[Chem. Pharm. Bull. 34(11)4613—4619(1986)]

Synthesis of Novel 13-Methyl-13-dihydroanthracyclines

TERUYO MATSUMOTO, MASAKO OHSAKI, MICHIYO SUZUKI, YOSHIKAZU KIMURA, and SHIRO TERASHIMA*

Sagami Chemical Research Center, 4–4–1, Nishi-Ohnuma, Sagamihara, Kanagawa 229, Japan

(Received May 20, 1986)

The title compounds, (+)-13-methyl-13-dihydro-4-demethoxydaunorubicin hydrochloride (**8**·HCl) and (+)-13-methyl-13-dihydrodaunorubicin hydrochloride (**9**·HCl), were prepared from (+)-4-demethoxydaunomycinone (**13**) and (+)-daunomycinone (**18**), respectively, by silylation of the C_7-hydroxy group, addition of methylmagnesium bromide to the C_{13}-carbonyl group, and direct glycosidation of the 7-*O*-silyl anthracyclinones with the daunosamine derivative (anthracycline numbering). In the P388 *in vitro* test, **8**·HCl was several hundred-fold more active than adriamycin hydrochloride (**1**·HCl). Notable anticancer activity, equivalent to that of adriamycin hydrochloride, was also observed in the P388 *in vivo* test of **8**·HCl.

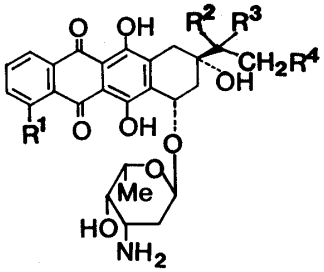
Keywords——anthracycline; anthracyclinone; 13-methyl-13-dihydroanthracycline; 13-methyl-13-dihydroanthracyclinone; chemoselective C_7-*O*-silylation; Grignard addition; glycosidation; *in vitro* cytotoxicity; *in vivo* anticancer activity

The anthracycline antibiotics, adriamycin (**1**) and daunorubicin (**2**), are widely used in the clinic for treating human leukemias and solid tumors.[1] Various studies on structure–activity relationships have so far been carried out in attempts to widen the range of tumor types susceptible to anthracycline chemotherapy and to reduce undesirable side effects of the parent drugs.[1,2]

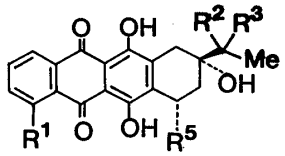
In the course of our studies on the synthesis and testing of analogues of **1** and **2**, we were interested in 13-dihydroanthracyclines and 4-demethoxyanthracyclines. The former analogues, represented by 13-dihydroadriamycin (**3**) and 13-dihydrodaunorubicin (**4**), have been reported to be produced as the principal metabolites of **1** and **2** in mammalian tissues and microorganisms,[3] and **4** exhibits significant antitumor activity, comparable to that of the parent compound (**2**). The latter 4-demethoxy analogues, the most notable of which are 4-demethoxyadriamycin (**5**) and 4-demethoxydaunorubicin (**6**),[1,4] are more potent and less toxic than the parent anthracyclines (**1** and **2**),[1,2,4] and are currently under clinical trial.[5] As in the cases of **1** and **2**, 13-dihydro-4-demethoxydaunorubicin (**7**) bearing (13*S*)-configuration has been found as a metabolite of **6**, and is anticipated to be partly responsible for the prominent anticancer activity of **6**.[6]

Recently, we have explored various novel synthetic routes to **5** and **6**[7–14] including an efficient glycosidation method to produce a desired α-glycoside as a sole product in a high yield.[13,14] Taking into account the significant anticancer activity of 13-dihydroanthracyclines (**3**, **4**, and **7**), the synthesis of (+)-13-methyl-13-dihydro-4-demethoxydaunorubicin hydrochloride (**8**·HCl), which has never been reported in spite of its seemingly readily accessible structure, was attempted. Since **8**·HCl was found to exhibit excellent *in vitro* cytotoxicity against P388 murine leukemia, (+)-13-methyl-13-dihydrodaunorubicin hydrochloride (**9**·HCl) was next prepared by a similar synthetic approach.

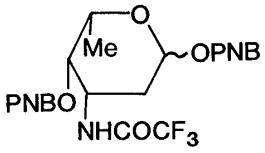
This report deals with the synthesis and preliminary evaluation of the anticancer activity of these novel 13-methyl-13-dihydroanthracyclines (**8** and **9**).



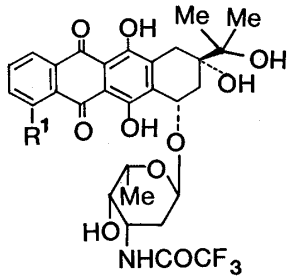
	R^1	R^2	R^3	R^4
1	OMe	=O		OH
2	OMe	=O		H
3	OMe	OH	H	OH
4	OMe	OH	H	H
5	H	=O		OH
6	H	=O		H
7	H	OH	H	H
8	H	OH	Me	H
9	OMe	OH	Me	H



	R^1	R^2	R^3	R^5
10	H	OH	Me	OH
11	H	=O		H
12	H	OH	Me	H
13	H	=O		OH
14	H	=O		OTBDMS
15	H	OH	Me	OTBDMS
18	OMe	=O		OH
19	OMe	=O		OTBDMS
20	OMe	OH	Me	OTBDMS
21	OMe	OH	Me	OH



16



17: R^1 = H
22: R^1 = OMe

TBDMS = Si–*tert*-BuMe$_2$, PNB = COC$_6$H$_4$–*p*-NO$_2$

Results and Discussion

At the outset, it was expected that preparation of optically active 13-methyl-13-dihydro-4-demethoxydaunomycinone (**10**) could be readily achieved by starting from (−)-7-deoxy-4-demethoxydaunomycinone (**11**), obtainable by optical resolution[7−9] or asymmetric synthesis.[10−12] While treatment of **11** with methylmagnesium bromide produced the desired (−)-tertiary alcohol (**12**) in 76% yield, introduction of the $C_{7\alpha}$-hydroxy group into **12** (anthracycline numbering) by sequential bromination and substitution according to the same procedure as that employed for preparing (+)-4-demethoxydaunomycinone (**13**) from **11**,[8] was found to afford only an 8% yield of **10**. On the other hand, when **13** was allowed to react with methyllithium,[15] the adduct **10** was formed in 41% yield. By comparing the yields of two types of the addition reactions, it appears evident that the derivative of **13**, in which the $C_{7\alpha}$-hydroxy group had been protected, should be used as a substrate of the addition reaction to obtain a better yield. Accordingly, chemoselective protection of the $C_{7\alpha}$-hydroxy group was next examined. After several unsuccessful attempts, it was found that treatment of **13** with 4-*tert*-butyldimethylsilyloxy-3-penten-2-one[16] in *N*,*N*-dimethylformamide gave the $C_{7\alpha}$-*O*-silyl derivative (**14**) in 95% yield. As expected, reaction of **14** with methylmagnesium bromide at −30——20 °C successfully afforded the adduct (**15**) in 78% yield. This yield compared well with that obtained in the preparation of **12** from **11**. Usual deprotection of **15** with aqueous hydrofluoric acid in acetonitrile or aqueous acetic acid in tetrahydrofuran gave **10** in an almost quantitative yield.

With **10** in hand, the glycoside formation was next attempted. Glycosidation of **10** with 3′-*N*-trifluoroacetyl-1,4-bis(*O*-*p*-nitrobenzoyl)-L-daunosamine (**16**)[14] under the same conditions as those previously explored,[13] gave the desired 3′-*N*-trifluoroacetyl-α-glycoside (**17**), mp 154.5—157 °C and $[\alpha]_D^{20}$ +152° (dioxane), in 66% yield after alkaline hydrolysis of the 4′-*O*-*p*-nitrobenzoyl group. However, it was found that **15** carrying the $C_{7\alpha}$-*O*-silyl group could also be used as a glycosidation substrate. Thus, the same successive treatments of **15** as those described for **10** afforded a 69% yield of **17**. *In situ* cleavage of the *tert*-butyldimethylsilyloxy group by the trifluoromethanesulfonate anion, which is presumably present as a counter anion of the oxonium ion derived from **16**, may explain the successful direct glycosidation of **15**. Further alkaline hydrolysis of the 3′-*N*-trifluoroacetamide group of **17** followed by salt formation gave rise to **8**·HCl, mp 209—211 °C and $[\alpha]_D^{20}$ +130° (methanol), in 64% yield.

Since **8**·HCl was found to exhibit prominent cytotoxicity against P388 murine leukemia cells (*vide infra*), preparation of **9**·HCl was carried out according to the same reaction scheme as that established for the synthesis of **8**·HCl. Chemoselective protection of (+)-daunomycinone (**18**), prepared by acid hydrolysis of commercially available **2**·HCl, with 4-*tert*-butyldimethylsilyloxy-3-penten-2-one[16] followed by addition of methylmagnesium bromide, produced the adduct (**20**) in a good overall yield. Deprotection of **20** readily gave (+)-13-methyl-13-dihydrodaunomycinone (**21**). Direct glycosidation of **20** with **16** in the same manner as that employed for **15**, followed by stepwise alkaline hydrolysis and salt formation, afforded **9**·HCl, mp 196.5—198.5 °C and $[\alpha]_D^{22}$ +155° (methanol), by way of the 3′-*N*-trifluoroacetyl derivative (**22**), mp 152—157 °C and $[\alpha]_D^{21}$ +196° (dioxane).

Next, these 13-methyl-13-dihydroanthracyclines (**8**·HCl and **9**·HCl) were subjected to P388 murine leukemia *in vitro* assay along with their 3′-*N*-trifluoroacetyl derivatives (**17** and **22**). The results are shown in Table I with those for **1**·HCl, **2**·HCl, and **6**·HCl. While **9**·HCl and **17** exhibited comparable cytotoxicity to **1**·HCl and **2**·HCl, **8**·HCl was several hundred-fold more active than **1**·HCl. Comparing the cytotoxicity of **2**·HCl and **6**·HCl with that of the corresponding 13-methyl-13-dihydro congeners (**9**·HCl and **8**·HCl), it appears evident that replacement of the C_9-acetyl group with a 1-hydroxy-1-methylethyl group is more effective for increasing the cytotoxicity of 4-demethoxyanthracyclines than that of natural 4-methoxyanthracyclines. In the P388 *in vivo* test, **8**·HCl and **17** showed the following T/C values at the optimal doses: **8**·HCl, T/C=206 (1.25 mg/kg); **17**, T/C=153 (40 mg/kg). The T/C value of **8**·HCl compares well with that of **1**·HCl (T/C=*ca.* 200 (2.5—3.0 mg/kg)).

Further studies aimed at characterizing the antitumor activity of **8**·HCl will be reported shortly.

TABLE I. *In Vitro* Cytotoxicity of 13-Methyl-13-dihydroanthracyclines against P388 Murine Leukemia

Compound	IC_{50} (μg/ml)[a]	IC_{90} (μg/ml)[a]
1·HCl	1.0×10^{-3}	4.7×10^{-3}
2·HCl	3.9×10^{-3}	—
6·HCl	1.1×10^{-5}	—
8·HCl	5.5×10^{-7}	5.5×10^{-5}
9·HCl	4.1×10^{-3}	—
17	6.1×10^{-4}	1.1×10^{-2}
22	1.9×10^{-2}	—

a) Cell growth inhibition (percent) after incubation for 48 h at 37 °C.

Experimental[17]

(*R*)-2-Acetyl-2,5,12-trihydroxy-1,2,3,4-tetrahydro-6,11-naphthacenedione ((−)-7-Deoxy-4-demethoxydaunomy-

cinone) (11)——A sample prepared according to the reported method[8,9] showed mp 217—218 °C and $[\alpha]_D^{20}$ −84.2° (c=0.095, $CHCl_3$) (lit.,[8] mp 218—219 °C and $[\alpha]_D^{20}$ −90.3° (c=0.106, $CHCl_3$); lit.,[9] mp 214—216 °C and $[\alpha]_D^{20}$ −90.6° (c=0.106, $CHCl_3$)).

(*R*)-2,5,12-Trihydroxy-2-(1-hydroxy-1-methylethyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (12)——An ethereal solution of methylmagnesium bromide (3.0 M solution, 0.20 ml, 0.60 mmol) was added to a stirred solution of **11** (41.3 mg, 0.12 mmol) in THF (4 ml) cooled at −20 °C, and the mixture was stirred at the same temperature for 1 h. A further amount of the solution of methylmagnesium bromide (3.0 M solution, 0.20 ml, total 1.2 mmol) was added, and stirring was continued at −20 °C for 1 h, then at 0 °C for 0.5 h. The mixture was poured into 1 M HCl, and extracted with EtOAc. The combined organic extracts were washed successively with H_2O and satd. NaCl, then dried over anhyd. Na_2SO_4. Filtration and concentration *in vacuo* followed by purification by column chromatography (SiO_2, C_6H_6–EtOAc 10:1→5:1) gave **12** as a red powder (32.8 mg, 76%). Recrystallization from C_6H_6 gave an analytical sample of **12**, mp 223—226.5 °C and $[\alpha]_D^{20}$ −109° (c=0.103, $CHCl_3$), $[\alpha]_D^{20}$ −89.7° (c=0.107, dioxane). IR (KBr): 3470, 1620, 1590 cm^{-1}. NMR ($CDCl_3$) δ: 1.36, 1.42 (6H, two s, $C(CH_3)_2$), 1.45—3.35 (8H, m, C_1-H_2, C_3-H_2, C_4-H_2, C_2-OH, and $C(OH)(CH_3)_2$), 7.63—7.98 (2H, m, aromatic protons), 8.13—8.50 (2H, m, aromatic protons), 13.53, 13.58 (2H, two s, phenolic OH×2). MS m/e: 368 (M^+), 310, 292. *Anal.* Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.24; H, 5.37.

(2*S*,4*S*)-2-Acetyl-2,4,5,12-tetrahydroxy-1,2,3,4-tetrahydro-6,11-naphthacenedione ((+)-4-Demethoxydaunomycinone) (13)——This was prepared from **11** according to the reported method.[8,9,11] A sample recrystallized from C_6H_6 showed mp 186—188 °C and $[\alpha]_D^{20}$ +167° (c=0.096, dioxane) (lit.,[8] mp 184—185.5 °C and $[\alpha]_D^{20}$ +157° (c=0.114, dioxane); lit.,[11] mp 183.5—184.5 °C and $[\alpha]_D^{20}$ +153° (c=0.09, dioxane); lit.,[18] mp 182.5—183 °C and $[\alpha]_D^{20}$ +164.5° (c=0.1, dioxane)).

(2*S*,4*S*)-2-Acetyl-4-*tert*-butyldimethylsilyloxy-2,5,12-trihydroxy-1,2,3,4-tetrahydro-6,11-naphthacenedione (14)——A catalytic amount of *p*-toluenesulfonic acid monohydrate was added to a mixture of **13** (38.0 mg, 0.10 mmol) and 4-*tert*-butyldimethylsilyloxy-3-penten-2-one[16] (165 mg, 0.77 mmol) in DMF (4 ml), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into H_2O, and extracted with EtOAc. The combined organic extracts were washed successively with H_2O and satd. NaCl, then dried over anhyd. Na_2SO_4. Filtration and concentration *in vacuo* gave a residue, which was purified by column chromatography (SiO_2, C_6H_6–EtOAc 50:1) to afford **14** as a red solid (47.1 mg, 95%). Recrystallization from C_6H_6–C_6H_{14} gave an analytical sample of **14**, mp 178—180 °C and $[\alpha]_D^{20}$ +243° (c=0.108, dioxane). IR (KBr): 3480, 1725, 1625, 1590 cm^{-1}. NMR ($CDCl_3$) δ: 0.20, 0.33 (6H, two s, $Si(CH_3)_2$), 0.90 (9H, three s, $SiC(CH_3)_3$), 2.05 (1H, dd, J=14, 4 Hz, $C_{3\,ax}$-H), 2.26 (1H, dt, J=14, 2 Hz, $C_{3\,eq}$-H), 2.45 (3H, s, $COCH_3$), 3.04 (1H, d, J=20 Hz, $C_{1\,ax}$-H), 3.27 (1H, d, J=20 Hz, $C_{1\,eq}$-H), 5.46 (1H, s, C_2-OH), 5.41—5.55 (1H, m, C_4-H), 7.76—7.98 (2H, m, aromatic protons), 8.26—8.49 (2H, m, aromatic protons), 13.35, 13.63 (2H, two s, phenolic OH×2). MS m/e: 467 ($[M-CH_3]^+$), 425 ($[M-C(CH_3)_3]^+$), 407 ($[M-C(CH_3)_3-H_2O]^+$), 333, 307, 291. *Anal.* Calcd for $C_{26}H_{30}O_7Si\cdot 0.25H_2O$: C, 64.11; H, 6.31. Found: C, 64.23; H, 6.24.

(2*S*,4*S*)-4-*tert*-Butyldimethylsilyloxy-2,5,12-trihydroxy-2-(1-hydroxy-1-methylethyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (15)——An ethereal solution of methylmagnesium bromide (3.0 M solution, 0.7 ml, 2.1 mmol) was added to a solution of **14** (201 mg, 0.42 mmol) in THF (21 ml) with stirring at −30 °C. Stirring was continued for 45 min at −20 °C, then a further amount of the Grignard reagent (0.7 ml, 2.1 mmol, total 4.2 mmol) was added, and the mixture was stirred at −20 °C for 1 h (total 1.75 h). The whole mixture was poured into a mixture of 1 M HCl and EtOAc. The upper organic phase was separated, and the lower aqueous phase was further extracted with EtOAc. The organic extracts were combined, washed successively with H_2O and satd. NaCl, then dried over anhyd. $MgSO_4$. Filtration and concentration *in vacuo* gave a solid, which was separated by column chromatography (SiO_2, C_6H_6–EtOAc 50:1) to give **15** as a red solid (162 mg, 78%). An analytical sample of **15** prepared by recrystallization from C_6H_6–C_6H_{14} showed mp 183.5—184.5 °C and $[\alpha]_D^{20}$ +232° (c=0.094, dioxane). IR (KBr): 3470, 1625, 1590 cm^{-1}. NMR ($CDCl_3$) δ: 0.17, 0.29 (6H, two s, $Si(CH_3)_2$), 0.87 (9H, three s, $SiC(CH_3)_3$), 1.27, 1.37 (6H, two s, $C(CH_3)_2$), 1.82 (1H, dd, J=14, 4 Hz, $C_{3\,ax}$-H), 2.50 (1H, dt, J=14, 2 Hz, $C_{3\,eq}$-H), 2.70 (1H, d, J=20 Hz, $C_{1\,ax}$-H), 2.79 (1H, br s, $C(OH)(CH_3)_2$), 3.22 (1H, d, J=20 Hz, $C_{1\,eq}$-H), 5.44 (1H, s, C_2-OH), 5.36—5.48 (1H, m, C_4-H), 7.73—7.94 (2H, m, aromatic protons), 8.23—8.42 (2H, m, aromatic protons), 13.37, 13.59 (2H, two s, phenolic OH×2). MS m/e: 483 ($[M-CH_3]^+$), 441 ($[M-C(CH_3)_3]^+$), 423 ($[M-C(CH_3)_3-H_2O]^+$), 339, 308, 291. *Anal.* Calcd for $C_{27}H_{34}O_7Si\cdot 0.25H_2O$: C, 64.45; H, 6.91. Found: C, 64.52; H, 6.72.

(2*S*,4*S*)-2,4,5,12-Tetrahydroxy-2-(1-hydroxy-1-methylethyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione ((7*S*,9*S*)-13-Methyl-13-dihydro-4-demethoxydaunomycinone) (10)——a) Preparation from **15**: A mixture of MeCN and 46% HF (95:5) (1.4 ml) was added to **15** (13.9 mg, 0.028 mmol), and the mixture was stirred at room temperature for 20 min. Water was added, and the aqueous mixture was extracted with a mixture of THF and EtOAc. The organic extracts were combined, washed successively with H_2O and satd. NaCl, then dried over anhyd. Na_2SO_4. Filtration and concentration *in vacuo* followed by purification by column chromatography (SiO_2, C_6H_6–EtOAc 2:1→1:1) and trituration with Et_2O, gave pure **10** as a red powder (10.6 mg, 99%), mp 228—230 °C and $[\alpha]_D^{20}$ +149° (c=0.047, dioxane). IR (KBr): 3480, 3340, 3275, 1625, 1590 cm^{-1}. NMR ($CDCl_3$) δ: 1.33, 1.40 (6H, two s, $C(CH_3)_2$), 1.92 (1H, ddd, J=14.7, 4.9, 0.9 Hz, $C_{3\,ax}$-H), 2.58 (1H, dt, J=14.7, 2.1 Hz, $C_{3\,eq}$-H), 2.53 (1H, s, $C(OH)(CH_3)_2$), 2.69 (1H, d, J=18.7 Hz, $C_{1\,ax}$-H), 3.27 (1H, dd, J=18.7, 2.1 Hz, $C_{1\,eq}$-H), 3.52 (1H, dd, J=4.2, 0.9 Hz, C_4-OH), 4.14 (1H, s, C_2-

OH), 5.37 (1H, dt, J=4.2, 0.9 Hz, C_4-H), 7.83—7.88 (2H, m, aromatic protons), 8.34—8.40 (2H, m, aromatic protons), 13.40, 13.63 (2H, two s, phenolic OH × 2). MS *m/e*: 384 (M^+), 366 ($[M-H_2O]^+$), 348 ($[M-2H_2O]^+$), 330 ($[M-3H_2O]^+$), 308.

When a mixture of AcOH, THF, and H_2O (4 : 2 : 1) (0.8 ml) was added to **15** (8.2 mg, 0.016 mmol) and the whole was heated at reflux for 2 h, **10** was obtained as a red powder (6.4 mg, 100%) after extractive isolation and purification by column chromatography (SiO_2, C_6H_6–EtOAc 2 : 1→1 : 1). The NMR spectrum of this was identical with that of the above sample.

b) Preparation from **13**: An ethereal solution of methyllithium (1.4 M solution, 0.12 ml, 0.17 mmol) was added to **13** (9.7 mg, 0.026 mmol) in THF (1 ml) with stirring at 0 °C. The reaction was continued at the same temperature for 45 min, then the mixture was poured into 1 M HCl, and extracted with EtOAc. The combined organic extracts were washed successively with H_2O and satd. NaCl, then dried over anhyd. Na_2SO_4. Filtration and concentration *in vacuo* followed by purification by column chromatography (SiO_2, C_6H_6–EtOAc 4 : 1) gave **10** as a reddish orange powder (4.1 mg, 41%). The NMR spectrum of this sample was identical with that of the product obtained in a).

c) Preparation from **12**: A solution of bromine in CCl_4 (0.16 M solution, 0.35 ml, 0.055 mmol) was added portionwise to a suspension of **12** (10.2 mg, 0.028 mmol) in a mixture of CCl_4 and CH_2Cl_2 (1 : 2) (1.5 ml), and the mixture was heated at reflux for 8.5 h under irradiation with a 60W tungsten lamp. After cooling, 2% NaOH was added at 0 °C and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with EtOAc and neutralized with 5% HCl. The upper organic phase was separated, washed successively with H_2O and satd. NaCl, then dried over anhyd. Na_2SO_4. Filtration and concentration *in vacuo* gave a red residue, which was separated by column chromatography (SiO_2, C_6H_6–EtOAc 3 : 1) to give **10** as an orange powder (0.9 mg, 8%). The NMR spectrum of this sample was identical with that of the product obtained in a).

(2*S*,4*S*)-4-*O*-α-3′-*N*-Trifluoroacetyl-L-daunosaminyl-2,4,5,12-tetrahydroxy-2-(1-hydroxy-1-methylethyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (17)——a) Preparation from **15**: Ether (5.6 ml) was added to a mixture of **16**[14] (91.1 mg, 0.17 mmol) and molecular sieves 4A (462 mg) in CH_2Cl_2 (7 ml) with stirring under an argon atmosphere. Trimethylsilyl trifluoromethanesulfonate (0.07 ml, 0.36 mmol) was added to the mixture cooled to −40 °C, and stirring was continued in an ice bath for 30 min. A solution of **15** (41.6 mg, 0.083 mmol) in THF (30 ml) was added to the stirred mixture cooled to −30 °C, and the whole was stirred at below −5 °C for 7 h. The mixture was poured into satd. $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were washed successively with H_2O and satd. NaCl, and dried over anhyd. Na_2SO_4. Filtration and concentration *in vacuo* gave a red solid (107 mg) which was dissolved in MeOH (80 ml). After 0.1 M NaOH (1.7 ml) was added under ice cooling, the methanolic solution was stirred at 0 °C for 20 min to hydrolyze the 4′-*O*-*p*-nitrobenzoyl group. Then 10% AcOH (4 drops), H_2O, and EtOAc were successively added to the reaction mixture, and the upper organic layer was separated. The lower aqueous phase was further extracted with EtOAc. The organic extracts were combined, washed successively with H_2O and satd. NaCl, then dried over anhyd. Na_2SO_4. Filtration and concentration *in vacuo* followed by purification of the residue by column chromatography (SiO_2, $CHCl_3$, then $CHCl_3$–Me_2CO 10 : 1), gave **17** as a red powder (35.1 mg, 69%), mp 154.5—157 °C and $[\alpha]_D^{20}$ +152° (c=0.096, dioxane). IR (KBr): 3500, 3450, 1725, 1715, 1625, 1590 cm^{-1}. NMR ($CDCl_3$) δ: 1.16—1.46 (9H, two s, and one d, $C(CH_3)_2$ and $C_{5'}$-CH_3), 1.75—2.85 (5H, m, C_3-H_2, $C_{2'}$-H_2, and $C(OH)(CH_3)_2$), 2.74 (1H, d, J=19 Hz, $C_{1\,ax}$-H), 3.26 (1H, d, J=19 Hz, $C_{1\,eq}$-H), 3.69 (1H, br d, J=7 Hz, $C_{4'}$-H), 3.90—4.45 (2H, m, $C_{3'}$-H and $C_{5'}$-H), 4.29 (1H, s, C_2-OH), 5.15—5.34 (1H, m, C_4-H), 5.45—5.60 (1H, m, $C_{1'}$-H), 6.74 (1H, br d, J=8 Hz, NH), 7.68—7.96 (2H, m, aromatic protons), 8.11—8.44 (2H, m, aromatic protons), 13.38, 13.57 (2H, two s, phenolic OH × 2). MS *m/e*: 610 ($[M+1]^+$), 385 ($[\mathbf{10}+1]^+$), 367, 349, 309. *Anal.* Calcd for $C_{29}H_{30}F_3NO_{10}\cdot H_2O$: C, 55.50; H, 5.14; N, 2.23. Found: C, 55.53; H, 5.30; N, 2.16.

b) Preparation from **10**: Ether (2 ml) was added to a mixture of **16**[14] (39.7 mg, 0.073 mmol) and molecular sieves 4A (155 mg) in CH_2Cl_2 (2.5 ml) with stirring under an argon atmosphere. Trimethylsilyl trifluoromethanesulfonate (0.03 ml, 0.16 mmol) was added to the mixture cooled to −40 °C, and the whole was stirred at 0 °C for 30 min. A solution of **10** (13.4 mg, 0.035 mmol) in THF (16 ml) was added to the mixture cooled to −30 °C with stirring, and the stirring was continued at below −5 °C for 4.5 h. Extractive isolation in the same manner as that described in a) gave a red solid (59.3 mg), which was dissolved in MeOH (35 ml). After 0.1 M NaOH (0.75 ml) was added under ice cooling, the methanolic solution was stirred at 0 °C for 25 min. The hydrolysis was quenched by adding 10% AcOH (2 drops). The mixture was worked up in the same manner as that described in a), giving **17** as a red powder (14.1 mg, 66%) after separation by column chromatography (SiO_2, $CHCl_3$, then, $CHCl_3$–Me_2CO 10 : 1). The NMR spectrum of this sample was identical with that described in a).

(2*S*,4*S*)-4-*O*-α-L-Daunosaminyl-2,4,5,12-tetrahydroxy-2-(1-hydroxy-1-methylethyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione Hydrochloride ((+)-13-Methyl-13-dihydro-4-demethoxydaunorubicin Hydrochloride) (8·HCl)——A 0.1 M sodium hydroxide solution (5.6 ml) was added to a solution of **17** (34.1 mg, 0.056 mmol) in THF (1 ml), and the mixture was stirred at room temperature for 20 min, neutralized (pH *ca.* 8) with 1 M HCl, and extracted with $CHCl_3$. The combined extracts were washed with H_2O, dried over anhyd. Na_2SO_4, filtered, then concentrated *in vacuo* to *ca.* 2 ml in volume. When 0.25 M HCl in MeOH (0.4 ml) and Et_2O (*ca.* 10 ml) were successively added to the concentrated chloroform solution, **8**·HCl separated as an orange powder, which was triturated with Et_2O. The product was dried over KOH to yield 19.8 mg (64%) of **8**·HCl, mp 209—211 °C and $[\alpha]_D^{20}$ +130° (c=0.120, MeOH). IR (KBr): 3450,

1625, 1590 cm^{-1}. NMR (DMSO-d_6) δ: 1.15 (3H, d, J=6.5 Hz, $C_{5'}$-CH_3), 1.230, 1.233 (6H, two s, $C(CH_3)_2$), 1.69 (1H, dd, J=12.5, 3.9 Hz, $C_{2'\,eq}$-H), 1.89 (1H, dt, J=12.5, 3.5 Hz, $C_{2'\,ax}$-H), 1.97 (1H, dd, J=14.8, 4.6 Hz, $C_{3\,ax}$-H), 2.28 (1H, d, J=14.8 Hz, $C_{3\,eq}$-H), 2.76 (1H, d, J=18.6 Hz, $C_{1\,ax}$-H), 2.97 (1H, d, J=18.6 Hz, $C_{1\,eq}$-H), 3.56—3.61 (1H, m, $C_{4'}$-H), 4.00 (1H, s, C(OH)$(CH_3)_2$), 4.19 (1H, q, J=6.5 Hz, $C_{5'}$-H), 4.54 (1H, s, C_2-OH), 4.91—4.96 (1H, m, C_4-H), 5.25—5.30 (1H, m, $C_{1'}$-H), 5.50 (1H, d, J=5.9 Hz, $C_{4'}$-OH), 7.94—8.01 (2H, m, aromatic protons), 8.21—8.29 (2H, m, aromatic protons), 7.6—8.5 (3H, br s, NH_3^+), 12.6—14.1 (2H, br s, phenolic OH×2). SIMS m/z: 514 ($[MH-HCl]^+$), 384, 367, 349, 291. *Anal.* Calcd for $C_{27}H_{32}ClNO_9 \cdot 2H_2O$: C, 55.34; H, 6.19; N, 2.39. Found: C, 55.43; H, 6.25; N, 2.45.

(2*S*,4*S*)-2-Acetyl-2,4,5,12-tetrahydroxy-7-methoxy-1,2,3,4-tetrahydro-6,11-naphthacenedione ((+)-Daunomycinone) (18)——This was prepared by heating a solution of commercially available **2**·HCl in 0.2 M HCl at 90—100 °C for 1 h.[19] A sample recrystallized from C_6H_6 showed mp 213-215 °C and $[\alpha]_D^{20}$ +197° (c=0.118, dioxane). (lit.,[19] mp 213—214 °C and $[\alpha]_D^{20\pm3}$ +193° (c=0.1, dioxane)).

(2*S*,4*S*)-2-Acetyl-4-*tert*-butyldimethylsilyloxy-2,5,12-trihydroxy-7-methoxy-1,2,3,4-tetrahydro-6,11-naphthacenedione (19)——The same treatments of **18** (60.1 mg, 0.15 mmol) as those described for **13** gave **19** as a red amorphous powder (75.3 mg, 98%) after purification by column chromatography (SiO_2, C_6H_6–EtOAc 20:1), $[\alpha]_D^{22}$ +316° (c=0.164, dioxane). IR (KBr): 3480, 1725, 1620, 1585 cm^{-1}. NMR ($CDCl_3$) δ: 0.17, 0.30 (6H, two s, $Si(CH_3)_2$), 0.89 (9H, three s, $SiC(CH_3)_3$), 2.06 (1H, dd, J=14, 4 Hz, $C_{3\,ax}$-H), 2.25 (1H, dt, J=14, 2 Hz, $C_{3\,eq}$-H), 2.43 (3H, s, $COCH_3$), 2.97 (1H, d, J=19 Hz, $C_{1\,ax}$-H), 3.21 (1H, d, J=19 Hz, $C_{1\,eq}$-H), 4.11 (3H, s, OCH_3), 5.47 (1H, s, C_2-OH), 5.40—5.54 (1H, m, C_4-H), 7.41 (1H, d, J=8 Hz, C_8-H), 7.78 (1H, dd, J=each 8 Hz, C_9-H), 8.03 (1H, d, J=8 Hz, C_{10}-H), 13.49, 14.24 (2H, two s, phenolic OH×2). MS m/e: 497 ($[M-CH_3]^+$), 455 ($[M-C(CH_3)_3]^+$), 437 ($[M-C(CH_3)_3-H_2O]^+$), 363, 337, 321. *Anal.* Calcd for $C_{27}H_{32}O_8Si \cdot 0.75H_2O$: C, 61.64; H, 6.42. Found: C, 61.54; H, 6.55.

(2*S*,4*S*)-4-*tert*-Butyldimethylsilyloxy-2,5,12-trihydroxy-2-(1-hydroxy-1-methylethyl)-7-methoxy-1,2,3,4-tetrahydro-6,11-naphthacenedione (20)——The silyl ether (**19**) (75.3 mg, 0.15 mmol) was treated with an ethereal solution of methylmagnesium bromide (3 M solution, 0.30 ml, 0.90 mmol) in the same manner as that described for **14**, giving **20** as a red solid (53.2 mg, 69%) after purification by column chromatography (SiO_2, C_6H_6–EtOAc 20:1). Recrystallization from Et_2O–C_6H_{14} gave an analytical sample of **20**, mp 217.5—219 °C and $[\alpha]_D^{22}$ +277° (c=0.120, dioxane). IR (KBr): 3450, 1615, 1580 cm^{-1}. NMR ($CDCl_3$) δ: 0.17, 0.30 (6H, two s, $Si(CH_3)_2$), 0.88 (9H, three s, $SiC(CH_3)_3$), 1.26, 1.37 (6H, two s, $C(CH_3)_2$), 1.83 (1H, dd, J=14, 4 Hz, $C_{3\,ax}$-H), 2.52 (1H, d, J=14 Hz, $C_{3\,eq}$-H), 2.76 (1H, d, J=19 Hz, $C_{1\,ax}$-H), 2.83 (1H, s, C(OH)$(CH_3)_2$), 3.24 (1H, d, J=19 Hz, $C_{1\,eq}$-H), 4.12 (3H, s, OCH_3), 5.52 (1H, s, C_2-OH), 5.45—5.63 (1H, m, C_4-H), 7.42 (1H, dd, J=8, 1 Hz, C_8-H), 7.81 (1H, dd, J=each 8 Hz, C_9-H), 8.10 (1H, dd, J=8, 1 Hz, C_{10}-H), 13.45, 14.07 (2H, two s, phenolic OH×2). MS m/e: 513 ($[M-CH_3]^+$), 471 ($[M-C(CH_3)_3]^+$), 453 ($[M-C(CH_3)_3-H_2O]^+$), 369, 338, 321. *Anal.* Calcd for $C_{28}H_{36}O_8Si \cdot 0.25H_2O$: C, 63.08; H, 6.90. Found: C, 62.88; H, 7.08.

(2*S*,4*S*)-2,4,5,12-Tetrahydroxy-2-(1-hydroxy-1-methylethyl)-7-methoxy-1,2,3,4-tetrahydro-6,11-naphthacenedione ((7*S*,9*S*)-13-Methyl-13-dihydrodaunomycinone) (21)——Deprotection of **20** (10.1 mg, 0.019 mmol) with a mixture of MeCN and 46% HF (95:5) in a similar manner to that described for the preparation of **10** gave **21** as a red powder (7.8 mg, 99%) after purification by column chromatography (SiO_2, C_6H_6–EtOAc 2:1→1:1) and trituration with Et_2O. The sample showed mp 236—239 °C.[20] IR (KBr): 3450, 1615, 1580 cm^{-1}. NMR ($CDCl_3$) δ: 1.31, 1.43 (6H, two s, $C(CH_3)_2$), 1.90 (1H, dd, J=15.0, 5.0 Hz, $C_{3\,ax}$-H), 2.58 (1H, dt, J=15.0, 2.1 Hz, $C_{3\,eq}$-H), 2.60 (1H, s, C(OH)$(CH_3)_2$), 2.66 (1H, d, J=18.7 Hz, $C_{1\,ax}$-H), 3.23 (1H, dd, J=18.7, 2.1 Hz, $C_{1\,eq}$-H), 3.50 (1H, br s, C_4-OH), 4.10 (3H, s, OCH_3), 4.26 (1H, s, C_2-OH), 5.37 (1H, m, C_4-H), 7.40 (1H, dd, J=8.0, 0.8 Hz, C_8-H), 7.79 (1H, dd, J=each 8.0 Hz, C_9-H), 8.05 (1H, dd, J=8.0, 0.8 Hz, C_{10}-H), 13.34, 13.99 (2H, two s, phenolic OH×2). MS m/e: 414 (M^+), 396 ($[M-H_2O]^+$), 378 ($[M-2H_2O]^+$), 360 ($[M-3H_2O]^+$), 338.

(2*S*,4*S*)-4-*O*-α-3′-*N*-Trifluoroacetyl-L-daunosaminyl-2,4,5,12-tetrahydroxy-2-(1-hydroxy-1-methylethyl)-7-methoxy-1,2,3,4-tetrahydro-6,11-naphthacenedione (22)——Glycosidation of **20** (15.5 mg, 0.029 mmol) with **16**[14] (32.3 mg, 0.060 mmol) by the use of trimethylsilyl trifluoromethanesulfonate (0.025 ml, 0.13 mmol) followed by alkaline hydrolysis of the 4′-*O*-*p*-nitrobenzoyl group in the same manner as that described for the preparation of **17**, gave **22** as a red powder (14.2 mg, 76%) after purification by column chromatography (SiO_2, $CHCl_3$, then $CHCl_3$–Me_2CO 30:1→15:1→8:1). The sample (**22**) showed mp 152—157 °C and $[\alpha]_D^{21}$ +196° (c=0.109, dioxane). IR (KBr): 3490, 3450, 1720, 1615, 1580 cm^{-1}. NMR ($CDCl_3$) δ: 1.13—1.48 (9H, two s and one d, $C(CH_3)_2$ and $C_{5'}$-CH_3), 1.60—2.75 (5H, m, C_3-H_2, $C_{2'}$-H_2, and C(OH)$(CH_3)_2$), 2.74 (1H, d, J=19 Hz, $C_{1\,ax}$-H), 3.25 (1H, d, J=19 Hz, $C_{1\,eq}$-H), 3.67 (1H, br d, J=7 Hz, $C_{4'}$-H), 4.09 (3H, s, OCH_3), 3.90—4.45 (2H, m, $C_{3'}$-H and $C_{5'}$-H), 4.27 (1H, s, C_2-OH), 5.20—5.40 (1H, m, C_4-H), 5.48—5.65 (1H, m, $C_{1'}$-H), 6.67 (1H, br d, J=8 Hz, NH), 7.41 (1H, d, J=8 Hz, C_8-H), 7.79 (1H, dd, J=each 8 Hz, C_9-H), 8.05 (1H, d, J=8 Hz, C_{10}-H), 13.38, 14.02 (2H, two s, phenolic OH×2). MS m/e: 639 (M^+), 414 (**21**$^+$), 396, 378, 338. *Anal.* Calcd for $C_{30}H_{32}F_3NO_{11} \cdot H_2O$: C, 54.80; H, 5.21; N, 2.13. Found: C, 55.02; H, 5.48; N, 2.13.

(2*S*,4*S*)-4-*O*-α-L-Daunosaminyl-2,4,5,12-tetrahydroxy-2-(1-hydroxy-1-methylethyl)-7-methoxy-1,2,3,4-tetrahydro-6,11-naphthacenedione Hydrochloride ((+)-13-Methyl-13-dihydrodaunorubicin Hydrochloride) (9·HCl)——Alkaline hydrolysis of **22** (22.6 mg, 0.035 mmol) under the same conditions as those employed for **17** gave **9**·HCl as a

red powder (12.9 mg, 63%) after salt formation with 0.25 M HCl in MeOH and trituration with Et_2O. The hydrochloride showed mp 196.5—198.5 °C and $[\alpha]_D^{22}$ +155° (c=0.102, MeOH). IR (KBr): 3450, 1615, 1580 cm^{-1}. NMR (DMSO-d_6) δ: 1.15 (3H, d, J=6.5 Hz, $C_{5'}$-CH_3), 1.22 (6H, two s, $C(CH_3)_2$), 1.69 (1H, dd, J=12.5, 4.0 Hz, $C_{2'\,eq}$-H), 1.89 (1H, dt, J=12.5, 3.6 Hz, $C_{2'\,ax}$-H), 1.96 (1H, dd, J=14.9, 4.8 Hz, $C_{3\,ax}$-H), 2.28 (1H, d, J=14.9 Hz, $C_{3\,eq}$-H), 2.78 (1H, d, J=18.4 Hz, $C_{1\,ax}$-H), 2.96 (1H, d, J=18.4 Hz, $C_{1\,eq}$-H), 3.56—3.61 (1H, m, $C_{4'}$-H), 3.97 (1H, s, $C(OH)(CH_3)_2$), 3.99 (3H, s, OCH_3), 4.18 (1H, q, J=6.5 Hz, $C_{5'}$-H), 4.47 (1H, s, C_2-OH), 4.95—5.01 (1H, m, C_4-H), 5.25—5.31 (1H, m, $C_{1'}$-H), 5.45 (1H, d, J=6.1 Hz, $C_{4'}$-OH), 7.62—7.68 (1H, m, C_8-H), 7.87—8.02 (5H, m, C_{10}-H, C_9-H, and NH_3^+), 13.30, 14.03 (2H, two s, phenolic OH × 2). SIMS m/z: 544 ($[MH-HCl]^+$), 414, 397, 379, 321. *Anal.* Calcd for $C_{28}H_{34}ClNO_{10}\cdot 2H_2O$: C, 54.59; H, 6.22; N, 2.27. Found: C, 54.34; H, 6.19; N, 2.17.

Acknowledgements 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.

References and Notes

1) *a*) F. Arcamone, *Lloydia*, **40**, 45 (1977); *b*) *Idem*, "Topics in Antibiotic Chemistry," Vol. 2, ed. by P. G. Sammes, Ellis Horwood, Chichester, 1978, pp. 99—239; *c*) *Idem*, "Anticancer Agents Based on Natural Product Models," ed. by J. M. Cassady and J. D. Douros, Academic Press, New York, 1980, pp. 1—41; *d*) *Idem*, "Doxorubicin Anticancer Antibiotics," Academic Press, New York, 1981.
2) M. B. Naff, J. Plowman, and V. L. Narayanan, "Anthracycline Antibiotics," ed. by H. S. El Khadem, Academic Press, New York, 1982, pp. 1—57.
3) See ref. 1*d*, p. 141 and p. 164.
4) *a*) F. Arcamone, L. Bernardi, P. Giardino, B. Patelli, A. DiMarco, A. M. Cassaza, G. Pratesi, and P. Reggiani, *Cancer Treat. Rep.*, **60**, 829 (1976); *b*) F. Arcamone, L. Bernardi, B. Patelli, P. Giardino, A. DiMarco, A. M. Cassaza, C. Soranzo, and B. Patelli, *Experientia*, **34**, 1255 (1978); *c*) B. Patelli, L. Bernardi, F. Arcamone, and A. DiMarco, Japan Tokkyo Koho JP., 58-40557 (1983); *d*) F. Arcamone, L. Bernardi, P. Giardino, and A. DiMarco, Japan Tokkyo Koho JP., 57-36919 (1982).
5) Abstracts of Papers 14th International Congress of Chemotherapy, Kyoto, Japan 1985, pp. 210—212.
6) M. J. Broadhurst, C. H. Hassall, and G. J. Thomas, *Tetrahedron Lett.*, **25**, 6059 (1984).
7) K. Tamoto and S. Terashima, *Chem. Pharm. Bull.*, **32**, 4328 (1984).
8) K. Tamoto, M. Sugimori, and S. Terashima, *Tetrahedron*, **40**, 4617 (1984).
9) Y. Kimura, M. Suzuki, T. Matsumoto, R. Abe, and S. Terashima, *Bull. Chem. Soc. Jpn.*, **59**, 415 (1986).
10) S-S. Jew, S. Terashima, and K. Koga, *Chem. Pharm. Bull.*, **27**, 2351 (1979).
11) N. Tanno and S. Terashima, *Chem. Pharm. Bull.*, **31**, 811, 821 (1983).
12) *a*) M. Suzuki, Y. Kimura, and S. Terashima, *Chem. Lett.*, **1985**, 367; *b*) *Idem, Tetrahedron Lett.*, **26**, 6481 (1985).
13) Y. Kimura, M. Suzuki, T. Matsumoto, R. Abe, and S. Terashima, *Bull. Chem. Soc. Jpn.*, **59**, 423 (1986).
14) Y. Kimura, T. Matsumoto, M. Suzuki, and S. Terashima, *Bull. Chem. Soc. Jpn.*, **59**, 663 (1986).
15) Addition reaction of methylmagnesium bromide to **13** in the same manner as that employed for **11** gave a complex mixture of products, in which the presence of a small amount of **10** could be detected by thin layer chromatography analysis.
16) T. Veysoglu and L. A. Mitscher, *Tetrahedron Lett.*, **22**, 1299, 1303 (1981).
17) All melting points were determined with a Yamato MP-21 melting point apparatus and are not corrected. Infrared (IR) spectral measurements were performed using a JASCO A-202 diffraction grating infrared spectrometer. Nuclear magnetic resonance (NMR) spectra were measured with a Varian EM-390 spectrometer (90 MHz), a Hitachi R-90H spectrometer (90 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). Mass spectra (MS) were taken with a Hitachi RMU-6MG mass spectrometer and a Hitachi M-80A mass spectrometer (SIMS). Measurements of optical rotations were carried out using a Horiba SEPA-200 automatic digital polarimeter. Wakogel C-200 was used as an adsorbent for column chromatography. All reactions were carried out using anhyd. solvents. In particular, tetrahydrofuran, ether, and dioxane freshly distilled from sodium benzophenone ketyl, and dichloromethane freshly distilled from calcium hydride were used. Trimethylsilyl trifluoromethanesulfonate purchased from Petrarch System Inc. (Chisso) was used without further purification. The following abbreviations are used for reagents and solvents: acetic acid (AcOH), acetone (Me_2CO), acetonitrile (MeCN), benzene (C_6H_6), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), ether (Et_2O), ethyl acetate (EtOAc), hexane (C_6H_{14}), methanol (MeOH), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF).
18) M. J. Broadhurst, C. H. Hassall, and G. H. Thomas, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2249.
19) F. Arcamone, G. Franceshi, P. Orezzi, G. Cassinelli, W. Barbieri, and R. Mondelli, *J. Am. Chem. Soc.*, **86**, 5334 (1964).
20) Measurement of the optical rotation of this sample was not carried out.