CHEMICAL MODIFICATION OF ANTHRACYCLINE ANTIBIOTICS IV
SYNTHESIS OF NEW ANTHRACYCLINES WITH TRISACCHARIDE

HIROSHI TANAKA, TAKEO YOSHIOKA, YASUTAKA SHIMAUCHI, YOSHIYUKI MATSUSHITA, YASUE MATSUZAWA, TOSHIKAZU OKI and TOMOYUKI ISHIKURA

Central Research Laboratories, Sanraku-Ocean Co., Ltd.
Johnan, Fujisawa, Kanagawa, Japan

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The glycosidation products of *O*-α-L-cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α,β-L-rhodosamine with aklavinone, daunomycinone, adriamycinone and carminomycinone were synthesized and the resulting products were deacylated, yielding anthracyclines having the trisaccharide. We further synthesized *N*-monomethyl and *N*-didemethyl derivatives of daunomycinone trisaccharide by photolysis with sunlight. 3''-*O*-Acyl derivatives of daunomycinone, carminomycinone and aklavinone glycosides showed a marked antitumor activity against L1210 leukemia in mice with ILS 110, 86 and 86%, respectively, while 3''-*O*-acetyladriamycinone glycoside was highly toxic with a ILS of 44%.

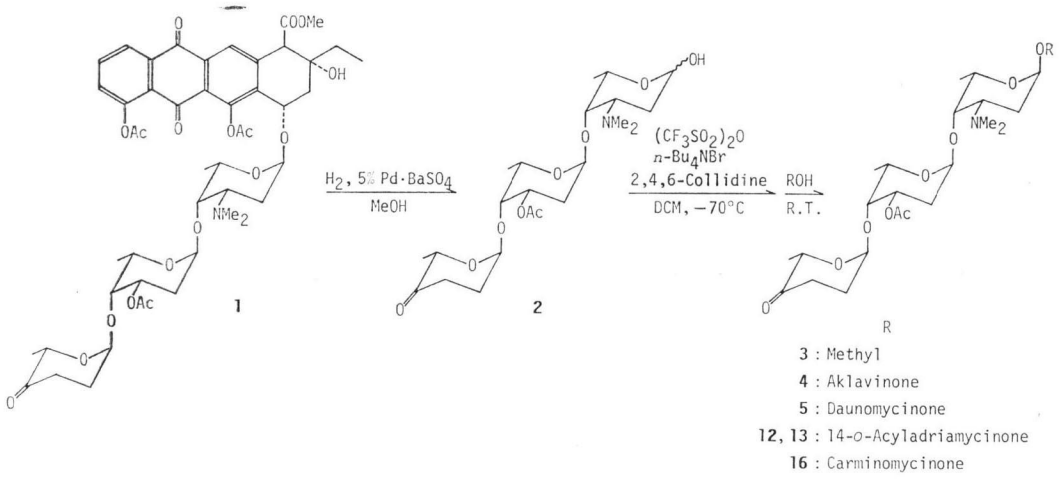
Our hope is to develop anthracyclines which have a lower cardiac toxicity than adriamycin (ADM)[1] but a similar degree of anticancer activity[2,3]. Trisarubicinol (L-cinerulosyl-2-deoxy-L-fucosyl-L-rhodosaminyl-13-dihydrocarminomycinone)[4] which was obtained by microbial glycosidation of carminomycinone[5] using *Streptomyces galilaeus* KE 303 showed a strong antitumor activity against L1210 leukemia in mice. It was different from carminomycin I (CM)[5] in its higher ratio of inhibition of RNA synthesis to DNA synthesis in L1210 cells. There are many reports on chemical glycosidation of anthracyclinones[6], but none with a trisaccharide. In the present investigation, the monoacetyl trisaccharide (**2**) obtained from 4,6,3''-*O*-triacetylaclacinomycin A (**1**) was reacted with anthracyclinones by a one-pot reaction including sulfonylation and bromination of a sugar[7], yielding new anthracyclines with the trisaccharide. These products and their deacetyl products were tested for the antitumor activity against L-1210 leukemia. *N*-Monomethyl and *N*-didemethyl derivatives of daunomycinone trisaccharide were also synthesized and tested the antitumor activity.

Synthesis of Anthracyclinone Trisaccharides

Treatment of aclacinomycin A (ACM)[8,9] with acetic anhydride and pyridine gave 4,6,3''-*O*-triacetylaclacinomycin A (**1**) in quantitative yield. Hydrogenolysis of **1** over 5% palladium-barium sulfate in methanol gave a monoacetyl trisaccharide (**2**) as a colorless powder in 98% yield. This product (**2**) gave only one grayish green spot (Rf 0.24) by 10% sulfuric acid coloration on a TLC plate using chloroform - methanol (7: 1). The ^{1}H NMR spectrum of **2** showed that **2** was an α,β-anomeric mixture of its rhodosamine moiety: *O*-α-L-cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α,β-L-rhodosamine. The ratio of α: β (*ca.* 2: 1) was obtained from the relative intensity of their C-1 anomeric proton resonances: broad singlet (W_H=6 Hz) at δ 5.40 in the α-anomer (**2a**) and broad doublet (W_H= 13.8 Hz) at δ 4.70 in the β-anomer (**2b**). The β-anomer was isolated as a colorless needles by crystallization from ethyl ether–*n*-hexane.

Glycosidation of **2** with alcohols was achieved by the one-pot reaction where a glycosyl trifluoromethanesulfonate (or methanesulfonate) and a glycosyl bromide were the reaction intermediates[7]. When

Fig. 1.

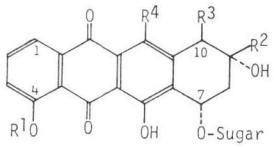


2 was treated with trifluoromethanesulfonic anhydride in the presence of 2,4,6-collidine and tetra-*n*-butylammonium bromide in dichloromethane at −70°C, followed by methanol (4 molar excess for **2**), only one product (**3**) was obtained. It was detected as a grayish green spot (Rf 0.36) on a TLC (above solvent system and coloration). This product (**3**) was determined to be methyl α-glycoside by ^{1}H NMR analysis. The methyl β-glycoside was not found. Compound **2b** under the same conditions gave also only one product, which was assignable to **3** by $[\alpha]_D$, ^{1}H NMR and IR spectroscopies. The β-anomer of **3** was not produced. These findings indicated that the trisaccharide **2** as well as **2b** were converted stereoselectively to an α-glycoside by this procedure (Fig. 1).

A similar coupling reaction of aklavinone[10] with a 3 molar excess of **2** afforded exclusively α-glycoside (**4**). Field desorption mass spectrum (FD-MS) of **4** indicated its molecular ion peak (M^+) at *m/z* 853. Compound **4** was characterized by analysis of its ^{1}H and ^{13}C NMR spectra. The broad singlet (W_H=*ca.* 5 Hz) at δ 5.51 was assigned to the C-1′ equatorial proton (α-configuration). Two aromatic hydroxyl protons were observed at δ 11.98 and 12.65, and the chemical shift of the C-7 in ^{13}C NMR was the same as that of ACM[9]. These evidences suggested that the monoacetyl trisaccharide (**2**) was attached to the C-7 oxygen of the aglycone. Thus, compound **4** was determined to be the 3′′-*O*-acetyl derivative of ACM.

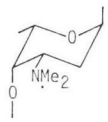
Daunomycinone[11], adriamycinone[1] and carminomycinone were coupled similarly with **2** (Table 1). Glycosidation of **2** with daunomycinone furnished the protected glycoside (**5**). In the case of adriamycinone, a suitable protection of the C-14 hydroxyl group was required for the regioselective glycosidation. Partial acetylation of the compound was performed by using boroacetic anhydride[12] and pyridine to give 14-*O*-acetyladriamycinone (**10**)[13]. By an alternative route, **10** could be prepared from daunomycin (DM)[11]. Namely, bromination of the C-14 methyl of DM with bromine in methanol - dioxane, followed by acid hydrolysis gave 14-bromodaunomycinone, which was esterified with a sodium carboxylate such as sodium acetate or phenylacetate in refluxing acetone, affording **10** or 14-*O*-phenylacetyladriamycinone (**11**)[13]. The coupling reaction of these anthracyclinones (**10** and **11**) with **2** provided the corresponding protected anthracycline glycosides (**12** and **13**). For the preparation of carminomycinone glycoside (**16**) methanesulfonyl chloride instead of trifluoromethanesulfonic anhydride was used.

Table 1. Structures of anthracycline glycosides.



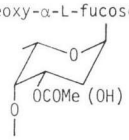
Compound	Aglycone	R^1	R^2	R^3	R^4	Sugar*
4	Aklavinone	H	CH_2CH_3	$COOCH_3$	H	Rho-AcDeFuc-CinA
5	Dauno-mycinone	CH_3	$COCH_3$	H	OH	Rho-AcDeFuc-CinA
6		CH_3	$COCH_3$	H	OH	Rho-DeFuc-CinA
7		CH_3	$COCH_3$	H	OH	Rho-DeFuc
8		CH_3	$COCH_3$	H	OH	MDau-DeFuc-CinA
9		CH_3	$COCH_3$	H	OH	Dau-DeFuc-CinA
12	Adria-mycinone	CH_3	$COCH_2OCOCH_3$	H	OH	Rho-AcDeFuc-CinA
13		CH_3	$COCH_2OCOCH_2C_6H_5$	H	OH	Rho-AcDeFuc-CinA
14		CH_3	$COCH_2OH$	H	OH	Rho-AcDeFuc-CinA
15		CH_3	$COCH_2OH$	H	OH	Rho-DeFuc-CinA
16	Carmino-mycinone	H	$COCH_3$	H	OH	Rho-AcDeFuc-CinA

*Rho : α-L-Rhodosamine



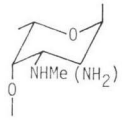
AcDeFuc : 3-O-Acetyl-2-deoxy-α-L-fucose

DeFuc : 2-Deoxy-α-L-fucose

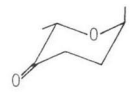


MDau : N-Methyl-α-L-daunosamine

Dau : α-L-Daunosamine



CinA : α-L-Cinerulose A



The yields in these reactions which gave exclusively target anthracycline glycosides were 20~40%, and unreacted anthracyclinones were recovered in each case.

The structure of the protected glycosides was determined by FD-MS, ^1H and ^{13}C NMR spectroscopies and elemental analysis. The stereochemistry of the C-1′ glycosidic linkage of each glycoside was assigned to the α-configuration on the basis on ^1H NMR analysis. The resonances of their C-1′ protons revealed a broad singlet with $W_H = ca.$ 5 Hz at $\delta \sim 5.5$, characteristic of an equatorial anomeric proton. The following data supported the assignment of the linkage of the sugar moiety to the C-7 position of the aglycone. All of the aromatic hydroxyl protons of the glycosides were observed in the offset region (δ 11.9~13.9). In the ^{13}C NMR spectra, the chemical shifts of the C-7 of **5** and **14** coincided with those[14] of DM and ADM, respectively (Table 2).

Protective groups of the resulting glycosides were removed by hydrolysis with potassium carbonate in aqueous methanol - acetone. Hydrolysis of **5** gave the target glycoside (**6**) and the disaccharide derivative (**7**) which formed by the C-1‴ glycosidic bond cleavage with the base. Both **12** and **13** were

Table 2. ^{1}H and ^{13}C NMR chemical shifts of 3''-*O*-acetylanthracycline glycosides.

Compound	Aglycone										Sugar				
	1	2	3	11	5	12	7	10	14	ArOH	1'	NMe_2	1''	COMe	1'''
4	7.81dd J=1.6, 8	7.66t J=8	7.27dd J=1.6, 8	7.66s	—	—	5.27bs W_H=7.5	4.12s	1.10t J=7	11.98b 12.65b	5.51bs W_H=5	2.17s	5.07bs W_H=5.6	2.06s	5.02bt W_H=12
	*120.1	137.3	124.8	120.9	192.7	181.2	70.6	57.2	6.7	—	101.6	43.3	99.0	21.4 170.4	99.4
5	7.99dd J=1.5, 8	7.74t J=8	7.36dd J=1.5, 8	—	—	—	5.25bs W_H=8	3.22d 2.88d J=20	2.42s	13.22s 13.88s	5.53bs W_H=5	2.19s	5.06bs W_H=5.2	2.09s	5.02t W_H=11
	*118.4	134.5	119.7	155.9	186.6	186.9	69.6	33.4	24.7	—	101.2	43.3	99.0	21.4 170.4	99.4
14	7.98dd J=1.5, 8	7.75t J=8	7.37dd J=1.5, 8	—	—	—	5.27bs W_H=7.4	3.26d 2.96d J=20	4.76s	13.28bs 13.89s	5.54bs W_H=5.2	2.26s	5.10bs W_H=7.3	2.09s	5.03bt W_H=11.2
	*118.5	135.7	119.8	155.6	186.6	186.9	69.2	33.6	65.5	—	101.1	43.2	99.0	21.4 170.4	98.4
16	7.84dd J=1.5, 8	7.68t J=8	7.27dd J=1.5, 8	—	—	—	5.23bs W_H=7.8	3.25d 2.92d J=20	2.42s	12.12b 12.93b 13.38bs	5.49bs W_H=5	2.22s	5.06bs W_H=6.3	2.07s	5.01bt W_H=11.4

In ppm (δ), obtained from $CDCl_3$ solutions containg TMS as internal reference. * ^{13}C NMR data. J and W_H: in Hz.

Table 3. Antitumor activity of new anthracycline glycosides against L1210 leukemia.

Compound	*In vivo* ILS (%) (dose: mg/kg/day)				*In vitro* IC_{50} (μg/ml)		
					Cyto-toxicity on day 2	DNA synthesis	RNA synthesis
ACM	86 (7.5)				0.06	1.30	0.10
DM	50 (1.25)				0.036	0.30	0.18
ADM	192 (1.25)				0.018	1.25	0.49
CM	—				0.005	0.20	0.29
4	80 (10)	86 (7.5)	68 (5)	14 (2.5)	0.03	0.65	0.085
5	32 (7.5)	110 (5)	50 (2.5)	44 (1.25)	0.01	0.30	0.02
6	Tox. (5)	44 (2.5)	44 (1.25)	26 (0.63)	0.01	0.17	0.017
7	20 (5)	50 (2.5)	44 (1.25)	14 (0.32)	0.01	0.28	0.04
8					0.02	0.62	0.065
9	57 (7.5)	52 (5)	35 (2.5)	21 (1.25)	0.09	1.15	0.165
12					0.01	0.50	0.014
13					0.03	4.0	0.15
14	32 (1.25)	44 (0.63)	44 (0.32)	20 (0.16)	0.01	0.32	0.012
15					0.01	0.15	0.011
16	54 (7.5)	60 (5)	86 (2.5)	54 (1.25)	0.01	0.36	0.04

In vivo antitumor activity: CDF_1 mice inoculated intraperitoneally with 10^5 L1210 leukemia cells were treated by intraperitoneal administration of the compound daily for 10 days starting 24 hours after inoculation. Death or survival of the test and the control mice was recorded daily for 30 days and the antitumor activity was evaluated in terms of the percentage increase in life span (ILS) over the control.

Cytotoxicity: L1210 cells (4×10^4 cells/ml) were cultured in RPMI 1640 medium containing 20% calf serum with test compounds (0.01~0.5 μg/ml) at 37°C under 5% CO_2–95% air atmosphere. The cell growth was periodically determined using a hemocytometer by counting viable cells staind with trypan blue (0.17%). Cytotoxicity was expressed as IC_{50} of the control growth on day 2.

Nucleic acid biosynthesis: After preincubation of L1210 cell suspension (5×10^5 cells/ml) with test compound (0.01~2.5 μg/ml) at 37°C for 15 minutes, [2-^{14}C]TdR or -UR was added with 0.05 μCi/ml, respectively, and incubated at 37°C for 60 minutes. The reaction was terminated by rapid chilling and adding 1 ml of cold 10% TCA to 1 ml of reaction mixture. The precipitate was washed twice with 2 ml of cold 5% TCA, and dissolved in 0.25 ml of 99% formic acid. The radioactivity was counted with a Aloka LSC-653 liquid scintillation spectrometer in BRAY's scintillator.

rapidly hydrolyzed to 3′′-*O*-acetyladriamycinone glycoside (**14**), and further hydrolysis furnished the fully deprotected glycoside (**15**). The absence of the protective groups in the resulting glycosides was proved by their ^{1}H NMR spectra.

Photo-catalized *N*-demethylation[9] of daunomycinone glycoside (**6**) with sunlight in chloroform gave a mixture. Preparative thin-layer chromatography of this mixture on silica gel afforded *N*-monomethyl (**8**) and *N*-didemethyl (**9**) derivatives. In their ^{1}H NMR and IR spectra, the signals of dimethylamino group (singlet at δ 2.19 and two weak absorption bands at 2760, 2810 cm^{-1} in **6**) were absent and the *N*-methyl of **8** revealed a singlet at δ 2.40.

Biological Activity

Antitumor activity of the new anthracycline glycosides were tested against L1210 leukemia in comparison with ACM, DM, ADM and CM. The results are shown in Table 3. Among the resulting glycosides, 3′′-*O*-acetyldaunomycinone trisaccharide (**5**) showed the best antitumor activity (ILS 110%) against L1210 leukemia-bearing mice. This activity was considerably higher than that of DM. Compound **4** and **16** showed the similar degree of antitumor activity as ≻CM. While adriamycinone glyco-

side (**14**) showed significant toxicity at 2.5 mg/kg dose level, it had a remarkably low activity in comparison with ADM.

On cultured L1210 leukemia cells, all of the glycosides except for **9** and **13** were more cytotoxic than the corresponding parent drugs. Their effects in inhibiting RNA synthesis were 7- to 36-fold stronger than those in inhibiting DNA synthesis. This preferential inhibition of RNA synthesis may be due to the length of sugar residue. Deprotection at 3''-*O*-acetyl group enhanced the inhibitory effects on DNA synthesis.

Experimental

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. IR and UV-Vis. spectra were recorded on a Hitachi 260–30 spectrophotometer and a Hitachi 200–20 spectrophotometer, respectively. Optical rotation was measured using a JASCO DIP-181 polarimeter. ^{1}H NMR and ^{13}C NMR data were obtained with a Varian XL-100 and EM-390 spectrophotometers. Chemical shifts are expressed in parts per million down field from internal tetramethylsilane. Abbreviations: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and b=broad. Mass spectra were taken on a Hitachi RMU-7 mass spectrometer. Silica gel thin-layer chromatography (TLC) was carried out using precoated plates of Silica Gel 60 F_{254} (E. Merck, Darmstadt). Column chromatography was performed on Silica Gel 60 (70~230 mesh, E. Merck, Darmstadt).

4,6,3''-*O*-Triacetylaclacinomycin A (**1**)

Acetylation of ACM (10.0 g) with Ac_2O (30 ml) and pyridine (20 ml) at 20°C for 15 hours gave the triacetate (**1**) in 96% yield: mp. 148~151°C; $[\alpha]_D$ +64.0° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 261 nm (416), 344 (65); ν_{max}^{KBr} 1780, 1735, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08 (3H, s, $COCH_3$), 2.43 (3H, s, $COCH_3$), 2.50 (3H, s, $COCH_3$).

O-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α,β-L-rhodosamine (**2**) and β-Anomer (**2b**)

Compound **1** (1.0 g) in methanol (50 ml) was hydrogenated over 5% Pd-$BaSO_4$ (1.0 g) for 1 hour at 20°C under atmospheric pressure. The solid was removed by filtration and the filtrate was evaporated to a brownish syrup. The syrup was dissolved with $CHCl_3$ (20 ml) and extracted with 1% (v/v) aqueous AcOH (20 ml). After washing with $CHCl_3$, the colorless aqueous layer was adjusted to pH 8 with $NaHCO_3$ and reextracted with $CHCl_3$ (20 ml×4). The combined extracts were dried (Na_2SO_4) and evaporated to give a white powder of **2** (479 mg, 98%): mp. 64~69°C, $[\alpha]_D^{24}$ −238° (*c* 0.5, $CHCl_3$). This product (**2**) was crystallized from Et_2O-*n*-hexane to afford colorless needles of **2b**: mp. 133~134°C; $[\alpha]_D^{22}$ −234° (*c* 0.5, $CHCl_3$); ν_{max}^{KBr} 1725, 1240, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.06 (3H, s, $COCH_3$), 2.27 (6H, s, $N(CH_3)_2$), 4.70 (1H, bd, W_H=13.8 Hz, C-1 H), 5.01 (2H, bs & bt, C-1′ H & C-1″ H), 5.18 (1H, m, C-3′ H).

Anal. Calcd. for $C_{22}H_{37}NO_9$: C 57.50, H 8.12, N 3.05.
Found: C 57.33, H 7.96, N 3.16.

Methyl *O*-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminide (**3**)

Trifluoromethanesulfonic anhydride $(CF_3SO_2)_2O$ (25 μl) was added to a solution of **2** (60 mg), *n*-Bu_4NBr (82 mg) and 2,4,6-collidine (50 μl) in CH_2Cl_2 (1.2 ml) at −70°C under N_2 atmosphere. After 10 minutes, MeOH (21 μl) was added and the mixture was stirred at 22°C for 1.5 hours. The solution was diluted with Et_2O and washed with 2% aqueous $NaHCO_3$. The ethereal solution was dried (Na_2SO_4) and the solvent was evaporated to give a syrup. This syrup was chromatographed on silica gel ($CHCl_3$-MeOH, 50: 1) to yield **3**, as a syrup. **2b** under the same conditions also gave **3**: $[\alpha]_D^{23}$ −275° (*c* 0.2, $CHCl_3$); $\nu_{max}^{CHCl_3}$ 1725, 1240, 1120, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (3H, s, $COCH_3$), 2.23 (6H, s, $N(CH_3)_2$), 3.30 (3H, s, OCH_3), 4.77 (1H, bs, W_H=5 Hz, C-1 H), 5.00 (2H, bs & bt, C-1′ H & C-1″ H).

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-L-rhodosaminyl]aklavinone (**4**)

To a solution of **2** (311 mg), 2,4,6-collidine (0.26 ml) and *n*-Bu_4NBr (425 mg) in CH_2Cl_2 (3 ml) containing Molecular sieves 4A (600 mg), and $(CF_3SO_2)_2O$ (0.13 ml) was added with stirring at −70°C under N_2 atmosphere. After 20 minutes, aklavinone (140 mg) in CH_2Cl_2 (6 ml) was added to the mixture and the reaction was continued for 5 hours at 20°C. The mixture was diluted with benzene (70 ml) and washed with 1% aqueous $NaHCO_3$, and 5% aqueous KH_2PO_4 and water. The benzene solution was dried and evaporated. The resulting residue was chromatographed on silica gel. Elution with $CHCl_3$ - MeOH (100: 1) gave unreacted aklavinone (42 mg) and subsequent elution with $CHCl_3$ - MeOH (200: 3) afforded **4** (112 mg, 39%): mp. 137~140°C; $[\alpha]_D^{24}$ −60.3° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 435 nm (166); ν_{max}^{KBr} 1735, 1675, 1625 cm^{-1}; FD-MS: *m/z* 853 (M^+).

Anal. Calcd. for $C_{44}H_{55}NO_{16}\cdot\frac{1}{2}H_2O$: C 61.25, H 6.54, N 1.62.
Found: C 61.18, H 6.72, N 1.81.

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl]daunomycinone (**5**)

By the same procedure described above, the coupling reaction of daunomycinone (100 mg) with **2** (230 mg) [$(CF_3SO_2)_2O$ (0.09 ml), 2,4,6-collidine (0.2 ml) and *n*-Bu_4NBr (322 mg) in CH_2Cl_2] gave **5** (69 mg 33%), after column chromatography on silica gel with $CHCl_3$-MeOH (100: 1): mp. 147~150°C; $[\alpha]_D^{24}$ −60.4° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 485 nm (134), 499 (137); ν_{max}^{KBr} 1730, 1625, 1585 cm^{-1}; FD-MS: *m/z* 840 (MH^+).

Anal. Calcd. for $C_{43}H_{53}NO_{16}\cdot\frac{3}{2}H_2O$: C 59.57, H 6.45, N 1.62.
Found: C 59.58, H 6.45, N 1.82.

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl] daunomycinone (**6**) and 7-*O*-[*O*-(2-Deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl]daunomycinone (**7**)

To a solution of **5** (100 mg) in Me_2CO (8 ml) and 70% aqueous MeOH (18 ml) was added 0.6 ml of 1 N K_2CO_3. The mixture was allowed to stand at 23°C for 5 hours under N_2 atmosphere. The mixture was neutralized with 5% aqueous KH_2PO_4 and concentrated. The resulting solution was extracted with $CHCl_3$ and the extract was evaporated to a reddish residue. Purification of the residue by chromatography on silica gel with $CHCl_3$ - MeOH, 60: 1 gave **6** (45 mg, 47%); mp. 145~148°C; $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 485 nm (145), 499 (149); ν_{max}^{KBr} 1725, 1620, 1580 cm^{-1}; FD-MS: *m/z* 798 (MH^+); ^1H NMR ($CDCl_3$) δ 5.01 (2H, m, C-1′ H & C-1″ H), 5.21 (1H, bs, C-7 H), 5.50 (1H, bs, C-1′ H).

Anal. Calcd. for $C_{41}H_{51}NO_{15}\cdot\frac{3}{2}H_2O$: C 59.70, H 6.53, N 1.69.
Found: C 59.84, H 6.41, N 1.87.

The successive elution with $CHCl_3$ - MeOH (5: 1) provided **7** (12 mg, 15%): mp. 157~161°C; $[\alpha]_D^{20}$ +104° (*c* 0.02, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 485 nm (149), 498 (153); ν_{max}^{KBr} 1715, 1620, 1580 cm^{-1}; FD-MS: *m/z* 686 (MH^+); ^1H NMR ($CDCl_3$) δ 5.00 (1H, bs, C-1″ H), 5.22 (1H, bs, C-7 H), 5.52 (1H, bs, C-1′ H).

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(2-deoxy-α-L-fucosyl)-(1→4)-(*N*-methyl-α-L-daunosaminyl)] daunomycinone (**8**) and 7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(2-deoxy-α-L-fucosyl)-(1→4)-α-L-daunosaminyl]daunomycinone (**9**)

A $CHCl_3$ (10 ml) solution of **6** (34 mg) was irradiated with sunlight at 30°C for 2.5 hours. The solution was evaporated and the resulting residue was purified by preparative TLC using $CHCl_3$ - MeOH (10: 1) to afford **8** (5.1 mg, 15%; Rf 0.27): mp. 149~153°C; $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 483 nm (130), 499 (134); ν_{max}^{KBr} 1710, 1610, 1570 cm^{-1}; FD-MS: *m/z* 784 (MH^+); ^1H NMR ($CDCl_3$) δ 2.40 (6H, s, $COCH_3$ & $NHCH_3$) and **9** (2.7 mg, 8%, Rf 0.25): mp. 161~164°C; $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 483 nm (118), 498 (121); ν_{max}^{KBr} 1710, 1610, 1575 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (3H, s, $COCH_3$).

14-*O*-Acetyladriamycinone (**10**)

A solution of adriamycinone (150 mg) in Ac_2O (5 ml) containing $B(OH)_3$ (500 mg) was stirred at 20°C. After 1 hour, pyridine (0.15 ml) was added to the solution and the reaction was further continued for 1 hour. The mixture was poured into ice-water and extracted with $CHCl_3$. The extract was evaporated and chromatographed on silica gel using $CHCl_3$ as an eluant to afford **10** (138 mg, 84%): mp. 240~243°C (Ref.[13] mp. 242~245°C); $[\alpha]_D^{24}$ +75.0° (*c* 0.02, $CHCl_3$): ν_{max}^{KBr} 1730, 1610, 1575 cm^{-1}.

14-*O*-Acetyladriamycinone (**10**) and 14-*O*-Phenylacetyladriamycinone (**11**) from DM

Acetylation of 14-bromodaunomycinone with AcONa in refluxing Me_2CO gave **10**[13]. When sodium phenylacetate was used instead of AcONa, phenylacetylation of 14-bromodaunomycinone (200 mg) furnished **11** (188 mg, 84%): mp. 175~176°C; $[\alpha]_D^{24}$ +75.6° (*c* 0.03, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 480 nm (246), 487 (237); ν_{max}^{KBr} 1730, 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.80 (2H, s, CH_2–C_6H_5), 7.32 (6H, s & dd, CH_2–C_6H_5 & C-1 H).

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl]-14-*O*-acetyladriamycinone (**12**)

By the same procedure described for **4**, glycosidation of **10** (200 mg) with **2** (600 mg) [$(CF_3SO_2)_2O$ (0.25 ml), 2,4,6-collidine (0.52 ml) and *n*-Bu_4NBr (835 mg) in CH_2Cl_2], followed by chromatography gave **12** (86 mg, 22%): mp. 141~144°C; $[\alpha]_D^{24}$ −57.9° (*c* 0.027, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 483 nm (105), 498 (105); ν_{max}^{KBr} 1735, 1620, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.07 (3H, s, $COCH_3$), 2.18 (3H, s, $COCH_3$), 2.21 (6H, s, $N(CH_3)_2$), 4.85~5.52 (7H, m, C-7 H, C-3″ H, C-1′ H, C-1″ H, C-1‴ H & $COCH_2$ $OCOCH_3$), 7.3~8.02 (3H, m, aromatic H), 13.22 (1H, bs, aromatic OH), 13.90 (1H, bs, aromatic OH).

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl]-14-*O*-phenylacetyladriamycinone (**13**)

The coupling reaction of **11** (100 mg) with **2** (258 mg) by the same procedure described above gave **13** (58 mg, 32%): mp. 127~131°C; $[\alpha]_D^{24}$ −56.0° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 483 nm (96), 498 (97); ν_{max}^{KBr} 1730, 1620, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.76 (2H, s, CH_2–C_6H_5), 7.30 (6H, s & dd, CH_2–C_6H_5 & C-1 H).

Anal. Calcd. for $C_{51}H_{59}NO_{18}\cdot 2H_2O$: C 60.65, H 6.28, N 1.39.
Found: C 60.25, H 6.18, N 1.49.

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl]adriamycinone (**14**)

To a solution of **13** (20 mg) in Me_2CO (2 ml) and 70% aqueous MeOH (5 ml) was added 0.1 ml of 1 N K_2CO_3. After 5 minutes, the solution was neutralized with aqueous KH_2PO_4. Work-up and purification with preparative TLC ($CHCl_3$ - MeOH, 15: 1) afforded **14** (12 mg, 68%): mp. 144~147°C; $[\alpha]_D^{24}$ −69.6° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 483 nm (125), 498 (126); ν_{max}^{KBr} 1725, 1615, 1580 cm^{-1}; FDMS: *m/z* 856 (MH^+).

Anal. Calcd. for $C_{43}H_{53}NO_{17}\cdot\frac{3}{2}H_2O$: C 58.50, H 6.33, N 1.59.
Found: C 58.51, H 6.30, N 1.60.

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl]adriamycinone (**15**)

Hydrolysis of **13** (34 mg) with 1 N K_2CO_3 (0.17 ml) in Me_2CO (1 ml) and 50% aqueous MeOH (6 ml) gave **15** (7.6 mg, 27%): mp. 154~157°C; ν_{max}^{KBr} 1720, 1615, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.72 (2H, s, $COCH_2OH$), 5.00 (2H, m, C-1″ H & C-1‴ H), 5.27 (1H, bs, C-7 H), 5.51 (1H, bs, C-1′ H).

7-*O*-[*O*-α-L-Cinerulosyl-(1→4)-*O*-(3-*O*-acetyl-2-deoxy-α-L-fucosyl)-(1→4)-α-L-rhodosaminyl]carminomycinone (**16**)

Compound **2** (358 mg) in CH_2Cl_2 (5 ml) was treated with methanesulfonyl chloride (0.084 ml) in the presence of 2,4,6-collidine (0.17 ml) at −30°C. After 10 minutes, this mixture was added to a cold suspension (−10°C) of carminomycinone (100 mg), *n*-Bu_4NBr (503 mg), 2,4,6-collidine (0.34 ml) and Molecular sieves 4A (4.0 g) in CH_2Cl_2 (40 ml) and the reaction mixture was then stirred overnight at 23°C. After work-up, the resulting material was chromatographed on silica gel using $CHCl_3$ - MeOH (75: 1) to yield **16** (43 mg, 20%): mp. 140~143°C; $[\alpha]_D^{22}$ −59.5° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 472 nm (153), 495 (199), 517 (141), 531 (137); ν_{max}^{KBr} 1725, 1600 cm^{-1}.

Anal. Calcd. for $C_{42}H_{51}NO_{16}\cdot H_2O$: C 59.77, H 6.33, N 1.66.
Found: C 59.57, H 6.41, N 1.69.

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