*N*-HCl)-7-*O*-(2',6'-dideoxy-2'-fluoro-α-L-talopyranosyl) Adriamycinone-14-^{14}C (DA-125-^{14}C). Initially, 44 mg (0.057 mmol) of 14-*O*-(3-*N*-*t*-butoxycarbonyl-β-alanyl)-7-*O*-(2',6'-dideoxy-2'-fluoro-3',4'-isopropylidine-α-L-talopyranosyl) adriamycinone (**B-12**) was dissolved in 1 mL of dry tetrahydrofuran and 1 mL of ether/HCl (saturated ether with HCl at -20°C) was then added. The reaction was then removed from an ice bath and stirred at room temperature for 2 h. The precipitated red solid was collected by centrifugation, washed with ether (5 × 10 mL), and dried under reduced pressure, yielding 35.9 mg (355 μCi, 6.63 mCi/mmol) of DA-125-^{14}C•HCl identical in all respect (NMR, TLC) to an authentic sample. TLC (System D) R_f = 0.64 (System E) R_f = 0.57.

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REFERENCES

1. S. K. Carter. Adriamycin. A review. *J. Natl. Cancer Inst.* **55**, 1265-1274 (1975).

2. K. Kitaura, Y. Watanabe, Y. Ishihara, and H. Takahira. Antitumor activity of adriamycin. *Jap. J. Antibiot.* **25**, 65-71 (1972).

3. J. Yang, W. B. Kim, K. S. Kim, and H. M. Rhu. Chemical physicochemical and pharmacological studies on DA-125, a new anticancer drug. Second Congress of New Drug Development, Abstr. p. 36, April 15, 1993, Seoul, South Korea.

   Private communication with Dong-A Research Laboratory, Korea

4. M. S. Kim, J. H. Kim, J. W. Park, M. H. Ceng, and N. G. Baek. Studies on the side-effects and the cardiotoxicity on DA-125, a new anthracycline antineoplastic agent. Second Congress of New Drug Development, Abstr., p. 38, April 15, 1993, Seoul, South Korea.

5. H. J. Shim, E. D. Lee, E. J. Yoon, S. D. Lee, W. B. Kim, J. Yang, and M. G. Lee. *Intl. J. of Pharmaceuticals* **103**, 147-154, 1994.

6. F. Arcamone, G. Cassinelli, G. Franceschi, S. Penco, C. Pol, S. Redaelli, and A. Selva. In *International Symposium on Adriamycin* (S. K. Carter, A. DiMarco, M. Ghione, I. H. Krakoff, and G. Mathé, Eds.), Springer-Verlag, Berlin and New York, 1972, p. 9.

7. S. Penco, G. Vicario, F. Angelucci, and F. Arcamone. *J. Antibiot.* **30**, 773 (1977).

8. B. R. Vishnuvajjala, T. Kataoka, F. D. Crazer, D. T. Wittiak, and L. Malspeis. *J. Labelled Compd. Radiopharm.* **14**, 77 (1978).

9. C. R. Chen, M. Tan Fong, A. N. Fujiwara, D. W. Henry, M. A. Leaffer, W. W. Lee, and T. H. Smith. *J. Labelled Compd. Radiopharm.* **14**, 111 (1978).

10. W. W. Lee and T. H. Smith. *J. Amer. Chem. Soc.* **98**, 1970 (1978).

11. D. Hodson, G. Holt, and D. K. Wall. *J. Chem. Soc.* **C** 971 (1970.

12. J. D. Cox and R. J. Wayne. *J. Chem. Soc.*, 1896 (1951).

13. (a) Org. Synthesis Coll. **14**, 943 (1963). (b) A. Stoll, J. Rutschmann, A. V. Wartburg, and J. Renz. *Helv. Chim. Acta* **39**, 993 (1956).

14. Y. Lapidot, S. Rapport, and Y. Wolman. *J. Lip. Res.* **8**, 142 (1967).

