1-Propyl-1,4-dihydronicotinamide was prepared according to the literature procedure:[24] mp 87 °C dec (lit.[24] mp 86 °C dec); NMR ($CDCl_3$) δ 0.92 (t, 3 H), 1.61 (q, 2 H), 3.14 (m, 4 H), 4.76 (m, 1 H), 5.60 (s, 2 H), 5.75 (m, 1 H), 6.92 (d, 1 H).

N-[(*R*)-α-Methylbenzyl]-1-propyl-1,4-dihydronicotinamide was prepared according to the literature procedure:[24] mp 111 °C dec (lit.[24] mp 110 °C dec); NMR ($CDCl_3$) δ 0.91 (t, 3 H), 7.55 (m, 5 H), 3.05 (t, 2 H), 3.15 (m, 2 H), 4.70 (d, 1 H), 5.4 (s, 2 H), 5.75 (d, 1 H), 6.78 (d, 1 H), 7.30 (m, 5 H); $[\alpha]^{20}_D$ -172.3° (CH_3CN) [lit.[24] $[\alpha]^{20}_D$ -172.9° (CH_3CN)].

General Procedure for the Reduction of the α-Halo Ketones. An aliquot of a solution of DHNA (0.10 M) (or for the reactions of the α-fluoro ketone, 0.20 M), the ketone (0.050 M), and the additive was placed in a Pyrex reaction ampule, degassed, and sealed under vacuum. When the reaction was catalyzed by irradiation a 275 watt G.E. Sunlamp was used, and the mixtures were thermostated in a Pyrex water bath. In the dark reactions the ampule was thermostated in an oil bath at 61 °C for the appropriate time. After the required reaction time, the ampule was opened, and a aliquot solution of the internal standard (0.04 M) was added. The product mixture from the reduction of the fluoro ketone was analyzed by GLPC using a 20 ft × $^1/_4$ in. glass column packed with 10% FFAP on Chromosorb WAW DMCS, 60/80 mesh, or for the other halo ketones a 20 ft × $^1/_4$ in. glass column packed with 5% OV-101 on Chromosorb WAW DMCS, 100/120 mesh. GLPC analyses were carried out with a HP 5840 A gas chromatograph interfaced to a HP 5840 A integrator. The area ratios were converted to mole ratios for quantitative determinations by using standard calibration curves constructed from known mixtures.

Products were identified by a comparison of their retention times, GLPC-mass spectra, GLPC-IR, and ^{1}H NMR with those of authentic samples. Duplicate experiments were run with each ketone.

The internal standard, 1,4-di-*tert*-butylbenzene, and the anticipated products from both the homolytic and heterolytic reactions were added to the solvent, degassed, sealed, and thermostated, 61 °C, for the required time. A GLPC analysis of the mixture showed that the products were stable under the reaction conditions.

Polarographic Reduction and Oxidation. The current-voltage curves for the polarographic reductions of the ketones and the oxidation of the dihydronicotinamides were obtained with a Princeton Applied Research (PAR) Model 174 A polarograph interfaced with a PAR 303 DME. The solutions were anhydrous acetonitrile containing $(Bu)_4N^+ClO_4^-$ (0.1 M) and the reactant (0.01 M). The $E_{1/2}$ values relative to $Ag/AgClO_4$ (0.1 M) are listed in Table III.

ESR Spectroscopy. The ESR spectra were obtained for the radical anion of 2,6-di-*tert*-butylbenzoquinone by allowing a solution 4.9×10^{-2} M in the quinone and $4.7\text{-}10.4 \times 10^{-2}$ M in BANAH to stand at room temperature for several minutes. The intensity of the signals grew with time and appeared to be persistent for days. A Brucker ER 200 E/D spectrometer was used to record the spectra.

NMR Studies of the Reaction Velocity. A degassed acetonitrile-d_3 solution (0.101 M) in BANAH and (0.055 M) in α-bromoacetophenone and 0.020 M in 1,4-di-*tert*-butylbenzene as an internal standard was allowed to react in the cavity of a Brucker 200-MHz ^{1}H NMR spectrometer, whose probe was thermostated at 61 °C. The integrated signals at δ 1.27 and 2.55 corresponded to the methyl protons of the internal standard and acetophenone, respectively, were recorded as a function of time. A plot of the results is given in Figure 1.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada and the University of Alberta for their generous support of this work.

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Substituent Effects on the Redox Chemistry of Anthracycline Antitumor Drugs

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Reduction of 11-deoxydaunomycin (**8**), adriamycin (**1**), 4-demethoxydaunomycin (**9**), and 4-methoxy-6-deoxydaunomycin (**10**) with *meso*- and *d,l*-3,3',5,5,5',5'-hexamethyl-2,2'-dioxo-3,3'-bimorpholinyl (**3** and **4**) is described. Quinone methide intermediates from glycosidic cleavage of reduced **1**, **8**, and **9** were characterized by UV-vis spectroscopy and the rate constants for their tautomerization to the respective 7-deoxyaglycons were determined. These rate constants together with those from earlier measurements, ranging from 0.013 to 0.000 095 s^{-1}, establish an order of nucleophilicity of the quinone methides from reductive glycosidic cleavage of five anthracyclines of biological interest. The dimerization of the quinone methide from reduction of 11-deoxydaunomycin was established and the rate constant determined for comparison with the rate constant for dimerization of the quinone methide from reduction of aclacinomycin A. Reduction of **10** did not yield glycosidic cleavage but only catalysis of the disproportionation of **4** most likely by hydride transfer from the hydroquinone of **10** to 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**5**), the product of oxidation of **4**. The rate constant for hydride transfer was measured as a function of pH and compared with the rate constant for hydride transfer from 7-deoxydaunomycinone hydroquinone to **5**.

Introduction

Adriamycin (**1**) and daunomycin (**2**) are clinically important anthracycline antitumor drugs produced by mutant strains of *Streptomyces peucetius*.[1,2] Since their discovery extensive investigation of their chemical and biochemical reactivity has occurred. These studies have in part been directed to the discovery of derivatives or methodology which will maximize tumor response and minimize side effects, especially the acute cardiotoxicity. An excellent recent review has been published by Abdella and Fisher.[3] The in vivo and in vitro redox chemistry of the drugs is complex and appears to be involved in the

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biological activity. Adriamycin and daunomycin as well as many of their derivatives are efficient catalysts for the production of reactive oxygen species via electron transfer to molecular oxygen. The catalytic production of reactive oxygen species may be responsible for the cardiotoxicity and/or the antitumor activity. Anthracycline interaction with nucleic acids and cell membranes have been observed and proposed as sites of drug activity. Under anaerobic conditions most of the anthracyclines appear to undergo reductive glycosidic cleavage to form quinone methide transients which have been proposed as reactive intermediates in the binding of the aglycon portion of the drugs to biological macromolecules, especially DNA. In this respect 1 and 2 have been described as bioreductively activated.[4,5] Reactivity of quinone methide transients from reductive glycosidic cleavage of anthracyclines with both nucleophiles[6,7] and electrophiles[8] has been observed. In the absence of a reactive macromolecular or small molecule substrate, the quinone methide tautomerizes to the 7-deoxyaglycon of the anthracycline which is an important metabolite.[9]

We have observed that the radical dimers *meso*- and *d,l*-3,3′,5,5,5′,5′-hexamethyl-2,2′-dioxo-3,3′-bimorpholinyl (*meso* and *d,l*-TM-3 dimers, **3** and **4**) react quantitatively with adriamycin and daunomycin to yield their 7-deoxyaglycons, daunosamine, and 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**5**).[10] The active reducing agent in these glycosidic cleavages is the captodative radical, 3,5,5-trimethyl-2-oxomorpholin-3-yl (TM-3,**6**), from bond homolysis of **3** and **4**.[11] TM-3 is a mild one-electron reducing agent for the anthracyclines which has proven to be a useful reagent for the sequential generation of their many redox states. Because TM-3 and its derivatives are relatively nontoxic and rapidly transform most of the anthracyclines to their nontoxic 7-deoxyaglycons, they have potential application as anthracycline antidotes. Apparent in vivo reduction of adriamycin to 7-deoxyadriamycinone (**7**) with **3** and **4** diminshes the toxicity of adriamycin in mice.[12] 3,3′,5,5′-Tetramethyl-5,5′-bis(hydroxymethyl)-2,2′-dioxo-3,3′-bimorpholinyl (DHM-3 dimer), a water-soluble derivative of TM-3 dimer, has been used effectively as a rescue drug in high adriamycin dose-rescue therapy in mice with L-1210 leukemia[13] and as an antidote for adriamycin[14] and other quinone antitumor drug extravasation necrosis in pigs.[15]

We describe here the results of mechanistic studies of the reduction of four anthracyclines, 11-deoxydaunomycin (**8**), adriamycin (**1**), 4-demethoxydaunomycin (**9**), and 4-demethoxy-6-deoxydaunomycin (**10**), with TM-3 dimer. In particular the quinone methide states of **1**, **8**, and **9** from glycosidic cleavage of their respective hydroquinone states

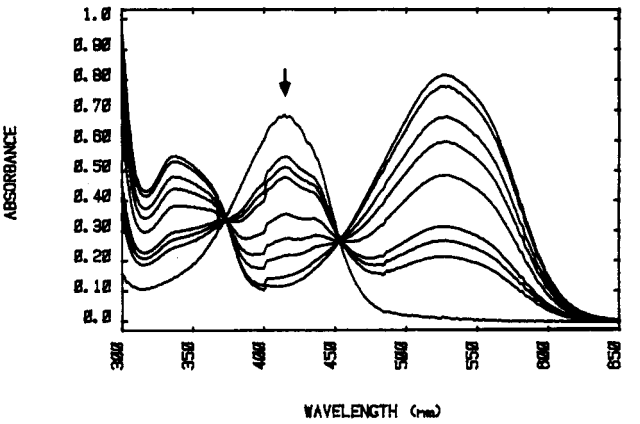

Figure 1. UV–vis absorption spectra of an oxygen-degassed (1:1) Tris–Tris·HCl buffered methanol solution, 6.4×10^{-5} M in 11-deoxydaunomycin (**8**), 6.4×10^{-4} M in *d,l*-TM-3 dimer (**4**), as a function of time at 25 °C. Scans were 1 s in duration and occurred at the following times after mixing: 22, 27, 32, 54, 74, 94, 136, and 216 s. The spectrum used for time 0, first one shown, was that for a buffered 6.4×10^{-5} M solution of **8**. Absorption at 340 and 528 nm resulted from formation of quinone methide **11**.

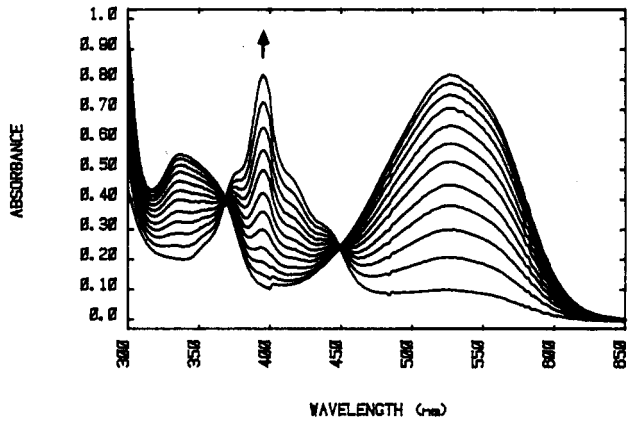

Figure 2. UV–vis absorption spectra of the reaction solution described in the caption to Figure 1 at the following times after mixing: 216, 458, 740, 1100, 1622, 2224, 2944, 4024, 5226, 7026, 10 028, and 17 000 s. Absorption at 395 nm resulted from formation of the hydroquinones of **12** and **13**.

were characterized and the rate constants for tautomerization to their 7-deoxyaglycons determined. The hydroquinone state of **10** was shown to be stable with respect to glycosidic cleavage and reacted via hydride transfer to oxazinone **5**. 11-Deoxyanthracyclines including 11-deoxydaunomycin and aclacinomycin A are of interest because they do not readily chelate metal ions such as ferrous or ferric ion, anthracycline metal chelation taking place at the oxygen functions at the 11- and 12-positions. Chelated iron has been proposed as a catalyst for the production of potentially cardiotoxic reactive oxygen species upon aerobic reduction of the anthracyclines.[16] 4-Demethoxydaunomycin[17] also shows a biological substituent effect in that it is both more active and more cardiotoxic than daunomycin.[18]

Results and Discussion

Reduction of 11-Deoxydaunomycin. Reaction of 11-deoxydaunomycin (**8**) with 10 equiv of *d,l*-

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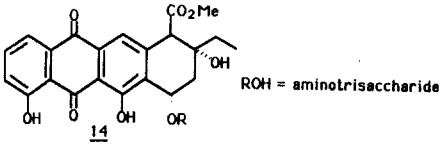
3,3′,5,5,5′,5′-hexamethyl-2,2′-dioxo-3,3′-bimorpholinyl (4) at 25 °C in freeze–thaw degassed methanol buffered to pH 8 with Tris buffer gives the spectral changes shown in Figures 1 and 2 as a function of time. Figure 1 covers the time period 22–216 s, and the spectral changes are characteristic of formation of the quinone methide 11. In this time regime the 420-nm band of 11-deoxydaunomycin decreases and the 340- and 528-nm bands assigned to the quinone methide increase. Figure 2 covers the time period 216–17 000 s and shows the transformation of the quinone methide to hydroquinone type products. The bands at 340 and 528 nm decrease, and the band at 395 nm, characteristic of the hydroquinone state, increases. The redox state assignments are made based upon the spectral properties of aclacinomycin A redox states;[19] aclacinomycin A bears the same chromophore as 11-deoxydaunomycin. The two products of the reduction upon exposure to air were isolated in approximately equal quantities and shown to be 7,11-dideoxydaunomycinone (12) and 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl (13) by mass spectrometry and in analogy with the products of reduction of aclacinomycin A (14). The dimer 13 was further characterized by ^{1}H NMR spectroscopy. The aglycon dimer 13 was the predominant product when the reduction was performed in deuterium oxide or methanol-*d* solvent, consistent with an earlier isotope effect observed in the analogous reduction of aclacinomycin A.[19] Formation of both 12 and 13 upon rat liver microsomal reduction of 8 has also been recently noted.[20]



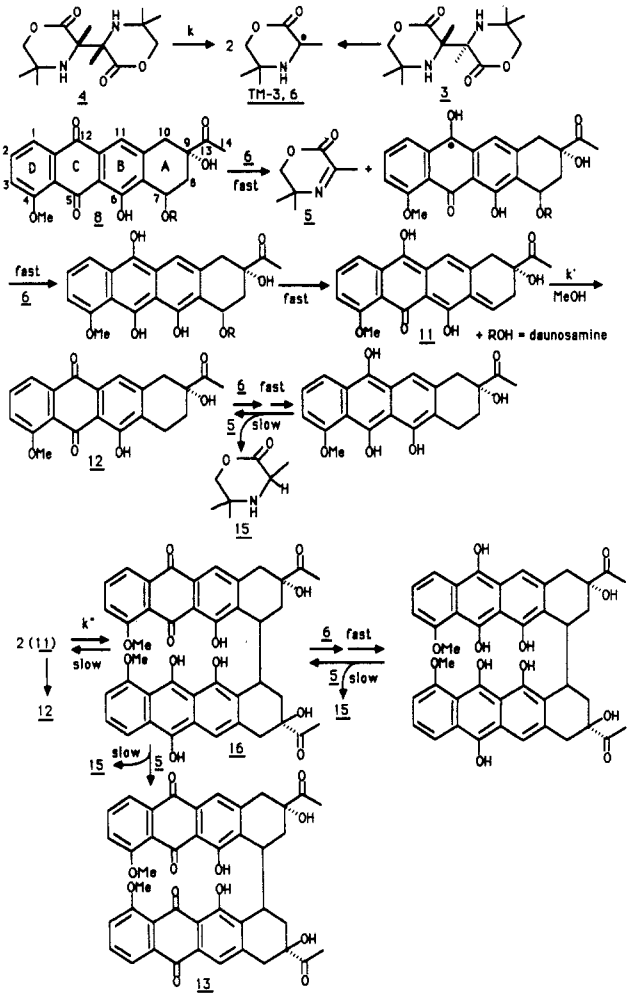
14

Reduction of 13 with *d,l*-TM-3 dimer (4) for 70 h gave upon exposure to air 4% cleavage to 7,11-dideoxydaunomycinone (12) and 96% recovered 13. GLC analysis of the TM-3 products showed a 50/50 mixture of 3,5,5-trimethyl-2-oxomorpholine (15) and oxazinone 5.

The results of these experiments are completely consistent with expectation based upon results of analogous experiments with aclacinomycin A and daunomycin.[11,19] The various redox processes taking place are shown in Scheme I. Under the conditions used to generate Figures 1 and 2, the slow steps are bond homolysis of 4, tautomerization of quinone methide 11 to 7,11-dideoxydaunomycinone (12), and dimerization of 11 to the mono hydroquinone of 13, shown as structure 16. With the large excess of 4 utilized, 12 and 16 are rapidly reduced to 7,11-dideoxydaunomycinone hydroquinone and the bis hydroquinone of 13, respectively, both absorbing at 390 nm. In the time period from 17 000 s to 70 h, these hydroquinones slowly reduce oxazinone 5 to morpholine 15. Temporary restoration of 16 leads to some reversal of the dimerization of quinone methide 11 followed by tautomerization of 11 to 7,11-dideoxydaunomycinone (12). Predominant formation of 13 when the reduction is performed in methanol-*d* solvent occurs because the deuterium kinetic isotope effect on tautomerization is much larger than on dimerization.[19]

The rate law for the formation and destruction of quinone methide 11 is first order (bond homolysis of TM-3)

Scheme I



followed by combined first and second order (tautomerization and dimerization of 11). Because a first-order followed by a combined first- and second-order rate law cannot be integrated, the reaction was run with a large excess of reducing agent to form 11 rapidly, and the decay of 11 was fitted to the combined first and second-order rate law, (eq 1) by using a nonlinear least-squares algorithm.

$$A_t = (A_0k')/[e^{k't}(k' + (A_0k''/\epsilon)) - (A_0k''/\epsilon)] \quad (1)$$

A_t is the absorbance of 11 at time t, A_0 is the absorbance at time 370 s, t is the time from 370 s, k' is the pseudounimolecular rate constant for tautomerization of 11, k'' is the bimolecular rate constant for dimerization of 11 and ϵ is the molar extinction coefficient of 11 at 528 nm. The initial delay time of 370 s was sufficient for production of enough TM-3 to reduce all the 11-deoxydaunomycin more than 7 times as determined from the first-order rate constant for bond homolysis of *d,l*-TM-3 dimer. The maximum absorbance for 11 in Figure 1 predicts that the molar extinction coefficient for 11 is at least 13 000. The rate constants k' and k'' are compared with those for other anthracyclines in Table I.

The quinone methide from reduction of daunomycin has been trapped as a nucleophile with benzaldehyde in an aldol-type reaction in competition with tautomerization. The yield of the aldol product was especially high when the reduction was performed in the presence of an excess of benzaldehyde in methanol- *d* solvent.[8,11] Similar reduction of 11-deoxydaunomycin in the presence of benzaldehyde gave only a trace of an additional product. The quantity was insufficient for characterization; however, the

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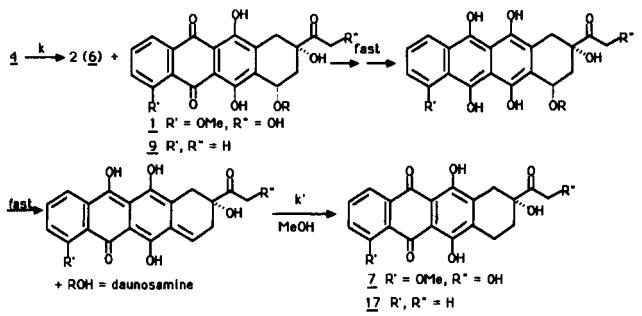
Table I. Extinction Coefficients for Quinone Methides and Rate Constants for Tautomerization (k') and Dimerization (k'') of Quinone Methides from Reductive Glycosidic Cleavage of Anthracyclines[a]

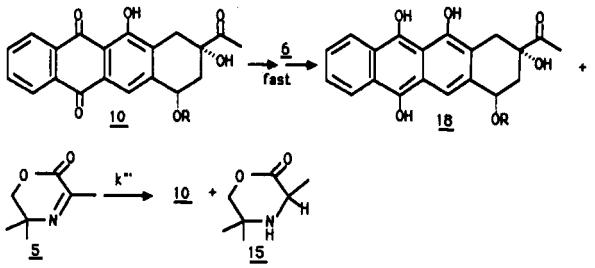
anthracycline	solvent	k', s^{-1}	k'', M^{-1} s^{-1}	ϵ for quinone methide, M^{-1} cm^{-1} (λ, nm)	ref
daunomycin (2)	water	0.046			21
	methanol	0.013		9800 (620)	11
adriamycin (1)	water	0.030			21
	methanol	0.011		9600 (620)	this work
4-demethoxydaunomycin (9)	methanol	0.0086		7600 (620)	this work
aclacinomycin A (14)	methanol	0.000 89	23[b]	9000 (600)[b]	19
				13000 (548)[b]	
11-deoxydaunomycin (8)	methanol	0.000 095	1	13000 (528)[c]	this work

[a] Rate constants were measured at pH 8 at 25 °C and are the average of two or three measurements. The average deviations from the mean for measurements of k' were less than 3%, and the standard deviations from the least-squares analyses were less than 6%. The average deviation from the mean for the measurement of k'' was 25%, and the standard deviations from the least-squares analyses were less than 8%. The standard deviations for the extinction coefficients of the quinone methides of 1, 2, and 9 from the least-squares analyses were less than 400 M^{-1} cm^{-1}. [b] This value is corrected relative to the value in ref 19 for the anticipated higher extinction coefficient for the quinone methide based upon the extinction coefficient for 11 and for an arithmetic error. [c] This extinction coefficient is assigned with the assumption that the concentration of the quinone methide at its maximum is equal to the starting concentration of 8.

Scheme II



Scheme III



new material was probably the analogous benzaldehyde adduct, based upon a similar observation by Fisher and co-workers for the reduction of 8 in aqueous medium in the presence of sodium *p*-carboxybenzaldehyde.[21]

Reduction of Adriamycin (1) and 4-Demethoxydaunomycin (9). Adriamycin and 4-demethoxydaunomycin were reduced with *d,l*-TM-3 dimer in degassed, Tris buffered methanol at 25 °C with spectroscopic monitoring of formation and destruction of their quinone methide states. The absorbance at 618–620 nm vs. time for the quinone methide resulting from reductive glycosidic cleavage of adriamycin was monitored and is shown in Figure 3 of the supplementary material. This absorbance change was completely analogous to that observed earlier for daunomycin.[11] Complete scans from 350 to 800 nm during the first 1020 s of the reduction of 4-demethoxydaunomycin (9) were obtained and are shown in Figures 4 and 5 of the supplementary material. The 480-nm band of 9 decreased as the 375- and 600-nm bands increased, reaching a maximum at 180 s, characteristic of quinone methide formation.[11] After 180 s the 480-nm absorbance increased and the absorbance at 375 and 600 nm decreased due to tautomerization to the 7-deoxyaglycon. The reductions gave only 7-deoxyadriamycinone and 4-demethoxy-7-deoxydaunomycinone, respectively, and in both cases the absorbance at 618–620 nm vs. time followed the consecutive first-order rate law shown in eq 2. The slow steps were bond homolysis of 4 and tautomerization of the quinone methide states as shown in Scheme II. The rate constants for tautomerization of the quinone methides are reported in Table I. A_t is the absorbance of the quinone

$$A_t = [\epsilon_{qm}k[\text{anthracycline}]_0/(k' - k)](e^{-kt} - e^{-k't}) \quad (2)$$

methide at time t, ϵ_{qm} is the average extinction coefficient for the quinone methide at 618 and 620 nm, k is the rate constant for bond homolysis of 4 and k' is the rate constant for tautomerization of the quinone methide.

Table II. Rate Constants for Reduction of Oxazinone 5 by Hydroquinones[a]

anthracycline	temp, °C	pH	k''', M^{-1} s^{-1}	ref
4-demethoxy-6-deoxydaunomycin	25	8	0.42	this work
	37	8	1.4	this work
	37	9	2.3	this work
7-deoxydaunomycinone	25	8	2.1	11, 23

[a] At the 95% confidence level the errors in k''' obtained from the least-squares analysis were less than 10%.

Reduction of 4-Demethoxy-6-deoxydaunomycin (10). Malatesta and co-workers reported that 10 did not undergo glycosidic cleavage when reduced electrochemically.[22] Reduction of 10 with *d,l*-TM-3 dimer in degassed methanol solvent similarly did not result in glycosidic cleavage at pH 8 at 25 or 37 °C or at pH 9 at 37 °C. Spectroscopic monitoring of these reductions showed formation of the hydroquinone of 10, assigned structure 18, at 400 nm followed by oxidation of 18 by oxazinone 5 back to starting anthracycline as shown in Figures 6 and 7 of the supplementary material. Chromatographic analysis showed only recovered 10 and a quantitative yield of oxazinone 5 and morpholine 15. This mode of reactivity shown in Scheme III is analogous to that described above for the hydroquinone of 7,7',11,11'-tetradeoxy-7,7'-bidaunomycinonyl (13) and earlier for the hydroquinone of 7-deoxydaunomycinone.[11] The decay at 400 nm after six half-lives of 4 followed second-order kinetics, first order in both hydroquinone and oxazinone. Nonlinear least-squares fitting of the data to the integrated rate law, correcting for absorption by 10 (eq 3), gave the rate constants for hydride transfer from 18 to 5 as a function of temperature and pH reported in Table II. A_t is the absorbance at 400 nm at time t from six half-lives of 4 ϵ_{18} and ϵ_{10} are the extinction coefficients for 18 and 10 at 400 nm,

$$A_t = \epsilon_{10}[10]_0 + (\epsilon_{18} - \epsilon_{10}) \left[e^{-k'''t[10]_0[4]_0} \left\{ \frac{A_0 - \epsilon_{10}[10]_0}{\epsilon_{18} - \epsilon_{10}} \right\} \Big/ [4]_0 + \left\{ \frac{A_0 - \epsilon_{10}[10]_0}{\epsilon_{18} - \epsilon_{10}} \right\} \{1 - e^{-k'''t[4]_0}\} \right] \quad (3)$$

k''' is the rate constant for reaction of **18** with **5**, $[10]_0$ is the initial concentration of **10**, $[4]_0$ is the initial concentration of **4**, and A_0 is the absorbance at 400 nm after six half-lives of **4**. The extinction coefficient ϵ_{18} for the hydroquinone was estimated at 7600 M^{-1} cm^{-1} in proportion to the extinction coefficients at λ_{max} for **10**, daunomycin, and 7-deoxydaunomycinone hydroquinone[11] ($\epsilon_{18} = \epsilon_{10} \times \epsilon_{\text{7-deoxydaunomycinone hydroquinone}}/\epsilon_{\text{daunomycin}}$).

Discussion of Substituent Effects. The values for the pseudo-first-order rate constant k' for the tautomerization of the quinone methide transients to the 7-deoxyaglycons shown in Table I vary by a factor of more than 140, with the quinone methide from glycosidic cleavage of daunomycin being most reactive and that from cleavage of 11-deoxydaunomycin being least reactive with Tris buffered methanol. This rate constant is a measure of the reactivity of the quinone methide with the electrophile, the proton, and possibly an inverse measure of the reactivity with a nucleophile. The comparison of k' shows that substituent effects are observed in the A,B, and D rings. Even the reactivity of quinone methides from daunomycin and adriamycin are slightly different, consistent with the same measurement by Fisher in aqueous medium with enzymatic reduction.[21] Clearly the absence of the 11-hydroxy substituent in the B ring has the most pronounced effect. This substituent is in direct conjugation with the enolic part of the quinone methide chromophore and its presence should increase the nucleophilicity and correspondingly the rate of protonation. The absence of the methoxy substituent in the 4-position exerts only a small effect on k', suggesting that the D ring is somewhat insulated from the quinone methide functionality. The intermediate value of k' for tautomerization of the quinone methide from **9** does not appear to correlate with either relative tumor responce or cardiotoxicity as determined by Mimnaugh and co-workers.[18] However, our recent measurement of the toxicity of quinone antitumor drugs in the pig skin necrosis model places **9** intermediate between adriamycin and aclacinomycin A as it appears in Table I.[15]

The proposal of Moore that the anthracyclines are bioreductively activated[4] included a prediction that the resulting quinone methides would be reactive with nucleophilic sites in critical biological molecules. Previous nucleophilic trapping experiments have shown that quinone methides from 11-deoxydaunomycin and aclacinomycin A, located at the bottom of Table I, are most reactive and could be trapped with ethyl xanthate as well as *N*-acetylcysteine;[6] whereas, the quinone methide from daunomycin, located at the top of Table I, could only be trapped with *N*-acetylcysteine.[7] Correspondingly, the quinone methide from daunomycin is most reactive with electrophilic aldehydes.[11,21]

The rate constant k'' for dimerization of quinone methides is probably a measure of the balance of electrophilic and nucleophilic reactivity plus some steric component. In this reaction one quinone methide is serving as a nucleophile and the other as an electrophile. This balance is only observed in the 11-deoxyanthracyclines. The order of magnitude difference in k'' parallels the order of magnitude difference in k' and suggests that the quinone methide from aclacinomycin A dimerizes faster because it is slightly more nucleophilic than the quinone methide from 11-deoxydaunomycin. The effect is small, however, and could also arise from the steric difference in the A rings.

The hydroquinone from reduction of 4-demethoxy-6-deoxydaunomycin (**10**) does not undergo glycosidic cleavage even at higher pH. Its reluctance to cleave has been rationalized by Malatesta and co-workers in terms of A ring conformation.[22] The absence of the 6-hydroxy substituent allows the daunosamine at the 7-position to be pseudoequatorial, which is unfavorable for continuous orbital overlap during the elimination. Not surprisingly the hydroquinone then reacts via the slower process of apparent hydride transfer first observed with the hydroquinone of 7-deoxydaunomycinone.[11,23] Hydride transfer from **18** to **5** is 5 times slower than the analogous transfer from the hydroquinone of 7-deoxydaunomycinone to **5**. This rate difference as well as the pH effect shown in Table II is consistent with the number and type of anthracene, electron-donating, oxygen substituents. The more electron rich the aromatic system the higher is the rate of hydride transfer. Although biological expression of the apparent hydride transfer reaction has not been established, a possibility is the oxidation by a quinone of the hydroquinone from nucleophilic addition to a quinone methide. This is a necessary step for stabilization of the nucleophilic addition product in the Fisher method of trapping quinone methides with sulfur nucleophiles.[6,7,21] Another possibility is the reduction of hydrogen peroxide to water observed with daunomycin hydroquinone.[24]

In summary we present spectral and kinetic characterization of some of the intermediates from reduction of four additional anthracyclines. The determination of the rate constants for tautomerization of the quinone methides from glycosidic cleavage under identical conditions yields an order of reactivity as a nucleophile of this presumably important intermediate as a function of various substituent changes. Clearly the biological activity of the anthracyclines is complex and nucleophilicity/electrophilicity of the quinone methide state is only one component of the complex puzzle.[3] However, as the puzzle is solved, knowledge of these substituent effects might be useful in the design of superior drugs.

Experimental Section

General Remarks. A Hewlett-Packard 8450 spectrometer was used for obtaining UV–vis spectral data. GLC analyses were performed with a Hewlett-Packard 5790 chromatograph equipped with Hewlett-Packard 3390A integrator. HPLC analyses were performed with a Hewlett-Packard 1090 chromatograph equipped with a diode array UV–vis detector and data processing unit or a Tracor HPLC with a Model 950 pump and a Model 970A variable wavelength UV–vis detector. Mass spectral data were obtained with a VG Instruments 7070 EQ-HF high-resolution mass spectrometer equipped with a FAB inlet system. NMR data were obtained with a Bruker 250-MHz spectrometer; chemical shifts

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are reported in ppm on the δ scale and coupling constants in hertz. Solvents were either reagent or spectroscopic grade. 11-Deoxydaunomycin hydrochloride was obtained from the National Cancer Institute, Drug Development Branch, Bethesda, MD, and adriamycin, 4-demethoxydaunomycin, 11-deoxydaunomycin, and 4-demethoxy-6-deoxydaunomycin hydrochlorides were obtained from Farmitalia Carlo Erba, Milan, Italy. *meso*- and *d,l*-3,3′,5,5,5′,5′-tetramethyl-2,2′-dioxo-3,3′-bimorpholinyl, *meso*- and *d,l*-TM-3 (**3** and **4**) were prepared by photoreductive dimerization of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**5**) and separated by low-temperature alumina flash chromatography.[25] 3,3′,5,5′-Tetramethyl-5,5′-bis(hydroxymethyl)-2,2′-dioxo-3,3′-bimorpholinyl, DHM-3 dimer, was prepared by photoreductive dimerization of 5,6-dihydro-5-(hydroxymethyl)-3,5-dimethyl-1,4-oxazin-2-one and used a mixture of diastereoisomers.[26] Tris, tris(hydroxymethyl)aminomethane, and Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride, were obtained from Sigma Chemical Co., St. Louis, MO. No metal needles were used in any of the syringe transfers to be described.

Spectroscopic Monitoring of the Reaction of 11-Deoxydaunomycin (8) with *d,l*-TM-3 Dimer (4). A two-compartment cell was used consisting of a 1-cm Pyrex cuvette from Spectrocell, Inc., Oreland, PA, fused to a degassing chamber 1.6 cm o.d. × 4.5 cm long and a 0.9 cm o.d. tube for attachment to a vacuum line via an Ultra Torr union. The angle between the cuvette and the 0.9-cm tube and the 1.6-cm chamber were 100° and 120°, respectively. *d,l*-TM-3 dimer (**4**) (3.42 mg, 1.20×10^{-5} mol) was dissolved in 5.0 mL of dichloromethane and 0.66 mL (1.59×10^{-6} mol) was transferred into the cuvette compartment of the cell. The dichloromethane was evaporated with a stream of nitrogen while the solution was cooled with ice–water. 11-Deoxydaunomycin hydrochloride (**8**) (1.46 mg, 2.66×10^{-6} mol) was dissolved in 25.0 mL of a methanolic solution 1.0×10^{-3} M in Tris and 1.0×10^{-3} M in Tris·HCl, and 3.0 mL of this solution was diluted to 5.0 mL with the methanolic buffer solution. Of the resulting solution, 2.5 mL, 1.60×10^{-7} mol, was added to the 1.6-cm chamber. The methanol solution was freeze (−196 °C)–pump(3×10^{-6} Torr)–thaw(0 °C)–sonnicate degassed through five cycles and the apparatus sealed under vacuum. The cell was then transferred to a thermostated cell holder at 25.2 ± 0.1 °C. The cell holder consisted of an aluminum block milled to accommodate the entire cell and connected to a thermostated circulator. The methanol solution was temperature equilibrated at 25.2 ± 0.1 °C for ca. 15 min by placing the cell holder at 90° to its normal position. The *d,l*-TM-3 dimer and the 11-deoxydaunomycin solution were then rapidly mixed by shaking the cell holder vigorously for 13 s. The absorbance of the solution from 300 to 650 nm as a function of time was recorded as shown in Figures 1 and 2. The decay of the absorbance at 528–530 nm over the time period 370–15 600 s was fitted to a combined first- and second-order integrated rate law by using a nonlinear least-squares analysis. After 68 h the cell was opened. Silica gel TLC analysis eluting with dichloromethane/methanol (97:3) showed one large spot with R_f 0.6 assigned to 7,11-dideoxydaunomycinone (**12**) and a smaller spot with R_f 0.3 assigned to 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl (**13**).

Reaction of 11-Deoxydaunomycin (8) with *meso*-TM-3 Dimer (3) in Methanol-*d* Solvent. A two-compartment Pyrex apparatus was fabricated; it consisted of a thick-wall 25-mL tube fused at 90° in the upper part to a smaller tube, 1.2 cm o.d. × 4.5 cm long, and at the top coaxially to a 0.9 cm o.d. tube for connection to the vacuum line with an Ultra Torr union. *meso*-TM-3 dimer (**3**) (85 mg, 3.0×10^{-4} mol) was dissolved in 1 mL of dichloromethane. The solution was quantitatively transferred to the small side arm of the apparatus and the solvent evaporated with a stream of nitrogen. 11-Deoxydaunomycin hydrochloride (**8**) (16.4 mg, 3.0×10^{-5} mol) was dissolved in 10 mL of a methanol-*d* solution of Tris (36 mg, 3.0×10^{-4} mol) and Tris·HCl (43 mg, 2.7×10^{-4} mol). The solution was introduced into the 25-mL tube. After freeze–thaw degassing as described above, the apparatus was sealed under vacuum. The TM-3 dimer was then dissolved in the orange-yellow solution of anthracycline by shaking. The color of the solution deepened to a dark red in about 1 min to then slowly fade to yellow in ca. 3 h. The tube was kept in the dark at ambient temperature for 87 h. During that time a few crystals of product had separated. The apparatus was opened and the solution decanted. The crystals were collected and washed five times with a total of 2 mL of methanol. Silica gel TLC analysis of the crystals showed only one spot, R_f 0.3, using dichloromethane/methanol (97:3) as eluent. The mother liquors showed a few orange-yellow tiny spots on TLC along with a large R_f 0.3 spot. The UV–vis spectrum showed the typical anthraquinone chromophore with an absorption maximum at 420 nm. The mother liquors were rotary evaporated under reduced pressure, and the residue was treated with 5 mL of water. The undissolved yellow solid was collected by suction and washed with water and a little methanol to give 8 mg of orange-yellow crystals, mp ca. 170 °C (dec). TLC analysis showed only traces of impurities contaminating the main compound with R_f 0.3. The positive ion FAB mass spectrum of the R_f 0.3 product showed multiple peaks in the region of m/z 735 with intensities relative to the 735 peak as follows: 733 (50, M + 3), 734 (70), 735 (100), 736 (85), 737 (80), 738 (65). Quinone reduction during FAB mass spectrometry has been observed earlier with 7-deoxyaglycon derivatives.[11] The best FAB spectrum was obtained with a sample dissolved in chloroform and mixed with glycerol and a small amount of dimethyl sulfoxide. The major product was assigned to the structure 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl (**13**) on the basis of the molecular weight and the ^{1}H NMR data reported below.

Reaction of 11-Deoxydaunomycin (8) with *d,l*-TM-3 Dimer (4) in Methanol Solvent. The side arm of an apparatus identical with the one described in the previous section was charged with 8.5 mg (3.0×10^{-5} mol) of *d,l*-TM-3 dimer (**4**) dissolved in 0.5 mL of dichloromethane, and the solvent was evaporated with a stream of nitrogen. 11-Deoxydaunomycin hydrochloride (**8**) (8.2 mg, 1.5×10^{-5} mol) was dissolved in 20.0 mL of a methanolic solution 2×10^{-2} M in both Tris and Tris·HCl. The solution was introduced into the 25-mL compartment of the apparatus, freeze–thaw degassed and sealed under vacuum. After mixing, the initially yellow reaction mixture turned to red with maximum intensity after ca. 5 min. to then slowly fade to yellow. After 18 h the apparatus was opened. The UV–vis spectrum of the solution showed the same anthraquinone chromophore as that of 11-deoxydaunomycin with λ_{max} 420 nm. Silica gel TLC analysis, using dichloromethane/methanol (97:3) as eluent, showed both the spot with R_f 0.3 corresponding to 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl (**13**) and the spot with R_f 0.6 assigned as 7,11-dideoxydaunomycinone (**12**). After rotary evaporation of the solvent, the solid residue was treated with water, collected by suction, washed with water, and dried to give 8 mg of orange crystals. The two products were cleanly separated by flash chromatography using 0.040–0.063 μm Merck silica gel and dichloromethane/methanol (98:2) as eluent, yielding 2 mg of each product. Positive ion FAB mass spectrometry of the R_f 0.6 product showed peaks clustered around m/z 368 as follows: 367 (M + 1, 43), 368 (100), 369 (intensity obscured by protonated glycerol tetramer), 370 (23).

Isolation and ^{1}H NMR Spectrum of 13 from Reduction of 11-Deoxydaunomycin with 3,3′,5,5′-Tetramethyl-3,3′-bis(hydroxymethyl)-2,2′-dioxo-3,3′-bimorpholinyl (DHM-3 Dimer) in Aqueous Medium. An aqueous solution (10 mL) of 11-deoxydaunomycin (16 mg, 3.0×10^{-5} mol) was adjusted to pH 7 with solid phosphate buffer (0.17 g of pHydrion buffer). The solution was transferred to a 20-mL compartment of a two-compartment reaction vessel with an attachment for connection to the high vacuum line. 3,3′,5,5′-Tetramethyl-3,3′-bis(hydroxymethyl)-2,2′-dioxo-3,3′-bimorpholinyl (DHM-3 dimer), a water-soluble derivative of TM-3 dimer, was employed as the reducing agent. DHM-3 dimer (9.2 mg, 3.1×10^{-5} mol) was dissolved in 1.5 mL of acetonitrile, and the solution was transferred to the second compartment which had a volume of 5 mL. The acetonitrile was evaporated with a stream of nitrogen. The cell was freeze–thaw degassed through six cycles and sealed. The reactants were mixed, and an immediate dull red color developed, which became light brown within 1 min. Turbidity ensued followed by formation of a gelatinous precipitate. Although the reaction

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appeared to be over within minutes, the reaction mixture was allowed to stand at ambient temperature for 4 h after which it was exposed to air and extracted with 10 mL of chloroform. The major product of the reaction was separated from other minor products, including traces of 7,11-dideoxydaunomycinone (**12**), by TLC (0.25-mm precoated silica gel eluted with methylene chloride/methanol, 96:4). After two chromatographies, 7,7′,11,11′-tetradeoxy-7,7′-daunomycinonyl (**13**) was isolated as a single product (3.8 mg, 37%) and further characterized from the following ^{1}H NMR data: ($CDCl_3$, 250 Mz) δ 12.97 (s, 1 H, 6-OH), 7.87 (d, J = 7.5, 1 H, 1-H), 7.63 (t, J = 7.5, 1 H, 2-H), 7.53 (s, 1 H, 11-H), 7.18 (d, J = 7.5, 1 H, 3-H), 4.59 (br s, 1 H, 9-OH), 4.42 (br s, 1 H, 7-H), 3.90 (s, 3 H, 4-OMe), 3.61 (d, J = 16, 1 H, 10-H_a), 3.23 (d, J = 16, 1 H, 10-H_e), 2.37, (d, J = 15, 1 H, 8-H_a), 2.07 (s, 3 H, 14-Me), 1.92 (br d, J = 15, 1 H, 8-H_e). Peaks at 12.97 and 4.59 ppm were exchanged with deuterium oxide, and the peak at 1.92 ppm was sharpened by irradiation at 4.42 ppm and vice versa. The simplicity of the NMR spectrum suggested that the major dimer was one of the two symmetrical possibilities.

Reaction of 7,7′,11,11′-Tetradeoxy-7,7′-bidaunomycinonyl (13) with *dl*-TM-3 Dimer. A 25-mL volumetric flask was charged with 1.03 mg (1.41×10^{-6} mol) of 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl and brought to volume with methanol. A 10-mL volumetric flask was charged with 4.8 mg (4.0×10^{-5} mol) of Tris and 6.26 mg (4.0×10^{-5} mol) of Tris·HCl and brought to volume with the above 5.64×10^{-5} M solution of **13**. Via a 1-mL syringe, 2.5 mL (1.41×10^{-7} mol) of this solution was transferred to the 1.6-cm chamber of the two compartment cell described above. The cuvette compartment was precharged with 0.142 mg (5.00×10^{-7} mol) of *d,l*-TM-3 dimer (**4**) also as described above. After freeze–thaw degassing, the cell was temperature equilibrated for 15 min at 25.1 ± 0.1 °C in the thermostated cell holder tilted at 90°. Upon mixing, the reaction was spectroscopically monitored from 300 to 800 nm periodically for 69.5 h. Hydroquinone absorption at 397 nm increased, reaching a maximum at 1620 s. From 1620 s to 69.5 h, absorbance at 397 nm decreased while quinone absorbance at 420 nm increased, ultimately to approximately its original magnitude. The cell was opened and the product solution quantitatively analyzed by HPLC and GLC. HPLC analysis was performed with a 10-μm phenyl reverse-phase column, 4.6 mm × 250 mm, eluting with 60% tetrahydrofuran–40% pH 4 formate buffered water at a flow rate of 1.5 mL/min and monitoring at 420 nm. Standard solutions of 5.64×10^{-5} M 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl (**13**) in methanol and 5.43×10^{-5} M 7,11-dideoxydaunomycinone (**12**) in methanol were used to calibrate the HPLC response as a function of injection size. The final concentrations of anthracycline products determined in this way were 4.29×10^{-6} M 7,11-dideoxydaunomycinone and 5.37×10^{-5} M 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl, representing a 4% conversion of **13** to **12**. GLC analysis was performed with a 25-m Hewlett-Packard cross-linked 5% PhMe silicone ultraperformance column with a 0.17 μm thick coating, eluting with hydrogen at 2 mL/min and temperature programming (60 °C for 1 min to 100 °C at 10 deg/min and then isothermal for 10 min). The GLC was calibrated with standard methanol solutions composed of 2.0×10^{-4} M 3,5,5-trimethyl-2-oxomorpholine (**15**) and 1.0×10^{-4} M methyl benzoate as an internal standard and 2.0×10^{-4} M 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**5**) and 1.0×10^{-4} M methyl benzoate. Analysis of the product mixture with methyl benzoate again as the internal standard gave concentrations of **15** and **5** both equal to 2.0×10^{-4} M.

Reaction of 11-Deoxydaunomycin (8) with *d,l*-TM-3 Dimer (4) in the Presence of Benzaldehyde. Two small-scale reductions were conducted in two compartment cells with cuvettes in methanol-*d*, with an without benzaldehyde present. *d,l*-TM-3 dimer (1.40 mg, 4.9×10^{-6} mol) was dissolved in 25 mL of dichloromethane, and 0.71 mL (1.4×10^{-7} mol) was transferred into the cuvette compartment. The solvent was evaporated with a steam of nitrogen. 11-Deoxydaunomycin hydrochloride (1.10 mg, 2.0×10^{-6} mol), Tris (3.0 mg, 2.5×10^{-5} mol), and Tris·HCl (4.0 mg, 2.5×10^{-5} mol) were dissolved in 25.0 mL of methanol-*d* solvent, and 2.5 mL (2.0×10^{-7} mol of **8**) was added to the 1.6-cm chamber of the cell. After freeze–thaw degassing and sealing under vacuum as described, the cell was temperature equilibrated at 25.1 ± 0.1 °C in the thermostated cell holder, the reagents were mixed by shaking, and the reaction was monitored at 550–552 nm as a function of time. Reaction in the presence of benzaldehyde was run in the same way except redistilled benzaldehyde (4.2 mg, 4.0×10^{-5} mol) was added to the 2.5 mL of buffered 11-deoxydaunomycin methanol-*d* solution. The spectral changes observed during the two reactions were approximately the same. The reaction product mixtures were also compared by silica gel TLC eluting with dichloromethane/methanol (97:3). No remarkable differences appeared; only a yellow-colored, very tiny additional spot at R_f 0.45 appeared for the reaction in the presence of benzaldehyde.

Spectroscopic Monitoring of the Reaction of Adriamycin (1) with *d,l*-TM-3 Dimer (4). A 10-mL volumetric flask was charged with 2.41 mg (4.16×10^{-6} mol) of adriamycin hydrochloride and brought to volume with 1.0×10^{-4} M Tris–1.0×10^{-4} M Tris·HCl buffered methanol. An aliquot (4.28 mL, 1.78×10^{-6} mol) of this solution was diluted to 10.0 mL with the buffered methanol. A 25-mL volumetric flask was charged with 1.80 mg (6.34×10^{-6} mol) of *d,l*-TM-3 dimer (**4**) and brought to volume with dichloromethane. A portion of the latter solution (0.88 mL, 2.23×10^{-7} mol) was delivered to the cuvette compartment of the cell (vide supra) chilled to 0 °C. The dichloromethane was then evaporated with a steady stream of nitrogen. An aliquot of the adriamycin solution (2.5 mL, 4.45×10^{-7} mol) was added to the 1.6-cm compartment. The sample was freeze–pump–thaw–sonicate degassed as described for the sample of 11-deoxydaunomycin and placed in a thermostated cell holder at 25.1 ± 0.1 °C for 10 min prior to mixing. Upon mixing the average absorbance at 618–620 nm (λ_{max} of the quinone methide transient) was monitored as a function of time (Figure 3, supplementary material). The resulting data were fit to a consecutive first-order rate law by a nonlinear least-squares analysis as shown in Table I. HPLC analysis of the reaction mixture with a 4.6 mm × 30 cm Alltech RSIL–phenyl column eluting with 40% tetrahydrofuran–60% buffered water (v/v) (buffer consisted of 0.1% ammonium formate adjusted to pH 4.0 with formic acid) at a flow rate of 2.0 mL/min and detecting at 480 nm showed adriamycin at a retention time of 3.0 min and 7-deoxyadriamycinone (**7**) at a retention time of 4.4 min. The area ratio was 0.95:1.0, respectively.

Spectroscopic Monitoring of the Reaction of 4-Demethoxydaunomycin Hydrochloride (9) with *d,l*-TM-3 Dimer (4). A 50-mL volumetric flash was charged with 2.70 mg (5.06×10^{-6} mol) of 4-demethoxydaunomycin hydrochloride and brought to volume with 2.0×10^{-3} M Tris–Tris·HCl buffered methanol. An aliquot of the anthracycline solution (2.48 mL, 2.50×10^{-7} mol) was added to the 1.6-cm chamber of the two compartment cell to which the cuvette compartment had been precharged with 0.0355 mg (1.25×10^{-7} mol) of *d,l*-TM-3 dimer as described above. The cell was freeze–thaw degassed and placed in the thermostated cell holder at 25.1 ± 0.1 °C for 35 min prior to mixing. Upon mixing, the reaction was monitored from 350 to 800 nm every 30 s for 1020 s (Figures 4 and 5, supplementary material). Time vs. absorbance at 618–620 nm data were fit to a consecutive first-order rate equation as shown in Table I. HPLC analysis as described for the reduction of adriamycin showed the presence of unreacted 4-demethoxydaunomycin at a retention time of 5.8 min and 4-demethoxy-7-deoxydaunomycinone (**17**) at a retention time of 12.8 min with an area ratio of 0.94:1.0, respectively.

Reaction of 4-Demethoxy-6-deoxydaunomycin Hydrochloride (10) with *d,l*-TM-3 Dimer at 25 °C and pH 8. A 25-mL volumetric flask was charged with 4.55 mg (8.78×10^{-6} mol) of 4-demethoxy-6-deoxydaunomycin hydrochloride and brought to volume with 2×10^{-3} M Tris–Tris·HCl buffered methanol. An aliquot of this solution (2.54 mL, 8.9×10^{-7} mol) was diluted to 10 mL using the buffered methanol. Of the resulting solution, 2.50 mL (2.23×10^{-7} mol) was transferred to the 1.6-cm compartment of the two compartment cell to which the cuvette compartment had been charged with 0.0632 mg (2.23×10^{-7} mol) of *d,l*-TM-3 dimer as described above. After freeze–thaw degassing, the cell was allowed to temperature equilibrate in the thermostated cell holder at 25.1 °C for 15 min. Upon mixing, the reaction was monitored spectroscopically for 19 h. The absorption at 400 nm increased reaching a maximum at 890 s, after which time the absorption decreased due to oxidation of hydroquinone by oxazinone **5**. Absorption vs. time after 6 half-lives

Table III. Absorption at 400 nm vs. Time Data for Reduction of 4-Demethoxy-6-deoxydaunomycin (10) by *d,l*-TM-3 Dimer (4) in Buffered Methanol Solvent[a]

conditions			
temp, °C	pH	time, s	absorpn
25	8	1190	0.896
		1490	0.885
		1790	0.878
		2090	0.873
		2390	0.865
		2690	0.860
		2990	0.848
37	8	290	0.825
		590	0.809
		890	0.796
		1190	0.786
		1490	0.775
		1790	0.764
		2090	0.755
		2390	0.747
		2690	0.742
		2990	0.736
		3290	0.732
37	9	290	0.769
		590	0.744
		890	0.721
		1190	0.700
		1490	0.683
		1790	0.664
		2090	0.652
		2390	0.642
		2690	0.631
		2990	0.625
		3290	0.619

[a] The concentrations of **10** and **4** utilized are reported in the respective experimental sections. These data were used to calculate the rate constants, k''' in eq 3, reported in Table II.

of **4** (1190 s) are reported in Table III. The cell was opened at 19 h and any residual hydroquinone air oxidized to quinone. HPLC analysis of the reaction solution showed only 4-demethoxy-6-deoxydaunomycin (**10**).

Reaction of 4-Demethoxy-6-deoxydaunomycin Hydrochloride (10) with *d,l*-TM-3 Dimer at 37 °C and pH 8. The two-compartment cell was charged and freeze–thaw degassed as described above to give a solution 1.00×10^{-4} M in 4-demethoxy-6-deoxydaunomycin hydrochloride and 5.0×10^{-5} M in *d,l*-TM-3 dimer upon mixing. After 15 min of temperature equilibration at 36.9 ± 0.1 °C, the reactants were mixed and the progress of the reaction monitored spectroscopically for 22 h. The elevated temperature yielded an increased rate of reduction along with a slight increase in the rate of oxidation of hydroquinone by oxazinone **5**. As a result the absorption at 400 nm maximized sooner, 230 vs. 890 s, than during the reduction at 25 °C. Absorption vs. time after 6 half-lives of **4** (290 s) are reported in Table III. HPLC analysis again showed that starting material was the only quinone present upon opening the cell.

Reaction of 4-Demethoxy-6-deoxydaunomycin Hydrochloride (10) with *d,l*-TM-3 Dimer (4) at 37 °C and pH 9. The two-compartment cell was charged and freeze–thaw degassed as described above to give a solution 8.90×10^{-5} M in 4-demethoxy-6-deoxydaunomycin hydrochloride in 2.0×10^{-3} M Tris–Tris·HCl buffered methanol adjusted to pH 9.0 and 8.90×10^{-5} M in *d,l*-TM-3 dimer upon mixing. The pH was obtained by addition of 80 μL of a 0.050 M methanolic sodium hydroxide solution to a 5.0 mL aliquot of the drug–Tris buffer solution. The cell was equilibrated at 37.2 ± 0.1 °C for 15 min in the thermostated cell holder. Upon mixing, UV–vis spectra were recorded periodically for 22 h. As a result of increasing the pH, the rate of oxidation of hydroquinone increased further. Absorption at 400 nm reached a maximum at 250 s. Oxidation of hydroquinone by oxazinone **5** was virtually complete by 21 h; consequently, no spectral change was observed upon opening the cell. Absorption vs. time after 6 half-lives (290 s) of **4** are reported in Table III. HPLC analysis again showed starting material as the only quinone product. A similar reaction was performed with 2.0×10^{-4} M 4-demethoxy-6-deoxydaunomycin and 2.0×10^{-4} M *d,l*-TM-3 dimer in pH 9.0 buffered methanol and analyzed by capillary GLC for the products of *d,l*-TM-3 dimer. The analysis was performed as described for the analysis of the products of reduction of 7,7′,11,11′-tetradeoxy-7,7′-bidaunomycinonyl (**13**) and showed 2.0×10^{-4} M 3,5,5-trimethyl-2-oxomorpholine (**15**) and 2.0×10^{-4} M 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**5**).

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Supplementary Material Available: Five figures (3–7) showing spectral changes during the reductions of adriamycin (**1**), 4-demethoxydaunomycin (**9**), and 4-demethoxy-6-deoxydaunomycin (**10**) with TM-3 dimer (5 pages). Ordering information is given on any current masthead page.