Synthesis of New Anthracycline Derivatives Containing Acetylsalicylic or Palmitic Acid Moiety

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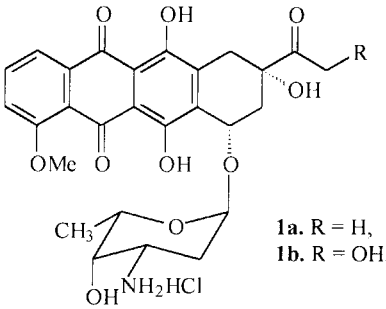
The potential anticancer agents new anthracycline derivatives (**2-9**) have been synthesized from daunomycin (**1a**) and doxorubicin (**1b**) as starting materials. Compounds **2** and **6** were prepared by the nucleophilic displacement type esterification of a 14-bromodaunomycin (**1c**) with a acetylsalicylic and palmitic acid in triethylamine, respectively. Compounds **3** and **7** were obtained from daunomycin (**1a**) by direct amidation with the corresponding acids in the presence of EDCI and PP as esterification reagents. Whereas **4** and **8** were prepared by reaction of doxorubicin (**1b**) with one equivalent of acetylsalicylic and palmitic acid using DCC/DMAP, respectively. **5** and **9** were obtained from **1b** by acylation with two equivalents of the corresponding acids using EDCI/PP reagents.

Keywords : Daunomycin, Doxorubicin, Anthracycline, Acetylsalicyclic acid, Palmitic acid.

Introduction

The substitution of one hydrogen atom in the daunorubicin (daunomycin, DM, **1a**) acetyl side chain with a hydroxyl group gives adriamycin (doxorubicin, DX, **1b**) (Figure 1). These anthracycline antibiotics, in particular, are the powerful antitumor agents in the treatment of a wide range of human cancers.[1,2] They have structural features that allow their intercalation into double helical DNA and poison cellular DNA topoisomerase II (top2).[3-7] But, their clinical use is often limited by severe cardiotoxicity and other undesirable side effects.[8] For these reasons, many drugs mixed with DM (**1a**) or DX (**1b**) with some amino acids in a proper ratio have been used recently and a number of patents related to the efficacy of the blended drugs have been published.[9-11] Because these amino acids have low recognition to DNA, these types of prescriptions have the drawback that they should be administered in large amount to exhibit drug efficacy. Therefore, we have begun to focus on the results that occur when using the prodrug, which was the coupling of DM (**1a**) or DX (**1b**) with various amino acids. Acetylsalicylic acid has been widely used in treatment of various diseases due to its anticancer activity.[12,13] Palmitic acid is also a promising material having strong adsorption property in a cell wall.[14]



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

Figure 1. Structures of two clinically used anthracyclines.

In the present study, we describe the preparation of some glycosides, new anthracycline analogues by coupling of DM (**1a**) or DX (**1b**) with two kinds of acid molecules. The results are expected to exhibit better effective therapies than anything else previously developed.

Results and Discussion

In previous publications, we frequently used the Michael type condensation[15-18] or the Friedel-Crafts acylation[19,20] for the total syntheses of some anthracyclinone derivatives. We reported the successful preparation of a new aglycon containing an ester linkage at C-14 position through a nucleophilic displacement coupling method.[20-22] Here, we endeavor to directly prepare some new anthracycline analogues from commercially available anticancer agents, such as daunomycin (**1a**) or doxorubicin (**1b**). Several new anthracycline derivatives were synthesized using two esterification methods (Scheme 1). The key feature for synthesis of the new analogues is the acylation of **1a-1b** with two kinds of acids, acetylsalicylic or palmitic acid. Numerous esterification methods have been developed using aliphatic or aromatic carboxylic acid and some alcohols.[23-27] However, these coupling reactions exhibited clearly different reaction patterns for the two kinds of acids in the esterification.

First, the synthesis of 14-bromo DM (**1c**) was accomplished by the application of the known procedure:[11] **1c** was synthesized in the best yield when using **1a**, 10% Br$_2$/

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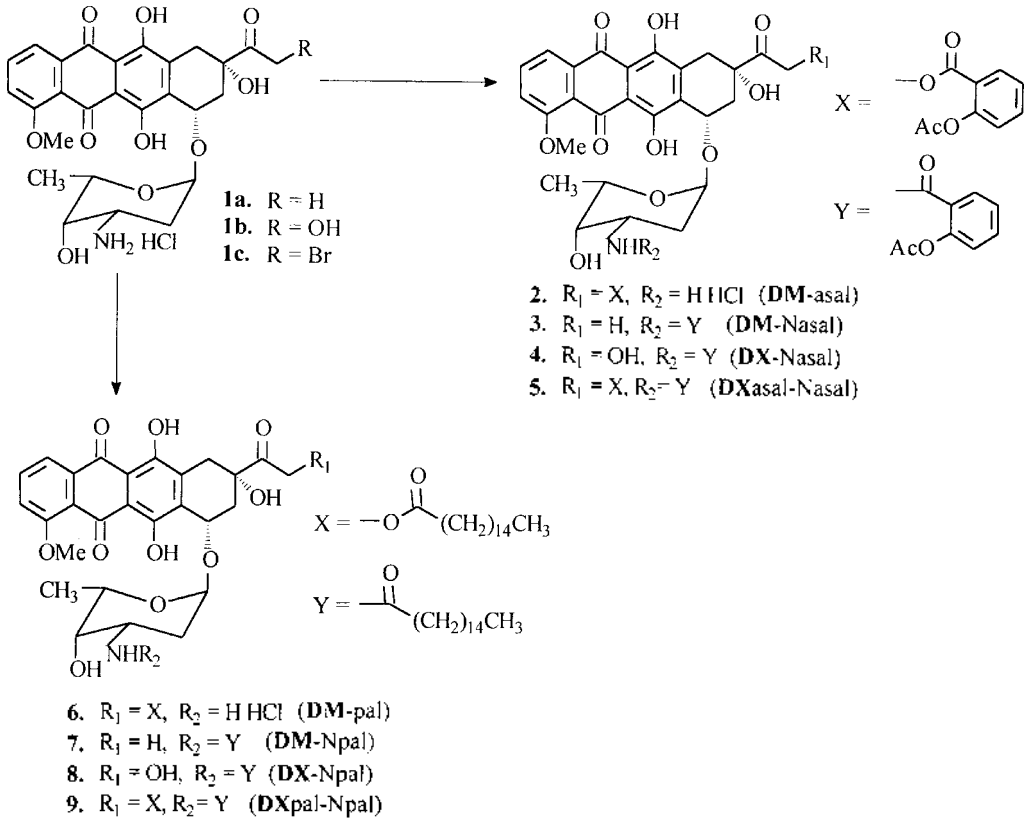

Scheme 1. Synthesis of new anthracycline analogues (**2-9**).

CH_2Cl_2, and trimethylorthoformate in a minimum quantity of co-solvent (methanol/1,4-dioxane, v/v=1 : 2), consequently the side product formation originating in dimethylketalization of ketone at C-13 site was exceedingly diminished. For prolonged the reaction time (*ca.* >40 min), the cleavage of a glycosidic bond from glycosides was increased due to the generation of HBr. So, after dissolving **1c** and the byproduct ketal intermediate in acetone without separating the reaction mixture, the reaction mixture was converted to **1c** by further stirring for 1 hr.

All these compounds (**2-9**) were obtained through the acylation of a hydroxyl group at C-14 site in the aglycon and/or amine group at C-3' position in the glycon with acetylsalicylic or palmitic acid. DM-asal (**2**) and DM-pal (**6**) were synthesized by the esterification of 14-bromo DM (**1c**) with a acetylsalicylic or palmitic acid sodium salt in acetone under gentle reflux at 50-60 °C, respectively. However, yields were very low due to the cleavage of the glycosidic bond from glycosides. Therefore, two kinds of mixed solution which were either ethanol/conc. H_2SO_4[24] or acetone/triethylamine solution,[25] were used to enhance the solubility of **1c**. When using these solutions, the reaction time was shortened to 4-12 hr and the yields were somewhat increased to 40-55%. But, DM-asal (**2**) and DM-pal (**6**) were obtained in the best yields (89-95%) when carrying out the reaction of **1c** with an acetylsalicylic or palmitic acid instead of the corresponding sodium salts in triethylamine for 3-4.5 hr.[28]

The synthesis of DM-Nasal (**3**) and DM-Npal (**7**) were tried *via* the direct coupling reaction of amine group at C-3' in DM (**1a**) with two kinds of acids in the presence of 1,3-dicyclohexylcarbodiimide (DCC)/4-(dimethylamino)pyridine (DMAP), according to the reported procedures.[26] These reactions were similar to completion on TLC (*ca.* 95%), but the yields of the final product were lower at 50-75% due to the difficulties in removing the solvent (DMF) and dicyclohexylurea (DCU). Therefore, the reaction in synthesis of DM-Nasal (**3**) and DM-Npal (**7**) was tried using **1a** and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI)/4-pyrrolidinopyridine (PP) in a minimum quantity of DMF,[27] and consequently the reaction time was shortened and the yields (91-96%) were increased.

The syntheses of DX-Nasal (**4**) and DX-Npal (**8**) with the amine group of DX (**1b**) were tried using both DCC/DMAP and EDCI/PP as coupling reagents. In both cases, it was observed that the acylation of amine groups at C-3' was faster than the acylation of hydroxyl groups at C-14 position. But, before completion of the coupling with amine group of DX (**1b**), preparation of DXasal-Nasal (**5**) and DXpal-Npal (**9**) was also started by esterification of C_{14}-OH in the glycosides. Consequently, the esterification of C-14 site always occurred accompanied by the amidation of C-3'. However, DX-Nasal (**4**) and DX-Npal (**8**) were obtained in effective yields by esterification, using DCC/DMAP for 3-5 hr. Whereas the reaction time was clearly shortened when using only DMF solvent, the reaction time was lengthened for increasing quantities of CH_2Cl_2 when using co-solvent (CH_2Cl_2/DMF). Therefore, the best ratio of mixed solvent (DMF/CH_2Cl_2) was 1 : 10 for the synthesis of DX-Nasal (**4**)

Table 1. Cytotoxic activities of anthracycline derivatives (**2-9**) in comparison with adriamycin

Agents	IC_{50}[c] (μM)			
	SNU-16[a]	SNU-16/Adr	MCF7[b]	MCF7/Adr
Adriamycin (**1b**)	0.16	0.35 (2.19)[d]	0.29	0.43 (1.48)
2	1.75	2.12 (1.21)	0.34	0.39 (1.15)
3	7.13	8.65	13.42	9.75
4	11.57	14.24	7.41	0.82
5	8.72	8.78	12.13	9.42
6	2.12	1.68 (0.79)	2.12	2.12 (1.00)
7	6.88	8.79	5.30	6.03
8	7.21	7.82	13.86	14.02
9	9.72	7.86	10.69	7.36

[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).

and 1 : 1 for the synthesis of DX-Npal (**8**).

Finally, the reactions for the syntheses of DXasal-Nasal (**5**) and DXpal-Npal (**9**) also used EDCI/PP as a coupling reagent. In these cases, whereas **4** and **8** were obtained in 76-84% when reacting for 3-5 hr, **5** and **9** were obtained in 82-92% for the longer reaction time (*ca.* 10-12 hr).

The cytotoxic activities of anthracycline derivatives **2-9** against two kinds of human tumor cells (SNU-16 and MCF-7) and their adriamycin-resistant cell lines are shown in Table 1. Compounds **2** and **6** were less cytotoxic against SNU-16 and MCF7 but exhibited a lower relative resistance value (IC_{50} of resistant cell lines/IC_{50} of parental cell lines) than adriamycin. In addition, the others (**3-5** and **7-9**) exhibited very low antitumor activity compared with the reference. These results indicate that amidation compounds of 3'-NH_2, such as **3-5** and **7-9**, unlike acylation compounds of C-14 OH such as **2** and **6** cause a decrease in the activity inherent in the parent anthracycline antibiotics.

In conclusion, we have synthesized new anthracycline analogues expected to exhibit biological activity as potential anticancer agents through acylation. Further detailed studies on the results of biological tests 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.[30] Bulk grade hexane was distilled before use. Merck pre-coated silica gel plates (Art. 5554) with fluorescent indicator were used as analytical TLC. In the development of chromatograms two mobile phases were used: methylene chloride/methanol/formic acid/water (100 : 15 : 2 : 1) and methylene chloride/hexane/methanol (12 : 6 : 1). Gravity column chromatography and flash column chromatography were carried out on silica gel (230-400 mesh from Merck). ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM EX-400 spectrometer. Chemical shifts were internally referenced to TMS for ^1H 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 DX-110/110A Tandem mass spectrometer (FAB$^-$). UV-VIS absorption spectra were recorded on a Hitachi-556 spectrophotometer. Optical rotations were determined using the Rudolph AUTOPOL apparatus with a 0-100-1.5 polarimeter sample tube. Melting points were obtained on a Büchi 510 melting point apparatus and were uncorrected.

14-Bromodaunomycin hydrochloride (1c). After trimethylorthoformate (0.20 mL, 1.94 mmol) was added to a solution of daunomycin hydrochloride (**1a**) (0.23 g, 0.41 mmol) dissolved in methanol/1,4-dioxane (v/v=1 : 2, 12 mL), the reaction mixture was stirred at room temperature for 20 min. To the reaction solution was added dropwise a Br_2/CH_2Cl_2 (v/v=1 : 9, 0.25 mL, 0.48 mmol) solution, and then stirred at 25 °C for 40 min. After the resulting mixture was poured into dry ether (200 mL), the solid residue was filtered with glass filter and washed with ether (2 × 50 mL). The solid was recrystallized from acetone/ether, filtered, washed with ether, and dried on phosphorus pentoxide under reduced pressure to give pure 14-bromo DM (**1c**) (0.22 g, 83%) as a red solid: mp 176-177 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 14.00 (bs, 1H, PhOH), 13.30 (bs, 1H, PhOH), 7.99 (bs, 2H, NH_2), 7.91 (m, 2H, ArH) 7.89 (m, 1H, ArH), 5.55 (m, 1H, C_4H), 5.28 (m, 1H, C_{7eq}H), 4.49 (m, 1H, C_1'H), 4.21 (q, 1H, J = 6.68 Hz, C_5H), 4.00 (s, 2H, C_{14}H), 3.97 (s, 3H, C_4OCH_3), 3.78 (bs, 1H, C_4OH), 3.60 (m, 1H, C_3H), 3.05 (d, 1H, J = 18.10 Hz, C_{10eq}H), 2.92 (d, 1H, J = 18.10 Hz, C_{10ax}H), 2.44 (d, 1H, J = 14.21 Hz, C_{8eq}H), 2.07 (dd, 1H, J = 14.21, 5.43 Hz, C_{8ax}H), 1.88 (dd, 1H, J = 12.70, 9.00 Hz, C_2H), 1.85 (d, 1H, J = 12.70 Hz, C_2H), 1.65 (d, 3H, J = 6.68 Hz, C_5CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.51, 186.11, 169.25, 161.20, 161.01, 160.12, 137.98, 135.80, 134.93, 132.21, 122.01, 120.10, 119.01, 109.90, 105.23, 98.54, 84.56, 73.47, 69.37, 68.01, 55.94, 54.11, 38.42, 37.04, 34.12, 21.20, 16.72.

Daunomycin-14-acetylsalicylate hydrochloride (2). To a solution of 14-bromo DM (**1c**) (0.51 g, 0.79 mmol) and acetylsalicylic acid (0.22 g, 1.22 mmol) in acetone (250 mL) were added triethylamine (0.45 mL, 3.20 mmol) followed by stirring for 3 hr. After completing the reaction monitored on TLC, the solvent was evaporated under reduced pressure. To the residue dissolved in dry THF (150 mL) was added ethereal HCl (1.0 M, 2.4 mL) followed by stirring at -20 °C for 2 hr. The organic solvent was concentrated by a rotary evaporator and recrystallized from methanol/diethyl ether to give DM-asal (**2**) (0.52 g, 89%) as a red powder: mp 207-209 °C; $[\alpha]_D^{20}$ +249.98° (c 0.004, CH_3OH); IR (KBr) 3433, 2939, 1732, 1615, 1578, 1418, 1289, 1209, 1123, 1086, 990, 760 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 2H, C_3'NH_2), 8.00 (dd, 1H, J = 7.81, 1.46 Hz, ArH), 7.91 (m, 2H ArH), 7.71 (td, 1H, J = 7.81, 1.46 Hz, ArH), 7.43 (t, 1H, J = 7.81 Hz, ArH), 7.26 (d, 1H, J = 7.81 Hz, ArH), 6.99 (t, 1H, J = 7.81 Hz, ArH), 5.53 (m, 1H, C_4H), 5.50 (d, 1H, J = 17.59 Hz, C_{14}H), 5.38 (d, 1H, J = 17.59 Hz, C_{14}H), 5.29 (m, 1H, C_{7eq}H), 4.97 (m, 1H, C_1'H), 4.23 (q, 1H, J = 6.35 Hz, C_5H),

3.97 (s, 3H, C_4OCH_3), 3.57 (m, 2H, C_9OH, C_3-H), 3.36 (d, 1H, J = 18.06 Hz, C_{10eq}H), 3.10 (dd, 1H, J = 18.06, 5.86 Hz, C_{10ax}H), 2.32 (d, J = 14.65 Hz, 1H, C_{8eq}H), 2.24 (s, 3H, OAc), 2.11 (dd, J = 14.65, 5.86 Hz, C_{8ax}H), 1.89 (td, 1H, J = 12.23, 2.97 Hz, C_2-H), 1.67 (dd, 1H, J = 12.23, 4.40 Hz, C_2-H), 1.18 (d, 3H, J = 6.35 Hz, C_5-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 207.15, 189.57, 186.28, 186.17, 182.85, 171.33, 168.80, 163.25, 163.22, 163.19, 163.17, 160.56, 160.51, 157.68, 150.11, 149.93, 146.17, 134.52, 131.16, 122.32, 119.77, 118.87, 110.63, 110.55, 110.49, 75.11, 66.11, 56.59, 45.57, 43.29, 40.32, 20.74, 20.67, 20.64, 16.64, 14.01; UV(CH_3OH): λ_{max} (logε) = 206 (1.39), 234 (1.94), 477 (0.52); Mass (FAB$^-$, Na) m/z 729 (M-HCl + Na)$^+$.

Daunomycin-3'-*N*-acetylsalicylate (3). After the mixture of acetylsalicylic acid (0.43 g, 2.39 mmol) and EDCI (0.92 g, 4.80 mmol) in dry DMF/CH_2Cl_2 (50 mL, v/v = 1 : 1) was stirred on an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added daunomycin hydrochloride (**1a**) (0.68 g, 1.21 mmol) and 4-pyrrolidinopyridine (0.36 g, 2.43 mmol), followed by stirring for 4 hr. The reaction mixture was dissolved with CH_2Cl_2 (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 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/MeOH=12 : 6 : 1) to give DM-Nasal (**3**) (0.76 g, 91%) as a red powder: mp 128-130 °C; $[\alpha]_D^{20}$ +79.92° (c 0.004, CH_3OH); IR (KBr) 3408, 3260, 2939, 1713, 1627, 1578, 1412, 1369, 1283, 1215, 1116, 1030, 984 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.94 (s, 1H, PhOH), 13.23 (s, 1H, PhOH), 8.03 (s, 1H, C_3-NH), 8.00 (dd, 1H, J = 7.32, 3.91 Hz, ArH), 7.85 (d, 1H, J = 7.32 Hz, ArH), 7.77 (td, 1H, J = 7.81, 3.91 Hz, ArH), 7.39 (d, 1H, J = 9.28 Hz, ArH), 7.37 (d, 1H, J = 8.30 Hz, ArH), 6.91 (d, 1H, J = 8.30 Hz, ArH), 6.83 (t, 1H, J = 7.32 Hz, ArH), 5.99 (d, 1H, J = 8.30 Hz, C_4-H), 5.48 (d, 1H, J = 3.42 Hz, C_{7eq}H), 5.31 (d, 1H, J = 3.90 Hz, C_1-OH), 4.22 (q, 1H, J = 6.35 Hz, C_5-H), 4.07 (s, 3H, C_4OCH_3), 3.78 (m, 1H, C_3-H), 3.63 (bs, 1H, C_4-OH), 3.22 (d, 1H, J = 19.53 Hz, C_{10eq}H), 2.91 (d, 1H, J = 19.53 Hz, C_{10ax}H), 2.42 (s, 3H, C_{14}H), 2.28 (d, J = 14.65 Hz, 1H, C_{8eq}H), 1.95 (s, 3H, OAc), 1.90 (dd, 1H, J = 14.65, 5.84 Hz, C_{8ax}H), 1.69-1.80 (m, 2H, C_2-H), 1.28 (d, 3H, J = 6.35 Hz, C_5-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.31, 185.88, 185.72, 168.68, 160.25, 155.82, 154.60, 135.29, 134.40, 134.27, 133.55, 119.82, 118.85, 118.43, 110.39, 110.27, 93.84, 78.21, 78.07, 77.56, 75.34, 69.07, 68.04, 66.74, 64.33, 56.18, 45.03, 40.13, 39.29, 38.87, 35.81, 32.56, 29.29, 29.19, 22.65, 16.74; UV (CH_3OH): λ_{max} (logε) = 236 (2.95), 250 (2.37), 495 (1.14); Mass (FAB$^-$, Na) m/z 713 (M + Na)$^+$.

Doxorubicin-3'-*N*-acetylsalicylate (4). After the mixture of acetylsalicylic acid (0.24 g, 1.33 mmol) and DCC (0.49 g, 2.38 mmol) in dry DMF/CH_2Cl_2 (v/v=1 : 10, 100 mL) was stirred on an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added doxorubicin hydrochloride (**1b**) (0.70 g, 1.21 mmol) and DMAP (0.16 g, 1.31 mmol), followed by stirring for 5 hr. The reaction mixture was dissolved with CH_2Cl_2 (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 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/MeOH=12 : 6 : 1) to give DX-Nasal (**4**) (0.65 g, 76%) as a red powder and DXasal-Nasal (**5**) (0.07 g, 8.0%) as a red powder. **4**: mp 145-147 °C; $[\alpha]_D^{20}$ +39.95° (c 0.004, CH_3OH); IR (KBr) 3445, 2939, 2384, 1744, 1621, 1578, 1418, 1289, 1215, 1116, 987, 760 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.98 (s, 1H, PhOH), 13.14 (s, 1H, PhOH), 7.96 (dd, 1H, J = 7.81, 1.46 Hz, ArH), 7.72 (t, 1H, J = 8.30 Hz, ArH), 7.53 (t, 1H, J = 8.30 Hz, ArH), 7.47 (td, 1H, J = 7.81, 1.46 Hz, ArH), 7.33 (d, 1H, J = 8.30 Hz, ArH), 6.97 (d, 1H, J = 7.81 Hz, ArH), 6.91 (t, 1H, J = 8.30 Hz, ArH), 6.15 (d, 1H, J = 8.30 Hz, C_4-H), 5.58 (d, 1H, J = 18.07 Hz, C_{14}H), 5.46 (s, 1H, C_9OH), 5.33 (d, 1H, J = 18.07 Hz, C_{14}H), 5.20 (m, 1H, C_{7eq}H), 4.75 (s, 1H, $C_{14}OH$), 4.33-4.23 (m, 1H, C_1-H), 4.23 (q, 1H, J = 6.6 Hz, C_5-H), 4.10-4.21 (m, 1H, C_3-H), 4.01 (s, 3H, C_4OCH_3), 3.64 (s, 1H, C_4-OH), 3.27 (d, 1H, J = 18.64 Hz, C_{10eq}H), 2.91(d, 1H, J = 18.64, C_{10ax}H), 2.49 (d, J = 14.65 Hz, 1H, C_{8eq}H), 2.11 (dd, J = 14.65, 3.91 Hz, C_{8ax}H), 1.95 (s, 3H, OAc), 1.79-1.85 (m, 2H, C_2-H), 1.32 (d, 3H, J = 6.6 Hz, C_5-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.78, 186.65, 186.24, 169.40, 169.18, 162.37, 161.46, 160.73, 156.04, 155.39, 135.90, 135.20, 133.52, 130.77, 128.67, 120.56, 119.65, 118.21, 117.42, 77.19, 69.70, 69.42, 67.34, 66.70, 56.60, 45.38, 36.55, 35.53, 33.53, 31.49, 29.88, 29.73, 23.37, 23.03, 16.85, 14.87; UV(CH_3OH): λ_{max} (logε) = 209 (2.03), 234 (1.93), 498 (0.52); Mass (FAB$^+$, Na) m/z 729 (M + Na)$^-$.

Doxorubicin-di(acetylsalicylate) (5). After the mixture of acetylsalicylic acid (0.54 g, 3.00 mmol) and EDCI (0.92 g, 4.80 mmol) in dry DMF/CH_2Cl_2 (100 mL, v/v=1 : 10) was stirred on an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added doxorubicin hydrochloride (**1b**) (0.70 g, 1.21 mmol) and 4-pyrrolidinopyridine (0.36 g, 2.43 mmol), followed by stirring for 12 hr. The reaction mixture was extracted with CH_2Cl_2 (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane/MeOH=12 : 6 : 1) to give DXasal-Nasal (**5**) (0.86 g, 82%) as a red powder and DX-Nasal (**4**) (0.08 g, 10%) as a red powder. **5**: mp 174-176 °C; $[\alpha]_D^{20}$ +64.96° (c 0.004, CH_3OH); IR (KBr) 3457, 2939, 1621, 1578, 1418, 1289, 1209, 1123, 990 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.78 (s, 1H, PhOH), 13.00 (s, 1H, PhOH), 8.01 (s, 1H, C_3-NH), 7.93 (d, 1H, J = 7.81 Hz, ArH), 7.86 (m, 1H, ArH), 7.79 (m, 1H, ArH), 7.63 (t, 1H, J = 7.81 Hz, ArH), 7.43 (t, 1H, J = 7.81 Hz, ArH), 7.33 (t, 1H, J = 7.81 Hz, ArH), 7.29 (d, 1H, J = 7.81 Hz, ArH), 7.20 (d, 1H, J = 8.30 Hz, ArH), 6.93 (d, 1H, J = 8.30 Hz, ArH), 6.88 (d, 1H, J = 7.81 Hz, ArH), 6.84 (d, 1H, J = 7.81 Hz, ArH), 6.74 (t, 1H, J = 6.84 Hz, C_4-H), 5.54 (d, 1H, J = 18.06 Hz, C_{14}H), 5.43 (m, 1H, C_{7eq}H), 5.30 (d, 1H, J = 18.06 Hz, C_{14}H), 4.22 (d, 1H, J = 6.35 Hz, C_1-H), 4.15 (d, 1H, J = 6.84 Hz, C_3-H), 4.05 (q, 1H, J = 5.86 Hz, C_5-H), 3.95 (s, 3H, C_4OCH_3), 3.64

(s, 1H, C_4-OH), 3.14 (d, 1H, J = 18.56 Hz, C_{10eq}H), 2.91 (d, 1H, J = 18.56 Hz, C_{10ax}H), 2.47 (d, 1H, J = 14.16 Hz, C_{8eq}H), 2.07 (d, 1H, J = 14.16 Hz, C_{8ax}H), 1.95 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.83 (m, 2H, C_2-H), 1.31 (d, 3H, J = 5.86 Hz, C_5-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.25, 186.58, 186.09, 169.17, 161.51, 160.77, 160.21, 155.75, 155.08, 135.91, 135.88, 135.81, 135.69, 135.22, 135.11, 134.88, 133.44, 130.15, 120.27, 119.83, 119.70, 119.24, 118.38, 117.50, 111.91, 111.29, 110.99, 110.40, 100.25, 99.61, 77.11, 75.95, 69.13, 68.38, 66.81, 62.15, 56.66, 35.41, 33.56, 29.27, 29.17, 24.42, 24.40, 22.78, 14.22; UV (CH_3OH): λ_{max} (logε) = 233 (2.65), 251 (2.00), 495 (0.95); Mass (FAB$^-$, Na) m/z 891 $(M + Na)^+$.

Daunomycin-14-palmitate hydrochloride (6). To a solution of 14-bromo DM (**1c**) (0.51 g, 0.79 mmol) and palmitic acid (0.41 g, 1.60 mmol) in acetone (250 mL) were added triethylamine (0.45 mL, 3.20 mmol) followed by stirring for 3 hr. After completing the reaction monitored on TLC, the solvent was evaporated under reduced pressure. To the residue dissolved in dry THF (150 mL) was added ethereal HCl (1.0 M, 2.4 mL) followed by stirring at -20 °C for 2 hr. The organic solvent was concentrated by a rotary evaporator and purified by column chromatography on silica gel (CH_2Cl_2/MeOH/HCO_2H/H_2O=100 : 15 : 2 : 1) to give DM-pal (**6**) (0.77 g, 95%) as a red powder: mp 146-148 °C; $[\alpha]_D^{20}$ +39.95° (c 0.004, CH_2Cl_2); IR (KBr) 3457, 2927, 2853, 1738, 1615, 1578, 1412, 1289, 1209, 1116, 987 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (bs, 1H, PhOH), 13.04 (bs, 1H, PhOH), 8.17 (bs, 2H, NH_2), 7.76 (m, 1H, ArH), 7.61 (m, 1H, ArH), 7.21 (m, 1H, ArH), 5.79 (m, 1H, C_4-H), 5.42 (m, 1H, C_{7eq}H), 5.30 (d, 1H, J = 18.55 Hz, C_{14}H), 5.15 (d, 1H, J = 18.55 Hz, C_{14}H), 4.90 (m, 1H, C_1-H), 4.19 (q, 1H, J = 5.86 Hz, C_5-H), 3.92 (s, 3H, C_4OCH_3), 3.65 (s, 1H, C_4-OH), 3.15 (d, 1H, J = 18.55 Hz, C_{10eq}H), 2.85 (d, 1H, J = 18.55 Hz, C_{10ax}H), 2.44 (t, 2H, J = 7.32 Hz, Pal$C_{16}CH_2$), 2.37 (d, 1H, J = 14.65 Hz, C_{8eq}H), 2.02 (dd, 1H, J = 14.65, 4.40 Hz, C_{8ax}H), 1.67-1.83 (m, 4H, C_2-H & Pal$C_{17}CH_2$), 1.29 (d, 3H, J = 5.86 Hz, C_5CH_3), 1.26 (m, 14H, Pal$C_{18\text{-}29}CH_2$), 0.88 (t, 3H, J = 6.84 Hz, Pal$C_{30}CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.23, 186.52, 186.49, 173.44, 166.85, 158.14, 157.65, 138.93, 137.68, 134.22, 133.02, 121.71, 118.52, 118.32, 112.53, 110.25, 102.62, 76.40, 69.66, 69.13, 68.62, 66.81, 51.88, 49.14, 45.93, 45.76, 45.39, 45.36, 36.23, 35.71, 33.21, 31.88, 29.69, 29.63, 29.53, 29.34, 29.27, 29.18, 28.24, 24.92, 22.66, 19.00, 15.62; UV(CH_3OH): λ_{max} (logε) = 210 (2.25), 235 (3.04), 250 (2.41); Mass (FAB$^+$, Na) m/z 805 (M-HCl + Na)$^-$.

Daunomycin-*N*-palmitate (7). After the mixture of palmitic acid (0.62 g, 2.42 mmol) and EDCI (0.92 g, 4.80 mmol) in dry DMF/CH_2Cl_2 (100 mL, v/v=1 : 10) was stirred on an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added daunomycin hydrochloride (**1a**) (0.68 g, 1.21 mmol) and 4-pyrrolidinopyridine (0.36 g, 2.43 mmol), followed by stirring for 4 hr. The reaction mixture was extracted with CH_2Cl_2 (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 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/MeOH=12 : 6 : 1) to give DM-Npal (**7**) (0.74 g, 96%) as a red powder: mp 96-98 °C; $[\alpha]_D^{20}$ +29.91° (c 0.004, CH_2Cl_2); IR (KBr) 3432, 2927, 2853, 1720, 1621, 1578, 1412, 1289, 1209, 1123, 987 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.91 (s, 1H, PhOH), 13.14 (s, 1H, PhOH), 7.95 (d, 1H, J = 7.81 Hz, ArH), 7.73 (t, 1H, J = 7.81 Hz, ArH), 7.33 (d, 1H, J = 7.81 Hz, ArH), 6.02 (d, 1H, J = 8.30 Hz, C_4-H), 5.44 (d, 1H, J = 2.93 Hz, C_{7eq}H), 5.12 (d, 1H, J = 1.96 Hz, C_1-H), 4.21 (q, 1H, J = 6.34 Hz, C_5-H), 4.15 (m, 1H, C_3-H), 4.01 (s, 3H, C_4OCH_3), 3.64 (s, 1H, C_4-OH), 3.13 (d, 1H, J = 18.55 Hz, C_{10eq}H), 2.75 (d, J = 18.55 Hz, C_{10ax}H), 2.40 (s, 3H, $C_{14}CH_3$), 2.26 (d, 1H, J = 14.65 Hz, C_{8eq}H), 2.10 (td, 2H, J = 7.32, 2.93 Hz, Npal$_2$$CH_2$), 2.04 (dd, 1H, J = 14.65, 4.40 Hz, C_{8ax}H), 1.73-1.85 (m, 2H, C_2-H), 1.53 (t, 2H, J = 6.84, 6.35 Hz, Npal$_3$$CH_2$), 1.28 (d, 3H, J = 6.34 Hz, C_5CH_3), 1.23 (m, 10H, Npal$_{4\text{-}8}$$CH_2$), 1.21 (m, 14H, Npal$_{9\text{-}15}$$CH_2$), 0.86 (t, 3H, J = 7.32, 6.35 Hz, Npal$_{16}$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.97, 186.49, 186.11, 172.41, 160.65, 156.16, 155.47, 135.47, 135.18, 134.24, 133.67, 123.94, 120.53, 119.58, 118.22, 111.17, 110.97, 100.59, 76.65, 69.86, 69.57, 67.14, 65.50, 56.52, 45.23, 42.60, 37.79, 36.79, 35.03, 33.33, 31.94, 29.95, 29.72, 29.67, 29.54, 29.41, 29.39, 29.32, 25.78, 24.96, 22.73, 16.81, 14.18; UV(CH_3OH): λ_{max} (logε) = 250 (2.88), 477 (1.50), 494 (1.50); Mass (FAB$^-$, Na) m/z 789 $(M + Na)^+$.

Doxorubicin-*N*-palmitate (8). After the mixture of palmitic acid (0.34 g, 1.33 mmol) and DCC (0.49 g, 2.38 mmol) in dry DMF/CH_2Cl_2 (100 mL, v/v=1 : 10) was stirred on an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added doxorubicin hydrochloride (**1b**) (0.70 g, 1.21 mmol) and DMAP (0.16 g, 1.33 mmol), with further stirring for 3 hr. The reaction mixture was extracted with CH_2Cl_2 (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane/MeOH=12 : 6 : 1) to give DX-Npal (**8**) (0.66 g, 84%) as a red powder and DXpal-Npal (**9**) (0.11 g, 11%) as a red powder. **8**: mp 168-170 °C; $[\alpha]_D^{20}$ +299.97° (c 0.004, CH_2Cl_2); IR (KBr) 3445, 2927, 2853, 1726, 1621, 1584, 1418, 1289, 1209, 1123, 1086, 1018, 987 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.88 (s, 1H, PhOH), 13.08 (s, 1H, PhOH), 7.94 (d, 1H, J = 7.81 Hz, ArH), 7.74 (t, 1H, J = 7.81 Hz, ArH), 7.34 (d, 1H, J = 7.81 Hz, ArH), 6.04 (d, 1H, J = 8.30 Hz, C_4-H), 5.45 (d, 1H, J = 3.42 Hz, C_{7eq}H), 5.14 (s, 1H, C_1-H), 4.75 (s, 2H, $C_{14}CH_2$), 4.55 (bs, 1H, C_{14}OH), 4.14 (m, 2H, C_3&C_5-H), 4.02 (s, 3H, C_4O CH_3), 3.63 (s, 1H, C_4-OH), 3.15 (d, 1H, J = 19.04 Hz, C_{10eq}H), 2.81 (d, 1H, J = 19.04 Hz, C_{10ax}H), 2.29 (d, 1H, J = 14.65 Hz, C_{8eq}H), 2.12 (t, 2H, J = 4.88 Hz, Npal$_2$$CH_2$), 2.09 (dd, 1H, J = 14.65, 4.40 Hz, C_{8ax}H), 1.73-1.84 (m, 2H, C_2-H), 1.53 (t, 3H, J = 7.33 Hz, Npal$_3$$CH_2$), 1.28 (d, 3H, J = 6.35 Hz, C_5CH_3), 1.23 (m, 10H, Npal$_{4\text{-}8}$$CH_2$), 1.20 (m, 14H, Npal$_{9\text{-}15}$$CH_2$), 0.86 (t, 3H, J = 7.33 Hz, Npal$_{16}$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.59, 186.46, 186.05, 172.46, 160.64, 155.96, 155.20, 135.53, 135.09, 133.48, 133.42, 120.44, 119.62, 118.28.

111.25, 111.06, 100.63, 76.40, 69.54, 69.48, 67.26, 65.49, 56.52, 45.12, 42.70, 42.15, 37.89, 36.76, 35.66, 33.82, 31.93, 29.89, 29.709, 29.67, 29.53, 29.40, 29.37, 29.31, 25.75, 24.95, 22.72, 16.88, 14.18; UV(CH_3OH): λ_{max} (logε) = 234 (3.05), 251 (2.38), 495 (1.12); Mass (FAB^+, Na) m/z 805 $(M + Na)^+$.

Doxorubicin-dipalmitate (9). After the mixture of palmitic acid (0.77 g, 3.00 mmol) and EDCI (0.92 g, 4.80 mmol) in dry DMF/CH_2Cl_2 (v/v=1 : 10, 200 mL) was stirred on an ice bath for 30 min and allowed to reach room temperature, to the stirred solution was added doxorubicin hydrochloride (**1b**) (0.68 g, 1.21 mmol) and 4-pyrrolidinopyridine (0.36 g, 2.43 mmol), followed by further stirring for 10 hr. The reaction mixture was extracted with CH_2Cl_2 (200 mL), washed with water (2 × 100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane/MeOH=12 : 6 : 1) to give DXpal-Npal (**9**) (0.94 g, 92%) as a red powder and DX-Npal (**8**) (0.06 g, 5%) as a red powder. **9**: mp 92-95 °C; $[\alpha]_D^{20}$ +139.94° (c 0.004, CH_2Cl_2); IR (KBr) 3432, 2927, 2865, 1744, 1627, 1578, 1412, 1289, 1215, 1116, 987 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.95 (s, 1H, PhOH), 13.20 (s, 1H, PhOH), 8.02 (d, 1H, J = 7.81 Hz, ArH), 7.77 (t, 1H, J = 7.81 Hz, ArH), 7.35 (d, 1H, J = 7.81 Hz, ArH), 5.80 (d, 1H, J = 8.30 Hz, C_4H), 5.48 (d, 1H, J = 3.42 Hz, $C_{7eq}H$), 5.33 (d, 1H, J = 18.07 Hz, $C_{14}H$), 5.25 (d, 1H, J = 1.47 Hz, C_1H), 5.08 (d, 1H, J = 18.07 Hz, $C_{14}H$), 4.22 (q, 1H, J = 6.35 Hz, C_5H), 4.14 (m, 1H, C_3H), 4.07 (s, 3H, C_4OCH_3), 3.64 (s, 1H, C_4OH), 3.26 (d, 1H, J = 19.04 Hz, $C_{10eq}H$), 2.97 (d, 1H, J = 19.04 Hz, $C_{10ax}H$), 2.46 (m, 3H, $C_{8eq}H$ & $PalC_{16}CH_2$), 2.04-2.42 (m, 3H, $C_{8ax}H$, $NPal_2CH_2$), 1.66-1.88 (m, 4H, C_2H & Pal $C_{17}CH_2$), 1.55 (t, 3H, J = 7.32, 6.84 Hz, $NPal_3CH_2$), 1.31 (d, 3H, J = 6.35 Hz, C_5CH_3), 1.26 (m, 24H, $PalC_{18-29}CH_2$), 1.23 (m, 24H, $NPal_{4-15}CH_2$), 0.88 (t, 3H, J = 7.32, 6.35 Hz, $PalC_{30}CH_3$), 0.87 (t, 3H, J = 6.84 Hz, $NPal_{16}Me$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 206.49, 186.81, 186.35, 179.08, 173.08, 173.04, 172.30, 160.81, 156.08, 155.61, 147.03, 138.79, 135.57, 135.38, 133.53, 127.34, 120.76, 119.73, 118.28, 111.40, 111.23, 108.05, 103.14, 102.50, 100.56, 100.50, 77.16, 69.80, 69.74, 69.70, 67.25, 65.89, 56.66, 45.10, 38.31, 38.24, 36.85, 35.57, 33.99, 33.90, 33.70, 31.99, 30.10, 29.78, 29.73, 29.72, 29.70, 29.55, 29.44, 29.42, 29.37, 29.32, 29.20, 25.78, 25.02, 23.56, 22.78, 16.82, 14.22; UV(acetone): λ_{max} (logε) = 237 (1.59), 477 (0.93), 495 (0.58); Mass (FAB^+, Na) m/z 1043 $(M + Na)^+$.

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