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Synthesis of 4-Demethoxyanthracyclines Carrying a Lipophilic Alkanoyl Group at the C₉-Position

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By employing various novel reactions developed in our synthetic studies on 4-demethoxyadriamycin and 4-demethoxydaunorubicin, three examples of the title compounds (**7a—c**) were prepared from (*R*)-2,5,12-trihydroxy-1,2,3,4-tetrahydro-6,11-naphthacenedione-2-carboxylic acid (**9**). While **7a—c** showed marked cytotoxicity against P388 *in vitro*, it was found that **7a** (carrying a nonanoyl group at the C₉-position) was ineffective against P388 *in vivo*.

Keywords—4-demethoxyanthracycline; 4-demethoxyanthracyclinone; C₉-lipophilic alkanoyl group; alkylmagnesium bromide; acylimidazole; 1,2-addition reaction; glycosidation; *in vitro* cytotoxicity; *in vivo* anticancer activity

The anthracycline antibiotics, adriamycin (**1**) and daunorubicin (**2**), are important anticancer agents.^{1,2)} Since various undesirable side effects, the most well-known and serious of which is dose-related cardiotoxicity, restrict the utility of **1** and **2** for cancer chemotherapy,³⁾ extensive studies have been carried out on the structure-activity relationships, culminating in the synthesis of various notable analogues of **1** and **2**, some of which exhibit superior anticancer activity in the P388 *in vivo* murine leukemia test system.^{1,2)}

We were interested in 3'-*N*-trifluoroacetyladiamycin 14-*O*-valerate (**3**), so-called AD-32,^{1,2,4)} and the 4-demethoxy congeners of **1** and **2**, 4-demethoxyadriamycin (**5**) and 4-demethoxydaunorubicin (**6**).^{1,2,5)} The former analogue (**3**) has been reported to show reduced cardio- and gastrointestinal toxicity.⁴⁾ Though the insolubility in water resulting from a highly lipophilic nature hampered the clinical application of **3**, 3'-*N*-trifluoroacetyladiamycin 14-*O*-

	R ¹	R ²
1	CH ₂ OH	H
2	Me	H
3	CH ₂ OCO(CH ₂) ₃ Me	COCF ₃
4	CH ₂ OCO(CH ₂) ₄ COOH	COCF ₃

	R ¹	R ²
5	CH ₂ OH	H
6	Me	H
7a	(CH ₂) ₇ Me	H
7b	(CH ₂) ₃ Me	H
7c	CHMe ₂	H
8a	(CH ₂) ₇ Me	COCF ₃
8b	(CH ₂) ₃ Me	COCF ₃
8c	CHMe ₂	COCF ₃

hemidiapate (**4**) has recently been developed as an improved analogue of **1** which shows good anticancer activity as compared with **3**, with greatly superior water solubility.⁶⁾ The latter 4-demethoxyanthracyclines (**5** and **6**) are well known because of their increased anticancer activity (5—10 times higher than those of natural **1** and **2**).⁵⁾ One of these analogues **6** is under clinical trials as an orally administrable anthracycline.⁷⁾

Taking into account the novel aspects of these analogues (**3—6**), syntheses of 4-demethoxyanthracyclines **7** carrying a lipophilic alkanoyl group at the C₉-position were attempted.⁸⁾ This report deals with the synthesis and preliminary evaluation of the anticancer activity of **7** and their 3'-*N*-trifluoroacetyl derivatives **8**, prepared by employing various efficient reactions previously developed in our synthetic studies on **5** and **6**.⁹⁻¹²⁾

Results and Discussion

Previously, it was reported that methylmagnesium bromide could effectively react with the (*R*)-acylimidazole (**10**) derived from optically pure (*R*)-2,5,12-trihydroxy-1,2,3,4-tetrahydro-6,11-naphthacenedione-2-carboxylic acid (**9**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) or boron trifluoride etherate (BF₃OEt₂), giving rise to (*R*)-7-deoxy-4-demethoxydaunomycinone in a good yield.¹⁰⁾ Syntheses of **7** commenced with examination of the same reaction by using various Grignard reagents in place of methylmagnesium bromide.

Thus, when **10** produced *in situ* from **9** was treated with 20 eq of octylmagnesium bromide at -40 °C for 4.5 h in the presence of TMSOTf, (*R*)-2,5,12-trihydroxy-2-nonanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione (**11a**) was obtained in 56% yield based on **9**. Similarly, the (*R*)-2-pentanoyl- and (*R*)-2-(2-methylpropanoyl) derivatives (**11b** and **11c**) could be produced from **9**, both in 50% yield. In contrast to the case of (*R*)-7-deoxy-4-demethoxydaunomycinone,⁹⁾ protection of **11a** with ethylene glycol failed, and **11a** was always recovered. This may be explained by the increased steric hindrance owing to the octyl group. However, it was found that **11a** could be well protected by treatment with 1,2-bis(trimethylsilyloxy)ethane in the presence of TMSOTf to afford the (*R*)-acetal (**12a**) in 95% yield. While **12b** was prepared in 89% yield in a similar manner to that described for **12a**, protection of **11c** could not be achieved even by employing these stronger reaction conditions probably because of the further increased steric hindrance. Bromination of **12a, b** with bromine under irradiation, followed by immediate treatment of the bromides under aqueous alkaline conditions to selectively introduce the C_{7α}-hydroxy group (anthracycline numbering) and by acidic removal of the acetal groups, gave optically pure 4-demethoxyanthracyclinones (**13a, b**), mp 158.5—159.5 °C, [α]_D²⁰ +134° (dioxane), and mp 138.5—140 °C, [α]_D²⁰ +96.7° (dioxane), both in 58% overall yield. The structures of **13a, b**, including the stereochemistry at the C₇-position, were definitely determined from the nuclear magnetic resonance (NMR) spectra. Similarly to the case of the synthesis of 4-demethoxydaunomycinone,⁹⁾ formation of the undesired C_{7β}-isomers (anthracycline numbering) was found to be negligible in the preparation of **13a, b**.¹³⁾ On the other hand, direct subsection of **12c** to the same sequential bromination and substitution reactions as those employed for **12a, b**, afforded a product which appeared to consist of **13c** and its C_{7β}-epimer (anthracycline numbering) in a ratio of 2:1.¹⁴⁾ Since it is well known that an anthracyclinone having a C_{7β}-equatorial hydroxy group can be epimerized by treatment with a strong acid,¹⁵⁾ the mixture was directly dissolved in trifluoroacetic acid to achieve equilibrium between **13c** and its C_{7β}-epimer. Separation of the equilibrated mixture, involving **13c** and its C_{7β}-epimer in a ratio of 5:1, by column chromatography afforded optically pure **13c**, mp 172—173 °C and [α]_D²⁰ +67.9° (dioxane), in 43% overall yield from **11c**.

With the three optically pure aglycones (**13a—c**) in hand, glycosidation with the

Chart 1

TABLE I. *In Vitro* Cytotoxicity of 4-Demethoxyanthracyclines Carrying a Lipophilic Alkanoyl Group at the C₉-Position against P388 Murine Leukemia

Compound	IC ₅₀ (μg/ml) ^{a)}
1	0.0034
7a	0.16
7b	0.14
7c	0.018
8a	2.2
8b	0.95
8c	0.19

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

daunosamine derivative was next carried out. According to the reported procedure,¹¹⁾ **13a** was allowed to react with 3-*N*-trifluoroacetyl-1,4-bis(*O*-*p*-nitrobenzoyl)-L-daunosamine¹²⁾ in the presence of TMSOTf in a mixture of ether and dichloromethane. The formed glycoside was immediately treated with dilute aqueous alkali to effect hydrolysis of the 4'-*O*-*p*-nitrobenzoyl group, giving the 3'-*N*-trifluoroacetyl- α -glycoside (**8a**) in 55% overall yield. Comparison of the NMR spectrum of **8a** with that of 3'-*N*-trifluoroacetyl-4-demethoxydaunorubicin¹¹⁾ rigorously established its α -glycoside structure. Further alkaline hydrolysis of the 3'-*N*-trifluoroacetyl group followed by salt formation with methanolic hydrogen chloride produced the glycoside hydrochloride (**7a**·HCl), mp 172—174 °C and $[\alpha]_D^{20} + 130^\circ$ (methanol), in 69% yield. In the same manner, **7b**, **c**·HCl, mp 171—172 °C, $[\alpha]_D^{20} + 121^\circ$ (methanol), and mp 198—200 °C, $[\alpha]_D^{20} + 193^\circ$ (methanol), could be prepared from **13b**, **c** by way of the corresponding 3'-*N*-trifluoroacetyl glycosides (**8b**, **c**).

Since the synthetic studies were completed as mentioned above, the cytotoxicity of **7** and **8** was next examined. The glycosides (**7** and **8**) were first subjected to *in vitro* cytotoxicity assay against P388 murine leukemia cells. The results are shown in Table I. As expected, the *in vitro* cytotoxicities of **7** and **8** were found to be distinctly lower than that of **1**, but comparable to those of **3** and **4**.^{4,6)} In view of its structural features, **7a** was anticipated to be most lipophilic, and it was subjected to P388 *in vivo* assay. Disappointingly, it was found that **7a** exhibited no effective T/C value at doses up to 80 mg/kg.

Experimental¹⁽⁶⁾

(R)-2,5,12-Trihydroxy-1,2,3,4-tetrahydro-6,11-naphthacenedione-2-carboxylic Acid (9)—This was prepared by resolving the *dl*-acid with (–)-*N*-methylephedrine according to the reported method.¹⁰⁾ The methyl ester derived from **9** showed mp 212–214 °C and $[\alpha]_D^{20}$ –58.0° ($c=0.10$, CHCl₃) (lit.,¹⁰⁾ mp 210.5–211.5 °C and $[\alpha]_D^{20}$ –60.0° ($c=0.10$, CHCl₃)).

(R)-(–)-2,5,12-Trihydroxy-2-nonanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione (11a)—A mixture of **9** (101 mg, 0.28 mmol), *N,N'*-carbonyldiimidazole (102 mg, 0.63 mmol), and HMPA (0.4 ml) in THF (20 ml) was stirred at room temperature under an argon atmosphere for 18.5 h, then cooled at –40 °C. TMSOTf (0.11 ml, 0.57 mmol) was added, and the stirring was continued for 20 min at the same temperature. An ethereal solution of octylmagnesium bromide (1.7 M solution, 1.7 ml, 2.9 mmol), which was prepared from octyl bromide and magnesium turnings in Et₂O according to the conventional procedure, was added to the reaction mixture, and the whole was stirred at –40 °C for 1 h. Two further portions of ethereal solution of octylmagnesium bromide (1.7 M solution, 0.8 ml) were added to the reaction mixture at intervals of 30 min (total 3.3 ml, 5.6 mmol). Stirring was continued for 2.5 h (total 4.5 h), then the reaction mixture was poured into a two-layer mixture of 1 M HCl and EtOAc. The upper organic layer was separated, and the lower aqueous phase was extracted with EtOAc. The organic extracts were combined, washed successively with H₂O and satd. NaCl, then dried over anhyd. MgSO₄. Filtration and concentration *in vacuo* followed by separation by column chromatography (SiO₂, C₆H₆) gave a red semisolid which was triturated with C₆H₁₄ to afford **11a** (72.0 mg, 56%). Recrystallization from C₆H₆–C₆H₁₄ gave an analytical sample of **11a** as a red powder, mp 184 °C and $[\alpha]_D^{20}$ –37.3° ($c=0.102$, CHCl₃). IR (KBr): 3500, 1705, 1625, 1590 cm^{–1}. NMR (CDCl₃) δ : 0.89 (3H, t, $J=6$ Hz, CH₃), 1.31 (12H, brs, COCH₂(CH₂)₆CH₃), 1.50–2.30 (2H, m, C₃–H₂), 2.67 (2H, t, $J=7$ Hz, COCH₂), 2.85–3.40 (4H, m, C₁–H₂ and C₄–H₂), 3.81 (1H, s, OH), 7.70–7.95 (2H, m, aromatic protons), 8.25–8.50 (2H, m, aromatic protons), 13.52 (2H, two s, phenolic OH $\times 2$). MS *m/e*: 450 (M⁺), 432, 309. *Anal.* Calcd for C₂₇H₃₀O₆: C, 71.98; H, 6.71. Found: C, 71.91; H, 6.72.

(R)-(–)-2,5,12-Trihydroxy-2-pentanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione (11b)—Similar treatments of **9** (53.6 mg, 0.15 mmol) to those described for the preparation of **11a**, except for the use of an ethereal solution of butylmagnesium bromide (1.9 M solution) and BF₃OEt₂ (0.02 ml, 0.16 mmol) in place of an ethereal solution of octylmagnesium bromide and TMSOTf, gave **11b** (30.0 mg, 50%) after extractive isolation and separation by column chromatography (SiO₂, C₆H₆–EtOAc 50:1). An analytical sample of **11b** was prepared as a red powder by recrystallization from C₆H₆, mp 187.5–188.5 °C and $[\alpha]_D^{20}$ –56.9° ($c=0.130$, CHCl₃). IR (KBr): 3470, 1715, 1625, 1590 cm^{–1}. NMR (CDCl₃) δ : 0.95 (3H, t, $J=6$ Hz, CH₃), 1.10–2.25 (6H, m, COCH₂(CH₂)₂CH₃ and C₃–H₂), 2.71 (2H, t, $J=7$ Hz, COCH₂), 2.85–3.40 (4H, m, C₁–H₂ and C₄–H₂), 3.84 (1H, s, OH), 7.73–7.98 (2H, m, aromatic protons), 8.26–8.49 (2H, m, aromatic protons), 13.53 (2H, two s, phenolic OH $\times 2$). MS *m/e*: 394 (M⁺), 376, 309. *Anal.* Calcd for C₂₃H₂₂O₆ · 0.25H₂O: C, 69.25; H, 5.68. Found: C, 69.31; H, 5.70.

(R)-(–)-2,5,12-Trihydroxy-2-(2-methylpropanoyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (11c)—The acid (**9**) (111 mg, 0.31 mmol) was treated in a similar manner to that described for the preparation of **11a**, except for the use of an ethereal solution of isopropylmagnesium bromide (2.1 M solution) in place of an ethereal solution of octylmagnesium bromide, to give **11c** (59.4 mg, 50%) after extractive isolation and separation by column chromatography (SiO₂, C₆H₆–EtOAc 50:1). Recrystallization from C₆H₆–C₆H₁₄ gave an analytical sample of **11c** as red crystals, mp 190–191.5 °C and $[\alpha]_D^{20}$ –53.7° ($c=0.134$, CHCl₃). IR (KBr): 3490, 1705, 1620, 1590 cm^{–1}. NMR (CDCl₃) δ : 1.20, 1.24 (6H, two d, J =each 7 Hz, CH₃ $\times 2$), 1.80–2.40 (2H, m, C₃–H₂), 2.70–3.20 (4H, m, C₁–H₂ and C₄–H₂), 3.25 (1H, dq, J =each 7 Hz, COCH), 3.88 (1H, s, OH), 7.72–7.98 (2H, m, aromatic protons), 8.23–8.50 (2H, m, aromatic protons), 13.46 (2H, s, phenolic OH $\times 2$). MS *m/e*: 380 (M⁺), 362, 309. *Anal.* Calcd for C₂₂H₂₀O₆ · 0.25H₂O: C, 68.65; H, 5.37. Found: C, 68.81; H, 5.50.

(R)-2,5,12-Trihydroxy-2-(2-octyl-1,3-dioxolan-2-yl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (12a)—TMSOTf (5 drops, *ca.* 0.13 mmol) was added to a mixture of **11a** (50.3 mg, 0.11 mmol) and 1,2-bis(trimethylsilyloxy)ethane (0.5 ml, 2.0 mmol) in CH₂Cl₂ (6 ml) cooled at 0 °C, and the whole was stirred at the same temperature for 0.5 h, then at room temperature for 15 h. The reaction mixture was poured into satd. NaHCO₃, and extracted with CH₂Cl₂. The combined extracts were washed successively with H₂O and satd. NaCl, dried over anhyd. MgSO₄, filtered, and concentrated *in vacuo*. The residue, obtained as a red solid, was purified by column chromatography (SiO₂, C₆H₆–EtOAc 30:1) to give **12a** as a red powder (52.2 mg, 95%), mp 179.5–182.5 °C. IR (KBr): 3460, 1620, 1590 cm^{–1}. NMR (CDCl₃) δ : 0.89 (3H, t, $J=6$ Hz, CH₃), 1.31 (12H, brs, (CH₂)₆CH₃), 1.50–2.40 (4H, m, C₃–H₂ and CH₂(CH₂)₆CH₃), 1.91 (1H, s, OH), 2.55–3.40 (4H, m, C₁–H₂ and C₄–H₂), 4.16 (4H, s, OCH₂CH₂O), 7.73–7.96 (2H, m, aromatic protons), 8.25–8.52 (2H, m, aromatic protons), 13.55, 13.58 (2H, two s, phenolic OH $\times 2$). MS *m/e*: 494 (M⁺), 185.

(R)-2,5,12-Trihydroxy-2-(2-butyl-1,3-dioxolan-2-yl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (12b)—The same treatments of **11b** (55.7 mg, 0.14 mmol) as those described for **11a** gave **12b** as a red powder (55.1 mg, 89%) after purification by column chromatography (SiO₂, C₆H₆–EtOAc 30:1), mp 215–217 °C. IR (KBr): 3500, 1625, 1590 cm^{–1}. NMR (CDCl₃) δ : 0.95 (3H, t, $J=6$ Hz, CH₃), 1.15–2.40 (8H, m, (CH₂)₃CH₃ and C₃–H₂), 1.95 (1H, s, OH), 2.60–3.35 (4H, m, C₁–H₂ and C₄–H₂), 4.20 (4H, s, OCH₂CH₂O), 7.79–8.05 (2H, m, aromatic protons), 8.33–

8.58 (2H, m, aromatic protons), 13.55, 13.58 (2H, two s, phenolic OH $\times$ 2). MS m/e : 438 (M^+), 129.

(2S,4S)-(+)-2,4,5,12-Tetrahydroxy-2-nonanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione (13a)—A solution of bromine in CCl_4 (0.16 M solution, 2.0 ml, 0.32 mmol) was added to a stirred mixture of **12a** (120 mg, 0.24 mmol) in a two-layer mixture of CCl_4 (20 ml) and H_2O (10 ml) over 45 min under reflux and irradiation with a 60W tungsten lamp. Stirring under reflux and irradiation was continued for a further 2 h, then the reaction mixture was cooled in an ice bath, diluted with 10% NaOH (0.6 ml, 1.5 mmol), and stirred for 40 min under ice cooling. After 5% HCl (3 ml) was added, the mixture was extracted with $CHCl_3$. 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 red residue (130 mg), to which THF (20 ml) and concd. HCl (4 ml) were added. After being stirred at room temperature for 15 h to hydrolyze the acetal group, the acidic mixture was diluted with H_2O and $CHCl_3$. The chloroform layer was separated, and the upper aqueous phase was further extracted with $CHCl_3$. The organic extracts were combined, washed successively with H_2O and satd. NaCl, dried over anhyd. Na_2SO_4 , filtered, then concentrated *in vacuo*. The red residue was purified by column chromatography (SiO_2 , C_6H_6 –EtOAc 40:1), giving **13a** (64.9 mg, 58%). Recrystallization from C_6H_6 – C_6H_{14} gave an analytical sample of **13a** as a red powder, mp 158.5–159.5 °C and $[\alpha]_D^{20} + 70.8^\circ$ ($c=0.096$, $CHCl_3$), $[\alpha]_D^{20} + 134^\circ$ ($c=0.076$, dioxane). IR (KBr): 3430, 1705, 1625, 1590 cm^{-1} . NMR ($CDCl_3$) δ : 0.89 (3H, t, $J=6$ Hz, CH_3), 1.32 (12H, brs, $(CH_2)_6CH_3$), 2.10–2.40 (2H, m, C_3 – H_2), 2.81 (2H, t, $J=7$ Hz, $COCH_2$), 3.00 (1H, d, $J=19$ Hz, C_{1ax} –H), 3.21 (1H, d, $J=19$ Hz, C_{1eq} –H), 3.80 (1H, d, $J=6$ Hz, C_4 –OH), 4.52 (1H, s, C_2 –OH), 5.26–5.51 (1H, m, C_4 –H), 7.75–8.00 (2H, m, aromatic protons), 8.28–8.55 (2H, m, aromatic protons), 13.37, 13.65 (2H, two s, phenolic OH $\times$ 2). MS m/e : 466 (M^+), 448, 430, 307. Anal. Calcd for $C_{27}H_{30}O_7 \cdot 0.25H_2O$: C, 68.85; H, 6.53. Found: C, 69.00; H, 6.57.

(2S,4S)-(+)-2,4,5,12-Tetrahydroxy-2-pentanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione (13b)—Successive treatments of **12b** (54.2 mg, 0.12 mmol) in the same manner as that described for **12a** gave **13b** (29.5 mg, 58%) after purification by column chromatography (SiO_2 , C_6H_6 –EtOAc 30:1). This was recrystallized successively from C_6H_6 – C_6H_{14} and C_6H_6 , giving an analytical sample of **13b** as a red powder, mp 138.5–140 °C and $[\alpha]_D^{20} + 97.0^\circ$ ($c=0.099$, dioxane). IR (KBr): 3450, 1710, 1625, 1590 cm^{-1} . NMR ($CDCl_3$) δ : 0.95 (3H, t, $J=6$ Hz, CH_3), 1.15–2.05 (4H, m, $(CH_2)_2CH_3$), 2.10–2.40 (2H, m, C_3 – H_2), 2.81 (2H, t, $J=7$ Hz, $COCH_2$), 3.01 (1H, d, $J=19$ Hz, C_{1ax} –H), 3.20 (1H, d, $J=19$ Hz, C_{1eq} –H), 3.80 (1H, d, $J=6$ Hz, C_4 –OH), 4.54 (1H, s, C_2 –OH), 5.25–5.47 (1H, m, C_4 –H), 7.75–8.00 (2H, m, aromatic protons), 8.28–8.52 (2H, m, aromatic protons), 13.37, 13.65 (2H, two s, phenolic OH $\times$ 2). MS m/e : 410 (M^+), 392, 374, 307. Anal. Calcd for $C_{23}H_{22}O_7 \cdot 0.25H_2O$: C, 66.58; H, 5.47. Found: C, 66.89; H, 5.62.

(2S,4S)-(+)-2,4,5,12-Tetrahydroxy-2-(2-methylpropanoyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (13c)—A solution of bromine in CCl_4 (0.16 M solution, 1.9 ml, 0.29 mmol) was added to a mixture of **11c** (79.3 mg, 0.21 mmol) in a two-layer mixture of CCl_4 (16 ml) and H_2O (8 ml) over 30 min under reflux and irradiation with a 60W tungsten lamp. Stirring under reflux and irradiation was continued for a further 45 min, then the reaction mixture was cooled in an ice bath, diluted with 10% NaOH (0.43 ml, 1.1 mmol), and stirred for 50 min at the same temperature. After 1 M HCl (1.3 ml, 1.3 mmol) was added, the mixture was worked up by the same procedure as that described for the preparation of **13a**, giving a red residue (78.2 mg) after extractive isolation followed by concentration of the combined chloroform extracts *in vacuo*. The residue was dissolved in trifluoroacetic acid (6.0 ml), and the solution was stirred for 17 h at room temperature to equilibrate the C_4 -hydroxy group.¹⁵ The reaction mixture was diluted with H_2O and $CHCl_3$. The lower chloroform layer was separated, and the upper aqueous phase was extracted with $CHCl_3$. The organic extracts were combined, washed successively with H_2O and satd. NaCl, dried over anhyd. Na_2SO_4 , filtered, then concentrated *in vacuo*. The residue, obtained as a red solid, was separated by column chromatography (SiO_2 , C_6H_6 –EtOAc 50:1 $\rightarrow$ 20:1), giving **13c** (35.6 mg, 43%). This was recrystallized twice from C_6H_6 – C_6H_{14} to afford an analytical sample of **13c** as a red powder, mp 172–173 °C and $[\alpha]_D^{20} + 67.9^\circ$ ($c=0.106$, dioxane). IR (KBr): 3450, 1715, 1625, 1590 cm^{-1} . NMR ($CDCl_3$) δ : 1.21, 1.22 (6H, two d, J =each 7 Hz, $C(CH_3)_2$), 2.10–2.40 (2H, m, C_3 – H_2), 3.04 (1H, d, $J=19$ Hz, C_{1ax} –H), 3.21 (1H, d, $J=19$ Hz, C_{1eq} –H), 3.46 (1H, dq, J =each 7 Hz, $COCH$), 3.77 (1H, d, $J=6$ Hz, C_4 –OH), 4.56 (1H, s, C_2 –OH), 5.23–5.50 (1H, m, C_4 –H), 7.77–8.01 (2H, m, aromatic protons), 8.25–8.53 (2H, m, aromatic protons), 13.38, 13.66 (2H, two s, phenolic OH $\times$ 2). MS m/e : 396 (M^+), 378, 360, 307. Anal. Calcd for $C_{22}H_{20}O_7$: C, 66.66; H, 5.09. Found: C, 66.43; H, 5.01.

(2S,4S)-(+)-4-O- α -3'-N-Trifluoroacetyl-L-daunosaminyl-2,4,5,12-tetrahydroxy-2-nonanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione (8a)—Ether (8 ml) was added to a mixture of 3-N-trifluoroacetyl-1,4-bis(*O*-*p*-nitrobenzoyl)-L-daunosamine¹² (119 mg, 0.22 mmol) and molecular sieves 4A (657 mg) in CH_2Cl_2 (10 ml), and the resulting mixture was cooled to $-40^\circ C$. TMSOTf (0.05 ml, 0.26 mmol) was added, and the whole mixture was stirred in an ice bath for 1 h, then cooled at $-20^\circ C$. A solution of **13a** (64.9 mg, 0.14 mmol) in CH_2Cl_2 (12 ml) was added to the mixture, and stirring was continued at -15 – $-10^\circ C$ for 6 h. The reaction mixture was poured into satd. $NaHCO_3$, 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 the crude glycoside as a red residue, which was dissolved in CH_2Cl_2 (2 ml). The dichloromethane solution was diluted with MeOH (100 ml), 0.1 M NaOH (2.2 ml) was added, and the whole was stirred at room temperature for 1 h. The reaction mixture was neutralized by adding AcOH (2 drops), then diluted successively with H_2O and EtOAc. The upper organic layer was separated, and the aqueous phase was further extracted with EtOAc. The organic extracts were combined, washed

successively with H₂O and satd. NaCl, then dried over anhyd. Na₂SO₄. Filtration and concentration *in vacuo* gave a red residue (151 mg), which was separated by column chromatography (SiO₂, CHCl₃, then CHCl₃-Me₂CO 40:1) to give **8a** as a red powder (52.5 mg, 55% overall yield), mp 210.5–213 °C and $[\alpha]_D^{20} + 161^\circ$ ($c=0.175$, dioxane). IR (KBr): 3500, 3450, 1720, 1715, 1625, 1590 cm⁻¹. NMR (CDCl₃) δ : 0.90 (3H, t, $J=6$ Hz, CH₃), 1.15–2.60 (19H, m, (CH₂)₆CH₃, C₃-H₂, C₂-H₂, and C₅-CH₃), 2.85 (2H, t, $J=7$ Hz, COCH₂), 3.04 (1H, d, $J=19$ Hz, C_{1ax}-H), 3.27 (1H, d, $J=19$ Hz, C_{1eq}-H), 3.53–3.85 (1H, m, C₄-H), 4.05–4.70 (2H, m, C₃-H and C₅-H), 4.27 (1H, C₂-OH), 5.29 (1H, t, $J=3$ Hz, C₄-H), 5.55 (1H, d, $J=3$ Hz, C₁-H), 6.67 (1H, br d, $J=9$ Hz, NH), 7.73–8.05 (2H, m, aromatic protons), 8.25–8.56 (2H, m, aromatic protons), 13.37, 13.65 (2H, two s, phenolic OH $\times 2$). MS m/e : 691 (M⁺), 466, 448, 307. Anal. Calcd for C₃₅H₄₀F₃NO₁₀·0.5H₂O: C, 60.00; H, 5.90; N, 2.00. Found: C, 60.09; H, 5.83; N, 2.02.

(2S,4S)-(+)-4-O- α -3'-N-Trifluoroacetyl-L-daunosaminyl-2,4,5,12-tetrahydroxy-2-pentanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione (8b)—Sequential glycosidation of **13b** (49.4 mg, 0.12 mmol) with 3-*N*-trifluoroacetyl-1,4-bis(*O*-*p*-nitrobenzoyl)-L-daunosamine¹²⁾ (101 mg, 0.19 mmol) and alkaline hydrolysis of the *p*-nitrobenzoyl group of the glycoside in the same manner as that described for the preparation of **8a**, gave **8b** as a red powder (51.3 mg, 67% overall yield), $[\alpha]_D^{20} + 171^\circ$ ($c=0.110$, dioxane), after separation by column chromatography (SiO₂, CHCl₃, then CHCl₃-Me₂CO = 30:1). IR (KBr): 3500, 3450, 1720, 1715, 1625, 1590 cm⁻¹. NMR (CDCl₃) δ : 0.96 (3H, t, $J=7$ Hz, CH₃), 1.31 (3H, d, $J=6$ Hz, C₅-CH₃), 1.15–2.55 (8H, (CH₂)₂CH₃, C₃-H₂, and C₂-H₂), 2.87 (2H, t, $J=7$ Hz, COCH₂), 3.03 (1H, d, $J=19$ Hz, C_{1ax}-H), 3.27 (1H, d, $J=19$ Hz, C_{1eq}-H), 3.55–3.85 (1H, m, C₄-H), 4.00–4.50 (2H, m, C₃-H and C₅-H), 4.29 (1H, s, C₂-OH), 5.28 (1H, t, $J=3$ Hz, C₄-H), 5.54 (1H, d, $J=3$ Hz, C₁-H), 6.72 (1H, br d, $J=9$ Hz, NH), 7.73–8.01 (2H, m, aromatic protons), 8.23–8.50 (2H, m, aromatic protons), 13.37, 13.65 (2H, two s, phenolic OH $\times 2$). MS m/e : 410 (M⁺), 392, 374, 307. Anal. Calcd for C₃₁H₃₂F₃NO₁₀·0.25H₂O: C, 58.17; H, 5.12; N, 2.19. Found: C, 58.14; H, 5.43; N, 2.07.

(2S,4S)-(+)-4-O- α -3'-N-Trifluoroacetyl-L-daunosaminyl-2,4,5,12-tetrahydroxy-2-(2-methylpropanoyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione (8c)—Glycosidation of **13c** (28.9 mg, 0.073 mmol) with 3-*N*-trifluoroacetyl-1,4-bis(*O*-*p*-nitrobenzoyl)-L-daunosamine¹²⁾ (63.3 mg, 0.12 mmol), followed by alkaline hydrolysis of the *p*-nitrobenzoyl group of the formed glycoside in the same manner as that described for the preparation of **8a**, gave **8c** as a red powder (25.0 mg, 55% overall yield), $[\alpha]_D^{20} + 164^\circ$ ($c=0.139$, dioxane), after separation by column chromatography (SiO₂, CHCl₃, then CHCl₃-Me₂CO 30:1). IR (KBr): 3520, 3450, 1720, 1715, 1625, 1590 cm⁻¹. NMR (CDCl₃) δ : 1.09–1.31 (6H, two d, CH(CH₃)₂), 1.30 (3H, d, $J=6$ Hz, C₅-CH₃), 1.75–2.50 (4H, m, C₃-H₂ and C₂-H₂), 3.07 (1H, d, $J=19$ Hz, C_{1ax}-H), 3.29 (1H, d, $J=19$ Hz, C_{1eq}-H), 3.35–3.90 (2H, m, COCH and C₄-H), 4.10–4.70 (2H, m, C₃-H and C₅-H), 4.29 (1H, s, C₂-OH), 5.29 (1H, t, $J=3$ Hz, C₄-H), 5.55 (1H, d, $J=3$ Hz, C₁-H), 6.69 (1H, br d, $J=9$ Hz, NH), 7.74–8.05 (2H, m, aromatic protons), 8.28–8.58 (2H, m, aromatic protons), 13.40, 13.67 (2H, two s, phenolic OH $\times 2$). MS m/e : 396 (M⁺), 378, 360, 307. Anal. Calcd for C₃₀H₃₀F₃NO₁₀·0.5H₂O: C, 57.14; H, 4.96; N, 2.22. Found: C, 57.00; H, 5.24; N, 1.99.

(2S,4S)-(+)-4-O- α -L-Daunosaminyl-2,4,5,12-tetrahydroxy-2-nonanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione Hydrochloride (7a·HCl)—A 0.1 M sodium hydroxide solution (4 ml) was added to a solution of **8a** (27.4 mg, 0.040 mmol) in THF (1 ml), and the mixture was stirred at room temperature for 40 min. The mixture was then neutralized at pH ~ 8 by adding 3% HCl, and extracted repeatedly with CHCl₃. The organic extracts were combined, washed with H₂O, and dried over anhyd. Na₂SO₄. After filtration, the filtrate was concentrated *in vacuo* to a volume of ca. 1 ml. Next, 0.25 M HCl in MeOH (0.3 ml) and Et₂O (15 ml) were successively added, and the orange powder that crystallized out was separated and triturated with Et₂O, giving **7a·HCl** (17.3 mg, 69%), mp 172–174 °C and $[\alpha]_D^{20} + 130^\circ$ ($c=0.134$, MeOH). IR (KBr): 3475, 1715, 1620, 1590 cm⁻¹. NMR (DMSO-*d*₆) δ : 0.84 (3H, t, $J=6.8$ Hz, (CH₂)₆CH₃), 1.16 (3H, d, $J=6.5$ Hz, C₅-CH₃), 1.23 (10H, brs, CH₂(CH₂)₅CH₃), 1.49 (2H, dt, J =each 6.7 Hz, COCH₂CH₂), 1.71 (1H, dd, $J=12.4, 4.1$ Hz, C_{2'eq}-H), 1.90 (1H, dt, $J=12.4, 3.4$ Hz, C_{2'ax}-H), 2.07 (1H, dd, $J=14.1, 5.6$ Hz, C_{3ax}-H), 2.16 (1H, dd, $J=14.1, 3.4$ Hz, C_{3eq}-H), 2.748, 2.751 (2H, two t, J =each 7.1 Hz, COCH₂), 2.95 (2H, brs, C₁-H₂), 3.62 (1H, br d, $J=6.1$ Hz, C₄-H), 4.22 (1H, q, $J=6.5$ Hz, C₅-H), 4.90 (1H, dd, $J=5.6, 3.4$ Hz, C_{4eq}-H), 5.28 (1H, d, $J=3.4$ Hz, C_{1'eq}-H), 5.49 (1H, d, $J=6.1$ Hz, C₄-OH), 5.53 (1H, s, C₂-OH), 7.90–8.15 (5H, m, aromatic protons and NH₃⁺), 8.20–8.27 (2H, m, aromatic protons), 13.28, 13.49 (2H, two s, phenolic OH $\times 2$). MS (SIMS) m/z : 596 (MH⁺), 466, 431, 291. Anal. Calcd for C₃₃H₄₂ClNO₉·1.25H₂O: C, 60.54; H, 6.85; N, 2.14. Found: C, 60.57; H, 6.61; N, 2.11.

(2S,4S)-(+)-4-O- α -L-Daunosaminyl-2,4,5,12-tetrahydroxy-2-pentanoyl-1,2,3,4-tetrahydro-6,11-naphthacenedione Hydrochloride (7b·HCl)—The same treatments of **8b** (34.0 mg, 0.053 mmol) as those described for **8a** gave **7b·HCl** as a red powder (27.0 mg, 88%), mp 170–172 °C and $[\alpha]_D^{20} + 121^\circ$ ($c=0.111$, MeOH). IR (KBr): 3520, 1715, 1620, 1590 cm⁻¹. NMR (DMSO-*d*₆) δ : 0.89 (3H, $J=7.3$ Hz, (CH₂)₂CH₃), 1.16 (3H, d, $J=6.4$ Hz, C₅-CH₃), 1.28 (2H, tq, J =each 7.4 Hz, CH₂CH₂CH₃), 1.49 (2H, tt, J =each 7.2 Hz, CH₂CH₂CH₂CH₃), 1.71 (1H, dd, $J=12.6, 4.0$ Hz, C_{2'eq}-H), 1.90 (1H, dt, $J=12.6, 3.6$ Hz, C_{2'ax}-H), 2.07 (1H, dd, $J=14.1, 5.8$ Hz, C_{3ax}-H), 2.18 (1H, dd, $J=14.1, 3.2$ Hz, C_{3eq}-H), 2.73, 2.80 (2H, two dt, J =each 18.2, 7.3 Hz, COCH₂), 2.93 (1H, d, $J=18.9$ Hz, C_{1ax}-H), 2.98 (1H, d, $J=18.9$ Hz, C_{1eq}-H), 3.62 (1H, br d, $J=6.1$ Hz, C₄-H), 4.23 (1H, q, $J=6.4$ Hz, C₅-H), 4.90 (1H, dd, $J=5.8, 3.2$ Hz, C_{4eq}-H), 5.28 (1H, d, $J=3.6$ Hz, C_{1'eq}-H), 5.49 (1H, d, $J=6.1$ Hz, C₄-OH), 5.51 (1H, s, C₂-OH), 7.88–8.04 (5H, m, aromatic protons and NH₃⁺), 8.22–8.29 (2H, m, aromatic protons), 13.28, 13.49 (2H, two s, phenolic OH $\times 2$). MS (SIMS) m/z : 540 (MH⁺), 410, 375, 291. Anal. Calcd for C₂₉H₃₄ClNO₉·2.5H₂O: C, 56.08; H, 6.33; N, 2.26. Found: C,

56.24; H, 6.29; N, 2.01.

(2S,4S)-(+)-4-O- α -L-Daunosaminy-2,4,5,12-tetrahydroxy-2-(2-methylpropanoyl)-1,2,3,4-tetrahydro-6,11-naphthacenedione Hydrochloride (7c·HCl)—The same treatments of **8c** (30.2 mg, 0.049 mmol) as those described for **8a** afforded **7c·HCl** as a red powder (17.8 mg, 65%), mp 198–200 °C and $[\alpha]_D^{20} + 193^\circ$ ($c=0.085$, MeOH). IR (KBr): 3450, 1715, 1625, 1590 cm^{-1} . NMR (DMSO- d_6) δ : 1.00, 1.06 (6H, two d, J =each 6.7 Hz, $\text{CH}(\text{CH}_3)_2$), 1.15 (3H, d, J =6.6 Hz, $\text{C}_5\text{-CH}_3$), 1.72 (1H, dd, J =12.6, 4.0 Hz, $\text{C}_{2'\text{eq}}\text{-H}$), 1.92 (1H, dt, J =12.6, 3.3 Hz, $\text{C}_{2'\text{ax}}\text{-H}$), 2.11–2.23 (2H, m, $\text{C}_3\text{-H}_2$), 2.99 (1H, d, J =18.3 Hz, $\text{C}_{1\text{ax}}\text{-H}$), 3.05 (1H, d, J =18.3 Hz, $\text{C}_{1\text{eq}}\text{-H}$), 3.49 (1H, dq, J =each 6.7 Hz, COCH), 3.58 (1H, br d, J =6.1 Hz, $\text{C}_4\text{-H}$), 4.20 (1H, q, J =6.6 Hz, $\text{C}_5\text{-H}$), 4.97 (1H, t, J =4.5 Hz, $\text{C}_{4\text{eq}}\text{-H}$), 5.30 (1H, d, J =3.3 Hz, $\text{C}_{1'\text{eq}}\text{-H}$), 5.47 (1H, d, J =6.1 Hz, $\text{C}_4\text{-OH}$), 5.54 (1H, s, $\text{C}_2\text{-OH}$), 7.82 (3H, brs, NH_3^+), 7.98–8.04 (2H, m, aromatic protons), 8.28–8.35 (2H, m, aromatic protons), 13.41–13.57 (2H, brs, phenolic OH $\times 2$). MS (SIMS) m/z : 526 (MH^+), 396, 361, 291. Anal. Calcd for $\text{C}_{28}\text{H}_{32}\text{ClNO}_9 \cdot 2\text{H}_2\text{O}$: C, 56.23; H, 6.07; N, 2.34. Found: C, 56.36; H, 5.98; N, 2.28.

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furans, ethers, and dioxanes freshly distilled from sodium benzophenone ketyl, and dichloromethane and acetone 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 solvents: acetic acid (AcOH), acetone (Me₂CO), benzene (C₆H₆), carbon tetrachloride (CCl₄), chloroform (CHCl₃), dichloromethane (CH₂Cl₂), dimethyl sulfoxide (DMSO), ether (Et₂O), ethyl acetate (EtOAc), hexamethylphosphoric triamide (HMPA), hexane (C₆H₁₄), methanol (MeOH), tetrahydrofuran (THF).