SYNTHESIS AND ANTITUMOR ACTIVITY OF OPTICALLY ACTIVE (+)-9-*O*-α-L-DAUNOSAMINYL-4-DEMETHOXYDAUNORUBICIN

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(Received for publication June 14, 1985)

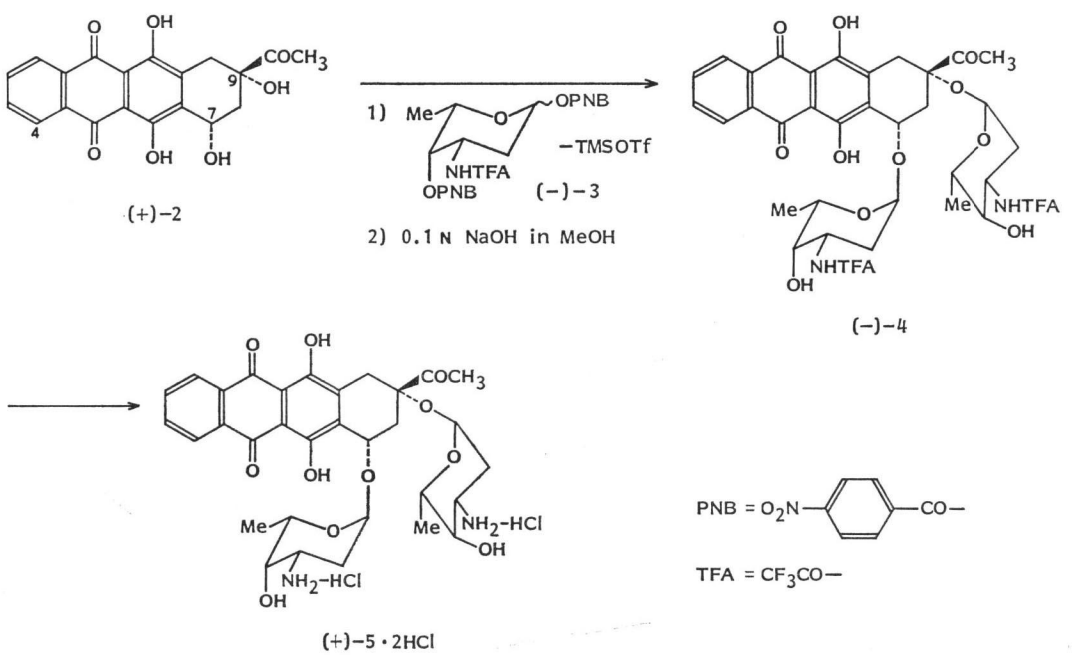
The anthracycline antibiotics, doxorubicin (**1a**) and daunorubicin (**1b**), are clinically useful antineoplastic agents[1,2]. While their utilization for cancer chemotherapy is limited by undesirable side effects, most notably cardiotoxicity, the unnatural 4-demethoxyanthracyclines represented by 4-demethoxyadriamycin (**1c**) and 4-demethoxydaunorubicin (**1d**), have been disclosed to exhibit more improved therapeutic indices than natural **1a,b**.[1,2]

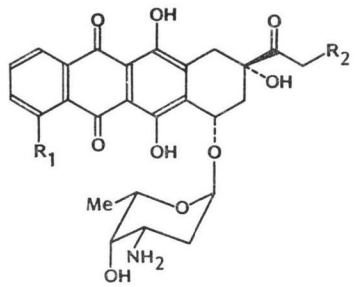
In our recent synthetic efforts on 4-demethoxyanthracyclines, we have succeeded in developing various simple and efficient synthetic schemes of optically pure (+)-4-demethoxydaunomycinone ((+)-**2**)[3~6] and novel glycosidation method[7]. By employing these synthetic processes, optically pure **1c** and **1d** could be unambiguously synthesized[7,8]. During studies on the scope and limitation of the glycosidation method previously explored in our laboratories, we have found that 1-*O*-acyl-daunosamine derivative treated by trimethylsilyl triflate (TMSOTf) is highly reactive species and can react with the tertiary 9-hydroxyl group of (+)-**2** in addition to the secondary 7-hydroxyl group (anthracycline numbering).

We wish to report a synthesis and a preliminary evaluation of antineoplastic activity of the novel 7-*O*, 9-*O*-bis-α-glycoside, (+)-9-*O*-α-L-daunosaminyl-4-demethoxydaunorubicin ((+)-**5**), which can be effectively produced by applying the explored double glycosidation reaction.

(−)-*N*-Trifluoroacetyl-1,4-di-*O*-*p*-nitrobenzoyl-L-daunosamine ((−)-**3**) prepared according to the reported method[9,10], was treated with TMSOTf in a mixture of dichloromethane and ether at 8~10°C for 0.5 hour, then allowed to react with (+)-**2**[3~6] at the same temp for 1 hour. Usual extractive isolation followed by mild alkaline hydrolysis of the two 4′-*O*-*p*-nitrobenzoyl groups, readily produced (−)-7-*O*, 9-*O*-bis(3′-*N*-trifluoroacetyl-α-L-daunosaminyl)-

Fig. 1.





1a R_1=OMe, R_2=OH
1b R_1=OMe, R_2=H
1c R_1=H, R_2=OH
1d R_1=H, R_2=H

4-demethoxydaunomycinone ((−)-**4**) in 85% yield, $[\alpha]_D^{20}$ −24.5° (dioxane). Further deprotection of the two 3′-*N*-trifluoroacetyl groups of (−)-**4** with 0.1 N aq sodium hydroxide solution at room temp, gave rise to the objective bisglycoside (**5**), which was isolated as its dihydrochloride ((+)-**5**·2HCl) in 67% yield, $[\alpha]_D^{20}$ +10.7° (*c* 0.112, MeOH).

In vitro antitumor activity of (+)-**5**·2HCl against P388 murine leukemia (IC_{50} 3×10^{-6} mM) was found to be almost the same as that of natural **1a**. In P388 *in vivo* test, (+)-**5**·2HCl exhibited the effective T/C value at optimal dose (T/C=163 at a dose of 5 mg/kg). Since the introduction of a daunosaminyl group into the 9-hydroxyl group has been reported to lower antitumor activity of parent natural anthracyclines[1,2], the significant T/C value observed for (+)-**5**·2HCl is worthy of remark.

Experimental

MP were determined with a Yanaco micro melting point apparatus and were uncorrected. Measurements of optical rotations were carried out with a Horiba SEPA-200 automatic digital polarimeter. ^{1}H NMR spectra were recorded with a Varian XL-100A spectrometer (100 MHz) and a Bruker AM-400 spectrometer (400 MHz). All signals were expressed as ppm downfield from TMS, used as an internal standard (δ value). IR spectra measurements were performed with a Jasco A-202 diffraction grating infrared spectrometer.

(+)-4-Demethoxydaunomycinone ((+)-**2**)

Prepared according to the reported method[3,4], mp 184~185°C, $[\alpha]_D^{20}$ +154° (*c* 0.10, dioxane) (ref[4]), mp 184~185.5°C, $[\alpha]_D^{20}$ +157° (*c* 0.114, dioxane)).

N-Trifluoroacetyl-1,4-di-*O*-*p*-nitrobenzoyl-L-daunosamine ((−)-**3**)

This was synthesized following the reported method[9,10], mp 201~202°C; $[\alpha]_D^{20}$ −117° (*c* 0.029, Me_2CO) (ref[10], mp 202~203°C, $[\alpha]_D^{20}$ −125° (*c* 0.03, 95% EtOH)).

(−)-7-*O*, 9-*O*-Bis(3′-*N*-trifluoroacetyl-α-L-daunosaminyl)-4-demethoxydaunomycinone ((−)-**4**)

TMSOTf (0.15 ml, 0.78 mmol) was added to a suspension of (−)-**3** (200 mg, 0.37 mmol) and molecular sieves 4A (0.9 g) in a mixture of dichloromethane (13 ml) and ether (10 ml) with stirring at −40°C under an argon atmosphere. The whole mixture was stirred at 8~10°C for 0.5 hour to give a clear solution. A dichloromethane solution (15 ml) of (+)-**2** (52.8 mg, 0.14 mmol) was added to the clear solution with stirring at the same temp. After stirring for 1 hour, the reaction mixture was poured onto a stirred mixture of EtOAc (100 ml) and satd $NaHCO_3$ (150 ml) to quench the glycosidation reaction. The upper organic layer was separated, washed with satd NaCl, dried over anhydrous $MgSO_4$, then concentrated *in vacuo*. The concentration residue was dissolved in MeOH (75 ml) and 0.1 N aq NaOH (3 ml) was added to the methanolic solution with stirring at 0°C. After stirring for 20 minutes at 0°C, the mixture was neutralized with AcOH, twice washed with satd NaCl, then dried over anhydrous Na_2SO_4. Filtration and concentration *in vacuo* followed by silica gel column chromatography ($CHCl_3$-Me_2CO, 19:1), afforded pure (−)-**4** (99.7 mg, 85% yield), mp >250°C; $[\alpha]_D^{20}$ −24.5° (*c* 0.098, dioxane); IR ν_{max} (KBr) cm^{-1} 3450, 1715, 1625, 1590; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.50 (3H, d, *J*=6 Hz, 6″-Me), 1.36 (3H, d, *J*=6 Hz, 6′-Me), 1.65~2.15 (5H, m, 2′-H_2+2″-H′$_2$+8-eq-H), 2.35 (3H, s, $COCH_3$), 2.45~2.75 (1H, m, 8-eq-H), 3.05 (1H, d, *J*=19 Hz, 10-ax-H), 3.65~4.05 (3H, m, 4′-H+4″-H+10-eq-H), 4.15~4.70 (4H, m, 3′,3″,5′,5″-H_4), 5.00 (2H, br s, 7-H+1′-H), 5.52 (1H, br d, *J*=3 Hz, 1′-H), 6.74 (1H, br d, *J*=8 Hz, NH), 6.83 (1H, br d, *J*=8 Hz, NH), 7.80~7.95 (2H, m, Ar), 8.36~8.52 (2H, m, Ar), 13.50 (1H, s, ArOH), 13.68 (1H, s, ArOH).

Anal Calcd for $C_{36}H_{36}F_6N_2O_{13}\cdot1.5H_2O$:
C 51.13, H 4.65, N 3.31.
Found: C 50.86, H 4.42, N 3.21.

(+)-9-*O*-α-L-Daunosaminyl-4-demethoxydaunorubicin Dihydrochloride ((+)-**5**·2HCl)

A solution of (−)-**4** (139.7 mg, 0.17 mmol) in 0.1 N aq NaOH (24 ml) was stirred for 40 minutes at 25°C under an argon atmosphere. The reaction mixture was neutralized to be pH 8 with 5 N HCl and extracted with $CHCl_3$ until a $CHCl_3$ extract showed no orange color. The combined $CHCl_3$ extracts were washed with H_2O (70 ml) and dried over anhydrous Na_2SO_4. Filtration and concentration *in vacuo* gave an orange residue, which was dissolved in a mixture of MeOH-$CHCl_3$, 1:9 (10 ml). Hydrogen chloride in MeOH (0.25 N) (1.5 ml) and Et_2O (60 ml) were successively added to the solution of the deprotected glycoside to give (+)-**5**·2HCl as an orange powder (80.2 mg, 67%), mp 198~199°C; $[\alpha]_D^{20}$ +10.7° (*c* 0.112, MeOH); IR ν_{max} (KBr) cm^{-1} 3420, 1720, 1630, 1595; ^1H NMR (400 MHz, DMSO-d_6) δ 0.37 (3H, d, J=6.5 Hz, 6″-Me), 1.25 (3H, d, J=6.5 Hz, 6′-Me), 1.77~2.00 (5H, m, 8-ax-H+2′-H_2+2″-H_2), 2.30 (3H, s, $COCH_3$), 2.45 (1H, d, J=13.6 Hz, 8-eq-H), 5.72 (1H, d, J=18.4 Hz, 10-ax-H), 3.18 (2H, br s, 3′-H+3″-H), 3.43 (1H, q, J=6.5 Hz, 5″-H), 3.51 (1H, br s, 4″-H), 3.53 (1H, d, J=18.4 Hz, 10-eq-H), 3.82 (1H, br s, 4′-H), 4.27 (1H, q, J=6.5 Hz, 5′-H), 4.89 (1H, d, J=4.9 Hz, 7-H), 5.00 (1H, d, J=6.0 Hz, 4′-OH), 5.64 (1H, d, J=6.0 Hz, 4″-OH), 7.98~8.05 (2H, m, Ar), 8.17 (6H, br s, NH_3×2), 8.27~8.34 (2H, m, Ar), 13.40 (1H, br s, ArOH), 13.55 (1H, br s, ArOH).

Anal Calcd for $C_{32}H_{40}N_2O_{11}Cl_2 \cdot 1.5H_2O$: C 52.90, H 5.97, N 3.86.
Found: C 52.70, H 5.68, N 3.43.

Acknowledgment

The authors are grateful to Dr. K. SAKAI and Miss K. YAMADA, Sagami Chemical Research Center, and Drs. S. TSUKAGOSHI and T. TASHIRO, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, for evaluation of the anticancer activity of (+)-**5**·2HCl.

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