Synthesis and Antitumor Activity of New Anthracycline Analogues

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Received May 4, 2001

New anthracycline analogues **2**-**9** as potential anticancer agents have been synthesized from daunomycin (**1a**) and doxorubicin (**1b**). Compounds **2** and **6** were prepared by the nucleophilic displacement type esterification of 14-bromodaunomycin (**1c**) with *N*-benzoyl-(2*R*,3*S*)-phenylisoserine and L-pyroglutamic acid in triethylamine, respectively. Compounds **3**, **7** and **4**, **8** were prepared by the reaction of either daunomycin (**1a**) or doxorubicin (**1b**) with one equivalent of the corresponding acids in the presence of EDCI/PP. Compounds **5**, **9** were obtained from **1b** by reaction with 2.2 equivalents of the corresponding acids in the same manner. The cytotoxic activities of the analogues in comparison with adrimycin on cultured SNU-16 and MCF7 cell were described.

Keywords : Daunomycin, Doxorubicin, Anthracycline derivatives, *N*-Benzoyl-(2*R*,3*S*)-phenylisoserine, L-Pyroglutamic acid.

Introduction

Anthracycline antibiotics, particularly doxorubicin (**DX**, **1b**) and daunomycin (**DM**, **1a**), have been used extensively in the treatment of human malignancies.[1] Doxorubicin stands apart for its activity against hard tumours, such as breast and lung cancers.; daunomycin shows a particular efficacy in the treatment of lymph cancers and leukemia. The antibiotic compounds affect DNA replication by donating electrons and bonding directly to the DNA chain.[2] However, cardiotoxicity and multidrug resistance are significant problems that limit the clinical effectiveness of such agents[3-4] (Figure 1).

The clinical utility of daunomycin (**1a**) or doxorubicin (**1b**) is frequently restricted by the appearance of cardiotoxicity from the damage of normal cells by the oxygen radical.[5-8] As a result, numerous efforts with synthetic treatments have been tried to overcome these disadvantages, culminating in the development of daunomycin (**1a**) or doxorubicin (**1b**) derivatives.[9-12] Examples are reported of coupling **1a** or **1b** with some amino acids as well as blending **1a** or **1b** with some amino acids.[9] *N*-Benzoyl-(2*R*,3*S*)-phenylisoserine plays a very important role as the C-13 side chain in Taxol.[13-14] L-Pyroglutamic acid (called 2-oxo-pyrrolidone carboxylic acid, or PCA), naturally occurring in vegetables, fruits, and dairy products, and normally present in large amounts in the human brain, is a non-toxic amino acid to the human body.[15] 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 in an attempt to obtain compounds that are more effective therapies than anything previously developed.

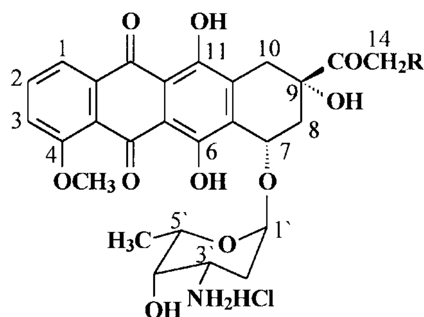

Figure 1. Structures of doxorubicin (**1b**, R = OH) and daunomycin (**1a**, R = H).

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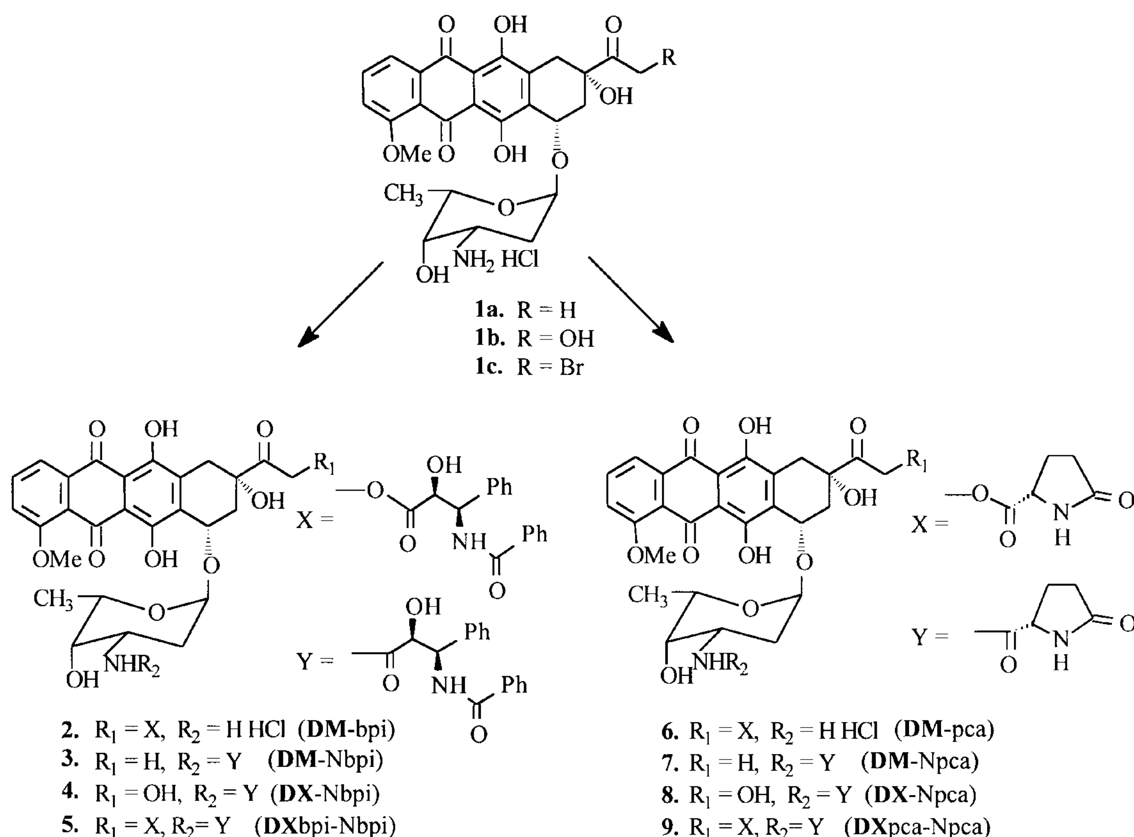
Results and Discussion

In previous papers, we described the total synthesis of anthracyclinone derivatives through Michael type condensation.[16-19] or Friedel-Crafts acylation.[20,21] We reported the successful preparation of a new aglycon, containing an ester linkage at C-14, through a nucleophilic displacement esterification method.[20,22] In the present study, we attempt to directly prepare some new anthracycline analogues from the commercially available anticancer agents, daunomycin (**1a**) and doxorubicin (**1b**). Several new anthracycline derivatives were synthesized separately using two different acylation methods (Scheme 1). The synthesis of 14-bromo DM (**1c**) was accomplished by the known procedure.[22,23] All compounds **2**-**9** were obtained through acylation of the C-14 hydroxyl group in the aglycon and/or the amino group at C-3′ in the glycon with *N*-benzoyl-(2*R*,3*S*)-phenylisoserine and L-pyroglutamic acid.

DM-bpi (**2**) and DM-pca (**6**), potential prodrugs, were



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

prepared by the reaction of 14-bromo DM (**1c**) with a *N*-benzoyl-(2*R*,3*S*)-phenylisoserine or L-pyroglutamic acid. To compare the activity of **2** and **6**, the carboamidation compounds DM-Nbpi (**3**), DX-Nbpi (**4**), DM-Npca (**7**), and DX-Npca (**8**) were synthesized by amidation of the amino group at C-3′ of the sugar moiety in **1a** or **1b** with the corresponding acids. In addition, the *N*-acylation compounds DXbpi-Nbpi (**5**) and DXpca-Npca (**9**) were prepared through esterification of the C_{14}-OH in DX (**1b**) with the corresponding acids followed by the amidation of the amino group at the sugar moiety with the corresponding acids.

First, the synthesis of 14-bromo DM (**1c**) was accomplished by the application of the known procedure.[22-24] **1c** was synthesized in best yield, using a minimum quantity of co-solvent (methanol/1,4-dioxane, v/v = 1 : 2), which diminished the formation of side product from dimethylketalization of ketone at C-13. The rate of bromination depends on the reaction temperature and time. The optimal conditions are bromination at 30 °C for 40 min.

DM-bpi (**2**) was synthesized as follow: To a 14-bromo DM (**1c**) prepared by introducing Br atom at C-14 of **1a** was added a solution on *N*-benzoyl-(2*R*,3*S*)-phenylisoserine and triethylamine in acetone; the mixture was stirred at room temperature for 13 hr.[25] After removing the solvent under reduced pressure, the residue was dissolved in THF, to which was added etheral HCl, followed by stirring at −20 °C for 2 hr and further stirring at room temperature for 3 hr, giving DM-bpi (**2**). DM-pca (**6**) was synthesized from DM (**1c**), L-pyroglutamic acid, and triethylamine in acetone as described for the preparation of DM-bpi (**2**).

Many attempts to prepare DM-Nbpi (**3**) and DX-Nbpi (**4**) through direct coupling of the amino group in daunomycin (**1a**) or doxorubicin (**1b**) with *N*-benzoyl-(2*R*,3*S*)-phenylisoserine, using 1,3-dicyclohexylcarbodiimide (DCC)/4-(dimethylamino)pyridine (DMAP) failed.[26] Reactants and DCU (dicyclohexylurea) were observed as the main products. Eventually, DM-Nbpi (**3**) was synthesized by the coupling of the NH_2HCl in **1a** with *N*-benzoyl-(2*R*,3*S*)-phenylisoserine, using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of catalytic amounts of 4-pyrrolidinopyridine (PP).[27-30] DX-Nbpi (**4**) was synthesized from DX (**1b**) as described for the preparation of DX-Nbpi (**3**). However, for the reaction of **1b** competition between the C-14 hydroxyl group and the amine group at C-3′ was observed. Both products, DX-Nbpi (**4**) and DXbpi-Nbpi (**5**), were formed, with the product ratio depending on the amounts of *N*-benzoyl-(2*R*,3*S*)-phenylisoserine and EDCI used in the reaction. DXbpi-Nbpi (**5**) was prepared using 2.2 equivalent of the corresponding acid and EDCI under similar conditions.

Synthesis of DM-Npca (**7**) and DX-Npca (**8**) was carried out as follows: L-pyroglutamic acid and EDCI (1.2 equivalent) were dissolved in dry DMF and the mixture stirred at 0 °C for 30 min; to the reaction mixture was added DM (**1a**) or DX (**1b**) and catalytic amounts of PP. The mixture was then stirred at room temperature for 4 hr to give DM-Npca

Table 1. The cytotoxic activity of the novel anthracycline derivatives (**2**-**9**) was tested "*in vitro*" in comparison with adriamycin on cultured SNU-16 and MCF7 cells

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	1.15	1.22(0.94)	2.18	2.25(0.97)
3	9.45	9.81	8.52	8.55
4	8.18	8.02	7.56	7.92
5	10.55	11.17	13.87	12.87
6	3.03	2.99(1.01)	2.10	2.05(1.02)
7	9.74	9.69	9.42	8.51
8	8.31	8.56	9.20	9.45
9	12.23	12.04	13.12	12.58

[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 DX-Npca (**8**).

DXpca-Npca (**9**) was synthesized from **1b** as described for the preparation of **8** by increasing the amounts of L-pyroglutamic acid (2.2 eq) and EDCI (2.2 eq).

Cytotoxic activity. The cytotoxic activities of anthracycline derivatives **2-9** against two kinds of human tumor cells (SNU-16 and MCF7) 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 other compounds (**3-5** and **7-9**) exhibited very low antitumor activity compared with the reference. These results suggest that the acylation of C-14 OH (**2**, **6**) maintains the activity inherent in the parent anthracycline antibiotics, whereas amidation of 3′-NH_2 (**3-5** and **7-9**) causes a decrease in antibiotic activity.

We have synthesized new anthracycline analogues expected to exhibit biological activity as potential anticancer agents. Further detailed studies on the results of the biological tests will be reported as they are completed.

Experimental Section

All reactions were carried out under argon atmosphere in dried glassware. All solvents were carefully dried and distilled as reported.[31] 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 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-(*N*)-benzoyl-(2*R*,3*S*)-phenylisoserinate hydrochloride (2). 14-Bromodaunomycin hydrochloride (**1c**, 0.20 g, 0.31 mmol) and *N*-benzoyl-(2*R*,3*S*)-phenylisoserine (0.11 g, 0.39 mmol) were dissolved in acetone (300 mL). To the mixture was added triethylamine (0.05 mL, 0.36 mmol), and the mixture was then stirred at room temperature for 13 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 = 100 : 15 : 2 : 1) gave daunomycin-14-(*N*)-benzoyl-(2*R*,3*S*)-phenylisoserinate hydrochloride (**2**, 0.21 g, 80%) as a red powder: mp 184-186 °C; $[\alpha]_D^{20}$ +174.96° (c 0.004, CH_3OH); IR (KBr) 3432, 2939, 1806, 1627, 1584, 1418, 1375, 1289, 1215, 1123, 1073, 707 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.89 (s, 1H, PhOH), 13.21 (s, 1H, PhOH), 8.96 (d, 1H, J = 8.7 Hz, C_{17}NH), 8.32 (d, 1H, J = 8.3 Hz, ArH) 7.80 (s, 2H, sugarNH_2), 7.40 (d, 2H, J = 7.3 Hz, serineArH), 6.78-7.16 (m, 2H, ArH, 8H, serineArH), 5.67 (d, 1H, J = 5.8 Hz, $C_{4'}$H), 5.63 (dd, 1H, J = 3.4, 8.7 Hz, C_{17}H), 5.31 (d, 1H, J = 3.9 Hz, C_{7eq}H), 4.91 (m, 1H, $C_{1'}$H), 4.91 (s, 1H, C_9OH), 4.78 (d, 1H, J = 18.0 Hz, C_{14}H), 4.68 (d, 1H, J = 3.4 Hz, C_{16}H), 4.37 (d, 1H, J = 18.0 Hz, C_{14}H), 4.12(q, 1H, J = 6.3 Hz, $C_{5'}$H), 4.09 (s, 3H, C_4OCH_3), 3.62 (m, 1H, $C_{4'}$OH), 3.37 (m, 1H, $C_{3'}$H), 3.18 (d, 1H, J = 17.5 Hz, C_{10eq}H), 2.93 (d, 1H, J = 17.5 Hz, C_{10ax}H), 2.20 (d, J = 14.6 Hz, 1H, C_{8eq}H), 2.14 (dd, 1H, J = 3.9, 14.6 Hz, C_{8ax}H), 1.63-1.92 (m, 2H, $C_{2'}$H), 1.19 (d, 3H, J = 6.3 Hz, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 208.09, 185.99, 185.52, 170.79, 165.97, 165.83, 164.17, 160.54, 155.59, 139.28, 136.07, 134.63, 134.44, 134.30, 133.53, 131.41, 128.13, 127.88, 127.29, 127.13, 126.78, 119.75, 119.58, 118.83, 113.12, 110.53, 99.60, 79.41, 74.96, 72.41, 69.87, 68.45, 66.01, 65.89, 56.62, 55.80, 54.57, 46.38, 32.13, 30.62, 29.62, 28.34, 15.58; UV (CH_3OH): λ_{max} (log ε) = 204 (2.33), 233 (1.58), 249 (0.94); Mass (FAB$^+$, Na) m/z 834 (M-HCl + Na)$^+$.

Daunomycin-3′-*N*-(*N*)-benzoyl-(2*R*,3*S*)-phenylisoserincarboamide (3). The mixture of *N*-benzoyl-(2*R*,3*S*)-phenylisoserine (0.18 g, 0.63 mmol) and EDCI (0.12 g, 0.63 mmol) in dry DMF (50 mL) was stirred on an 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, after which the mixture was stirred for 24 hr. The reaction 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*-(*N*)-benzoyl-(2*R*,3*S*)-phenylisoserincarbo-

amide (**3**, 0.29 g, 69%) as a red powder: mp 153-155 °C; $[\alpha]_D^{20}$ +74.98° (c 0.004, CH_2Cl_2); IR (KBr) 3408, 2939, 1824, 1720, 1658, 1584, 1529, 1486, 1418, 1289, 1215, 1110, 1036, 990, 707, 621, 572 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.91 (s, 1H, PhOH), 13.20 (s, 1H, PhOH), 8.66 (d, 1H, J = 8.79 Hz, serineNH), 8.31 (d, 1H, J = 8.3 Hz, ArH), 7.80-7.98 (m, 2H, serineArH), 7.73 (dd, 1H, J = 7.8, 8.3 Hz, ArH), 7.24-7.52 (m, 1H, ArH, 8H, serineArH), 6.87 (d, 1H, J = 8.3 Hz, sugarNH), 5.59 (dd, 1H, J = 3.4, 8.7 Hz, serineH), 5.40 (d, 1H, J = 5.8 Hz, $C_{4'}H$), 5.29 (d, 1H, J = 3.9 Hz, $C_{7eq}H$), 5.14 (m, 1H, $C_{1'}H$), 4.50 (s, 1H, C_9OH), 4.44 (d, 1H, J = 3.4 Hz, serineH), 4.06 (q, 1H, J = 6.3 Hz, $C_{5'}H$), 4.01 (s, 3H, C_4OCH_3), 3.62 (s, 1H, $C_{4'}OH$), 3.27 (m, 1H, $C_{3'}H$), 3.17 (d, 1H, J = 19.0 Hz, $C_{10eq}H$), 2.87 (d, 1H, J = 19.0 Hz, $C_{10ax}H$), 2.38 (s, 3H, $C_{14}CH_3$), 2.27 (d, 1H, J = 14.6 Hz, $C_{8eq}H$), 2.28 (dd, 1H, J = 3.9, 14.6 Hz, $C_{8ax}H$), 1.75 (dt, 1H, J = 3.9, 13.1 Hz, $C_{2'eq}H$), 1.63 (dd, 1H, J = 4.8, 13.1 Hz, $C_{2'ax}H$), 1.17 (d, J = 6.3 Hz, 3H, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.68, 186.65, 186.33, 171.70, 167.18, 166.97, 160.78, 156.24, 155.56, 137.63, 135.33, 134.24, 133.96, 133.43, 131.83, 131.77, 128.56, 128.06, 127.45, 127.36, 127.06, 127.01, 120.78, 119.64, 118.10, 111.30, 111.12, 100.60, 76.60, 72.93, 69.94, 68.58, 66.86, 56.60, 45.55, 36.57, 35.10, 33.88, 33.32, 29.76, 29.15, 24.85, 16.76; UV (CH_2Cl_2): λ_{max} (log ε) = 206 (1.66), 233 (1.75), 495 (0.43); Mass (FAB^+, Na) m/z 818 $(M + Na)^+$.

Doxorubicin-3′-*N*-(*N*)-benzoyl-(2*R*,3*S*)-phenylisoserincarboamide (4). The mixture of *N*-benzoyl-(2*R*,3*S*)-phenylisoserine (0.12 g, 0.42 mmol) and EDCI (0.08 g, 0.42 mmol) in dry DMF (50 mL) was stirred on 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 then stirred for 26 hr. The reaction 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*-(*N*)-benzoyl-(2*R*,3*S*)-phenylisoserincarboamide (**4**, 0.18 g, 64%) as a red powder: mp 177-179 °C; $[\alpha]_D^{20}$ +25.00° (c 0.004, CH_2Cl_2); IR (KBr) 3420, 2939, 1720, 1633, 1578, 1529, 1418, 1289, 1215, 1123, 1080, 987, 793, 704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.93 (s, 1H, PhOH), 13.22 (s, 1H, PhOH), 8.68 (d, 1H, J = 8.7 Hz, serineNH), 8.28 (d, 1H, J = 8.3 Hz, ArH), 7.76-7.79 (m, 2H, serineArH), 7.73 (dd, 1H, J = 7.8, 8.3 Hz, ArH), 7.23-7.56 (m, 1H, ArH, 8H, serineArH), 6.99 (d, 1H, J = 8.3 Hz, sugarNH), 5.99 (d, 1H, J = 5.8 Hz, $C_{4'}H$), 5.50 (dd, 1H, J = 3.4, 8.7 Hz, serineH), 5.42 (d, 1H, J = 3.9 Hz, $C_{7eq}H$), 5.12 (m, 1H, $C_{1'}H$), 4.93 (s, 1H, C_9OH), 4.75 (s, 2H, $C_{14}H$), 4.38 (d, 1H, J = 3.4 Hz, serineH), 4.11 (q, 1H, J = 6.3 Hz, $C_{5'}H$), 4.05 (s, 3H, C_4OCH_3), 3.63 (s, 1H, $C_{4'}OH$), 3.28 (m, 1H, $C_{3'}H$), 3.15 (d, 1H, J = 18.5 Hz, $C_{10eq}H$), 2.88 (d, 1H, J = 18.5 Hz, $C_{10ax}H$), 2.33 (d, 1H, J = 14.6 Hz, $C_{8eq}H$), 2.13 (dd, 1H, J = 4.4, 14.6 Hz, $C_{8ax}H$), 1.87 (dt, 1H, J = 3.9, 13.1 Hz, $C_{2'eq}H$), 1.69 (dd, 1H, J = 4.4, 13.1 Hz, $C_{2'ax}H$), 1.20 (d, 3H, J = 6.3 Hz, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.05, 185.79, 185.53, 170.65, 165.46, 161.94, 160.04, 155.53, 154.58, 138.63, 134.35, 133.40, 133.10, 130.60, 127.57, 126.54, 126.43, 126.33, 119.77, 118.80, 117.91, 110.36, 110.20, 100.01, 75.44, 72.58, 68.82, 67.95, 66.56, 64.52, 55.96, 55.26, 44.38, 39.99, 39.78, 39.57, 39.36, 39.15, 38.94, 38.73, 32.73, 29.02, 16.34; UV (CH_2Cl_2): λ_{max} (log ε) = 235 (0.29), 251 (0.17), 481 (0.10); Mass (FAB^+, Na) m/z 834 $(M + Na)^+$.

Doxorubicin-14, 3′-*N*-di(*N*)-benzoyl-(2*R*,3*S*)-phenylisoserinate (5). The mixture of *N*-benzoyl-(2*R*,3*S*)-phenylisoserine (0.22 g, 0.77 mmol) and EDCI (0.15 g, 0.78 mmol) in dry DMF (50 mL) was stirred on 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 **5** (0.23 g, 62%) as a red powder: mp 135-137 °C; $[\alpha]_D^{20}$ +125.28° (c 0.004, CH_2Cl_2); IR (KBr) 3425, 2940, 1720, 1630, 1577, 1530, 1418, 1280, 1215, 1124, 985, 782, 705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.94 (s, 1H, PhOH), 13.23 (s, 1H, PhOH), 8.92 (d, 1H, J = 8.7 Hz, $C_{17}NH$), 8.65 (d, 1H, J = 8.7 Hz, serineNH), 8.33 (d, 1H, J = 8.3 Hz, ArH), 6.94-8.12 (m, 2H, ArH, 10H, serineArH), 6.91 (d, 1H, J = 8.3 Hz, sugarNH), 5.87 (d, 1H, J = 5.8 Hz, $C_{4'}H$), 5.67 (dd, 1H, J = 3.4, 8.7 Hz, $C_{17}H$), 5.60 (dd, 1H, J = 3.4, 8.7 Hz, serineH), 5.51 (s, 1H, C_9OH), 5.30 (d, 1H, J = 3.9 Hz, $C_{7eq}H$), 5.02 (m, 1H, $C_{1'}H$), 4.81 (d, 1H, J = 18.0 Hz, $C_{14}H$), 4.39 (d, 1H, J = 18.0 Hz, $C_{14}H$), 4.30 (d, 1H, J = 3.4 Hz, $C_{16}H$), 4.22 (d, 1H, J = 3.4 Hz, serine H), 4.15 (q, 1H, J = 6.3 Hz, $C_{5'}H$), 4.01 (s, 3H, C_4OCH_3), 3.64 (s, 1H, $C_{4'}OH$), 3.39 (m, 1H, $C_{3'}H$), 3.20 (d, 1H, J = 18.5 Hz, $C_{10eq}H$), 2.81 (d, 1H, J = 18.5 Hz, $C_{10ax}H$), 2.22 (d, 1H, J = 14.6 Hz, $C_{8eq}H$), 2.16 (dd, 1H, J = 4.4, 14.6 Hz, $C_{8ax}H$), 1.82 (dt, 1H, J = 3.9, 13.18 Hz, $C_{2'eq}H$), 1.65 (dd, 1H, J = 4.4, 13.1 Hz, $C_{2'ax}H$), 1.18 (d, J = 6.3 Hz, 3H, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.02, 186.23, 186.12, 174.21, 173.15, 165.78, 165.13, 161.13, 160.05, 155.57, 155.25, 140.07, 140.01, 137.94, 134.22, 134.16, 133.27, 133.23, 130.28, 128.85, 128.58, 128.35, 128.14, 127.24, 127.16, 126.59, 126.55, 126.52, 126.36, 119.78, 119.02, 117.11, 110.23, 110.15, 100.12, 76.13, 75.65, 74.19, 73.52, 72.40, 68.75, 67.94, 67.66, 65.53, 55.85, 55.69, 55.23, 43.32, 41.58, 41.55, 39.75, 39.25, 39.16, 39.11, 38.55, 38.43, 33.83, 28.22, 15.44; UV (CH_2Cl_2): λ_{max} (log ε) = 253 (0.25), 261 (0.22), 454 (0.62); Mass (FAB^+, Na) m/z 1101 $(M + Na)^+$.

Daunomycin-14-pyroglutamate hydrochloride (6). 14-Bromodaunomycin hydrochloride (**1c**, 0.20 g, 0.31 mmol) and L-pyroglutamic acid (0.05 g, 0.39 mmol) was dissolved in acetone (200 mL). To the mixture was added triethylamine (0.05 mL, 0.36 mmol), and the mixture was then stirred at room temperature for 5 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 daunomycin-14-pyroglutamate hydrochloride (**6**, 0.20 g, 93%) as a red powder: mp 134-136 °C; $[\alpha]_D^{20}$ −49.98° (c 0.004, H_2O); IR (KBr) 3432, 2940, 1732, 1615, 1421, 1375, 1350, 1283, 1215, 1116, 1086, 987, 766 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 13.85 (s, 1H, PhOH), 13.11 (s, 1H, PhOH), 8.31 (s, 2H, NH_2), 8.11 (s, 1H, PAC NH), 7.77 (m, 2H, ArH), 7.54 (m, 1H, ArH), 5.76 (d, 1H, J = 5.8 Hz, $C_{4'}H$), 5.55 (s, 1H, C_9OH), 5.34 (s, 2H, $C_{14}H$), 5.24 (d, 1H, J = 3.9 Hz, $C_{7eq}H$), 4.88 (m, 1H, $C_{1'}H$), 4.29 (q, 1H, J = 6.3 Hz, $C_{5'}H$), 4.05 (m, 1H, $C_{16}H$), 3.91 (s, 3H, C_4OCH_3), 3.70 (m, 1H, $C_{4'}OH$), 3.42 (m, 1H, $C_{3'}H$), 3.05 (d, 1H, J = 18.0 Hz, $C_{10eq}H$), 2.69 (d, 1H, J = 18.0 Hz, $C_{10ax}H$), 2.22 (m, 2H, $C_{18}H$), 1.96-2.50 (m, 2H, $C_{17}H$), 1.87 (m, 2H, C_8H), 1.86 (m, 2H, $C_{2'}H$) 1.13 (d, 3H, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 208.04, 186.02, 185.92, 178.21, 172.33, 165.20, 160.54, 155.89, 138.43, 136.02, 134.58, 134.47, 119.77, 119.48, 118.85, 110.58, 110.44, 107.99, 75.08, 69.48, 66.18, 66.01, 56.58, 54.94, 46.56, 36.09, 31.94, 29.06, 28.48, 25.10, 20.65, 16.79; UV (H_2O): λ_{max} (log ε) = 233 (0.95), 253 (0.62), 487 (0.29); Mass (FAB$^+$, Na) m/z 678 $(M\text{-}HCl + Na)^+$.

Daunomycin-3′-*N*-pyroglutamiccarboamide (7). The mixture of L-pyroglutamic acid (0.30 g, 0.53 mmol) and EDCI (0.12 g, 0.63 mmol) in dry DMF (50 mL) was stirred on an ice bath for 30 min and then 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 stirred for 4 hr. The resulting mixture was extracted with 5% CH_3OH in $CHCl_3$ (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was then removed under reduced pressure. The residue was purified by column chromatography ($CH_2Cl_2/CH_3OH/HCO_2H/H_2O$ = 100 : 15 : 2 : 1) to give daunomycin-3′-*N*-pyroglutamiccarboamide (**7**, 0.32 g, 94%) as a red powder: mp 122-124 °C; $[\alpha]_D^{20}$ +124.97° (c 0.004, H_2O); IR (KBr) 3402, 2931, 1689, 1618, 1579, 1421, 1288, 1209, 1123, 989, 809, 675 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 13.84 (s, 1H, PhOH), 13.03 (s, 1H, PhOH), 8.15 (s, 1H, sugarNH), 7.72 (s, 1H, PAC NH), 7.66 (m, 2H, ArH), 7.45 (m, 1H, ArH), 5.74 (d, 1H, J = 5.8 Hz, $C_{4'}H$), 5.54 (s, 1H, C_9OH), 5.19 (s, 1H, $C_{7eq}H$), 4.81 (m, 1H, $C_{1'}H$), 4.20 (q, 1H, J = 6.3 Hz, $C_{5'}H$), 3.99 (m, 1H, PCA-αH), 3.88 (s, 3H, C_4OCH_3), 3.60 (m, 1H, $C_{4'}OH$), 3.35 (m, 1H, $C_{3'}H$), 3.15 (d, 1H, J = 18.5 Hz, $C_{10eq}H$), 2.79 (d, 1H, J = 18.5 Hz, $C_{10ax}H$), 2.28 (s, 3H, $C_{14}CH_3$), 1.85-2.20 (m, 8H, C_8H, $C_{2'}H$, PCA-γ, βH), 1.14 (d, 3H, J = 6.3 Hz, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 221.70, 185.77, 185.67, 177.21, 171.36, 162.99, 160.35, 155.91, 135.83, 135.23, 134.17, 134.10, 119.42, 119.27, 118.60, 110.29, 110.18, 100.32, 75.05, 70.16, 67.86, 66.64, 56.42, 55.51, 45.22, 35.95, 31.46, 29.75, 29.29, 25.32, 24.21, 17.10; UV (H_2O): λ_{max} (log ε) = 232 (0.85), 253 (0.57), 485 (0.25); Mass (FAB$^+$, Na) m/z 622 $(M + Na)^+$.

Doxorubicin-3′-*N*-pyroglutamiccarboamide (8). The mixture of L-pyroglutamic acid (0.05 g, 0.39 mmol) and EDCI (0.08 g, 0.42 mmol) in dry DMF (50 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**, 0.20 g, 0.35 mmol) and catalytic amounts of 4-pyrrolidinopyridine, and the mixture was then stirred for 4 hr. The resulting mixture was extracted with 5% CH_3OH in $CHCl_3$ (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was then removed under reduced pressure. The residue was purified by column chromatography ($CH_2Cl_2/CH_3OH/HCO_2H/H_2O$ = 100 : 15 : 2 : 1) to give doxorubicin-3′-*N*-pyroglutamiccarboamide (**8**, 0.22 g, 97%) as a pale red powder: mp 141-143 °C; $[\alpha]_D^{20}$ +122.98° (c 0.004, H_2O); IR (KBr) 3408, 2952, 1689, 1622, 1578, 1418, 1283, 1209, 1116, 1086, 1018, 988 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 13.79 (s, 1H, PhOH), 13.01 (s, 1H, PhOH), 8.12 (s, 1H, sugar NH), 7.90 (s, 1H, PCA NH), 7.68 (m, 2H, ArH), 7.40 (m, 1H, ArH), 5.69 (d, 1H, J = 5.8 Hz, $C_{4'}H$), 5.49 (s, 1H, C_9OH), 5.18 (s, 1H, $C_{7eq}H$), 4.88 (m, 1H, $C_{1'}H$), 4.59 (s, 2H, $C_{14}H$), 4.16 (q, 1H, J = 6.3 Hz, $C_{5'}H$), 4.04 (m, 1H, PCAα H), 3.85 (s, 3H, C_4OCH_3), 3.66 (m, 1H, $C_{4'}OH$), 3.42 (m, 1H, $C_{3'}H$), 2.88 (d, 1H, J = 18.0 Hz, $C_{10eq}H$), 2.68 (d, 1H, J = 18.0 Hz, $C_{10ax}H$), 1.85-2.32 (m, 8H, C_8H, $C_{2'}H$, PCA-γ, βH), 1.13 (d, 3H, J = 5.86 Hz, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 213.91, 185.84, 185.72, 177.15, 174.37, 163.07, 160.48, 154.37, 135.97, 135.12, 134.16, 133.84, 119.50, 119.40, 110.43, 110.32, 100.54, 75.01, 69.94, 68.02, 66.81, 64.01 56.53, 55.77, 55.01, 48.77, 45.41, 29.44, 29.30, 25.46, 24.83, 17.22; UV (H_2O): λ_{max} (log ε) = 233 (0.56), 252 (0.38), 489 (0.17); Mass (FAB$^+$, Na) m/z 678 $(M + Na)^+$.

Doxorubicin-14, 3′-dipyroglutamate (9). The mixture of L-pyroglutamic acid (0.10 g, 0.77 mmol) and EDCI (0.15 g, 0.78 mmol) in dry DMF (50 mL) was stirred in an ice bath for 30 min, after which it was 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 5% CH_3OH in $CHCl_3$ (200 mL), washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and the solvent was then removed under reduced pressure. The residue was purified by column chromatography on silica gel ($CH_2Cl_2/CH_3OH/HCO_2H/H_2O$ = 100 : 15 : 2 : 1) to give compound **9** (0.23 g, 87%) as a pale red solid: mp 128-130 °C; $[\alpha]_D^{20}$ +105.79° (c 0.004, H_2O); IR (KBr) 3409, 2950, 1785, 1636, 1568, 1424, 1283, 1207, 1122, 1083, 1015, 987 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 13.72 (s, 1H, PhOH), 13.05 (s, 1H, PhOH), 8.15 (s, 1H, sugarNH), 8.13 (s, 1H, C_{16} NH), 7.96 (s, 1H, PCA NH), 7.76 (m, 2H, ArH), 7.48 (m, 1H, ArH), 5.62 (d, 1H, J = 5.8 Hz, $C_{4'}H$), 5.51 (s, 1H, C_9OH), 5.35 (s, 1H, $C_{7eq}H$), 4.96 (m, 1H, $C_{1'}H$), 4.92 (d, 1H, J = 18.0 Hz, $C_{14}H$), 4.33 (d, 1H, J = 18.0 Hz, $C_{14}H$), 4.18 (q, 1H, J = 6.3 Hz,

$C_{5'}H$), 4.08 (m, 1H, $C_{16}H$), 4.02 (m, 1H, PCA-αH), 3.99 (s, 3H, C_4OCH_3), 3.61 (m, 1H, $C_{4'}OH$), 3.38 (m, 1H, $C_{3'}H$), 3.21 (d, 1H, J = 18.1 Hz, $C_{10eq}H$), 2.92 (d, 1H, J = 18.1 Hz, $C_{10ax}H$), 1.81-2.55 (m, 10H, C_8H, $C_{17,18}H$, PCA-γ, βH), 1.66 (m, 2H, $C_{2'}H$), 1.19 (d, 3H, J = 5.8 Hz, $C_{5'}CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 210.15, 187.63, 187.2, 178.35, 17.98, 171.12, 170.32, 162.29, 161.32, 155.94, 137.25, 135.33, 133.94, 133.85, 118.75, 118.26, 118.01, 110.45, 110.32, 100.27, 74.53, 65.59, 64.41, 64.12, 57.25, 56.68, 53.24, 47.78, 46.21, 39.82, 32.93, 29.16, 29.02, 25.18, 25.09, 20.66, 17.08; UV (CH_3OH): λ_{max} (log ε) = 235 (0.52), 257 (0.29), 489 (0.74); Mass (FAB^+, Na) m/z 789 $(M + Na)^+$.

Acknowledgment. This work was supported by a grant of Korea Basic Science Institute (KBSI) and Woosuk University. We gratefully acknowledged KBSI for the NMR and mass analyses.

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