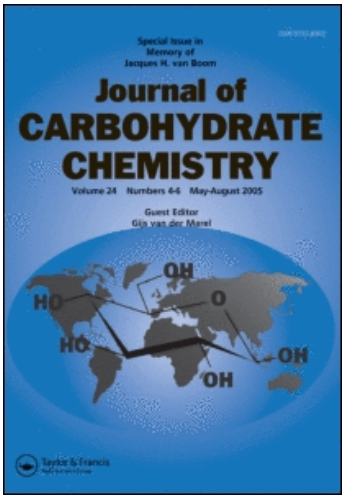

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PHOTOLYTIC N-MONO-DEMETHYLATION OF RHODOSAMINYLANTHRACYCLINONE TYPE ANTHRACYCLINES[1]

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ABSTRACT

It was found that rhodosaminylanthraclinone-type anthracyclines are readily N-mono-demethylated upon irradiation with visible light, leading to 3'-N-methyl-α-L-daunosaminylanthracyclinones in good yields. The reaction is preferentially observed with rhodosaminylanthracyclinones in which OH-4' of the sugar (rhodosamine) moiety is not further glycosylated. 3'-N-methyl-α-L-daunosaminyl anthracyclinones provide key compounds that can readily be further derivatized.

INTRODUCTION

Anthracycline antibiotics play a major role in the effective treatment of a number of neoplastic diseases and present one of the most investigated classes of antitumor agents.[2] Of all chemotherapeutic agents used today, doxorubicin (adriamycin) has the widest spectrum of antitumor activity. It is used with a high degree of efficacy in many human cancers and is probably the most utilized antitumor drug worldwide.[3]

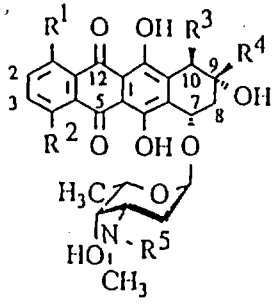
Doxorubicin and its 14-deoxy-analogue, daunorubicin, both carrying a daunosamine moiety at C-7 of their respective aglycone, adriamycinone and daunomycinone, are known to be susceptible to photolytic reactions,[4-8] resulting in breakdown of the naphthacenequinone nucleus[5] and the building up of polymers.[7]

Two other anthracyclines, each carrying the trisaccharide, α-L-cinerulosyl-(1→4)-2-deoxy-α-L-fucosyl-(1→4)-α-L-rhodosaminyl, at C-7 of their respective aglycone (aklavinone[9] and daunomycinone[10]), were found to undergo photolytic reactions. In these two cases, however, a photocatalyzed N-demethylation reaction was observed, which lead to the corresponding N-mono-demethylated and N-di-demethylated derivatives, although only at very low yields.[9,10]

We have now found that rhodosaminyl anthracyclinone type anthracyclines can most readily be N-mono-demethylated by irradiation with visible light, provided their sugar (rhodosamine) moiety is not further glycosylated. 3'-N-methyl-α-L-daunosaminylanthracyclinones provide key compounds that can readily be further derivatized to gain new cytostatic compounds.[11]

RESULTS AND DISCUSSION

Photolytic N-mono-demethylation of rhodosaminylanthracyclinone type anthracyclines appears as a reaction of



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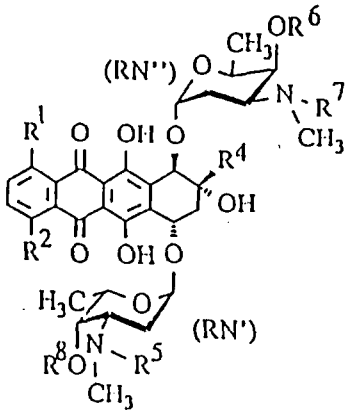

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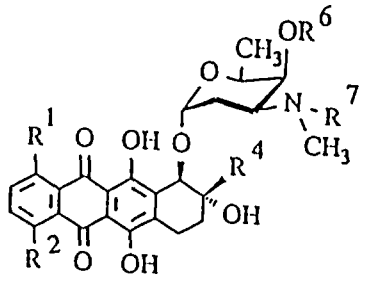

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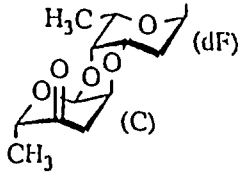

IV

		R^1	R^2	R^3	R^4	R^5 a	R^5 b	R^6	R^7 a	R^7 b	R^8	aglycone
I	1a,b	H	OH	OH	CH_2CH_3	CH_3	H	-	-	-	-	β-RMN
I	2a,b	OH	OH	$COOCH_3$	CH_2CH_3	CH_3	H	-	-	-	-	ϵ-isoRMN
I	3a,b	OH	OH	OH	CH_2CH_3	CH_3	H	-	-	-	-	β-isoRMN
I	4a,b	H	OCH_3	H	$COCH_3$	CH_3	H	-	-	-	-	DMN
I	5a,b	H	OCH_3	H	$CHOHCH_3$	CH_3	H	-	-	-	-	13-dihydro-DMN
II	6a,b	H	OH	-	CH_2CH_3	CH_3	H	H	CH_3	H	H	β-RMN
II	7a,b	H	OH	-	CH_2CH_3	CH_3	CH_3	H	CH_3	H	IV	β-RMN
II	8a,b	H	OH	-	CH_2CH_3	CH_3	H	IV	CH_3	CH_3	H	β-RMN
III	9a,b	H	OH	-	CH_2CH_3	-	-	H	CH_3	H	-	γ-RMN
III	10	H	OH	-	CH_2CH_3	-	-	IV	CH_3		-	γ-RMN
I	11	H	OH	OH	CH_2CH_3	CHO		-	-	-	-	β-RMN

wide validity and practicability, leading to a new class of cytostatically active anthracyclines, the 3'-N-methyl-α-L-daunosaminyl anthracyclinones. Although such kind of reaction has earlier been described in the literature for two anthracyclines carrying the trisaccharide α-L-cinerulosyl-(1→4)-2-deoxy-α-L-fucosyl-(1→4)-α-L-rhodosaminyl at C-7 of their respective aglycone, i.e. aklavinone[9] and daunomycinone,[10] its further investigation has been neglected, most likely because of the very low yields achieved.

When originally working with β-rhodomycin-I[12] 1a (also known as rhodomycin B[13,14] or betaclamycin T[15]), and 7-O-α-L-rhodosaminyl-ε-isorhodomycinone[16] 2a, we recognized that both compounds, when dissolved in chloroform, slowly decomposed during standing on the shelf. After finding out that the decomposition was due to exposure to visible light, we have focused on the isolation of the decomposition products, which were subsequently identified as the corresponding N-mono-demethylated derivatives, 1b and 2b, respectively.

In order to evaluate the reaction on a broader base, three additional anthracyclines were synthesized and tested: β-isorhodomycin-I (3a) was gained by acid hydrolysis of β-isorhodomycin-II[13] (6a); N,N-dimethyldaunomycin (4a) was synthesized from daunorubicin by reaction with formaldehyde in presence of sodium cyanoborohydride;[17] and N,N-dimethyldaunomycin-13-ol (5a) was isolated as a side-product of the latter reaction.[17]

The 7-O-α-L-rhodosaminyl anthracyclinones 1a-3a were smoothly N-mono-demethylated, providing products 1b-3b in good yields. Compounds 4a and 5a were also demethylated, although requiring longer irradiation times. In these two latter experiments yields were lower, which may be due to breakdown of the naphthacenequinone nucleus.[5] Also it was apparent that differences in the aglycone influence the demethylation rate.

When two rhodosamine moieties were present at C-7 and C-10 of the aglycone, as in the case of β-rhodomycin-II[13] (6a), both rhodosamine units were efficiently N-mono-demethylated, leading to derivative 6b in good yield.

Surprisingly, however, if one of the two rhodosamine units of 6a was carrying another sugar moiety at OH-4', N-demethylation was hampered at the rhodosamine unit carrying the additional sugar moiety. Thus rodorubicin 7a (formerly called cytorhodin S[18]), a new tetraglycosidic anthracycline that has entered clinical trials,[19] was smoothly N-mono-demethylated at the rhodosamine unit located at C-10 of the aglycone, β-rhodomycinone, leading to 7b. And similarly, the rodorubicin-isomer 8a, called cytorhodin T,[18] was again N-mono-demethylated at the free rhodosamine unit located at C-7 of the aglycone, leading to 8b.

The structure of 7b was unequivocally ascertained by hydrogenolysis,[13] leading to 10-O-(3'-N-methyl-α-L-daunosaminyl)-γ-rhodomycinone (9b), which was alternatively gained from 9a (γ-rhodomycin-I[13] or iremycin[20]) by photolytic N-mono-demethylation. As the resonances of protons of the rhodosaminyl units of 7a at both C-7 and C-10 have unequivocally been assigned by derivatization experiments,[12] the regioselective preference of the N-demethylation reaction could also be ascertained by 400 MHz ^{1}H NMR spectroscopy. While the shifts of the two dimethylamino groups of 7a were assigned to be 2.14 ppm for C-7-rhodosaminyl and to 2.20 ppm for C-10-rhodosaminyl, for 7b the shifts were determined to be 2.14 ppm and 2.34 ppm, respectively, demonstrating a downfield shift of 0.14 ppm for the methylamino group of the C-10-sugar unit. By analogy, N-mono-demethylation of iremycin (9a), exhibiting the signal of the dimethylamino group at 2.26 ppm, led to 9b which provided the N-CH_3 signal at 2.40 ppm, again accounting for a shift difference of 0.14 ppm.

The regioselective preference of the N-demethylation reaction of 8a, leading to 8b, was ascertained similarly.

While the shifts of the two dimethylamino groups of 8a were assigned to 2.17 ppm for C-7-rhodosaminyl and to 2.12 ppm for C-10-rhodosaminyl, for 8b the shifts were determined to be 2.31 ppm and 2.12 ppm, respectively, demonstrating a similar downfield shift of 0.14 ppm for the methylamino group of the C-7-rhodosaminyl unit. In each of the cases described, N-mono-demethylation was accompanied by the same downfield shift of the $N-CH_3$ signal of about 0.14 ppm relative to the signal of the corresponding $N(CH_3)_2$ group of the corresponding educt.

The structure of 8b was also ascertained by hydrogenolytic degradation of 8b to 10, a compound first described by Uchida et al.[21]

Comparing N-demethylation of the red anthracycline 1a and the violet 1-OH-analogue, 3a, it was obvious that demethylation of 3a was much faster, probably due to its absorption at longer wave lengths.

Interestingly, our original attempts to achieve complete N-demethylation by prolonged irradiation of β-rhodomycin-I (1a) to 7-O-α-L-daunosaminyl-β-rhodomycinone[22] in chloroform or chloroform/methanol as a solvent were unsuccessful.[23] Instead, 7-O-(3'-N-formyl-3'-N-methyl-α-L-daunosaminyl)-β-rhodomycinone (11) and β-rhodomycinone aglycone were identified as side products. The formyl derivative was characterized by FAB mass spectrometry ($M+H^+$, m/z = 558) and 400 MHz ^1H NMR spectroscopy, providing doubling of most of the signals due to the presence of rotational isomers of the N-formyl moiety. For example, two signals for $N-CH_3$ were observed at 2.92 and 3.00 ppm, and two signals were observed for the formyl proton at 8.01 and 8.12 ppm. Compounds of the type $R-CO-NR^1R^2$ give rise to two diastereoisomers with different populations that are separated by their rotational barrier. As the free energy of the two isomers is different, different signals are observed in the NMR spectrum,[24] while at elevated temperature the rotational barrier of the C-N bond collapses at the coalescence point. Indeed, the NMR

spectrum of 11, measured in DMSO at 400 MHz, revealed a coalescence temperature of 11 of about 150°C, which is in the normal range for asymmetric formamides.[24] At 175°C an NMR spectrum with very sharp signals was obtained, whereas at 180°C decomposition of 11 was observed.

The formation of N-formyl derivative 11 is most likely due to decomposition of chloroform, resulting in the appearance of dichlorocarbene. Dichlorocarbene, as an electrophile, is subsequently caught by the methylamino function of 1b, and the C-Cl bonds are hydrolyzed due to the presence of traces of water. Indeed, acidification was observed during the course of the reaction, which facilitates and better explains the cleavage of the glycosidic bond, resulting in the liberation of β-rhodomycinone. When chloroform was substituted by dichloromethane or dichloroethane (in a mixture with methanol), the formation of the formyl derivative was indeed suppressed. Although, under these conditions, the N-di-demethylated compound could be identified by thin layer chromatography, its formation was dramatically hampered, and its isolation from the reaction mixture was tedious. Even when the pure N-mono-demethylated derivative 1b was used for further photolytic demethylation, 7-O-α-L-daunosaminyl-β-rhodomycinone was isolated only in low yields. As decolorisation was observed during the reaction, decomposition reactions similar to the ones described for daunorubicin and doxorubicin[4-8] may be anticipated.

Interestingly, in all cases where N-di-demethylated product could unequivocally be identified, i.e., during photolysis of 1a, 3a, and 9a, it was found that the R_F values of the compounds using solvent systems A-C, increased in the order: starting material a < N-di-demethylated product < N-mono-demethylated product b, which may closely relate to the lipophilicity of the drugs.

Further research is required and currently going on in order to elucidate the mechanism of the photolytic N-demethylation reaction in more detail and to evaluate

these anthracyclines as well as a large number of newly gained N-substituted derivatives[11] as inhibitors of tumor growth in vitro and in vivo.

EXPERIMENTAL

General Procedures. ^{1}H NMR spectra were recorded, if not stated otherwise, at 300 MHz or 400 MHz with a Bruker AC-300 or a Bruker AM-400 NMR spectrometer, respectively, using deuterated chloroform or deuterated dimethylsulfoxide or a mixture of both as solvent and tetramethylsilane as internal standard. Phenolic OH-groups of the aglycone were assigned according to Vigevani et al.[25] Reactions were monitored by thin layer chromatography on silica gel 60 plates F 254 (Merck), and spots were determined by their inherent color or by ultraviolet light. Preparative chromatography was performed on silica gel of diameter 20-45 µm (Amicon) or 0.063 mm - 0.200 mm (Merck), if not stated otherwise. The solvent systems used are summarized in Table 1.

After column chromatography the fractions containing the desired product(s) were neutralized by the addition of a saturated aqueous solution of sodium bicarbonate, and the products were extracted with chloroform, thus providing the anthracyclines each as a free base. Evaporations were conducted in vacuo. Specific rotations were determined with a Perkin Elmer Polarimeter-241 in glacial acetic acid as the solvent of choice.

The mass spectra were recorded with a MS-902S, AEI, mass spectrometer using a FAB ion source and 3-nitrobenzyl alcohol as matrix.

Small scale photolysis was performed in Petri dishes of diameter 19 cm, using a mixture of chloroform/methanol, dichloromethane/methanol or dichloroethane/methanol (20/1 (v/v)), each, as solvent system. The Petri dishes were placed on a reflecting underlay and irradiated with one or

Table 1. Solvent System Used for TLC Assays

solvent system composition (%) (v/v)	A	B	C
Chloroform	70	89	77
Methanol	18	7.4	14
Acetic acid	8.5	3	7
Water	3.5	0.6	2

two 500 watt photographic bulbs while gently stirring, at a distance of about 25 cm, and the reactions were followed by thin layer chromatography.

For large scale photolysis, a special apparatus was developed, allowing, for example, demethylation of 1a in a 3-5 g quantity within a few hours, using a mercury diving-lamp (TQ 150 Z2, Hanau).[26]

Yields are not optimized.

7-O-(3'-N-Methyl-α-L-daunosaminyl)-β-rhodomycinone (1b). A solution of 500 mg = 0.92 mmol of 7-O-α-L-rhodosaminyl-β-rhodomycinone (1a) in a mixture of chloroform (1200 mL) and methanol (50 mL) was distributed over 8 Petri dishes of diameter 19 cm and irradiated on a reflecting underlay for 5 h with two 500 watt emitters, stirring gently, at a distance of about 25 cm, following the reaction by thin layer chromatography. The solutions were then combined, and the solvent was removed on a rotary evaporator. The residue was dissolved in the minimum amount of methanol, water was added, the pH was adjusted to 4 with 1% hydrochloric acid, and the mixture was extracted several times with chloroform, during which the desired product remained in the aqueous phase. This "chloroform phase I" was concentrated and further processed as described for the isolation of formyl derivative 11. The aqueous phase was adjusted to pH 7-8 with a saturated aqueous solution of sodium bicarbonate, and the

desired product was extracted with chloroform. The chloroform extracts were concentrated to dryness, and the remainder was purified by column chromatography (solvent system C) to give, after neutralization by sodium bicarbonate and extraction into chloroform, 264 mg (54 %) of compound 1b as an amorphous solid: $[\alpha]_D^{25}$ 301° (c 0.1, acetic acid). 300 MHz ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, $J_{13,Me-4}$ = 7.4 Hz, Me-14), 1.40 (d, 3H, $J_{5',Me-6'}$ = 6.6 Hz, Me-6'), 1.6-1.9 (m, 4H, CH_2-13 and CH_2-2'), 2.12 (dd, 1H, $J_{8a,8b}$ = 15 Hz, $J_{7,8a}$ = 4 Hz, H-8a), 2.27 (d, 1H, $J_{8a,8b}$ = 15 Hz, H-8b), 2.34 (s, 3H, N-CH_3), 2.71 (m, 1H, H-3'), 3.62 (bs, 1H, H-4'), 4.04 (s, 1H, OH-9), 4.11 (q, 1H, $J_{5',Me-6'}$ = 6.5 Hz, H-5'), 4.91 (s, 1H, H-10), 5.15 (m, 1H, H-7), 5.46 (bd, 1H, $J_{1',2'}$ = 3 Hz, H-1'), 7.32 (dd, 1H, $J_{2,3}$ = 8.3 Hz, $J_{1,3}$ = 1 Hz, H-3), 7.72 (t, 1H, $J_{1,2} = J_{2,3}$ = 8 Hz, H-2), 7.88 (dd, 1H, $J_{1,2}$ = 7.5 Hz, $J_{1,3}$ = 1 Hz, H-1). FAB-MS, m/z = 530 ($M+H^+$).

7-O-(3'-N-Methyl-α-L-daunosaminyl)-ε-isorhodomycinone (2b). 62 mg (0.10 mmol) of 7-O-α-L-rhodosaminyl-ε-isorhodomycinone (2a) were demethylated in analogy to 1a, and the reaction was followed by thin layer chromatography. Once the starting compound had been consumed, the solvent was removed on a rotary evaporator, and the reaction product was subjected to repeated column chromatography (10 g of silica gel 60 for HPLC, 25-40 μm, Merck; mobile phase: dichloromethane/methanol/water (80/8/1)). Residues of silica gel were removed by extracting the purified solid product several times with chloroform, recovering 2b as an amorphous solid in 27 mg (46 %) yield. $[\alpha]_D^{25}$ 310° (c 0.025, acetic acid). 270 MHz ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, $J_{13,\ Me-14}$ = 7.4 Hz, Me-14), 1.37 (d, 3H, $J_{5',\ Me-6'}$ = 6.5 Hz, Me-6'), 1.3-1.55 (m, 1H, H-13a), 1.7-1.95 (m, 3H, CH_2-2' and H-13b), 2.23 (dd, 1H, $J_{8a,8b}$ = 14.5 Hz, $J_{7,8a}$ = 4.2 Hz, H-8a), 2.37 (d, 1H, $J_{8a,8b}$ = 14.5 Hz, H-8b), 2.39 (s, 3H, N-CH_3), 2.82 (bd, 1H, $J_{2',3'}$ = 10 Hz, H-3'), 3.68 (bs, 1H, H-4'), 3.72 (s, 3H, $COOCH_3$), 4.10 (q, 1H, J

$J_{5',Me\text{-}6'}$ = 6.5 Hz, H-5'), 4.28 (s, 1H, H-10), 4.43 (bs, 1H, OH-9), 5.25 (m, 1H, H-7), 5.49 (bs, 1H, H-1'), 7.30 (s, 2H, H-2 and H-3). FAB-MS, m/z = 588 ($M+H^+$).

7-O-(3'-N-Methyl-α-L-daunosaminyl)-β-isorhodomycinone (3b). 280 mg (0.50 mmol) of 7-O-α-L-rhodosaminyl-β-isorhodomycinone were dissolved in 60 mL of methanol, 1000 mL of chloroform were added, and the solution was irradiated in analogy to 1a, however, only for 90 min. The solvent was then removed on a rotary evaporator, the residue was taken up in a little methanol, the pH was adjusted to 1 with 1 % hydrochloric acid, and the mixture was diluted to about 400 mL with water and extracted several times with chloroform, during which the desired product remained in the aqueous phase. Finally, the pH was adjusted to 7 with a saturated aqueous solution of sodium bicarbonate, and the desired product was extracted with chloroform, providing 3b as an amorphous solid in 234 mg (86 %) yield. $[\alpha]_D^{25}$ (c 0.1, acetic acid). 300 MHz ^{1}H NMR ($CDCl_3$/D_6-DMSO) δ 1.08 (t, 3H, $J_{13,\ Me\text{-}14}$ = 7 Hz, Me-14), 1.32 (d, 3H, $J_{5',\ Me\text{-}6'}$ = 6.3 Hz, Me-6'), 1.7-1.9 (m, 4H, CH_2-13 and CH_2-2'), 2.15 (bs, 2H, CH_2-8), 2.34 (s, 3H, N-CH_3), 2.70 (m, 1H, H-3'), 3.64 (bs, 1H, H-4'), 3.86 (bs, 1H, OH-9), 4.10 (q, 1H, $J_{5',Me\text{-}6'}$ = 6 Hz, H-5'), 4.68 (s, 1H, H-10), 5.02 (m, 1H, H-7), 5.36 (bs, 1H, H-1'), 7.17 (s, 2H, H-2 and H-3). FAB-MS, m/z = 546 ($M+H^+$).

7-O-(3'-N-Methyl-α-L-daunosaminyl)-daunomycinone (3'-N-methyl-daunomycin) (4b). 95 mg (0.17 mmol) of N,N-dimethyldaunomycin (4a) were demethylated in analogy to 1a, irradiating, however, for about 12 h, and following the reaction by TLC. The solvent was then evaporated, and the remainder was purified by column chromatography, using solvent system A as mobile phase, which gave 4b as an amorphous solid in 31 % yield (29 mg). $[\alpha]_D^{25}$ 358° (c 0.1, acetic acid). 400 MHz ^{1}H NMR ($CDCl_3$) δ 1.37 (d, 3H, $J_{5',Me\text{-}6'}$ = 6.6 Hz, H-5'), 1.6-1.8 (m, 2H, H-2'), 2.10 (dd, 1H, $J_{8a,8b}$ = 14.5 Hz, $J_{7,8a}$ = 4 Hz, H-8a), 2.36 (s, 3H, N-CH_3),[27] 2.41 (s, 3H, Me-14),[27] 2.73 (m, 1H, H-3'),

2.97 (d, 1H, $J_{10a,10b}$ = 19 Hz, H-10a), 3.22 (d, 1H, $J_{10a,10b}$ = 19 Hz, H-10b), 3.62 (bs, 1H, H-4'), 4.07 (s, 3H, OCH_3),[28] 5.29 (m, 1H, H-7), 5.50 (bs, 1H, H-1'), 7.38 (d, 1H, $J_{2,3}$ = 8.5 Hz, H-3), 7.77 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 8 Hz, H-2), 8.02 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1). FAB-MS, m/z = 542 ($M+H^+$).

7-O-(3'-N-Methyl-α-L-daunosaminyl)-daunomycinone-13-ol (3'-N-methyl-13-dihydrodaunorubicin) (5b). 50 mg (0.09 mmol) of N,N-dimethyl-13-dihydrodaunorubicin (5a) were demethylated in analogy to 4a, providing 5b as an amorphous solid in 13 % yield (6.5 mg). 400 MHz ^1H NMR ($CDCl_3$)[29] δ 1.33 (d, $J_{13,Me-14}$ = 6.3 Hz, Me-14), 1.38 (d, $J_{5',Me-6'}$ = 6.6 Hz, Me-6'), 1.6-1.9 (m, CH_2-2'), 2.35 (s, N-CH_3), 2.60 (d, $J_{10a,10b}$ = 19 Hz, H-10a), 3.19 (d, $J_{10a,10b}$ = 19 Hz, H-10b), 3.61 (bs, H-4'), 3.69 (q, $J_{5',Me-6'}$ = 6.4 Hz, H-5'), 4.08 (s, OCH_3), 5.30 (m, H-7), 5.52 (bs, H-1'), 7.38 (d, $J_{2,3}$ = 8.4 Hz, H-3), 7.77 (t, $J_{1,2}$ = $J_{2,3}$ = 8 Hz, H-2), 8.03 (dd, $J_{1,2}$ = 7.7 Hz, $J_{1,3}$ = 1 Hz, H-1). FAB-MS, m/z = 544 ($M+H^+$).

7,10-O-Bis-(3'-N-Methyl-α-L-daunosaminyl)-β-rhodomycinone (6b). 120 mg (0.17 mmol) of 7,10-O-bis-α-L-rhodosaminyl-β-rhodomycinone (6a) were irradiated for 3 h in analogy to 1a. The solvent was then removed on a rotary evaporator. The residue was taken up in a little methanol, water was added, and the mixture was extracted with chloroform at pH 2, 4, 6 and 8 successively. The product in the chloroform extracts obtained at pH 6 and 8 was chromatographed on a silica gel column, using solvent system A as mobile phase. After renewed extraction by shaking with aqueous sodium bicarbonate/chloroform, 6b was isolated as an amorphous solid in 59 % yield (66 mg). $[\alpha]_D^{25}$ 358° (c 0.1, acetic acid). 400 MHz ^1H NMR ($CDCl_3$)[30,31] δ 1.12 (t, 3H, $J_{13,Me-14}$ = 7.4 Hz, Me-14), 1.33 (d, 3H, $J_{5'',Me-6''}$ = 6.6 Hz, Me-6''), 1.39 (d, 3H, $J_{5',Me-6'}$ = 6.6 Hz, Me-6'), 1.63 (m, 1H, H-13a), 1.74 (m, 1H, H-13b), 1.7-1.9 (m, CH_2-2' and CH_2-2''), 2.25 (m, CH_2-8), 2.33 and 2.34 (s, 3H, N-CH_3, each), 2.65-2.73 (m, 2H, H-3' and

H-3''), 3.57 (bs, 1H, H-4''), 3.60 (bs, 1H, H-4'), 3.65 (bs, 1H, OH-9), 3.93 (q, 1H, H-5''), 4.07 (q, 1H, H-5'), 4.99 (s, 1H, H-10), 5.51 (m, 1H, H-7), 5.41 (bd, 1H, $J_{1'',2''}$ = 3.6 Hz, H-1''), 5.44 (bd, 1H, $J_{1',2''}$ = 3.7 Hz, H-1'), 7.32 (dd, 1H, $J_{2,3}$ = 8.4 Hz, $J_{1,3}$ = 1 Hz, H-3), 7.72 (t, 1H, $J_{1,2} = J_{2,3}$ = 8 Hz, H-2), 7.91 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{1,3}$ = 1 Hz, H-1). FAB-MS, m/z = 673 ($M+H^+$).

3'-N-Mono-demethylated rodorubicin (7b). 400 mg (0.425 mmol) of rodorubicin (7a) were photolyzed in analogy to 1a. Once 7a was no longer detectable by TLC, the solvent was evaporated, and the remainder was purified by column chromatography, using solvent system A as mobile phase. The combined fractions containing 7b were neutralized by the addition of 15 % aqueous ammonia prior to extraction and work up as described for 1b, providing 7b as an amorphous solid in 21 % yield (84 mg).[32] $[\alpha]_D^{25}$ 133° (c 0.1, acetic acid). 300 MHz ^{1}H NMR ($CDCl_3$)[33] δ 1.11 (t, 3H, $J_{13,Me-14}$ = 7.4 Hz, Me-14), 1.23, 1.28 1.34 and 1.36 (d, 3H, J = 6.5-7 Hz, each, Me-6'(dF'), Me-6'(C'), Me-6' (RN') and Me-6''(RN'')), 2.14 (s, 6H, $N(CH_3)_2$ (RN')), 2.34 (s, 3H, N-CH_3 (RN'')), 3.57 (bs, 1H, H-4'' (RN'')), 3.76 (bs, 2H, H-4' (RN') and H-4' (dF')), 3.92 and 4.01 (q, 1H, J = 6.5 Hz, each, H-5'(RN') and H-5''(RN'')), 4.66 (q, 1H, $J_{5',Me-6'}$ = 6.5 Hz, H-5' (dF')), 4.79 (q, 1H, $J_{5',Me-6'}$ = 6.5 Hz, H-5'(C')), 4.98 (s, 1H, H-10), 5.11 (bm, 2H, H-1' (dF') and H-7), 5.20 (d, 1H, $J_{1',2'}$ = 3 Hz, H-1'(C')), 5.41 (bd, 1H, $J_{1'',2''}$ = 3Hz, H-1'' (RN'')), 5.46 (bs, 1H, H-1' (RN'), 7.33 (dd, 1H, $J_{2,3}$ = 8.4 Hz, $J_{1,3}$ = 1 Hz, H-3), 7.73 (t, 1H, $J_{1,2} = J_{2,3}$ = 8 Hz, H-2), 7.91 (dd, 1H, $J_{1,2}$ = 7.5 Hz, $J_{1,3}$ = 1 Hz, H-1). FAB-MS, m/z = 927 ($M+H^+$).

3'-N-Mono-demethylated cytorhodin T (8b). 50 mg (0.053 mmol) of cytorhodin T (8a) were photolyzed in analogy to 7a, providing 8b as an amorphous solid in 16 mg (32 %) yield. 270 MHz ^{1}H NMR ($CDCl_3$)[34,35] δ 1.11 (t, 3H, $J_{13,Me-14}$ = 7.5 Hz, Me-14), 1.2-1.4 (Me-6' (RN') and Me-6'' of RN'', dF'' and C''), 2.12 (s, 6H, $N(CH_3)_2$

(RN''), 2.31 (s, 3H, N-CH_3 (RN')), 3.59 (bs, 1H, H-4' (RN')), 3.70 (bs, 1H, H-4'' (RN''), 3.85 (q, 1H, $J_{5'',Me-6''}$ = 6.5 Hz, H-5'' (RN'')), 3.96 (bs, 1H, H-4'' (dF''), 4.07 (q, 1H, $J_{5',Me-6'}$ = 6.5 Hz, H-5' (RN')), 4.62 (q, 1H, $J_{5'',Me-6''}$ = 6.5 Hz, H-5'' (dF''), 4.76 (q, 1H, $J_{5'',Me-6''}$ = 6.5 Hz, H-5'' (C'')), 5.02 (s, 1H, H-10), 5.09 (bs, 1H, H-1'' (dF''), 5.16 (bm, 2H, H-1'' (C'') and H-7), 5.41 (bm, 2H, H-1' (RN') and H-1'' (RN'')), 7.31 (d, 1H, $J_{2,3}$ = 8 Hz, H-3), 7.72 (t, 1H, $J_{1,2} = J_{2,3}$ = 8 Hz, H-2), 7.91 (d, 1H, $J_{1,2}$ = 8 Hz, H-1).

10-O-(3'-N-Methyl-α-L-daunosaminyl)-γ-rhodomycinone (9b). a) Preparation of 9b from 9a. Iremycin (9a) (51 mg, 0.10 mmol) was photolyzed in analogy to 1a, and 9b was isolated as described for the isolation of 1b, using solvent system C as eluant. Pure 9b was isolated as an amorphous solid in 5 mg yield; pure N-di-demethylated product was isolated in 3 mg yield; and a mixture of N-mono-demethylated product 9b and N-di-demethylated product was isolated in 25 mg yield. 9b: $[\alpha]_D^{25}$ 136° (c 0.1, acetic acid). 300 MHz ^{1}H NMR ($CDCl_3$) δ 1.09 (t, 3H, $J_{13,Me-14}$ = 7.4 Hz, Me-14), 1.34 (d, 3H, $J_{5'',Me-6''}$ = 6.6 Hz, Me-6''), 1.5-2.2 (m, 6H, CH_2-8, CH_2-13 and CH_2-2''), 2.40 (s, 3H, N-CH_3), 2.8-3.0 (m, 3H, CH_2-7 and H-3''), 3.68 (bs, 1H, H-4''), 3.99 (q, 1H, $J_{5'',Me-6''}$ = 6.5 Hz, H-5''), 4.94 (s, 1H, H-10), 5.38 (bd, 1H, $J_{1'',2''}$ = 3 Hz, H-1''), 7.30 (dd, 1H, $J_{2,3}$ = 8.3 Hz, $J_{1,3}$ = 1 Hz, H-3), 7.72 (t, 1H, $J_{1,2} = J_{2,3}$ = 8 Hz, H-2), 7.87 (dd, 1H, $J_{1,2}$ = 7.5 Hz, $J_{1,3}$ = 1 Hz, H-1). FAB-MS, m/z = 514 ($M+H^+$).

b) Preparation of 9b from 7b. 7b (15 mg, 0.016 mmol), dissolved in methanol (5 mL), was hydrogenated in the presence of 10 % palladium on charcoal (8 mg) at room temperature and atmospheric pressure for 30 min. The reaction mixture was filtered and the solvent evaporated. The residue was extracted (chloroform/water) into chloroform and purified by preparative TLC, using solvent system C, providing 9b as an amorphous solid in 3 mg yield. The NMR data were in agreement with those of product 9b, isolated according to procedure a.

7-Deoxy-7-derhodosaminyl-cytorhodin T (10). 8b (4 mg), dissolved in dry methanol (5 mL), containing glacial acetic acid (0.1 mL), was hydrogenated and the product isolated as described for the preparation of 9b from 7b, providing 10 as an amorphous solid in 1 mg yield. 270 MHz ^{1}H NMR ($CDCl_3$)[34,35] δ 1.07 (t, 3H, $J_{13,Me-14}$ = 7.5 Hz, Me-14), 1.2-1.35 (Me-6'' of RN'', dF'' and C''), 2.12 (s, 6H, $N(CH_3)_2$ (RN'')), 2.93 (bm, 2H, CH_2-7), 3.70 (bs, 1H, H-4'' (RN'')), 3.79 (bs, 1H, H-4'' (dF'')), 3.87 (q, 1H, $J_{5'',Me-6''}$ = 6.5 Hz, H-5'' (RN'')), 4.62 (q, 1H, $J_{5'',Me-6''}$ = 6.5 Hz, H-5'' (dF'')), 4.76 (q, 1H, $J_{5'',Me-6''}$ = 6.5 Hz, H-5'' (C'')), 4.96 (s, 1H, H-10), 5.08 (d, 1H, H-1'' (dF'')), 5.17 (d, 1H, H-1'' (C'')), 5.44 (d, 1H, H-1'' (RN''), 7.29 (d, 1H, $J_{2,3}$ = 8 Hz, H-3), 7.69 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 8 Hz, H-2), 7.89 (d, 1H, $J_{1,2}$ = 8 Hz, H-1).

7-O-(3'-N-Formyl-3'-N-methyl-α-L-daunosaminyl)-β-rhodomycinone (11). Various product mixtures of "chloroform phase I" obtained, as described during the preparation of 1b, were combined (390 mg) and separated on a silica gel column (diameter 3.5 cm, packed height 10 cm; mobile phase: dichloromethane/ methanol (19/1)) under slight pressure (with the aid of compressed air). The fractions containing pure substance of R_F 0.29 in dichloromethane/methanol (19/1) were collected, providing 11 as an amorphous solid (80 mg). 400 MHz ^{1}H NMR ($CDCl_3$) δ 1.12 and 1.13 (t, $J_{13,Me-14}$ = 7.4 Hz, Me-14, each), 1.30 and 1.32 (d, $J_{5',Me-6'}$ = 7 and 8 Hz, respectively, Me-6', each), 1.76 (m, H-13a and H-2'a), 1.86 (m, H-13b), 2.14 (m, H-8a), 2.25 (d, $J_{8a,8b}$ = 15 Hz, H-8b), 2.35-2.55 (m, H-2'b), 2.75 and 2.86 (d, OH, each), 2.92 and 3.00 (s, N-CH_3, each), 3.56 (bd, $J_{2',3'}$ = 14.3 Hz, H-3'), 3.66 and 3.75 (s, OH-9, each), 3.70 and 3.86 (d, $J_{4',OH-4'}$ = 7.1 and 5.1 Hz, respectively, H-4', each), 4.2-4.4 (m, H-5'), 4.90 and 4.92 (d, $J_{10,OH-10}$ = 3.8 and 4.3 Hz, respectively, H-10, each), 5.12 and 5.15 (m, H-7, each), 5.55 (bd, $J_{1',2'}$ = 3.7 Hz, H-1', each), 7.27 and 7.33 (d, $J_{2,3}$ = 8.4 Hz, H-3, each), 7.69 and 7.72 (t, $J_{1,2}$ = $J_{2,3}$ = 8.5

Hz, H-2, each), 7.85 and 7.88 (d, $J_{1,2}$ = 8 Hz, H-1, each), 8.01 and 8.12 (s, formyl-H, each), 12.06 and 12.08 (s, OH-4, each), 12.82 and 12.85 (s, OH-6, each), 13.52 and 13.58 (s, OH-11, each). FAB-MS, m/z = 558 ($M+H^+$).

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23. 7-O-α-L-daunosaminyl-β-rhodomycinone[22] was, however, obtained by photolytic N-demethylation of purified 1b, requiring longer irradiation times (P. Hermentin and E. Raab, unpublished results).

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27. The signal of H-8b is superimposed.

28. The signal of H-5' is superimposed.

29. The product consisted of a 3:1 epimeric mixture; communicated are the data of the major epimer without integration data.

30. Assigned by derivatization experiments.[12]

31. The RN units at C-7 and C-10 are termed prime and two prime, respectively.

32. Prolonged irradiation may favor the decrease of educt 7a, however, subsequent separation of the various N-mono-, N-di- and N-multiply-demethylated products formed was no longer satisfactorily achieved.

33. The sugar units of the trisaccharide chain at C-7 are termed RN', dF' and C', the rhodosamine unit at C-10 is termed RN''; RN = rhodosamine, dF = 2-deoxy-fucose, C = cinerulose.

34. Measured after shaking the $CDCl_3$ solution with a 5 % solution of sodium carbonate in 99.5 % D_2O.

35. The sugar units of the trisaccharide chain at C-10 are termed RN'', dF'' and C'', the rhodosamine unit at C-7 is termed RN'; for abbreviations see footnote.[33]