VOL. 55 NO. 2, FEB. 2002 THE JOURNAL OF ANTIBIOTICS pp. 181 — 190

Preparation and Cytotoxic Activity of Some New Rhodomycin Derivatives Bearing Modifications in the Sugar Moiety

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(Received for publication August 2, 2001)

The synthesis and structural determination of a number of new rhodomycin derivatives, modified in the sugar part are described. The cytotoxicity against leukemic L1210 cells of these compounds is reported, along with β-rhodomycinone and two regioisomers of the above compounds, which were isolated during the synthetic procedure.

The anthracycline quinone antibiotics doxorubicin (**I**, Figure 1) and daunomycin (**II**, Figure 1) are powerful antitumor agents and are extensively used in the chemotherapy of a wide range of human cancers.[1] However, their clinical effectiveness is limited, due to a number of undesired side-effects, the most serious being the dose-related cardiotoxicity and the ability to induce multidrug resistance (MDR).[2,3] On the other hand the rhodomycins, which bear structural similarities to the anthracyclines, have limited clinical applicability, due to their strong toxicities.[4,5] Oxaunomycin (**III**, Figure 1) showed very strong cytotoxicity against murine leukemia L1210 cells, being 100-fold more active than doxorubicin and was found to possess a significant antitumor effect in some experimental tumors.[6] However, it was considered too toxic for further development.

As precursors of reactive quinone methides, many natural and synthetic quinones function as bioreductive alkylating agents and have antitumor activity.[7~10] The cytotoxicity of quinones may be due to two competing mechanisms: soft electrophilic arylation and redox cycling oxidation.[11~13] However, most quinone antitumor agents used clinically, such as anthracycline antibiotics, mitomycin C and benzoquinone derivatives, have a complex chemical structure with a number of active functional groups and the exact contribution of the quinoid ring to their antitumor activity remains uncertain.[14~17] The anthacycline antibiotics **I** and **II** covalently bind to and intercalate with DNA, inhibit DNA replication and RNA transcription, are DNA topoisomerase II poisons, induce DNA breakage and chromosomal aberrations, produce oxidative stress and damage biomembranes, trigger apoptosis and have a wide spectrum of anticancer activity.[14~22]

The discovery of less toxic rhodomycins has renewed interest in the preparation of novel derivatives, as potential antitumor agents. It should be noted that, in contrast to the

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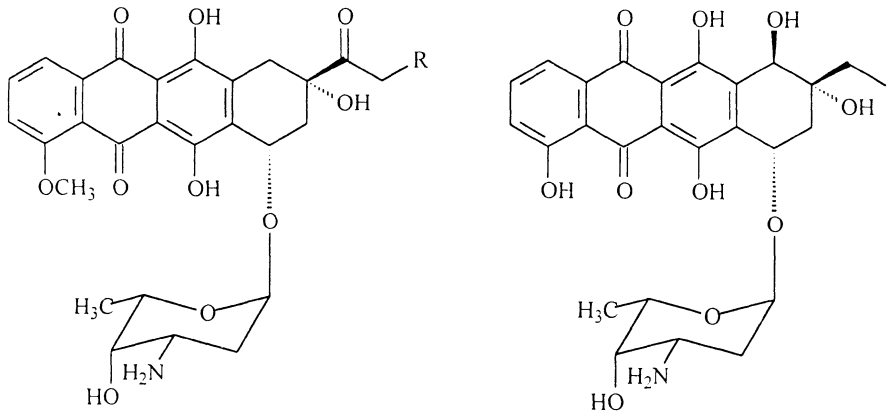
Fig. 1.



lead compounds **I**, **II** or **III**, some new anthracycline and rhodomycin analogs with increased lipophilicity may remain active against MDR tumor sublines.[23~25] As part of our continuing search for new anthracyclines, with an improved pharmacological profile, we report here the results of our studies on a number of new rhodomycin derivatives.

Material and Methods

General Remarks

^{1}H-NMR spectra and 2-D spectra were recorded on a Bruker Avanche 400 instrument, whereas ^{13}C-NMR specra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS (δ scale). The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2D NMR techniques: COSY, HMQC and HMBC. Mass spectra were recorded with a Nermag R 10-10C spectrometer using ES-MS techniques. Flash chromatography was performed on Merck silica gel 60 (0.040~0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates.

General Method of Glycosidation

To a stirred solution of the glycosyl donors (0.190 mmol) and molecular sieves 4A (300 mg) in dry CH_2Cl_2 (6 ml) and dry Et_2O (2 ml), was added trimethylsilyl trifluoromethanesulfonate (0.154 mmol) under argon at -40°C. The mixture was stirred for 1 hour at -5°C and then cooled to -15°C and a solution of β-rhodomycinone or 10-*O*-acetyl-β-rhodomycinone (0.059 mmol) in dry CH_2Cl_2 (2 ml) was added. After being stirred for 12 hours under the same conditions, the mixture was poured into a stirred mixture of AcOEt (10 ml) and saturated aqueous $NaHCO_3$ (3 ml). The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The rhodomycin glycosides were purified by preparative TLC (CH_2Cl_2/MeOH 99/1 or 98/2).

Deprotection of Phenylboronate Derivatives of Compounds **17** and **18**

The boronate derivative (0.035 mmol) was dissolved in a mixture of CH_2Cl_2 (3 ml)/Me_2CO (2 ml)/AcOH (0.05 ml), and 2-methyl-2,4-pentanediol (0.1 ml) was added. After being stirred for 6 hours at room temperature the reaction mixture was washed with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. Compounds **17** and **18** were purified by preparative TLC (CH_2Cl_2/MeOH 99/1 or 98/2).

10-*O*-Acetyl-7-*O*-(3-chloro-4-trifluoroacetamido-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl) Rhodomycinone (**11**)

Syrup, Yield: 56%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.4 (1H, s, OH-11), 12.9 (1H, s, OH-6), 12.0 (1H, s, OH-4), 7.88 (1H, d, J=8.0 Hz, H-1), 7.71 (1H, t, J=8.0 Hz, H-2), 7.31 (1H, d, J=8.0 Hz, H-3), 6.42 (IH, d, J=9.0 Hz, NH), 6.27 (1H, s, H-10), 5.47 (1H, d, J=3.8 Hz, H-1′), 5.13

(1H, dd, J=4.2/1.5 Hz, H-7), 4.48 (1H, m, H-3′), 4.38 (1H, d, J=9.0 Hz, H-4′), 4.22 (1H, m, H-5′), 3.48 (1H, s, OH-9), 2.28 (1H, d, J=14.8 Hz, H-8a), 2.22 (1H, dd, J=13.4/5.2 Hz, H-2′a), 2.05~2.00 (4H, m, CH_3COO-10/H-8b), 1.92 (1H, td, J=13.4/3.8 Hz, H-2′b), 1.76 (1H, m, H-13a), 1.48 (1H, m, H-13b), 1.18 (3H, d, J=6.3 Hz, H-6′), 1.03 (3H, t, J=7.3 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.2 (C-5), 186.3 (C-12), 169.2 (CH_3COO-10), 162.8 (C-4), 157.0 (C-11), 156.0 (C-6), 137.6 (C-2), 135.1 (C-6a), 134.3 (C-10a), 133.3 (C-12a), 125.1 (C-3), 119.9 (C-1), 115.8 (C-4a), 111.9 (C-5a/C-11a), 100.4 (C-1′), 71.6 (C-7/C-9), 70.5 (C-5′), 67.3 (C-10), 54.6 (C-3′), 52.4 (C-4′), 35.1 (C-2′), 32.0 (C-8), 29.0 (C-13), 20.7 (CH_3COO-10), 17.2 (C-6′), 6.40 (C-14). ES-MS m/z: 695 (M+Na).

10-*O*-Acetyl-7-*O*-(4-*O*-acetyl-3-bromo-2,3,6-trideoxy-α-L-arabino-hexopyranosyl) Rhodomycinone (**12**)

Syrup, Yield: 63%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.2 (1H, s, OH-11), 12.9 (1H, s, OH-6), 12.0 (1H, s, OH-4), 7.92 (1H, d, J=7.8 Hz, H-1), 7.74 (1H, t, J=7.8 Hz, H-2), 7.34 (1H, d, J=7.8 Hz, H-3), 6.27 (1H, s, H-10), 5.36 (1H, d, J=3.5 Hz, H-1′), 5.16 (1H, dd, J=4.2/1.8 Hz, H-7), 4.93 (1H, t, J=9.4 Hz, H-4′), 4.11~4.03 (2H, m, H-3′/H-5′), 3.56 (1H, s, OH-9), 2.56 (1H, dd, J=13.2/5.2 Hz, H-2′a), 2.45~2.30 (2H, m, H-8a/H-2′b), 2.24~1.90 (7H, m, CH_3COO-4′/CH_3COO-10/H-8b), 1.81 (1H, m, H-13a), 1.49 (1H, m, H-13b), 1.26 (3H, d, J=6.3 Hz, H-6′), 1.04 (3H, t, J=7.2 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 189.8 (C-5), 186.1 (C-12), 169.8 (CH_3COO-4′), 169.0 (CH_3COO-10), 162.5 (C-4), 157.4 (C-11), 156.0 (C-6), 137.4 (C-2), 135.2 (C-6a), 134.7 (C-10a), 133.3 (C-12a), 125.0 (C-3), 119.9 (C-1), 115.8 (C-4a), 112.0 (C-5a/C-11a), 100.6 (C-1′), 76.4 (C-4′), 71.3 (C-7/C-9), 68.7 (C-5′), 66.6 (C-10), 45.3 (C-3′), 41.1 (C-2′), 31.9 (C-8), 28.9 (C-13), 20.9 (CH_3COO-4′), 20.4 (CH_3COO-10), 17.3 (C-6′), 6.14 (C-14). ES-MS m/z: 686 (M+Na).

10-*O*-Acetyl-7-*O*-(4-*O*-acetyl-3-chloro-2,3,6-trideoxy-α-L-arabino-hexopyranosyl) Rhodomycinone (**13**)

Syrup, Yield: 51%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.4 (1H, s, OH-11), 12.8 (1H, s, OH-6), 12.1 (1H, s, OH-4), 7.86 (1H, d, J=8.1 Hz, H-1), 7.70 (1H, t, J=8.1 Hz, H-2), 7.30 (1H, d, J=8.1 Hz, H-3), 6.25 (1H, s, H-10), 5.41 (1H, d, J=3.4 Hz, H-1′), 5.15 (1H, dd, J=4.0/1.4 Hz, H-7), 4.82 (1H, t, J=9.8 Hz, H-4′), 4.15~3.90 (2H, m, H-3′/H-5′), 3.63 (1H, s, OH-9), 2.36 (1H, d, J=15.1 Hz, H-8a), 2.31 (1H, dd, J=13.2/4.9 Hz, H-2′a), 2.20~1.95 (8H, m, CH_3COO-4′/CH_3COO-10/H-8b/H-2′b), 1.76 (1H, m, H-13a), 1.48 (1H, m, H-13b), 1.24 (3H, d, J=6.0 Hz, H-6′), 1.04 (3H, t, J=7.3 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.0 (C-5), 186.0 (C-12), 170.1 (CH_3COO-4′), 169.2 (CH_3COO-10), 162.7 (C-4), 157.2 (C-11), 156.0 (C-6), 137.4 (C-2), 135.4 (C-6a), 134.5 (C-10a), 133.3 (C-12a), 125.0 (C-3), 119.9 (C-1), 116.0 (C-4a), 112.0 (C-5a/C-11a), 101.1 (C-1′), 76.3 (C-4′), 71.5 (C-7/C-9), 68.1 (C-5′), 67.0 (C-10), 54.8 (C-3′), 40.1 (C-2′), 32.1 (C-8), 29.0 (C-13), 20.9 (CH_3COO-4′), 20.8 (CH_3COO-10), 17.5 (C-6′), 6.42 (C-14). ES-MS m/z: 642 (M+Na).

10-*O*-Acetyl-7-*O*-(4-*O*-acetyl-3-azido-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl) Rhodomycinone (**14**)

Syrup, Yield: 57%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.4 (1H, s, OH-11), 12.8 (1H, s, OH-6), 12.0 (1H, s, OH-4), 7.82 (1H, d, J=7.8 Hz, H-1), 7.67 (1H, t, J=7.8 Hz, H-2), 7.26 (1H, d, J=7.8 Hz, H-3), 6.24 (1H, s, H-10), 5.53 (1H, d, J=2.9 Hz, H-1′), 5.22~5.17 (2H, br s, H-7/H-4′), 4.26 (1H, q, J=6.4 Hz, H-5′), 3.69 (1H, m, H-3′), 3.65 (1H, s, OH-9), 2.35 (1H, d, J=15.1 Hz, H-8a), 2.18 (3H, s, CH_3COO-4′), 2.02 (3H, s, CH_3COO-10), 2.20~1.90 (3H, m, H-8b/H-2′a/H-2′b), 1.75 (1H, m, H-13a), 1.47 (1H, m, H-13b), 1.19 (3H, d, J=6.4 Hz, H-6′), 1.02 (3H, t, J=7.3 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 190.8 (C-5), 185.9 (C-12), 170.4 (CH_3COO-4′), 169.1 (CH_3COO-10), 162.6 (C-4), 157.3 (C-11), 156.1 (C-6), 137.4 (C-2), 135.6 (C-6a), 134.7 (C-10a), 133.2 (C-12a), 124.9 (C-3), 119.8 (C-1), 115.7 (C-4a), 112.1 (C-5a/C-11a), 101.3 (C-1′), 71.4 (C-9), 71.2 (C-7), 69.8 (C-4′), 66.9 (C-10), 66.3 (C-5′), 54.4 (C-3′), 32.0 (C-8), 29.6 (C-2′), 29.1 (C-13), 20.9 (CH_3COO-4′), 20.7 (CH_3COO-10), 16.6 (C-6′), 6.42 (C-14). ES-MS m/z: 648 (M+Na).

10-*O*-Acetyl-7-*O*-(4-*O*-acetyl-3-azido-2,3,6-trideoxy-α-L-arabino-hexopyranosyl) Rhodomycinone (**15**)

Syrup, Yield: 55%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.4 (1H, s, OH-11), 12.8 (1H, s, OH-6), 12.0 (1H, s, OH-4), 7.86 (1H, d, J=8.0 Hz, H-1), 7.70 (1H, t, J=8.0 Hz, H-2), 7.30 (1H, d, J=8.0 Hz, H-3), 6.25 (1H, s, H-10), 5.45 (1H, d, J=3.0 Hz, H-1′), 5.17 (1H, br s, H-7), 4.69 (1H, t, J=9.6 Hz, H-4′), 4.02 (1H, m, H-5′), 3.69 (1H, s, OH-9), 3.65 (1H, m, H-3′), 2.37 (1H, d, J=15.0 Hz, H-8a), 2.20 (1H, dd, J=13.2/4.4 Hz, H-2′a), 2.11 (3H, s, CH_3COO-4′), 2.04 (4H, m, CH_3COO-10/H-8b), 1.90~1.40 (3H, H-13a/H-13b/H-2′b), 1.21 (3H, d, J=6.2 Hz, H-6′), 1.04 (3H, t, J=7.3 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.0 (C-5), 186.1 (C-12), 170.1 (CH_3COO-4′), 169.2 (CH_3COO-10), 162.8 (C-4), 157.4 (C-11), 156.2 (C-6), 137.5 (C-2), 137.5 (C-6a), 134.7 (C-10a), 133.4 (C-12a), 125.0 (C-3), 119.9 (C-1), 115.9 (C-4a), 112.2 (C-5a/C-11a), 100.9 (C-1′), 75.2 (C-4′), 71.6 (C-7/C-9), 67.3 (C-5′), 67.1 (C-10), 54.5 (C-3′), 35.5 (C-2′), 32.2 (C-8), 29.1 (C-13), 21.0

(CH_3COO-4′), 20.9 (CH_3COO-10), 17.3 (C-6′), 6.51 (C-14). ES-MS *m/z*: 648 (M+Na).

10-*O*-Acetyl-7-*O*-(4-*O*-acetyl-3-trifluoracetamido-2,3,6-trideoxy-α-L-arabino-hexopyranosyl) Rhodomycinone (**16**)

Syrup, Yield: 64%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.5 (1H, s, OH-11), 13.0 (1H, s, OH-6), 12.2 (1H, s, OH-4), 7.90 (1H, d, J=7.8 Hz, H-1), 7.72 (1H, t, J=7.8 Hz, H-2), 7.32 (1H, d, J=7.8 Hz, H-3), 6.60 (1H, d, J=7.4 Hz, NH), 6.28 (1H, s, H-10), 5.43 (1H, d, J=3.8 Hz, H-1′), 5.17 (1H, dd, J=4.4/1.7 Hz, H-7), 4.59 (1H, t, J=9.9 Hz, H-4′), 4.25~4.10 (2H, m, H-5′/H-3′), 3.60 (1H, s, OH-9), 2.44~2.36 (2H, m, H-8a/H-2′a), 2.09 (3H, s, CH_3COO-4′), 2.04 (4H, m, CH_3COO-10/H-8b), 1.82~1.72 (2H, m, H-13a/H-2′b), 1.48 (1H, m, H-13b), 1.27 (3H, d, J=6.14 Hz, H-6′), 1.06 (3H, t, J=7.2 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.2 (C-5), 186.1 (C-12), 172.3 (CH_3COO-4′), 169.5 (CH_3COO-10), 162.8 (C-4), 157.1 (C-11), 157.1 (C-6), 137.4 (C-2), 136.9 (C-6a), 134.8 (C-10a), 133.5 (C-12a), 125.0 (C-3), 119.9 (C-1), 116.2 (C-4a), 112.2 (C-5a), 111.8 (C-11a), 100.7 (C-1′), 74.3 (C-4′), 71.9 (C-7), 71.3 (C-9), 66.9 (C-5′), 64.5 (C-10), 49.4 (C-3′), 35.6 (C-2′), 32.9 (C-8), 29.8 (C-13), 21.0 (CH_3COO-4′), 20.6 (CH_3COO-10), 17.2 (C-6′), 6.69 (C-14). ES-MS *m/z*: 718 (M+Na).

10-*O*-Acetyl-7,9-di-*O*-(4-*O*-acetyl-3-trifluoracetamido-2,3,6-trideoxy-α-L-arabino-hexopyranosyl) Rhodomycinone (**16a**)

Syrup, Yield: 12%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.5 (1H, s, OH-11), 13.0 (1H, s, OH-6), 12.2 (1H, s, OH-4), 7.88 (1H, d, J=7.7 Hz, H-1), 7.70 (1H, t, J=7.7 Hz, H-2), 7.35 (1H, d, J=7.7 Hz, H-3), 7.30 (1H, d, J=7.1 Hz, CF_3CONH-3″), 6.92 (1H, d, J=7.0 Hz, CF_3CONH-3′), 6.80 (1H, s, H-10), 5.49 (1H, d, J=3.9 Hz, H-1′), 5.40 (1H, d, J=3.9 Hz, H-1″), 4.93 (1H, br d, J=5.3 Hz, H-7), 4.70 (1H, t, J=10.1 Hz, H-4′), 4.50 (1H, t, J=10.1 Hz, H-4″), 4.30~4.10 (3H, m, H-5′/H-3′/H-3″), 3.30 (1H, m, H-5″), 2.45 (1H, d, J=14.8 Hz, H-8a), 2.35~2.00 (11H, m, CH_3COO-4′/CH_3COO-4″/CH_3COO-10/H-8b/H-2′a/H-2′b), 1.97 (1H, m, H-13a), 1.48 (1H, m, H-13b), 1.25 (3H, d, J=6.3 Hz, H-6′), 0.98 (3H, t, J=7.3 Hz, H-14), 0.60 (3H, d, J=6.4 Hz, H-6″). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.2 (C-5), 186.1 (C-12), 172.5 (CH_3COO-4″), 172.3 (CH_3COO-4′), 169.5 (CH_3COO-10), 162.8 (C-4), 157.1 (C-11), 157.1 (C-6), 137.4 (C-2), 136.9 (C-6a), 134.8 (C-10a), 133.5 (C-12a), 125.0 (C-3), 119.9 (C-1), 116.2 (C-4a), 112.7 (C-5a), 111.8 (C-11a), 100.7 (C-1′), 90.8 (C-1″), 76.8 (C-9), 74.7 (C-4′), 74.5 (C-4″), 70.5 (C-7), 66.9 (C-5′/C-5″), 64.5 (C-10), 49.4 (C-3′), 49.1 (C-3″), 35.6 (C-2′/C-2″), 32.9 (C-8), 24.5 (C-13), 21.0/20.6/20.5 (CH_3COO-10/CH_3COO-4′)/CH_3COO-4″), 17.4 (C-6′), 17.2 (C-6″), 6.70 (C-14). ES-MS *m/z*: 875 (M+Na).

10-*O*-(4-*O*-Acetyl-3-chloro-2,3,6-trideoxy-α-L-arabino-hexopyranosyl) Rhodomycinone (**17**)

Syrup, Yield: 38%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.7 (1H, s, OH-11), 12.9 (1H, s, OH-6), 12.0 (1H, s, OH-4), 7.88 (1H, d, J=7.8 Hz, H-1), 7.71 (1H, t, J=7.8 Hz, H-2), 7.32 (1H, d, J=7.8 Hz, H-3), 5.40 (1H, d, J=2.6 Hz, H-1′), 5.23 (1H, br s, H-7), 4.92 (1H, br s, H-10), 4.75 (1H, t, J=9.8 Hz, H-4′), 4.05 (1H, m, H-3′), 3.85 (1H, m, H-5′), 3.50 (1H, m, OH-9), 3.30 (1H, s, OH-7), 2.35~1.95 (4H, m, H-8a/H-8b/H-2′a/H-2′b), 1.90~1.65 (2H, m, H-13a/H-13b), 1.22 (3H, d, J=6.3 Hz, H-6′), 1.12 (3H, t, J=7.3 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 191.0 (C-5), 186.7 (C-12), 170.4 (CH_3COO-4′), 162.8 (C-4), 155.7 (C-11), 155.7 (C-6), 137.9 (C-6a), 137.4 (C-2), 136.5 (C-10a), 133.4 (C-12a), 125.0 (C-3), 119.9 (C-1), 115.8 (C-4a), 112.2 (C-5a/C-11a), 97.3 (C-1′), 76.7 (C-4′), 71.6 (C-10/C-9), 68.1 (C-5′), 62.7 (C-7), 55.2 (C-3′), 40.8 (C-2′), 33.9 (C-8), 30.8 (C-13), 20.8 (CH_3COO-4′), 17.7 (C-6′), 6.46 (C-14). ES-MS *m/z*: 600 (M+Na).

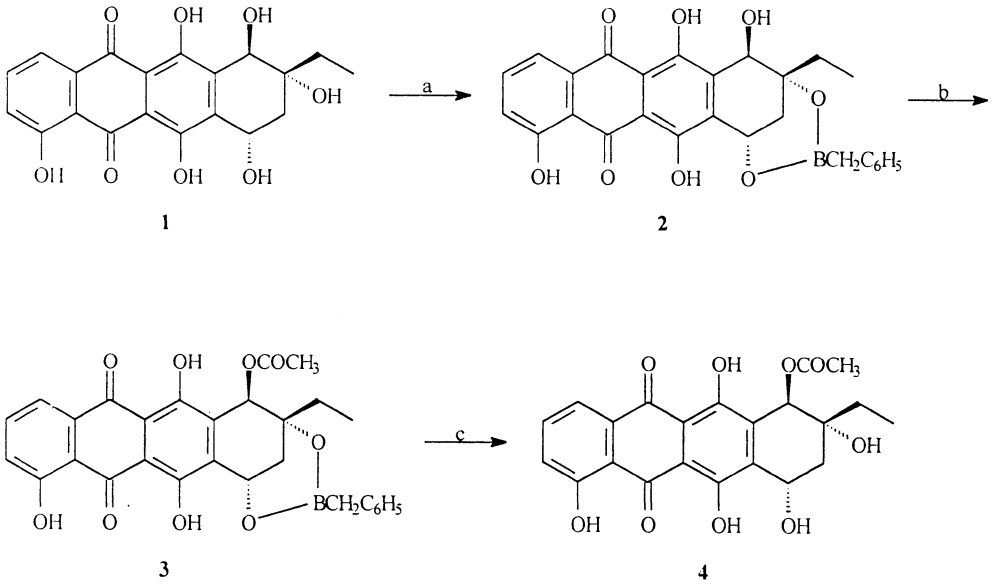
10-*O*-(4-*O*-Acetyl-3-azido-2,3,6-trideoxy-α-L-arabino-hexopyranosyl) Rhodomycinone (**18**)

Syrup, Yield: 44%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.6 (1H, s, OH-11), 12.8 (1H, s, OH-6), 12.0 (1H, s, OH-4), 7.85 (1H, d, J=7.8 Hz, H-1), 7.70 (1H, t, J=7.8 Hz, H-2), 7.26 (1H, d, J=7.8 Hz, H-3), 5.42 (1H, d, J=2.5 Hz, H-1′), 5.22 (1H, br s, H-7), 4.92 (1H, br s, H-10), 4.61 (1H, t, J=9.8 Hz, H-4′), 3.85 (1H, m, H-5′), 3.63 (2H, m, OH-9/H-3′), 3.30 (1H, s, OH-7), 2.30~1.50 (9H, m, H-8a/H-8b/H-13a/H-13b/H-2′a/H-2′b/CH_3COO-4′), 1.23 (3H, d, J=6.4 Hz, H-6′), 1.11 (3H, t, J=7.3 Hz, H-14). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 190.9 (C-5), 186.2 (C-12), 170.0 (CH_3COO-4′), 162.7 (C-4), 157.4 (C-11), 155.7 (C-6), 137.9 (C-6a), 137.4 (C-2), 136.6 (C-10a), 133.3 (C-12a), 125.0 (C-3), 119.9 (C-1), 115.8 (C-4a), 112.1 (C-5a/C-11a), 97.0 (C-1′), 75.4 (C-4′), 71.7 (C-10/C-9), 67.1 (C-5′), 62.4 (C-7), 57.5 (C-3′), 35.9 (C-2′), 33.9 (C-8), 30.8 (C-13), 20.9 (CH_3COO-4′), 17.4 (C-6′), 6.46 (C-14). ES-MS *m/z*: 606 (M+Na).

Cell Culture and Drug Treatments

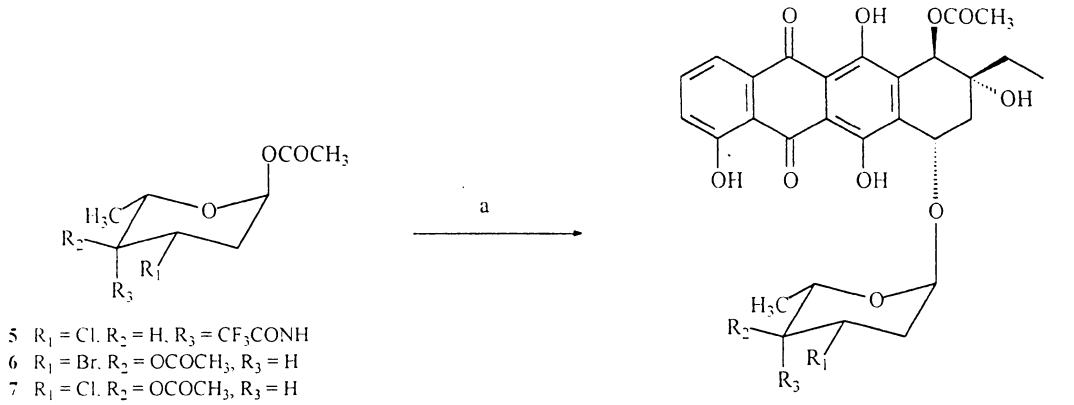
All solutions of synthetic compounds and daunomycin (from Sigma Chemical Co., St. Louis, MO) were dissolved in dimethyl sulfoxide (DMSO). Sequential dilutions were prepared in order to test compounds **1**, **4**, **11**~**18** (Schemes 1~3) over final concentrations ranging from 40.96 nM to

Scheme 1.



a: $PhB(OH)_2$, CH_3CO_2H.glacial, toluene.anh, **b:** Isoprenyl acetate, cat. H_2SO_4, **c:** 2-methyl-2,4-pentanediol, CH_3CO_2H

Scheme 2.

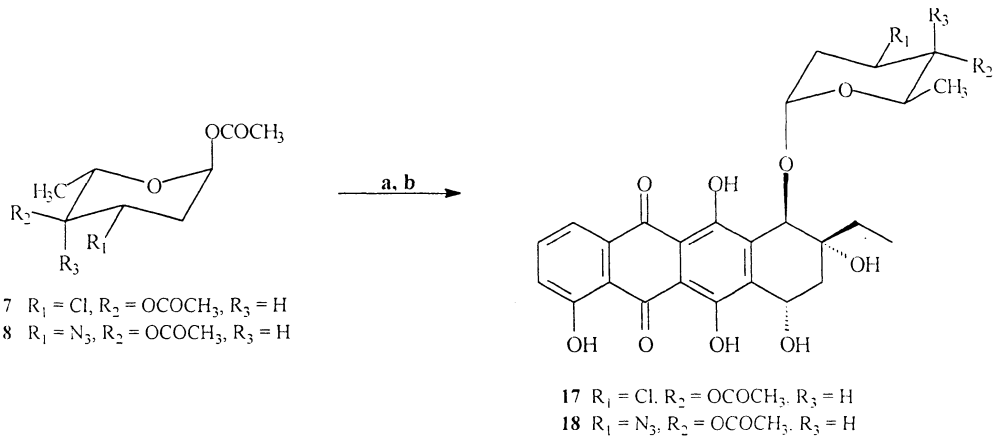


5 R_1 = Cl, R_2 = H, R_3 = CF_3CONH
6 R_1 = Br, R_2 = $OCOCH_3$, R_3 = H
7 R_1 = Cl, R_2 = $OCOCH_3$, R_3 = H
8 R_1 = N_3, R_2 = $OCOCH_3$, R_3 = H
9 R_1 = N_3, R_2 = H, R_3 = $OCOCH_3$
10 R_1 = CF_3CONH, R_2 = $OCOCH_3$, R_3 = H

11 R_1 = Cl, R_2 = H, R_3 = CF_3CONH
12 R_1 = Br, R_2 = $OCOCH_3$, R_3 = H
13 R_1 = Cl, R_2 = $OCOCH_3$, R_3 = H
14 R_1 = N_3, R_2 = $OCOCH_3$, R_3 = H
15 R_1 = N_3, R_2 = H, R_3 = $OCOCH_3$
16 R_1 = CF_3CONH, R_2 = $OCOCH_3$, R_3 = H

a: 10-*O*-acetyl-*β*-rhodomycinone (**4**), trimethylsilyltriflate, CH_2Cl_2 / Et_2O

Scheme 3.



7 R_1 = Cl, R_2 = $OCOCH_3$, R_3 = H
8 R_1 = N_3, R_2 = $OCOCH_3$, R_3 = H

17 R_1 = Cl, R_2 = $OCOCH_3$, R_3 = H
18 R_1 = N_3, R_2 = $OCOCH_3$, R_3 = H

a: Compound **2**, TMSOTf, CH_2Cl_2.anh, Et_2O.anh, b: 2-methyl-2,4-pentanediol, AcOH

25 μM and daunomycin over final concentrations ranging from 2.62 to 640 nM *in vitro*. Suspension cultures of murine L1210 lymphocytic leukemia cells (American Type Culture Collection, Rockville, MD) were maintained in continuous exponential growth by twice a week passage in RPMI 1640 medium supplemented with 8.25% fortified bovine calf serum (HyClone Laboratories, Logan, UT) and penicillin (100 IU/ml)- streptomycin (100 μg/ml) and incubated in the presence or absence of drugs at 37°C in a humidified atmosphere containing 5% CO_2. Since drugs were supplemented to the culture medium in 1-μl aliquots, the concentration of DMSO in the final incubation volume (0.5 ml) never exceeded 0.2% and did not affect the rates of macromolecule syntheses, growth and survival in control L1210 cells incubated with vehicle in the absence of drugs.[26~28]

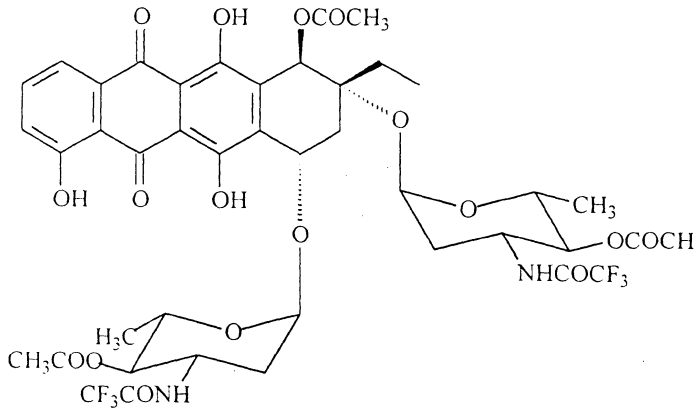
Tumor Cell Viability Assay

L1210 cells were seeded in triplicate in 48-well Costar cell culture plates at an initial density of 10,000 cells/0.5 ml of RPMI 1640 medium and grown at 37°C for 4 days in the presence or absence (control) of increasing concentrations of compounds **1**, **4** and **1~18** to evaluate drug cytotoxicity. The anthracycline quinone antibiotic daunomycin was tested in the same experiments for the sake of comparison. The viability of drug-treated cells was assessed from their ability to bioreduce the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) reagent (Promega, Madison, WI) in the presence of phenazine methosulfate (PMS; Sigma) into a water-soluble formazan product which absorbs at 490 nm.[29] After 4 days in culture, cell samples (about 10^6/0.5 ml/well for controls) were further incubated at 37°C for 3 hours in the dark in the presence of 0.1 ml of MTS : PMS (2 : 0.1) reagent and their relative cell viability was estimated by recording the absorbance at 490 nm, using a Cambridge model 750 automatic microplate reader (Packard, Downers Grove, IL).[26~28] Cell viability results (means±S.D.; n=3) were expressed as % of the net absorbance of MTS/formazan after bioreduction by vehicle-treated control cells at day 4 ($A_{490\,nm}$=1.825±0.129; 100±7%). Blank values at day 4 ($A_{490\,nm}$=0.296±0.016) for culture medium supplemented with MTS : PMS reagent in the absence of cells were substracted from the results. The concentrations of drugs required to inhibit by 50% the viability of L1210 tumor cells at day 4 (IC_{50} values) were calculated from linear regression of the slopes of the log-transformed concentration-survival curves.

Results and Discussion

In our efforts to gain access to semisynthetic β-rhodomycins which could be effective as antitumor agents, we originally focused on the glycosidation of β-rhodomycinone (**1**, Scheme 1) with appropriate glycosyl donors, using the trimethylsilyl triflate procedure[24]. It is known that direct glycosidation of unprotected β-

Fig. 2.



16a

rhodomycinone results in an inseparable mixture of the corresponding C-7 and C-10 *O*-glycosides.[30] Therefore the C-10-*O*-acetylated compound **4** was used for the regioselective glycosidation of C-7 hydroxyl group (Scheme 1). For the synthesis of this intermediate the boronate **2** was readily prepared from *β*-rhodomycinone (**1**) and phenylboric acid in glacial acetic acid and toluene.[31] It was first acetylated with isoprenyl acetate in the presence of catalytic amount of concentrared sulfuric acid to provide the 10-*O*-acetyl derivative **3**[24] and then treated with 2-methylpentane-2,4-diol and acetic acid, to afford the corresponding deboronated derivative **4**,[24] as depicted in Scheme 1. The glycosidation of **4** with the glycosyl donors **5**~**10**,[23,32~34] using trimethylsilyl triflate in a mixture of dry dichloromethane and ether at −15°C, in the presence of molecular sieves 4A, resulted in compounds **11**~**16** respectively (Scheme 2). Interestingly, during the synthesis of **16**, we have isolated from the reaction mixture a small amount of the diglycoside **16a** (Figure 2).

The synthesis of the compounds **17** and **18** was accomplished by glycosidation of **2** with **7** and **8**, followed by deprotection under conditions similar to those described above for the synthesis of the compounds **11**~**16** (Scheme 3). The structure of the coupling derivatives **11**~**16a**, **17** and **18** has unambiguously been established by the use of 1D and 2D NMR experiments. We have also assigned the ^{13}C chemical shifts for compounds **1**~**4** (Table 1), whose synthesis and ^{1}H-NMR data have been previously reported.[24]

Since several important cancer chemotherapeutic agents contain a quinone ring in their chemical structure,[14~16] it is significant to notice in the present study that all new synthetic rhodomycin and anthracycline derivatives tested, have antileukemic activities *in vitro*. Their ability to decrease tumor cell viability (mitochondrial function of the cell) after several days of drug treatment may be a better predictor of their anticancer potential than antiproliferation, since growth delay may allow survivors to resume division and clonal expansion once the drug is catabolized or eliminated and its effect is waning.[35]

All compounds tested are cytotoxic and the concentrations which decrease L1210 cell viability by 50% (IC_{50} values) after 4 days in culture are compared in Table 2. The cytotoxicities of *β*-rhodomycinone, the aglycone part of rhodomycin, and its 10-*O*-acetyl-*β*-rhodomycinone analog in L1210 cells at day 4 are characterized by IC_{50} values of 1.02 and 1.27 μM, respectively. Interestingly, four rhodomycin glycosides (**11**~**13** and **15**, IC_{50} values: 2.48~5.60 μM) are 2.0~5.5 times less effective than these aglycones, while **16** which is a relatively close analog of oxaunomycin, is about as effective as the above aglycones in decreasing tumor cell viability. The rhodomycin glycoside **14** (IC_{50} value: 0.52 μM) is the most active in this series, being 2.0~2.4 times more potent than the two aglycones. This preliminary study suggests that, even though the antitumor activity resides mostly with the aglycone part of rhodomycin, the bioactivity of such molecule can be substantially modulated by the nature of its glycoside substituent.

It is of interest to notice that the 10-*O*-glycosides **17** and **18**, possess a certain degree of activity, when compared to the other derivatives, even if they are regioisomers of the

Table 1. Carbon chemical shifts of compounds **1**~**4**.

Carbon No	**1**	**2**	**3**	**4**
1	119.8	119.6	119.6	119.6
2	137.6	137.0	137.1	137.1
3	124.9	125.0	124.8	124.8
4	162.7	162.7	162.6	162.5
4a	115.7	115.9	115.7	115.7
5	191.0	191.2	190.6	190.7
5a	111.6	111.8	112.1	111.6
6	156.0	155.7	155.1	155.9
6a	136.8	136.1	137.1	138.6
7	62.1	60.4	60.2	62.0
8	33..9	31.7	30.4	32.9
9	72.0	73.0	72.7	72..3
10	66.1	66..9	67.2	67.1
10a	136.5	137.1	137.2	133.8
11	157.3	157.0	157.4	157.4
11a	112.2	112.3	112.3	112.0
12	186.0	188.2	185.9	185.4
12a	133.2	133.9	133.9	133.2
13	30.7	30.6	29.2	29.6
14	6.39	6.45	6.50	6.47
1′	-	127.5	127.5	-
2′	-	133.9	133.9	-
3′	-	127.5	127.5	-
4′	-	130.9	131.1	-
5′	-	127.5	127.5	-
6′	-	133.9	133.9	-

Table 2. Comparison of the relative cytotoxicities of rhodomycin derivatives to that of daunomycin in the L1210 leukemic cell system *in vitro*.

Compound	IC_{50} (μM)
Daunomycin	0.019 ± 0.002
1	1.02 ± 0.08
4	1.27 ± 0.09
11	5.60 ± 0.47
12	3.85 ± 0.26
13	2.48 ± 0.13
14	0.52 ± 0.03
15	4.76 ± 0.28
16	1.16 ± 0.03
17	0.93 ± 0.06
18	1.01 ± 0.05

classical anthracyclines. Furthermore, **17** is approximately 2.5 times more potent compared to the corresponding 7-*O*-glycoside **13**. In conclusion, all the rhodomycinone glycosides reported here appear to be cytotoxic *in vitro*, but they are significantly less potent than daunomycin.

Acknowledgments

This study was supported by grants from the National Institutes of Health (National Cancer Institute # 1RO1CA86842-01 and Center of Biomedical Research Excellence # 1P20RR15563-01 with matching funds from the State of Kansas), BioServe Space Technologies (NASA # NAGW-1197), the Howard Hughes Medical Institute (Biological Sciences Education Grant), Kansas State University (Center for Basic Cancer Research) and the National Scholarship Foundation of Greece.

References

1) WEISS, R. B.; G. SAROSY, K. GLAGETT-CARR, M. RUSSO & B. LEYLAND-JONES: Anthracycline analogs: the past, present and future. Cancer Chemother. Pharmacol. 18: 185~187, 1986
2) GOTTESMAN, M. M.: How cancer cells evade chemotherapy: sixteenth Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res. 53: 747~754, 1993
3) KAYE, S. B.: The multidrug resistance phenotype. Br. J. Cancer 58: 691~694, 1988
4) ARCAMONE, F.: New antitumor anthracyclines. Lloydia 40: 45~66, 1977
5) ARCAMONE F., *In* Anticancer Agents Based on Natural product Models, *Ed.*, CASSIDY J. M., DOUROS, J. D., New York, 1980, p. 1
6) YOSHIMOTO, A.; S. FUJII, O. JOHDO, K. KUBO, T. ISHIKURA, H. NAGANAWA, T. SAWA, T. TAKEUCHI & H. UMAZAWA: Intensely potent anthracycline antibiotic oxaunomycin produced by a blocked mutant of the daunorubicin-producing microorganism. J. Antibiotics 39: 902~909, 1986
7) THOMSON, R. H.: Naturally occuring quinones III: recent advances. New York, Chapman and Hall, 1987
8) MOORE, H. W.: Bioactivation as a model for drug design bioreductive alkylation. Science 197: 527~532, 1977
9) LIN, A. J.; L. A. COSBY, C. W. SHANSKY & A. C. SARTORELLI: Potential bioreductive alkylating agents. 1. Benzoquinone derivatives. J. Med. Chem. 15: 1247~1252, 1972
10) LIN, A. J.; R. S. PARDINI, L. A. COSBY, B. J. LILLIS, C. W. SHANSKY & A. C. SARTORELLI: Potential bioreductive alkylating agents. 2. Antitumor effect and biochemical studies of naphthoquinone derivatives. *J.* Med. Chem. 16: 1268~1271, 1973
11) BRUNMARK, A. & E. CADENAS: Redox and addition chemistry of quinoid compounds and its biological implications. Free Radical Biol. Med. 7: 435~477, 1989
12) O'BRIEN, P. J.: Molecular mechanisms of quinone cytotoxicity. Chem.-Biol. Interact 80: 1~41, 1991
13) MONKS, T. J.; R. P. HANZLIK, G. M. COHEN, D. ROSS & D. G. GRAHAM: Quinone chemistry and toxicity. Toxicol. Appl. Pharmacol. 112: 2~16, 1992
14) MYERS, C. E. & B. A. CHABNER: Anthracyclines. *In* Cancer chemotherapy: principles and practice. *Ed.*, CHABNER B. A., COLLINS J. M., Philadelphia: Lippincott, 1990, pp. 356~381
15) BEGLEITER, A.; M. K. LEITH, G. MCCLARTY, S. BEENKEN, G. J. GOLDENBERG & J. A. WRIGHT: Characterization of L5178Y murine lymphoblasts resistant to quinone antitumor agents. Cancer Res. 48: 1727~1735, 1988
16) QIU, X. B.; A. H. SCHONTHAL & E. CADENAS: Anticancer quinones induce pRb-preventable G_2/M cell cycle arrest and apoptosis. Free Radical Biol. Med. 24: 848~854, 1998
17) ROSSI, L.; G. A. MOORE, S. ORRENIUS & P. J. O'Brien: Quinone toxicity in hepatocytes without oxidative stress. Arch. Biochem. Biophys. 251: 25~35, 1986
18) LIU, L. F.: DNA topoisomerase poisons as antitumor drugs. Ann. Rev. Biochem. 58: 351~375, 1989
19) MIMNAUGH, E. G.; K. A. KENNEDY, M. A. TRUSH & B. K. SINHA: Adriamycin-enhanced membrane lipid peroxidation in isolated rat nuclei. Cancer Res. 45: 3296~3304, 1985
20) GANAPATHI, R.; D. GRABOWSKI, G. HOELTGE & R. NEELON: Modulation of doxorubicin-induced chromosomal damage by calmodulin inhibitors and its relationship to cytotoxicity in progressively doxorubicin-resistant tumor cells. Biochem. Pharmacol. 40: 1657~1662, 1990
21) LING, Y.-H.; W. PRIEBE & R. PEREZ-SOLER: Apoptosis induced by anthracycline antibiotics in P388 parent and multidrug-resistant cells. Cancer Res. 53: 1845~1852, 1993
22) RAMACHANDRAN, C.; W. YOU & A. KRISHAN: *Bcl-2* and *mdr-1* gene expression during doxorubicin-induced

apoptosis in murine leukemic P388 and P388/R84 cells. Anticancer Res. 17: 3369~3376, 1997

23) ALIGIANNIS, N.; N. POULI, P. MARAKOS, A. L. SKALTSOUNIS, S. LEONCE, A. PIERRE & G. ATASSI: Synthesis and biological activity of 4′-azido- and 4′-trifluoracetamido-3′-chloro-3′-deamino-4′-deoxydaunorubicin. Bioorganic Medicinal Chem. Lett. 6: 2473~2476, 1996

24) KITA, Y.; H. MAEDA, M. KIRIHARA, Y. FUJII, T. NAKAJIMA, H. YAMAMOTO, Y. TAMURA & H. FUJIOKA: Synthesis of a potent rhodomycin, oxaunomycin, and its analogs. Chem. Pharm. Bull. 40: 61~65, 1992

25) COLEY, H. M.; P. R. TWENTYMAN & P. WORKAN: Further examination of 9-alkyl- and sugar-modified anthracyclines in the circumvention of multidrug resistance. Anti-Cancer Drug. Design 7: 471~481, 1992

26) PERCHELLET, E. M.; J. B. LADESICH, M. J. MAGILL, Y. CHEN, D. H. HUA & J. P. PERCHELLET: Tricyclic pyrone analogs: a new synthetic class of bifunctional anticancer drugs that inhibit nucleoside transport, microtubule assembly, the viability of leukemic cells in vitro and the growth of solid tumors *in vivo*. Anti-Cancer Drugs 10: 489~504, 1999

27) PERCHELLET, E. M.; M. J. MAGILL, X. HUANG, C. E. BRANTIS, D. H. HUA & J. P. PERCHELLET: Triptycenes: a novel synthetic class of bifunctional anticancer drugs that inhibit nucleoside transport, induce DNA cleavage and decrease the viability of leukemic cells in the nanomolar range *in vitro*. Anti-Cancer Drugs 10: 749~766, 1999

28) PERCHELLET, E. M.; M. J. MAGILL, X. HUANG, D. M. DALKE, D. H. HUA & J. P. PERCHELLET: 1,4-Anthraquinone: an anticancer drug that blocks nucleoside transport, inhibits macromolecule synthesis, induces DNA fragmentation and decreases the growth and viability of L1210 leukemic cells in the same nanomolar range as daunorubicin *in vitro*. Anti-Cancer Drugs 2000: in press

29) CORY, A. H.; T. C. OWEN, J. A. BARLTROP & J. G. CORY: Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. Cancer Commun. 3: 207~212, 1991

30) KITA, Y.; H. MAEDA, M. KIRIHARA, Y. FUJII, T. NAKAJIMA, H. YAMAMOTO, Y. TAMURA & H. FUJIOKA: The first total synthesis of a potent β-rhodomycin, oxaunomycin: regioselective glycosidation of the C-7 hydroxyl group of β-rhodomycinone. Tetrahedron Lett. 31: 7173~7174, 1990

31) BERTOUNESQUE, E.; J. C. FLORENT & C. MONNERET: Total synthesis of 4-demethoxyfeudomycinone C. Synthesis 270~272, 1991

32) MITAKU, S.; A. L. SKALTSOUNIS, F. TILLEQUIN & M. KOCH: Synthesis and glycosidation reactions of 3-halo-2,3,6-trideoxy-L-*arabino*-hexopyranoses. Synthesis 1068~1070, 1992

33) FLORENT, J. C. & C. MONNERET: Stereocontrolled route to 3-amino-2,3,6-trideoxy-hexopyranosides. K-10 Montmorillonite as a glycosidation reagent for acosaminide synthesis. J. Chem. Soc. Chem. Commun. 1171~1172, 1987

34) KOLAR, C.; K. DEHMEL, H. MOLDENHAUER & M. GERKEN: Semisynthetic β-rhodomycins: A new approach to the synthesis of 4-*O*-methyl-β-rhodomycins. J. Carbohydr. Chem. 9: 873~890, 1990

35) BROWN, J. M.: NCI's anticancer drug screening program may not be selecting for clinically active compounds. Oncology Res. 9: 213~215, 1997