Synthesis of New Anthracycline Derivatives Including Butyric or Retinoic Acid Moiety

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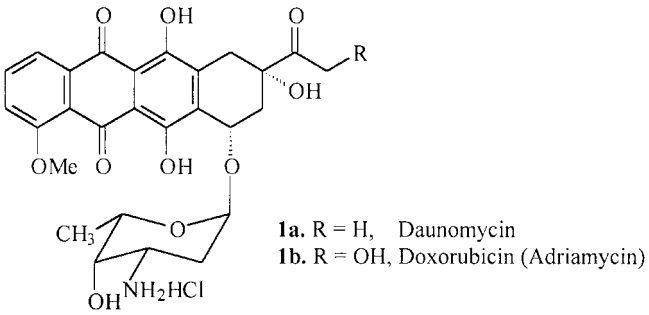
The potential anticancer agents, new anthracycline analogues (**2**-**9**) have been synthesized from the glycosides daunomycin (**1a**) and doxorubicin (**1b**). Compounds **2** and **6** were prepared by nucleophilic displacement esterification of a 14-bromodaunomycin (**1c**) with sodium or potassium salts of butyric and all *trans* retinoic acid, respectively. Compounds **3** and **7** were obtained from daunomycin (**1a**) by direct amidation with a butyric and all *trans* retinoic acid in the presence of EDCI and PP, respectively. Compounds **4** and **8** were obtained from doxorubicin (**1b**) by reaction with the corresponding acids in the same manner. Compounds **5** and **9** were prepared from doxorubicin (**1b**) by acylation with two equivalents of the corresponding acids under the same reaction conditions.

Keywords : Daunomycin, Doxorubicin, Anthracycline derivatives, Butyric and retinoic acid, Acylation.

Introduction

The anthracycline antibiotics daunomycin (**1a**) and adriamycin (**1b**) (Figure 1) are clinically effective anticancer chemotherapeutic agents against several types of human cancers as well as various experimental tumors.[1-4] However, their uses for cancer chemotherapy are seriously hampered by their side effects, especially peroxyl radical effected cardiotoxicity.[5] The cardiotoxicity that appeared as an acute or chronic disease has affected a depression of systole function, arrhythmia, hypotension, *etc*. There are many hypotheses on the source of cardiotoxicity, but the hypothesis that cardiotoxicity is related with the formation of oxygen radicals and the oxidation of lipids has been supported recently.

Many published results show the reduction of cardiotoxicity by diminishing peroxidative damages through blending retinoic acid and doxorubicin.[6] A lot of patents concerning the sodium or potassium salts of butyric acid, which arrest the proliferation of the human gastric cancer, breast cancer, and colon cancer have been approved.[7-8] Recently, we reported the synthesis of a new anthracyclinone by the coupling of 14-OH of the aglycon in **1b** with a butyric acid.[9] In connection with the recent studies, we attempted to prepare some new glycosides starting directly from commercially available drugs, not intermediates. Here, we report in the present study the synthesis of new anthracycline derivatives *via* coupling of C_{14}-OH and $C_{3'}$-NH_2 in DM (**1a**) and DX (**1b**) with two kinds of acid molecules, butyric and retinoic acid expecting diminished cardiotoxicity and undesirable side effects.



1a. R = H, Daunomycin
1b. R = OH, Doxorubicin (Adriamycin)

Figure 1. Structures of two clinically important daunomycin (**1a**) and doxorubicin (**1b**).

Results and Discussion

In the previous papers, we describe the total synthesis of anthracyclinone derivatives through Michael-type condensation[10-13] or Friedel-Crafts acylation.[9,14] We reported the successful preparation of a new aglycon containing an ester linkage at C-14 position through a nucleophilic displacement esterification method.[9,15] In the present study we attempted to directly prepare some new anthracycline analogues from commercially available anticancer agents, such as daunomycin (**1a**) and doxorubicin (**1b**). Several new anthracycline derivatives were synthesized using two acylation methods (Scheme 1). The synthesis of 14-bromo DM (**1c**) was accomplished by the known procedure.[15,16] All compounds (**2**-**9**) were obtained through the acylation of a hydroxyl group at C-14 site in the aglycon and/or amino group at C-3' position in the glycon with sodium butyrate, butyric and all *trans* retinoic acid.

DM-bu (**2**) and DM-re (**6**), potential prodrugs, were prepared by the reaction of 14-bromo DM (**1c**) with a butyric or all *trans* retinoic acid in which could occur the cleavage of

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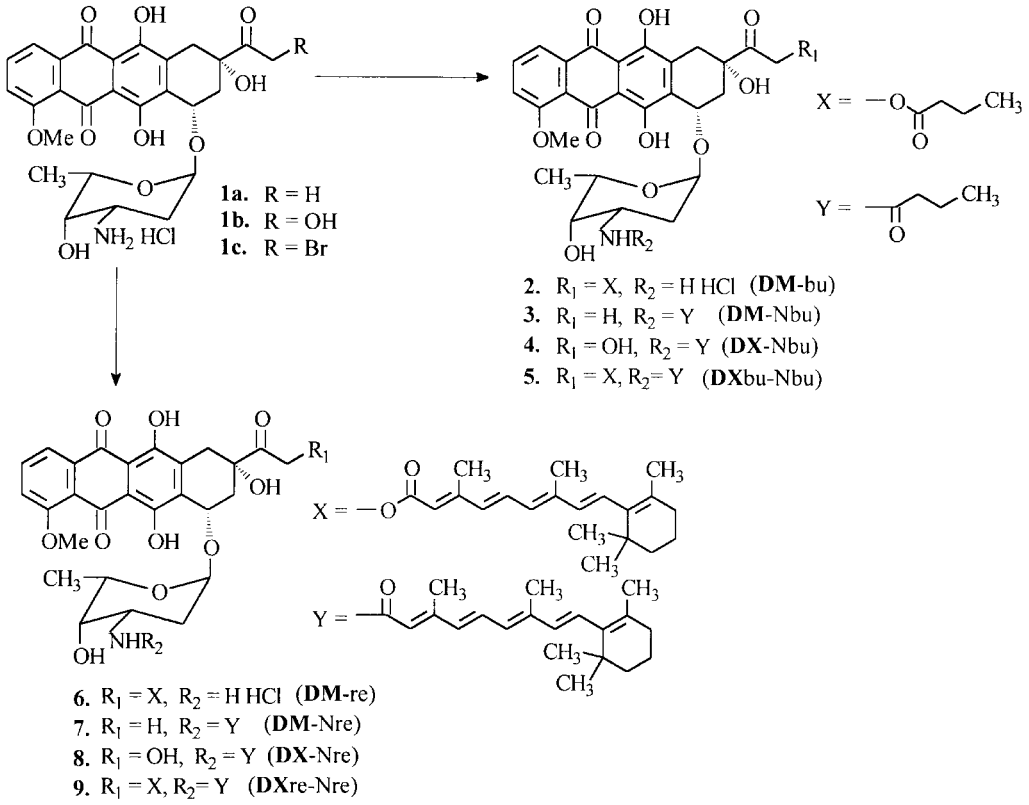

Scheme 1. Synthesis of new anthracycline analogues (**2**-**9**).

the ester bond at C-14 by an oxidation enzyme in the human body. For the purpose of comparing the activity of **2** and **6**, carboamidation compounds, DM-Nbu (**3**), DX-Nbu (**4**), DM-Nre (**7**), and DX-Nre (**8**), were synthesized by amidation of amino group at C-3' of sugar moiety in **1a** or **1b** with the corresponding acids. In addition, *N*-acylation compounds, DXbu-Nbu (**5**) and DXre-Nre (**9**), were prepared through the esterification of C_{14}-OH in DX (**1b**) with the corresponding acids followed by amidation of an amino group at the sugar moiety with the corresponding acids.

First, DM-bu (**2**) was synthesized as follow: To a 14-bromo DM (**1c**) prepared by introducing Br atom at C-14 position of **1a**[16] was added a solution of sodium butyrate in acetone; the solution was stirred at refluxing temperature for 5 hr.[17-20] After removing the solvent under reduced pressure, the residue was dissolved in THF, etheral HCl was added, the mixture was stirred at -20 °C for 2 hr and stirred at room temperature for 3 hr to afford DM-bu (**2**). However, because all *trans* retinoic acid was very sensitive to normal room lighting, it was easily transformed to *cis* form.[21,22] DM-re (**6**) was prepared without refluxing, unlike the procedure described for the preparation of **2**, as follow: To a solution of DM-Br (**1c**) and all *trans* retinoic acid dissolved in acetone with protection from light was added triethylamine (1.2 eq); the mixture solution was stirred at room temperature for 4 hr.[23] After removing the solvent under reduced pressure, the residue was dissolved in THF, to this was added etheral HCl, followed by stirring at -20 °C for 2 hr, and further stirring at room temperature for 3 hr to give DM-re (**6**).

Many attempts to prepare DM-Nbu (**3**) and DX-Nbu (**4**) through direct coupling of the NH_2HCl in daunomycin (**1a**) or doxorubicin (**1b**) with butyric acid using DCC/DMAP failed.[24] Reactants and DCU (dicyclohexylurea) were observed as main products. Eventually, DM-Nbu (**3**) was synthesized by coupling of the NH_2HCl in **1a** with butyric acid using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of catalytic amounts of 4-pyrrolidinopyridine (PP).[25-28] DX-Nbu (**4**) was synthesized from DX (**1b**) as described for the preparation of DM-Nbu (**3**). However, for the reaction of **1b** competition between the hydroxyl at C-14 and the amine group was observed. Both products DX-Nbu (**4**) and DXbu-Nbu (**5**) were formed, the products ratio depended on the amounts of butyric acid and EDCI. DXbu-Nbu (**5**) was prepared using 2.2 equivalent of the corresponding acid and EDCI.

Synthesis of DM-Nre (**7**) and DX-Nre (**8**) was carried out as follow: All *trans* retinoic acid and EDCI (1.2 equivalent) was dissolved in dry DMF with protection from light and stirred at 0 °C for 30 min; to the reaction mixture was added DM (**1a**) or DX (**1b**) and catalytic amounts of PP, and then the mixture was stirred at room temperature for 4 hr to give DM-Nre (**7**) and DX-Nre (**8**).

DXre-Nre (**9**) was synthesized from **1b** as described for the preparation of **8** by increasing the amounts of all *trans* retinoic acid (2.2 eq), EDCI (2.5 eq), and the reaction time (*ca.* 25 hr).

The cytotoxic activities of anthracycline derivatives (**2**-**9**) against two kinds of human tumor cells (SNU-16 and MCF-

Table 1. Comparison of the *in vitro* cytotoxicity of anthracycline derivatives (**2**-**9**) and adriamycin on human tumor cell lines

Agents	IC_{50}[c] (μM)			
	SNU-16[a]	SNU-16/Adr	MCF7[b]	MCF7/Adr
Adriamycin	0.16	0.35(2.19[d])	0.29	0.43(1.48)
2	8.86	7.89(0.89)	0.35	0.63(1.80)
3	14.42	28.82(1.99)	28.82	29.55(1.03)
4	9.65	9.51(0.98)	52.02	53.83(1.03)
5	9.72	8.76(0.90)	4.01	21.15(5.27)
6	5.34	4.22(0.79)	4.39	5.59(1.27)
7	8.75	8.32(0.95)	9.43	18.05(1.91)
8	8.31	8.56(1.03)	19.20	9.45(0.49)
9	9.23	9.04(0.98)	13.1	11.37(0.87)

[a]Human stomach adenocarcinoma. [b]Human breast adenocarcinoma. [c]Concentration inhibiting colony growth by 50%. [d]Relative resistance (IC_{50} of resistant cell lines/IC_{50} of parental cell lines).

7) and their adriamycin-resistant cell lines were shown in Table 1. Although compounds (**3**-**9**) show lower value of resistance index than adriamycin, the only **2** exhibited cytotoxic activity equivalent to adriamycin against MCF7. These results indicate that acylation of C-14 OH (**2**) maintains the activity inherent in the parent anthracycline antibiotics, whereas amidation of 3'-NH_2 (**3**-**5** and **7**-**9**) causes a decrease in the antibiotic activity.

We synthesized the new anthracycline analogues, which are expected to exhibit biological activity as potential anticancer agents. Further detail studies on the results of biological test will be reported in the future.

Experimental Section

All reactions were carried out under argon atmosphere with dried glassware. All solvents were carefully dried and distilled by literature procedure.[29] Bulk grade hexane was distilled before use. Merck pre-coated silica gel plates (Art. 5554) with fluorescent indicator were used as analytical TLC. Gravity column chromatography and flash column chromatography were carried out on silica gel (230-400 mesh from Merck). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM EX-400 spectrometer. Chemical shifts were internally referenced to TMS for ^{1}H or to solvent signals for ^{13}C. Infrared spectra were recorded on a Nicolet 5-DXB series FT-IR spectrophotometer. Mass spectra were obtained on a JEOL JMS HX-110/110A Tandem mass spectrometer (FAB^+, ESI). UV-VIS absorption spectra were recorded on a Hitachi-556 spectrophotometer. Optical rotations were determined using the Rudolph AUTOPOL IV apparatus with a 0-100-1.5 polarimeter sample tube. Melting points were obtained on a Büchi 510 melting point apparatus and are uncorrected.

Daunomycin-14-butyrate hydrochloride (2). A solution of 14-Bromodaunomycin hydrochloride (**1c**, 0.22 g, 0.34 mmol) prepared from daunomycin hydrochloride (**1a**)[5,16] and sodium butyrate (0.45 g, 4.13 mmol) in acetone (300 mL) was refluxed for 5 hr. Upon completion of the reaction the solvent was evaporated. The residue was dissolved in dry THF (150 mL), etheral HCl was added, and the mixture was stirred at -20 °C for 2 hr and further stirred at room temperature for 3 hr. The organic solvent was concentrated by a rotary evaporator and the residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH/HCO_2H/H_2O =88 : 15 : 2 : 1) to give daunomycin-14-butyrate hydrochloride (**2**, 0.17 g, 76%) as a red powder: mp 170-172 °C; $[\alpha]_D^{20}$ -24.997° (c 0.004, CH_3OH); IR (KBr) 3445, 2939, 1726, 1689, 1615, 1578, 1443, 1289, 1258, 1184, 1153, 1018, 987 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.00 (s, 1H, PhOH), 13.30 (s, 1H, PhOH), 8.15 (s, 2H, C_3NH_2), 8.02 (d, 1H, J = 7.81 Hz, ArH), 7.81 (dd, 1H, J = 8.30, 7.81 Hz, ArH), 7.42 (d, 1H, J = 8.30 Hz, ArH), 5.51 (s, 1H, C_{7eq}H), 5.30 (d, 1H, J = 18.07 Hz, C_{14}H), 5.22 (s, 1H, $C_{1'}$H), 5.16 (d, 1H, J = 18.07 Hz, C_{14}H), 4.96 (s, 1H, C_9OH), 4.16 (q, 1H, J = 6.35 Hz, $C_{5'}$H), 4.08 (s, 3H, C_4OCH_3), 3.80 (s, 1H, $C_{4'}$OH), 3.65 (m, 1H, $C_{3'}$H), 3.26 (d, 1H, J = 19.04 Hz, C_{10eq}H), 3.02 (d, 1H, J = 19.04 Hz, C_{10ax}H), 2.44 (t, 2H, J = 7.32 Hz, C_{16}H), 2.17 (dd, 2H, J = 16.11, 5.37 Hz, C_{8eq}H), 2.04 (t, 1H, J = 13.18, 3.91 Hz, $C_{2'eq}$H), 1.86 (dd, 1H, J = 16.11, 2.93 Hz, C_{8ax}H), 1.72 (m, 1H, J = 7.32 Hz, C_{17}H), 1.38 (dd, 1H, J = 13.18, 4.88 Hz, $C_{2'ax}$H), 1.32 (d, 3H, J = 6.35 Hz, $C_{5'}CH_3$), 1.01 (t, 3H, J = 7.32 Hz, C_{18}H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 207.54, 186.27, 186.16, 172.00, 163.38, 160.55, 154.14, 136.11, 134.52, 133.64, 119.79, 119,62, 118.85, 110.62, 110.54, 99.13, 74.97, 69.56, 66.05, 65.34, 56.57, 46.54, 45.57, 38.87, 36.02, 35.00, 31.82, 17.98, 16.65, 13.42; UV(CH_3OH): λ_{max} (logε) = 252 (1.04), 233 (2.16), 203 (1.34); Mass (FAB^+, Na) m/z 637 (M-HCl + Na)$^+$.

Daunomycin-3'-*N*-butyriccarboamide (3). After the mixture of butyric acid (0.06 mL, 0.64 mmol) and EDCI (0.20 g, 1.06 mmol) in dry DMF (200 mL) was stirred in ice bath for 30 min and allowed to warm to room temperature, to the stirred solution was added daunomycin hydrochloride (**1a**, 0.30 g, 0.53 mmol) and catalytic amounts of 4-pyrrolidinopyridine, and the mixture was then stirred for 12 hr. The resulting mixture was dissolved in CH_2Cl_2 (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane/CH_3OH= 12 : 6 : 1) to give daunomycin-3'-*N*-butyriccarboamide (**3**, 273.38 mg, 86%) as a red powder: mp 158-160 °C; $[\alpha]_D^{20}$ +50.00° (c 0.004, CH_3OH); IR (KBr) 3420, 2939, 1720, 1633, 1584, 1535, 1418, 1289, 1209, 1123, 987 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.93 (s, 1H, PhOH), 13.18 (s, 1H, PhOH), 7.87 (d, 1H, J = 7.81 Hz, ArH), 7.74 (dd, 1H, J = 8.30, 7.81 Hz, ArH), 7.34 (d, 1H, J = 8.30 Hz, ArH), 6.03 (d, 1H, J = 8.79 Hz, $C_{4'}$H), 5.46 (d, 1H, J = 2.93 Hz, C_{7eq}H), 5.17 (d, 1H, J = 1.46 Hz, $C_{1'}$H), 4.48 (s, 1H, C_9OH), 4.20 (q, 1H, J = 6.84 Hz, $C_{5'}$H), 4.03 (s, 3H, C_4OCH_3), 3.63 (s, 1H, $C_{4'}$OH), 3.15 (d, 1H, J = 18.55 Hz, C_{10eq}H), 2.88 (m, 1H, $C_{3'}$H), 2.79 (d, 1H, J = 18.55 Hz, C_{10ax}H), 2.40 (s, 3H, $C_{14}CH_3$), 2.28 (d, 1H, J = 14.65 Hz, C_{8eq}H), 2.09 (td, 2H, J = 7.33, 3.42 Hz, $BuCH_2$), 2.05 (dd, 1H, J = 14.65, 3.91 Hz,

$C_{8ax}H$), 1.89-1.75 (m, 2H, $C_{2'}H$), 1.57 (m, 2H, J = 7.33 Hz, $BuCH_2$), 1.28 (d, 3H, J = 6.35 Hz, $C_{5'}CH_3$), 0.88 (t, 3H, J = 7.33 Hz, $BuCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.94, 186.57, 186.18, 172.14, 162.33, 160.68, 156.57, 155.51, 135.46, 135.22, 134.25, 133.89, 120.59, 119.59, 118.22, 111.19, 111.00, 100.64, 76.57, 69.90, 69.52, 67.15, 56.55, 45.18, 38.61, 35.07, 33.34, 29.97, 19.16, 16.81, 13.72; UV(CH_3OH): λ_{max} (logε) = 252 (0.71), 230 (1.00), 203 (0.73); Mass (FAB^+, Na) m/z 621 $(M + Na)^+$.

Doxorubicin-3'-*N*-butyriccarboamide (4). After the mixture of butyric acid (0.04 mL, 0.41 mmol) and EDCI (0.13 g, 0.69 mmol) in dry DMF (200 mL) was stirred in an ice bath for 30 min and allowed to warm to room temperature, to stirred solution was added doxorubicin hydrochloride (**1b**, 0.20 g, 0.35 mmol) and catalytic amounts of 4-pyrrolidinopyridine, and the mixture was stirred for 3 hrs. The resulting mixture was dissolved in CH_2Cl_2 (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 12 : 1) to give doxorubicin-3'-*N*-butyric carboamide (**4**, 0.18 g, 80%) as a red powder: mp 162.5-165 °C; $[\alpha]_D^{20}$ +124.98° (c 0.004, CH_3OH); IR (KBr) 3432, 2927, 2853, 1726, 1633, 1584, 1418, 1289, 1209, 1116, 1018, 987 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.96 (s, 1H, PhOH), 13.20 (s, 1H, PhOH), 8.02 (d, 1H, J = 7.81 Hz, ArH), 7.78 (dd, 1H, J = 8.30, 7.81 Hz, ArH), 7.38 (d, 1H, J = 8.30 Hz, ArH), 5.85 (d, 1H J = 8.79 Hz, $C_{4'}H$), 5.45 (d, 1H, J = 3.91 Hz, $C_{7eq}H$), 5.25 (d, 1H, J = 1.46 Hz, $C_{1'}H$), 4.76 (s, 2H, $C_{14}H$), 4.53 (s, 1H, C_9OH), 4.16 (q, 1H, J = 6.84 Hz, $C_{5'}H$), 4.07 (s, 3H, C_4OCH_3), 3.63 (s, 1H, $C_{4'}OH$), 3.25 (d, 1H, J = 18.55 Hz, $C_{10eq}H$), 3.04 (m, 1H, $C_{3'}H$), 2.96 (d, 1H, J = 18.55 Hz, $C_{10ax}H$), 2.32 (d, 1H, J = 12.14 Hz, $C_{8eq}H$), 2.16 (dd, 1H, J = 12.14, 3.91 Hz, $C_{8ax}H$), 2.10 (td, 2H, J = 7.33, 2.93 Hz, $BuCH_2$), 1.83 (dt, 1H, J = 13.65, 4.88 Hz, $C_{2'eq}H$), 1.73 (dd, 1H, J = 13.65, 3.60 Hz, $C_{2'ax}H$), 1.60 (m, 2H, J = 7.33 Hz, $BuCH_2$), 1.29 (d, 3H, J = 6.84 Hz, $C_{5'}CH_3$), 0.90 (t, 3H, J = 7.33 Hz, $BuCH_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 213.71, 186.89, 186.45, 172.19, 160.88, 156.07, 155.50, 135.66, 135.38, 133.50, 133.47, 120.72, 119.78, 118.35, 111.49, 111.31, 100.709, 78.27, 69.72, 69.73, 67.22, 65.55, 56.65, 45.02, 38.65, 35.73, 33.98, 30.05, 19.14, 16.88, 13.72; UV(CH_3OH): λ_{max} (logε) = 251 (0.84), 233 (1.26), 203 (0.79); Mass (FAB^+, Na) m/z 637 $(M + Na)^+$.

Doxorubicin-14,3'-*N*-dibutyrate (5). After the mixture of butyric acid (0.07 mL, 0.76 mmol) and EDCI (0.17 g, 0.86 mmol) in dry DMF (200 mL) was stirred in an ice bath for 30 min and allowed to warm to room temperature, to the stirred solution was added doxorubicin hydrochloride (**1b**, 0.20 g, 0.35 mmol) and catalytic amounts of 4-pyrrolidinopyridine, and the mixture was stirred for 28 hr. The resulting mixture was extracted with CH_2Cl_2 (300 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane/CH_3OH=12 : 2 : 1) to give doxorubicin-14,3'-*N*-dibutyrate (**5**, 0.19 g, 81%) as a red powder: mp 120-122 °C; $[\alpha]_D^{20}$ +74.99° (c 0.004, CH_3OH); IR (KBr) 3444, 2927, 2953, 1738, 1633, 1584, 1418, 1289, 1209, 1116, 1018, 987 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.92 (s, 1H, PhOH), 13.15 (s, 1H, PhOH), 7.99 (d, 1H, J = 7.81 Hz, ArH), 7.75 (dd, 1H, J = 8.30, 7.81 Hz, ArH), 7.36 (d, 1H, J = 8.30, Hz, ArH), 5.90 (d, 1H, J = 8.30 Hz, $C_{4'}H$), 5.47 (s, 1H, $C_{7eq}H$), 5.33 (d, 1H, J = 18.06 Hz, $C_{14}H$), 5.21 (s, 1H, $C_{1'}H$), 5.09 (d, 1H, J = 18.06 Hz, $C_{14}H$), 4.58 (s, 1H, C_9OH), 4.21 (q, 1H, J = 6.34 Hz, $C_{5'}H$), 4.04 (s, 3H, C_4OCH_3), 3.64 (s, 1H, $C_{4'}OH$), 3.23 (d, 1H, J = 19.04 Hz, $C_{10eq}H$), 2.95 (d, 1H, J = 19.04 Hz, $C_{10ax}H$), 2.88 (m, 1H, $C_{3'}H$), 2.45 (t, 2H, J = 7.32 Hz, $C_{16}H$), 2.29 (d, 1H, J = 12.14 Hz, $C_{8eq}H$), 2.13 (dd, 1H, J = 12.14, 3.91 Hz, $C_{8ax}H$), 2.09 (td, 2H, J = 7.33, 3.42 Hz, $BuCH_2$), 1.83 (m, 2H, $C_{2'}H$), 1.75 (m, 2H, J = 7.33 Hz, $C_{17}H$), 1.59 (m, 2H, J = 7.35 Hz, $BuCH_2$), 1.32 (d, 3H, J = 6.84 Hz, $C_{5'}CH_3$), 1.01 (t, 3H, J = 7.33 Hz, $C_{18}H$), 0.89 (t, 3H, J = 7.33 Hz, $BuCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.49, 186.67, 186.23, 172.83, 172.13, 172.13, 160.74, 156.04, 155.50, 135.55, 135.27, 133.79, 133.53, 120.62, 119.68, 118.27, 111.32, 111.14, 100.61, 77.13, 69.82, 69.67, 67.27, 65.87, 50.61, 45.12, 38.66, 35.81, 35.50, 33.60, 30.04, 19.18, 18.51, 16.80, 13.75, 13.73; UV(CH_3OH): λ_{max} (logε) = 251 (1.04), 233 (1.49), 203 (1.01); Mass (FAB^+, Na) m/z 707 $(M + Na)^+$.

Daunomycin-14-retinoate hydrochloride (6). After 14-bromodaunomycin hydrochloride (**1c**, 0.22 g, 0.34 mmol) and all *trans* retinoic acid (0.12 g, 0.41 mmol) was dissolved in acetone (300 mL), to the mixture was added triethyl amine (0.06 mL, 0.41 mmol), and the mixture was then stirred at room temperature for 4 hr. After removing the solvent by a rotary evaporator, an etheral HCl in dry THF (200 mL) was added to the reaction mixture. The resulting mixture was stirred at -20 °C for 2 hr, further stirred at room temperature for 3 hr, and then the solvent was removed under reduced pressure. Purification of the residue by column chromatography (CH_2Cl_2/CH_3OH/HCO_2H/H_2O=88 : 15 : 2 : 1) gave pure daunomycin-14-retinoate hydrochloride (**6**, 0.25 g, 85%) as a red powder: mp 181-182 °C; $[\alpha]_D^{20}$ +124.98° (c 0.004, CH_3OH); IR (KBr) 3334, 2952, 1723, 1670, 1560, 1381, 1024, 984 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.40 (s, 1H, PhOH), 12.74 (s, 1H, PhOH), 7.45 (m, 1H, ArH), 7.35 (m, 1H, ArH), 7.00 (t, 1H, J = 12.21 Hz, $C_{19}H$), 6.90 (m, 1H, ArH), 6.28 (d, 2H, J = 15.62 Hz, $C_{18,23}H$), 6.14 (d, 2H, J = 16.11 Hz, $C_{20,22}H$), 5.87 (s, 1H, $C_{16}H$), 5.30 (s, 2H, $C_{14}CH_2$), 5.13 (s, 1H, $C_{1'}H$), 4.61 (s, 1H, $C_{7eq}H$), 4.13 (s, 1H, $C_{5'}H$), 3.82 (m, 2H, $C_{3'}H$), 3.71 (s, 3H, C_4OCH_3), 3.04 (s, 1H, $C_{10eq}H$), 2.74 (s, 1H, $C_{10ax}H$), 2.32 (s, 3H, $C_{17}CH_3$), 2.03 (m, 3H, $C_{2'eq}H$&$C_{28}CH_2$), 1.99 (s, 3H, $C_{21}CH_3$), 1.89 (m, 1H, $C_{2'ax}H$), 1.79 (s, 3H, $C_{25}CH_3$), 1.62 (m, 2H, $C_{27}CH_2$), 1.48 (m, 2H, $C_{28}CH_2$), 1.25 (s, 3H, $C_{5'}CH_3$), 1.04 (s, 6H, $C_{29}2CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 206.84, 206.75, 185.37, 185.06, 167.77, 165.92, 160.12, 155.78, 154.81, 153.96, 139.69, 137.56, 137.16, 134.89, 134.37, 133.89, 133.66, 131.33, 129.89, 129.46, 128.60, 119.68, 118.93, 118.93, 118.07, 117.34, 110.62, 110.37, 99.85, 77.20, 76.54, 69.47, 67.07, 66.46,

65.38, 56.41, 47.48, 39.68, 35.52, 34.33, 33.20, 30.99, 29.76, 29.06, 21.88, 19.33, 16,64, 14.13, 13.04; UV(CH_3OH): λ_{max} (logε) = 210 (1.65), 327 (1.24), 478 (1.28); Mass (FAB^+, Na) m/z 849 (M-HCl + Na)$^+$.

Daunomycin-3'-*N*-retincarboamide (7). After the mixture of all *trans* retinoic acid (0.19 g, 0.64 mmol) and EDCI (0.20 g, 1.06 mmol) in dry DMF (200 mL) was stirred in an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added daunomycin hydrochloride (**1a**, 0.30 g, 0.53 mmol) and catalytic amounts of 4-pyrrolidinopyridine, and the mixture was then 4 hr of stirring. The resulting mixture was extracted with CH_2Cl_2 (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/hexane/CH_3OH=12 : 6 : 1) to give daunomycin-3'-*N*-retincarboamide (**7**, 0.40 g, 92%) as a red powder: mp 150-152 °C; $[\alpha]_D^{20}$ +98.99° (c 0.004, acetone); IR (KBr) 3432, 2939, 1720, 1627, 1578, 1529, 1418, 1289, 1209, 1123, 990 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.89 (s, 1H, PhOH), 13.12 (s, 1H, PhOH), 7.93 (d, 1H, *J* = 7.81 Hz, ArH), 7.71 (dd, 1H, *J* = 8.30, 7.81 Hz, ArH), 7.31 (d, 1H, *J* = 7.81 Hz, ArH), 6.83 (dd, 1H, *J* = 15.14, 11.23 Hz, $RA_{11}H$), 6.22 (d, 1H, *J* = 16.11 Hz, $RA_{12}H$), 6.12 (d, 1H, *J* = 15.14 Hz, RA_7H), 6.09 (d, 1H, *J* = 15.14 Hz, RA_8H), 6.04 (d, 1H, *J* = 12.70 Hz, $RA_{10}H$), 5.61 (s, 1H, $RA_{14}H$), 5.46 (s, 1H, $C_{7eq}H$), 5.12 (s, 1H, $C_{1'}H$), 5.46 (s, 1H, C_9OH), 4.22 (m, 2H, *J* = 6.34 Hz, $C_{3'}$&$C_{5'}H$), 3.99 (s, 3H, C_4OCH_3), 3.69 (d, 1H, *J* = 6.84 Hz, $C_{4'}OH$), 3.12 (d, 1H, *J* = 18.55 Hz, $C_{10eq}H$), 2.81 (d, 1H, *J* = 7.81 Hz, $C_{3'}H$), 2.72 (d, 1H, *J* = 18.55 Hz, $C_{10ax}H$), 2.40 (s, 3H, $C_{14}CH_3$), 2.24 (s, 3H, $RA_{13}CH_3$), 2.06-1.99 (m, 2H, C_8H), 1.93 (s, 3H, RA_9-CH_3), 1.89-1.78 (m, 2H, $C_{2'}H$), 1.69 (s, 3H, RA_5CH_3), 1.60 (m, 2H, RA_3CH_2), 1.45 (m, 2H, RA_2CH_2), 1.28 (d, 3H, *J* = 6.84 Hz, $C_{5'}CH_3$), 1.01 (s, 6H, RA_12CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.02, 186.38, 185.99, 166.19, 160.61, 156.16, 155.42, 148.64, 138.52, 137.50, 137.11, 135.40, 135.23, 135.12, 134.23, 133.92, 129.61, 129.59, 129.39, 127.99, 121.03, 120.47, 119.53, 118.20, 111.11, 110.92, 100.72, 76.55, 69.92, 69.50, 67.20, 56.46, 45.23, 39.60, 35.02, 34.24, 33.30, 33.10, 29.92, 28.96, 24.95, 21.76, 19.26, 16.82, 14.17, 13.56, 12.87; UV(acetone): λ_{max} (logε) = 209 (0.51), 328 (0.32), 336 (0.32); Mass (FAB^+, Na) m/z 833 (M + Na)$^+$.

Doxorubicin-3'-*N*-retincarboamide (8). After the mixture of all *trans* retinoic acid (0.12 g, 0.41 mmol) and EDCI (0.13 g, 0.69 mmol) in dry DMF (200 mL) was stirred in an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added doxorubicin hydrochloride (**1b**, 0.20 g, 0.0.35 mmol) and catalytic amounts of 4-pyrrolidinopyridine, and the mixture was stirred for 2 hr. The resulting mixture was extracted with CH_2Cl_2 (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/hexane/CH_3OH=12 : 6 : 1) to give doxorubicin-3'-retincarboamide (**8**, 0.26 g, 91%) as a pale red powder: mp 157-159 °C; $[\alpha]_D^{20}$ +74.99° (c 0.004, acetone); IR (KBr) 3442, 2945, 1729, 1621, 1584, 1528, 1420, 1289, 1209, 1116, 1078, 984 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.96 (s, 1H, PhOH), 13.13 (s, 1H, PhOH), 7.94 (d, 1H, *J* = 7.81 Hz, ArH), 7.73 (dd, 1H, *J* = 8.30, 7.81 Hz, ArH), 7.34 (d, 1H, *J* = 8.30 Hz, ArH), 6.84 (dd, 1H, *J* = 15.14, 11.23 Hz, $RA_{11}H$), 6.22 (d, 1H, *J* = 16.11 Hz, $RA_{12}H$), 6.16 (d, 1H, *J* = 15.14 Hz, RA_7H), 6.08 (d, 1H, *J* = 15.14 Hz, RA_8H), 6.05 (d, 1H, *J* = 11.23 Hz, $RA_{10}H$), 5.61 (s, 1H, $RA_{14}H$), 5.47 (s, 1H, $C_{7eq}H$), 5.17 (s, 1H, $C_{1'}H$), 4.76 (s, 2H, $C_{14}H$), 4.59 (s, 1H, C_9OH), 4.17 (bd, 2H, *J* = 5.86 Hz, $C_{3'}$&$C_{5'}H$), 4.02 (s, 3H, C_4OCH_3), 3.67 (s, 1H, $C_{4'}OH$), 3.16 (d, 1H, *J* = 18.55 Hz, $C_{10eq}H$), 2.83 (d, 1H, *J* = 18.55 Hz, $C_{10ax}H$), 2.30 (d, 1H, *J* = 14.16 Hz, $C_{8eq}H$), 2.24 (s, 3H, $RA_{13}CH_3$), 2.15 (d, 1H, *J* = 14.16 Hz, $C_{8ax}H$), 2.01 (m, 1H, $C_{2'eq}H$), 1.94 (s, 3H, $RA_{13}CH_3$), 1.84 (m, 1H, $C_{2'ax}H$), 1.69 (s, 3H, RA_5CH_3), 1.60 (m, 2H, RA_3CH_2), 1.45 (m, 2H, RA_2CH_2), 1.28 (d, 3H, *J* = 6.35 Hz, $C_{5'}CH_3$); ^{13}H NMR (100 MHz, $CDCl_3$) δ 213.63, 186.48, 186.07, 166.22, 160.68, 155.98, 155.24, 148.86, 138.63, 137.51, 137.10, 135.52, 135.20, 135.11, 133.54, 133.42, 129.73, 129.64, 129.37, 128.07, 120.92, 120.47, 119.61, 118.29, 111.26, 111.06, 100.76, 76.43, 69.60, 69.48, 67.32, 65.51, 56.54, 45.12, 39.60, 35.67, 34.26, 33.83, 33.11, 29.92, 29.72, 28.92, 21.78, 19.27, 16.92, 13.58, 12.89; UV(acetone): λ_{max} (logε) = 210 (1.06), 327 (0.24), 478 (0.19); Mass (FAB^+, Na) m/z 849 (M + Na)$^+$.

Doxorubicin-diretinoate (9). After the mixture of all *trans* retinoic acid (0.23 g, 0.76 mmol) and EDCI (0.17 g, 0.86 mmol) in dry DMF (200 mL) was stirred in an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added doxorubicin hydrochloride (**1b**, 0.20 g, 0.35 mmol) and catalytic amounts of 4-pyrrolidinopyridine, and the mixture was stirred for 10 hr. The resulting mixture was extracted with CH_2Cl_2 (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane/CH_3OH=12 : 6 : 1) to give compound **9** (0.34 g, 89%) as a pale red solid: mp 138-140 °C; $[\alpha]_D^{20}$ +25.00° (c 0.004, acetone); IR (KBr) 3449, 2938, 1723, 1621, 1584, 1528, 1418, 1289, 1209, 1123, 984 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.91 (s, 1H, PhOH), 13.15 (s, 1H, PhOH), 7.98 (d, 1H, *J* = 7.81 Hz, ArH) 7.75 (dd, 1H, *J* = 8.30, 7.81 Hz, ArH), 7.36 (d, 1H, *J* = 8.30 Hz, ArH), 7.07-6.99 (m, 1H, $C_{19}H$), 6.85 (dd, 1H, *J* = 15.14, 11.23 Hz, $RA_{11}H$), 6.35-5.94 (m, 8H, $RA_{7,8,10,12}$ & $C_{18,20,22,23}H$), 5.81 (s, 1H, $C_{16}H$), 5.62 (s, 1H, $RA_{14}H$), 5.50 (s, 1H, $C_{7eq}H$), 5.38 (d, 1H, *J* = 18.07 Hz, $C_{14}H$), 5.22 (s, 1H, $C_{1'}H$), 5.13 (d, 1H, *J* = 18.07 Hz, $C_{14}H$), 4.63 (s, 1H, C_9OH), 4.26 (q, 1H, *J* = 6.34 Hz, C_5H), 4.11 (m, 1H, $C_{3'}H$), 4.03 (s, 3H, C_4OCH_3), 3.69 (s, 1H, $C_{4'}OH$), 3.23 (d, 1H, *J* = 18.06 Hz, $C_{10eq}H$), 2.89 (d, 1H, *J* = 18.06 Hz, $C_{10ax}H$), 2.51 (d, 1H, *J* = 14.16 Hz, $C_{8eq}H$), 2.38 (s, 3H, $C_{17}CH_3$), 2.26 (s, 3H, $RA_{13}CH_3$), 2.12 (d, 1H, *J* = 14.16 Hz, $C_{8ax}H$), 2.01 (s, 3H, $C_{21}CH_3$), 1.95 (s, 3H, RA_9CH_3), 1.76-1.88 (m, 2H, $C_{2'}H$), 1.72 (s, 3H, $C_{28}H$ & RA_4CH_2), 1.34 (s, 3H, *J* = 5.86 Hz, $C_{5'}CH_3$), 1.04 (s, 6H, $C_{29}2CH_3$), 1.01 (s, 6H, RA_12CH_3); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 206.85, 186.62, 186.17, 166.15, 165.89, 160.72, 156.10, 155.53, 148.88, 139.71, 138.59, 137.54, 137.15, 135.53, 135.25, 134.85, 133.95, 133.64, 131.30, 130.19, 129.92, 129.70, 129.43, 128.64, 128.05, 120.96, 120.59, 119.66, 118.31, 117.28, 115.27, 111.26, 111.09, 100.72, 76.67, 69.90, 69.70, 67.33, 65.46, 56.59, 45.17, 39.64, 35.57, 34.31, 34.28, 33.55, 33.17, 33.13, 31.63, 30.06, 29.74, 29.02, 28.99, 22.71, 21.82, 21.80, 21.12, 19.30, 16.86, 14.20, 14.13, 13.60, 13.27, 13.02, 12.92; UV(acetone): λ_{max} (logε) = 210 (0.73), 327 (0.36), 478 (0.24); Mass (FAB^+, Na) m/z 1131 $(M + Na)^+$.

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