Reaction of 2a with Methyllithium and Quenching of 5a with Protic Acids (or Protic Solvents) *in a Flask*. To a solution of **2a** (100 mg, 0.16 mmol) in 10 mL of THF *in a flask* was added methyllithium (20% excess of a 1.3-1.6 M ether solution) at room temperature with stirring under N_2. After 30 min of stirring, the mixture was quenched with 5-10 equiv of protic acids. The products were separated by TLC (ethyl acetate-*n*-hexane), and the ratio of **2a** to **2e** was determined by integration of CF_3 signals.

Protonolysis of 5bA with Various Protic Acids. To a solution of **2a** (150 mg, 0.24 mmol) in 15 mL of THF at room temperature was added 1.1 equiv of *p*-$CF_3C_6H_4Li$ (0.14 M hexane solution) with stirring under N_2 at room temperature. After 10 min of stirring, ca. 0.5 mL of the solution was transferred to an NMR tube and quenched with 100 equiv of protic acids. The ratio of **2a** to **2h** was calculated from the $CF_3C_6H_4$ signal of **2h** and the CF_3 signal (bidentate ligand) of **2a** and **2h**. The products were determined by TLC separation.

3,3-Bis(trifluoromethyl)-1,1-bis(*p*-methylphenyl)-1-[*p*-(trifluoromethyl)phenyl]-3*H*-2,1-benzoxastibole (2h): mp 182.5-183.5 °C; ^1H NMR ($CDCl_3$) 2.37 (s, 6 H), 7.24 (d, 4 H, J = 8 Hz), 7.10-8.10 (m, 8 H); ^{19}F NMR (THF) -75.0 (s, 6 F), -63.7 (s, 3 F). Anal. Calcd for $C_{30}H_{22}F_9OSb$: C, 52.13; H, 3.21. Found: C, 51.87; H, 3.20.

Protonolysis of a Mixture of 5b with 100 equiv of Acetic Acid. To a solution of **2h** (116 mg, 0.17 mmol) in 10.7 mL of THF was added 1 equiv of *p*-$CH_3C_6H_4Li$ (1.27 M ether solution) with stirring under N_2 at 0 °C. After 5 min of stirring, ca. 0.5 mL of the solution was transferred to an NMR tube at appropriate time intervals. The ratio of the three ate complexes (**5bA**:**5bB**:**5bC**) was measured by ^{19}F NMR, and the solution was quenched with 100 equiv of acetic acid. The ratio of **2a** to **2h** was determined by ^{19}F NMR.

Protonolysis of 5cA, 5dA, 5eA, and 5gA with 100 equiv of Acetic Acid or a Large Excess of Water. To a solution of **2c** (103 mg, 0.13 mmol) in 3 mL of ether was added 1.5 equiv of *p*-$CH_3C_6H_4Li$ with stirring under N_2 at -78 °C. After 30 min of stirring at room temperature the mixture was quenched with 100 equiv of acetic acid or a large excess of water. The ratio of **2c** to **2g** was determined by ^{19}F NMR and/or HPLC analysis. The products were separated by TLC (chloroform-*n*-hexane 3:8).

3,3-Bis(trifluoromethyl)-1-(*p*-methylphenyl)-1,1-bis[*p*-(trifluoromethyl)phenyl]-3*H*-2,1-benzoxastibole (2g): mp 166.5-169 °C; ^1H NMR ($CDCl_3$) 2.40 (s, 3 H), 7.28 (d, 2 H, J = 8 Hz), 7.49 (d, 2 H, J = 8 Hz), 7.12-8.04 (m, 12 H); ^{19}F NMR (THF) -75.0 (s, 6 F), -63.8 (s, 6 F). Anal. Calcd for $C_{30}H_{19}F_{12}OSb$: C, 48.35; H, 2.57. Found: C, 48.33; H, 2.60.

3,3-Bis(trifluoromethyl)-1-(*p*-methoxyphenyl)-1,1-bis(*p*-methylphenyl)-3*H*-2,1-benzoxastibole (2i): mp 169.5-170.0 °C; ^1H NMR ($CDCl_3$) 2.37 (s, 6 H), 3.82 (s, 3 H), 6.94 (d, 2 H, J = 9 Hz), 7.20 (d, 4 H, J = 8 Hz), 7.45 (d, 4 H, J = 8 Hz), 7.55 (d, 2 H, J = 9 Hz), 7.3-8.0 (m, 4 H). Anal. Calcd for $C_{30}H_{25}F_6O_2Sb$: C, 55.16; H, 3.86. Found: C, 54.92; H, 3.78.

3,3-Bis(trifluoromethyl)-1-(*p*-methoxyphenyl)-1,1-bis[*p*-(trifluoromethyl)phenyl]-3*H*-2,1-benzoxastibole (2j): mp 113.5-115 °C; ^1H NMR ($CDCl_3$) 3.84 (s, 3 H), 6.99 (d, 2 H, J = 9 Hz), 7.57 (d, 2 H, J = 9 Hz), 7.0-8.0 (m, 12 H); ^{19}F NMR ($CDCl_3$) -74.6 (s, 6 F), -63.4 (s, 6 F). Anal. Calcd for $C_{30}H_{19}F_{12}O_2Sb$: C, 47.34; H, 2.52. Found: C, 47.63; H, 2.48.

3,3-Bis(trifluoromethyl)-1-(*p*-chlorophenyl)-1,1-bis[*p*-(trifluoromethyl)phenyl]-3*H*-2,1-benzoxastibole (2k): mp 159.5-161 °C; ^1H NMR ($CDCl_3$) 7.69 (s, 8 H), 7.0-8.1 (m, 8 H); ^{19}F NMR ($CDCl_3$) -74.7 (s, 6 F), -63.5 (s, 6 F). Anal. Calcd for $C_{29}H_{16}OF_{12}ClSb$: C, 45.49; H, 2.11. Found: C, 45.58; H, 2.15.

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Formation and Reaction of the Quinone Methide from Reductive Cleavage of the Antitumor Drug Menogaril[1]

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Abstract: Anaerobic reduction of menogaril (**1**), a semisynthetic antitumor drug in clinical trials, with *d,l*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer) in methanol gave 7-deoxynogarol (**5**) and stereoisomers of bi(7-deoxynogarol-7-yl) (**6**) and, in the presence of *N*-acetylcysteine, 7-(*N*-acetylcysteinyl)-7-deoxynogarol (**10**) via an observed quinone methide intermediate (**8**). In the presence of excess reducing agent, **5** was formed relatively rapidly as the major product in its hydroquinone state. The rate-controlling step, tautomerization of the quinone methide, was autocatalyzed; the product, the hydroquinone of **5**, catalyzed the reaction. In fact, several anthracycline-derived hydroquinones were effective catalysts. Uncatalyzed tautomerization of the quinone methide yielded little if any **5**, in contrast with facile unimolecular formation of 7-deoxyaglycons from reduction of other anthracyclines. In the absence or presence of excess reducing agent, the rate of formation of **6** or formation of **6** in its bishydroquinone state, respectively, was second order in quinone methide concentration and relatively slow. The rate constants for the autocatalyzed tautomerization and the dimerization of the quinone methide are 27 ± 2 and 11 ± 1 M^{-1} s^{-1}, respectively. Reduction of menogaril in aqueous medium gave predominantly 7-deoxynogarol (**5**) relatively rapidly with excess reducing agent and a mixture of **5** and the aglycon dimer **6** slowly with substoichiometric amounts of reducing agent. Under both sets of conditions, the quinone methide transient was not observed. Reduction in aqueous medium with 0.3 equiv of reducing agent in the presence of *N*-acetylcysteine gave high yields of adduct **10**, suggesting a relatively long lifetime for the unobservable quinone methide transient even in aqueous medium in the absence of hydroquinones and reactive nucleophiles. A possible in vivo consequence of the relatively slow uncatalyzed tautomerization of the quinone methide is efficient nucleophilic trapping.

Menogaril, 7-*con*-O-methylnogarol (**1**), is a semisynthetic antitumor drug of the anthracycline class synthesized from nogalamycin, a product of the organism *Streptomyces nogalater*.[3] The molecular structure and absolute stereochemistry result from

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chemical, spectroscopic,[3,4] and X-ray studies.[5] Menogaril is presently in clinical trials[6] in part because it has a cardiotoxicity $^1/_{15}$ that of doxorubicin in chronically treated rabbits with only a 5–10 times less potency.[7] Because menogaril bears an alkoxy group in the 7-position, it has the potential for bioreductive activation via elimination of methoxide in a reduced state leading to an intermediate that reacts in vivo with a critical biological macromolecule.[8] Reductive elimination of alkoxide groups from either semiquinone or hydroquinone states of the anthracyclines and mitomycins[9-11] continues to be debated. Abdella and Fisher and Powis have extensively reviewed this and other aspects of the biological activity of the anthracyclines.[12] We[13-16] and others[17-20] have presented evidence that reduction of daunomycin, adriamycin, aclacinomycin A, 11-deoxydaunomycin, and 5-imidodaunomycin leads to formation of reactive quinone methide states via the respective hydroquinone states. The quinone methide states react in vitro via nucleophilic[17,18] and/or electrophilic[13] addition at the 7-position. The principal example of electrophilic addition is reaction with a proton to yield the 7-deoxyaglycons which are also important in vivo metabolites.[21] The quinone methide state also dimerizes at the 7-position with one quinone methide serving as an electrophile and the other as a nucleophile.[14,15] The occurrence of the dimerization reaction, which appears to be unique to the 11-deoxyanthracyclines including 11-deoxydaunomycin, aclacinomycin A, and now menogaril, has prompted some[22] to support elimination at the semiquinone state to form the quinone methide radical, which undergoes radical combination at the 7-position.

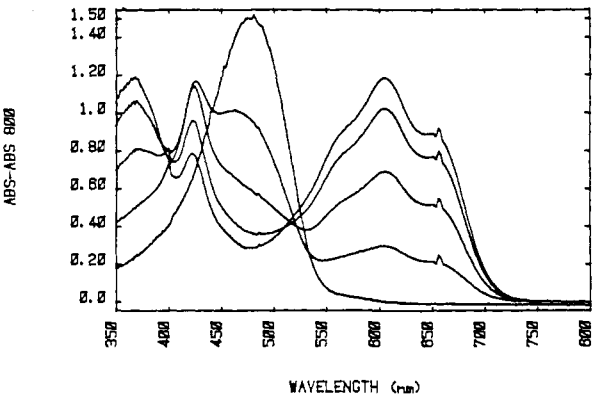
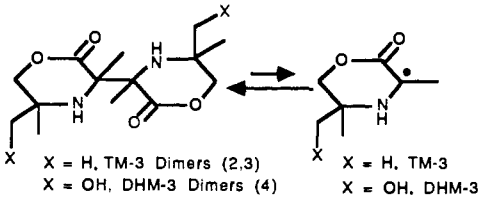


Figure 1. UV–vis spectra of a Trizma-buffered, methanol solution initially 1.0×10^{-4} M in menogaril and 2.0×10^{-4} M in TM-3 dimer at 25 °C as a function of time. The buffer consisted of 1.0×10^{-4} M tris(hydroxymethyl)aminomethane and 1.0×10^{-4} M tris(hydroxymethyl)aminomethane hydrochloride. Spectral scans occurred at times 0, 50, 100, 190, and 250 s after mixing the reagents. Menogaril (**1**) absorbs at 475 nm, menogarilhydroquinone at 420 nm, and quinone methide **8** at 375 and 604 nm.

A useful tool for generating the redox states of the anthracyclines in vivo and in vitro has been the oxomorpholinyl radicals from spontaneous bond homolysis of oxomorpholinyl radical dimers upon dissolution. The oxomorpholinyls that have proven to be most useful are 3,5,5-trimethyl-2-oxomorpholin-3-yl (TM-3) from



bond homolysis of either *d,l*- or *meso*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**2** or **3**)[23] and the water-soluble version, 3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl (DHM-3), from bond homolysis of a mixture of the stereoisomers of bi[3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl] (**4**).[24] The radicals in solution exist in equilibrium with their respective dimers unless a reducible substrate such as one of the quinone antitumor drugs is present. In the presence of a reducible substrate they react rapidly, most likely by one-electron transfer.[13,25] With many of the quinone antitumor drugs, the various redox states have been generated in succession by reaction with TM-3 dimer.[13-15] Because the oxomorpholinyl radical dimers show low animal toxicity and apparent in vivo redox activity with the anthracyclines and mitomycin C to produce ultimately inactive and nontoxic states, they also have potential as antidotes for extravasation necrosis[26,27] and high dose rescue therapy[28] with these quinone antitumor drugs.

Fisher and co-workers have reported that reduction of menogaril with spinach ferrodoxin–$NADP^+$ reductase in aqueous medium gives 7-deoxynogarol (**5**) with only the hydroquinone state of **1** as an observable transient.[17] Our ability to observe and study the reactivity of anthracycline quinone methide states generated by reduction with TM-3 dimer in methanol solvent prompted this investigation. We now report that reduction of menogaril in

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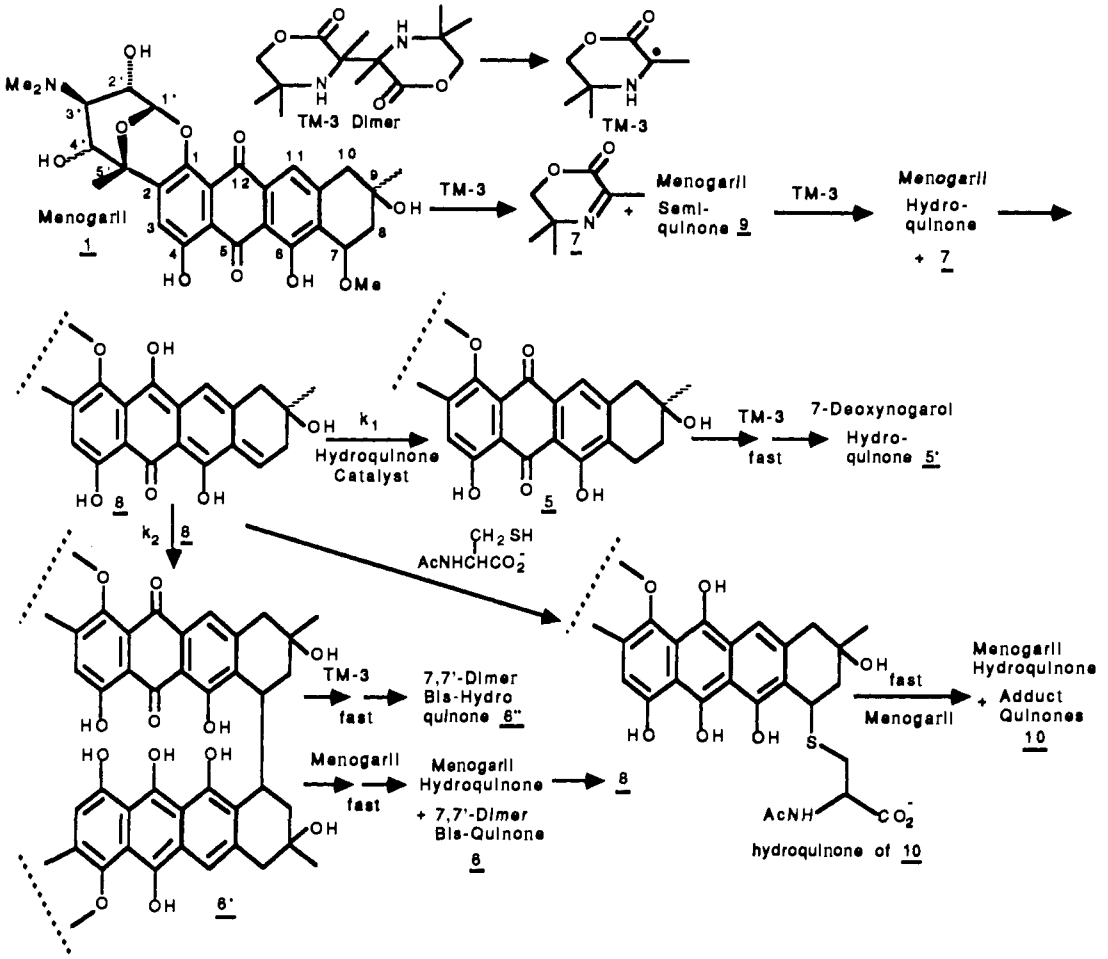
Scheme I



methanol solvent generates a long-lived quinone methide state, the lifetime of which is significantly shortened by the presence of hydroquinones, especially with water also present. In the absence of hydroquinones the long-lived quinone methide state is easily trapped by a good nucleophile.

Results and Discussion

As communicated earlier,[29] reduction of menogaril with 2 equiv or more of TM-3 dimer in apparent pH 8 buffered methanol at ambient temperature gave 85% 7-deoxynogarol (**5**) and 15% bi(7-deoxynogarol-7-yl) (**6**) as well as the product of oxidation of TM-3, 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**7**). Similar reduction with 0.5 equiv of TM-3 dimer gave 30% **5**, 40% **6**, and 30% recovered **1**. In both cases the yield of **6** is reported in terms of quinone units. The molar yield is half the number given. The products were isolated by reverse-phase, flash chromatography and characterized from spectroscopic data and, in the case of **5**, by comparison with an authentic sample. The reactions and a kinetic mechanism for formation of **5** and **6** as discussed below are shown in Scheme I.

UV–visible monitoring of the reduction of **1** by TM-3 dimer showed initial formation of the hydroquinone state absorbing at λ_{max} 420 nm followed by formation of the quinone methide state **8** absorbing at λ_{max} 375 and 604 nm with a shoulder at 648 nm as shown in Figure 1. The absorption bands were assigned based upon similar absorptions for the hydroquinone and quinone methide states of other anthracyclines.[13-15] With the subsequent disappearance of the quinone methide was appearance of the band for quinones **5** and **6** at λ_{max} 475 nm when substoichiometric quantities of reducing agent were employed and of the band for the hydroquinones of **5** and **6** at λ_{max} 420 nm when excess reducing agent was employed. In fact, with excess reducing agent the initially formed quinone products were rapidly reduced to their hydroquinone states. The decay of the 604-nm band of **8** was significantly dependent upon whether final products were formed in quinone or hydroquinone states as shown in Figure 1 of the earlier communication.[29] When final products were formed in hydroquinone states, the decay of the 604-nm band, significantly past its maximum absorbance, appeared deceptively first order; however, the apparent first-order rate constant, which was independent of the initial TM-3 dimer concentration, was proportional to the initial menogaril concentration. When final products were formed in quinone states, the decay of the 604-nm band was much slower, second order, and independent of the initial menogaril and TM-3 dimer concentrations.

A very low concentration of most like the menogaril semiquinone state (**9**) was observed by EPR spectroscopy upon reduction with TM-3 dimer in 10% dimethylformamide–90% methanol-*d* solvent at 5 °C. Because of the high modulation amplitude required, the signal was broad and the only hyperfine splittings that could be resolved were those from coupling with the benzylic protons. The coupling constants, assigned to one pseudoaxial and two pseudoequatorial protons, and the *g* value reported in the Experimental Section were consistent with those observed for other anthracycline semiquinones.[13,14,30] The concentration of the semiquinone of menogaril maximized under these conditions at about the same time as the quinone methide as determined by alternate EPR and UV–visible monitoring of the reaction mixture. In the analogous reduction of daunomycin, the semiquinone concentration maximized before the quinone methide concentration maximized.[13]

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Table I. Rate Constants (k_1) for Catalyzed Decay of Quinone Methide **8** to the Hydroquinone **5'** of 7-Deoxynogarol in Methanol[a]

[**1**], M × 10^4	reducing species (mol/mol of **1**)	added species (mol/mol of **1**)	k_1, M^{-1} s^{-1}	av $\tau_{1/2}$,[e] s
0.50	**2** (2)		28.3	700
0.60	**2** (5)		25.9	625
0.60[b]	**2** (5)		61.0	300
0.60[c]	**2** (5)		115	170
1.0	**2** (2)		26.0	385
1.0	**2** (5)		25.4	385
1.5	**2** (5)		28.0	230
1.5	**2** (10)		25.9	240
1.5	**3** (10)		28.7	220
1.5	**4** (10)		29.2	225
2.0	**2** (10)		25.9	165
1.0	**2** (2)	EDTA (1.1)	28.7	350
1.0	**2** (2)	Desferal (1.0)	28.1	335
1.0	**2** (2)	**7** (2.8)	22.9	420
1.0	**2** (3)	**5** (0.5)	27.9	205
1.0	**2** (5)	**5** (1.0)	27.6	140
1.0	**2** (12.5)	**5** (1.5)	25.4	115
0.50	**2** (3)	**6** (1.0[d])	25.3	345
0.80	**2** (4)	7-deoxydaunomycinone (1.0)		250
1.0	**2** (4)	alizarin (1.0)		230
1.0	**2** (5)	1,4-benzoquinone (1.0)		360

[a] Rate constants were calculated from eq 1. Data were obtained by measuring the decay of the quinone methide absorbance at 646–650 nm as a function of time, within a range of decay of 80–15% of the maximum value of the absorbance. The temperature was 25 ± 0.1 °C. The ϵ value for **8** at 646–650 nm was 10^4. The standard deviations from the least-squares analyses were less than 1.5%. [b] At pH 7.2. [c] At pH 6.0. [d] Actual molar concentration of **6** was 2.5×10^{-5} as two anthracycline units per mole of **6** were considered in calculations according to eq 1. [e] Determined from a first-order fit of the decay of the quinone methide band.

Although the UV–visible spectroscopic changes that occurred during reduction of menogaril were completely analogous to those observed during reduction of other anthracyclines,[13-15] the dependence of the apparent first-order rate constant for disappearance of the quinone methide on the initial menogaril concentration was unique. A rationale was evident from the observation that the decay of the 604-nm band in the presence of excess reducing agent was faster when the reaction was run with either product, **5** or **6**, present at time zero as also shown in Figure 1 of ref 29. The product present at time zero was rapidly reduced to its hydroquinone state and served as a catalyst for the tautomerization of the quinone methide to **5**. In the absence of initial product the reaction was autocatalyzed since the hydroquinone states of **5** and **6** were formed as the reaction proceeded. Further evidence for hydroquinones serving as catalysts for the decay of the quinone methide was the effect of adding TM-3 dimer or menogaril to the rate of decay of preformed quinone methide. Addition of 2 equiv of TM-3 dimer to quinone methide formed initially with 0.5 equiv of TM-3 dimer led to rapid formation of additional quinone methide and then a much faster rate of decay of the quinone methide, equal to that observed when quinone methide was formed initially with excess TM-3 dimer. Addition of 2 equiv of menogaril to quinone methide formed initially with 2 equiv of TM-3 dimer led to formation of additional quinone methide and then a much slower rate of decay of the quinone methide, equal to that observed when quinone methide was formed initially with substoichiometric amounts of TM-3 dimer. The decay of the 604-nm band in the presence of excess reducing agent was fit by nonlinear least-squares analysis to the integrated rate law (eq 1) for catalyzed decay of **8** to the hydroquinone of **5** and

$$A_t = [A_0k_1([\mathbf{1}]_0 + [\mathbf{5}\text{ or }\mathbf{6}]_0)]/[\exp(k_1([\mathbf{1}]_0 + [\mathbf{5}\text{ or }\mathbf{6}]_0)t) \times \{k_1([\mathbf{1}]_0 + [\mathbf{5}\text{ or }\mathbf{6}]_0) - A_0(k_1 - k_2)/\epsilon\} + A_0(k_1 - k_2)/\epsilon] \quad (1)$$

uncatalyzed dimerization of **8** to the bishydroquinone of **6** where A_t is the absorbance at 648 nm at time t from a time zero selected such that all menogarilhydroquinone had eliminated methanol to form quinone methide **8**, A_0 is the absorbance at the selected time zero, $[\mathbf{1}]_0$ is the initial concentration of menogaril, $[\mathbf{5}\text{ or }\mathbf{6}]_0$ is the initial concentration of the catalyst 7-deoxynogarol (**5**) or bi(7-deoxynogarol-7-yl) (**6**) with units of moles of quinone per liter, k_1 is the second-order rate constant for formation of **5** catalyzed by hydroquinones, k_2 is the second-order rate constant for dimerization of **8**, and ϵ is the extinction coefficient for **8** at 648 nm estimated to be 10^4 on the basis of observations described in the Experimental Section. Absorbance at 648 nm rather than 604 nm as a function of time was employed to minimize any contribution to the absorption by any hydroquinone species. The rate constants k_1 and k_2 were 27 ± 2 and 11 ± 1 M^{-1} s^{-1}, respectively, with initial concentrations of menogaril varying from 0.5×10^{-4} M to 2.0×10^{-4} M and with initial concentrations of **5** varying from 0 to 1.5×10^{-4} M as reported in Table I.

Table II. Rate Constants (k_1) for Catalyzed Decay of Quinone Methide **8** to the Hydroquinone **5'** of 7-Deoxynogarol in Water–Methanol[a]

% water, v/v	[**1**], M × 10^4	k_1, M^{-1} s^{-1}	av $\tau_{1/2}$,[b] s
5	1.0	33	260
5	1.5	33	170
10	1.0	46	190
20	1.0	63	130

[a] The quinone methide **8** was obtained by reduction of menogaril (**1**) with a 15-fold molar excess of *d,l*-TM-3 dimer (**2**). Rate constants were calculated from eq 1. Data were obtained by measuring the decay of the quinone methide absorbance at 646–650 nm as a function of time, within a range of decay of 60–20% of the maximum value of the absorbance. Temperature was 25 ± 0.1 °C, and the pH was 8.2. The ϵ value for **8** at 646–650 nm was 10^4. The standard deviations from the least-squares analyses were less than 7%. The rate constant for the uncatalyzed dimerization of **8** to the bishydroquinone of **6** (k_2 of eq 1) was set at 11 M^{-1} s^{-1}, the same value as for the reaction in pure methanol. [b] Determined from a first-order fit of the decay of the quinone methide band.

The differential form of the rate law (eq 2) was derived as follows. The important steps contributing to the kinetics were assumed to be as follows:

quinone methide (**8**) + hydroquinone (catalyst) →
7-deoxynogarol (**5**) + hydroquinone (catalyst) (slow, k_1)

7-deoxynogarol (**5**) + 2TM-3 →
7-deoxynogarolhydroquinone (**5'**) (fast)

2 quinone methide (**8**) →
7,7'-dimer–quinone–hydroquinone (**6'**) (slow, k_2)

7,7'-dimer–quinone–hydroquinone (**6'**) + 2TM-3 →
7,7'-dimer–bishydroquinone (**6''**) (fast)

Then $d[\mathbf{8}]/dt = -k_1[\mathbf{8}]_t[\text{hydroquinone}]_t - k_2[\mathbf{8}]_t^2$ where $[\text{hydroquinone}]_t \cong [\mathbf{8}]_0 + [\mathbf{5}\text{ or }\mathbf{6}]_0 - [\mathbf{8}]_t$. The expression for hydroquinone concentration at time t assumes that all hydroquinone units catalyzed tautomerization equally effectively and that under the reaction conditions all quinones were rapidly reduced to hydroquinones. The validity of these assumptions was established by experiment; in particular, the k_1's observed with either **5** or **6** present at the beginning of the reduction were similar within experimental error (see Table I). Then

$$d[\mathbf{8}]_t/dt = -k_1([\mathbf{1}]_0 + [\mathbf{5}\text{ or }\mathbf{6}]_0)[\mathbf{8}]_t - (k_2 - k_1)[\mathbf{8}]_t^2 \quad (2)$$

With 0.5 equiv of TM-3 dimer as the amount of reducing agent, the acceptable integrated rate law for decay of the 604-nm band was simply the integrated second-order rate law, and the rate constant was 5.5 ± 1 M^{-1} s^{-1}, half the constant observed in the presence of excess reducing agent. The rate constant was half as large because the overall process destroyed two quinone methides by dimerization and quickly regenerated one quinone methide. The regeneration of **8** was accomplished through a very fast reduction of menogaril to its hydroquinone state by the semireduced form of **6**, shown as **6'** in Scheme I, followed by rapid cleavage of menogarilhydroquinone to **8**. This process was observed in an independent experiment that showed rapid quinone methide formation upon addition of menogaril to partially reduced **6**. In the presence of excess TM-3 dimer the overall process of

dimerization destroyed two quinone methides (see Scheme I) as no more menogaril was available.

The kinetic analysis described above explains the unusual observations of the rate of disappearance of quinone methide as a function of reaction conditions. With excess reducing agent the decay of the 604-nm band appeared first order because the exponential term dominates under these conditions. The apparent first-order rate constant was proportional to the initial menogaril concentration because the initial menogaril concentration appears in the exponential term of the integrated autocatalyzed rate law as shown in eq 1. When the decay of the 604-nm band under autocatalyzed reaction conditions was fit by nonlinear least squares to an integrated rate law that also included an uncatalyzed unimolecular tautomerization of the quinone methide (a process known to occur with other anthracyclines[13,15]), the fitting procedure made the first-order rate constant very small, less than 5×10^{-5} s^{-1}. Furthermore, when the decay in the absence of hydroquinones was fit to a combined first- and second-order integrated rate law, the fitting procedure again made the first-order rate constant very small, less than 5×10^{-6} s^{-1}. Consequently, at an apparent pH of 8 the quinone methide from reduction of menogaril does not undergo appreciable unimolecular tautomerization to 7-deoxynogarol. Formation of the 7-deoxynogarol, observed with 0.5 equiv of TM-3 dimer as the initial amount of reducing agent, must then have occurred via menogarilhydroquinone serving as a catalyst for the tautomerization of the quinone methide in the early stages of the reaction. In fact, UV–visible monitoring of the reduction under these conditions showed the presence of menogarilhydroquinone as the concentration of quinone methide was rising. Significant quantities of **5** were formed because the catalyzed process is relatively rapid. A possible explanation for the catalysis of tautomerization is that a head-to-head encounter places the acidic 6-hydroxyl of the hydroquinone in the vicinity of the basic 7-position of the quinone methide. Similar though less effective catalysis by 7-deoxydaunomycinone or alizarinhydroquinones was also observed; however, the hydroquinone of 1,4-benzoquinone was ineffective. Although eq 1 may not be used for the determination of k_1 with these auxiliary catalysts, catalysis or the lack of catalysis was evident from the average half-life determined from a first-order fit of the decay of the quinone methide absorption band (see Table I).

Table I also reports the effect of several other additivities. In the process of arriving at the autocatalytic mechanism, the effects of pH, of changing the reducing agent to DHM-3 dimer (**4**), of metal chelating agents, and of the presence of the product of oxidation of TM-3 (oxazinone **7**) were explored. The autocatalytic rate law fit the data under all of these conditions. The rate constant k_1 increased with decreasing pH relative to the value of 27 M^{-1} s^{-1}. The metal chelating agents, EDTA and Desferal, as well as changing the reducing agent to DHM-3 dimer had no effect. The presence of additional oxazinone **7** at time zero decreased the apparent k_1, possibly by decreasing the effective hydroquinone concentration. Oxazinone **7** has been observed to oxidize hydroquinones to quinones with reduction to 3,5,5-trimethyl-2-oxomorpholine.[13-15]

Examination of the structure of menogaril (**1**) reveals a similarity between C5′ and C7, as both are benzylic carbons carrying a potential leaving group. Under the reaction conditions menogaril undergoes reductive cleavage at C7 but not at C5′. The presence of a phenolic OH at C6 likely renders the methoxy group at C7 a better leaving group through hydrogen bonding, whereas this functionality is absent at C1.

Reduction of 7-deoxynogarol (**5**) and bi(7-deoxynogarol-7-yl) (**6**) with excess TM-3 dimer was also explored briefly. In both reductions the respective hydroquinone and bishydroquinone were formed. The only apparent reaction of these hydroquinones was most likely tautomerization to a leuco form. Slow formation of leuco isomers from reduction of 7-deoxydaunomycinone has been described by Brand and Fisher.[31] These are keto tautomers of hydroquinones. The leuco isomers of **5** and **6** were not isolated because they could not be produced in sufficient amounts. The primary evidence for their formation was absorption at shorter wavelength and reversion to **5** and **6**, respectively, upon air oxidation.[31]

In our earlier correlation of extravasation necrosis from subcutaneous injection of the anthracyclines with the rate constant for tautomerization of the respective quinone methide, we reported a first-order rate constant for tautomerization of quinone methide **8** in methanol solvent of 2.5×10^{-3} s^{-1}.[27] This rate constant was obtained from first-order fit of the decay of quinone methide generated with excess TM-3 dimer prior to our discovery of the autocatalysis of the tautomerization. As discussed above, we can now only estimate the rate constant at less than 5×10^{-6} s^{-1}. Such a small value is actually more consistent with the low skin toxicity observed for menogaril.

Because Fisher and co-workers[17] were unable to observe the quinone methide as a transient upon enzymatic reduction of menogaril to 7-deoxynogarol and did not observe formation of the aglycon dimer **6**, we explored the effect of addition of water on reduction of menogaril by TM-3 dimer. With addition of small amounts of water to the methanol solvent (5–20%, v/v) formation of the quinone methide could still be observed, and the autocatalytic rate law (eq 1) fit the data. With 20% water the rate constant more than doubled. When the reduction was performed at pH 8 with 95% water–5% dimethylformamide (DMF) as the solvent, the quinone methide **8** could not be observed spectroscopically. The product mixture consisted predominantly of 7-deoxynogarol (**5**; >90%); formation of only 1–2% of aglycon dimer **6** was observed. The DMF was present to facilitate dissolution of the menogaril. The kinetics of tautomerization in 95% water–5% DMF could not be determined because under these conditions tautomerization was not the slow step. The effect of addition of water on the decay of quinone methide preformed in methanol was also examined. Addition of 4.0 mL of buffered water to 1.0 mL of quinone methide in buffered 95% methanol–5% DMF, formed by reduction of menogaril with 10 equiv of DHM-3 dimer, resulted in the decay of the quinone methide absorbance at 648 nm from 2.7 to 0.14 in 12 s. The dilution accounted for a drop in the absorbance to 0.5 and the reactions of **8**, to 0.14. With the condition of a substoichiometric amount of reducing agent where **8** has a very long lifetime in methanol solvent, formation of **8** was barely if at all observable in 95% water–5% DMF. However, substantial amounts of aglycon dimer **6** were formed, suggesting that the quinone methide lifetime is sufficient for bimolecular reaction under these conditions. Even with a relatively long lifetime, the quinone methide would not be observed if its rate of reaction significantly exceeded its rate of formation. Water in the medium seems to slow formation of the quinone methide from reduction of menogaril, much more so than formation of the quinone methide from reductive cleavage of daunomycin.[13,17] The slow step in the formation of **8** by reduction of menogaril with DHM-3 or with an NADPH enzyme[17] is the cleavage of menogarilhydroquinone.

The observation of the autocatalytic decay mechanism for the tautomerization of the quinone methide state of menogaril in the presence of hydroquinones raised the question of applicability to the tautomerization of quinone methides from reductive cleavage of other anthracyclines. The redox chemistry of aclacinomycin A was shown earlier to be similar to that of menogaril in that a 7-deoxyaglycon dimer as well as the 7-deoxyaglycon is formed.[14] The earlier report stated that the decay of the quinone methide from reductive cleavage of aclacinomycin A followed mixed first- and second-order kinetics consistent with first-order formation of the 7-deoxyaglycon and second-order formation of the 7-deoxyaglycon dimer. A brief reinvestigation now indicates that the decay kinetics are more complicated and that an autocatalytic tautomerization is operating at least in part under conditions of excess reducing agent. A similar reinvestigation of the reductive cleavage of daunomycin did not show any evidence for the autocatalytic tautomerization of its quinone methide state.

The long lifetime of the quinone methide **8** upon reduction with substoichiometric amounts of reducing agent suggested a high

(31) Brand, D. J.; Fisher, J. *J. Am. Chem. Soc.* **1986**, *108*, 3088.

Table III. Reduction of Menogaril (1) with d,l-TM-3 Dimer (2) in the Presence of N-Acetyl-L-cysteine[a]

[2], M × 10^5	[N-acetyl-cysteine], M × 10^2	concn, % unreact. 1	5	6	10	others
4.2	0	36	22	33		<10
4.2	2.5	~5	23	<5	59	<10
2.1	2.5	~10	~5	<5	80	<10
1.1	2.5	15	<5	<5	75	<10

[a] The reactions were run for 24 h at 25 °C in methanol buffered with 5.2 × 10^-2 M Tris and 2.5 × 10^-2 Tris·HCl with [1] = 1.0 × 10^-4 M.

probability for nucleophilic trapping with sulfur nucleophiles. The concept of bioreductive activation of the anthracyclines calls for addition of a nucleophilic site in a critical biological macromolecule to the quinone methide.[8] Reduction of **1** in the presence of *N*-acetylcysteine gave **5**, **6**, and a mixture of the diastereomers of 7-*S*-(*N*-acetylcysteinyl)-7-deoxynogarol (S adducts, **10**) in amounts depending upon the initial stoichiometry as reported in Table III. The yield of S adducts **10** was highest with 0.1–0.2 equiv of reducing agent. This is consistent with the Fisher chain mechanism for nucleophilic trapping of anthracycline quinone methides.[17,18] In the Fisher mechanism nucleophilic addition yields initially an adduct in the hydroquinone redox state that reforms the quinone methide unless it is oxidized to the quinone redox state by starting quinone. The mixture of diastereomeric S adducts was characterized by acid-catalyzed esterification with methanol and comparison with the S adducts isolated from reduction of menogaril in the presence of *N*-acetylcysteine methyl ester. The methyl ester of **10** was isolated as a 3:1 mixture of diastereomers and characterized from spectroscopic data.

Because the presence of *N*-acetylcysteine completely suppressed the formation of the aglycon dimer **6** even with amounts of reducing agent that would normally favor dimer formation as shown in Table III, the possibility of *N*-acetylcysteine reacting with **6** upon partial reduction was examined. The reason for partial reduction was the earlier observation that the analogous aglycon dimer from reductive cleavage of aclacinomycin A in its half-quinone–half-hydroquinone redox state formed some 7-deoxyalklavinone, the 7-deoxyaglycon of aclacinomycin A, most likely via cleavage to two molecules of quinone methide followed by tautomerization.[14] Partial reduction of **6** in the presence of *N*-acetylcysteine upon workup gave only recovered **6**. The lack of dimer formation then most likely results from nucleophilic trapping being much faster than dimerization. Less efficient suppression of 7-deoxynogarol (**5**) formation by *N*-acetylcysteine probably results from some autocatalytic tautomerization of the quinone methide by the hydroquinones present during the chain mechanism. Interestingly, *N*-acetylcysteine can displace methanol from the 7-position of menogaril even in the absence of reducing oxomorpholinyl radicals; however, this displacement occurs at a rate much slower than substitution in the presence of reducing species.

Formation of aglycon dimer **6** upon reduction with 0.5 equiv of DHM-3 dimer in aqueous medium suggested that nucleophilic trapping of the quinone methide should also be successful in aqueous medium even though the quinone methide concentration remains very low during reduction. Reduction of menogaril with 0.33 equiv of DHM-3 dimer in the presence of excess *N*-acetylcysteine gave an 80% yield of a mixture of the stereoisomers of adduct **10** and 9% of 7-deoxynogarol (**5**).

The relatively long lifetime for quinone methide **8** in methanol and water solvents in the absence of hydroquinone catalysts suggests that if **8** is formed in vivo, the probability for covalent binding to nucleophilic sites in critical macromolecules may be higher than the probability for covalent binding of the quinone methide from reduction of daunomycin or adriamycin. Under similar conditions, the half-life of the quinone methide from reduction of daunomycin is only 53 s[13] and from reduction of adriamycin, only 63 s.[15] The slow uncatalyzed tautomerization of the quinone methide **8** and its reactivity with nucleophiles may explain the low recovery of menogaril and its metabolites in animal[32] and human[33] metabolic studies. Only menogaril and *N*-demethylmenogaril were detected in human plasma, bile, and urine. Biliary and urinary excretion accounted for only 2–4% and 5%, respectively, of the daily dose.[33] Recovered drug and fluorescent metabolites in organs and extracellular fluid accounted for only 10% of the menogaril administered to rabbits.[32] Egorin and co-workers suggested the possible formation of less or nonfluorescent metabolites as an explanation for the low recovery. Efficient covalent binding of the quinone methide **8** to biological macromolecules, which would interfere with extraction of molecules bearing the fluorescent chromophore, is an alternate explanation.

In summary, reduction of menogaril (**1**) with TM-3 dimer in methanol solvent yields the quinone methide **8** as an observable transient. The quinone methide tautomerizes to 7-deoxynogarol (**5**) and dimerizes to the half-quinone–half-hydroquinone state of bi(7-deoxynogarol-7-yl) (**6**). The tautomerization is catalyzed by hydroquinones, especially the hydroquinones of **1**, **5**, and **6** which are present when excess reducing agent is employed, and is very slow in the absence of hydroquinones. Trapping of **8** with the nucleophile *N*-acetylcysteine is competitive with tautomerization and dimerization under conditions of substoichiometric amounts of reducing agent where hydroquinones are in low concentration and menogaril in its quinone oxidation state is available for oxidizing adduct **10** in its hydroquinone state. Although formation of **8** cannot be observed in aqueous medium even with substoichiometric amounts of reducing agent because its rate of destruction exceeds its rate of formation, **8** must be relatively long lived in aqueous medium because it can be efficiently trapped with *N*-acetylcysteine.

Experimental Section

General Remarks. EPR spectra were recorded with a Varian Associates Model 109E spectrometer equipped with a field-frequency lock and UV–visible spectra with a Hewlett-Packard 8450A or 8452A diode array spectrometer. ^{1}H NMR spectra were obtained with Bruker WM 250 and Varian Gemini 300 spectrometers. Chemical shifts are reported (in ppm) on the δ scale from internal tetramethylsilane and coupling constants are given in hertz. Mass spectra were obtained with a VG Instruments 7070 EQ-HF high-resolution mass spectrometer equipped with FAB inlet system. HPLC was performed with a Hewlett-Packard Model 1090 chromatograph equipped with a diode array UV–visible detector and data processing unit. A 2.1 × 100 mm Hypersil ODS 5-μm reverse-phase column was used for analytical analyses. The initial composition of the eluting solution was 60% v/v aqueous pH 4 buffer (3% ammonium formate adjusted to pH 4.0 with formic acid) and 40% methanol. The solvent delivery system was programmed to change gradually to 35% buffer in 12 min and then to 20% buffer in 8 min, at a flow rate of 0.5 mL/min. A Rainin, Dynamax Macro C-18, 60 A 8-μm column was used for preparative work. The eluting solution was a mixture of an aqueous pH 4 buffer (see above) and methanol in different ratios programmed as follows [v/v% buffer, time (min)]: 40, 0; 35, 5; 20, 15; 20, 30; 0, 35. The flow rate was 4 mL/min. A guard module (21.4 × 50 mm) preceded the column. The percentages of anthracyclines reported from HPLC analyses are the area percentages of the peaks resulting from compounds absorbing in the 420–500-nm region. TLC was performed with EM reagents' precoated silica gel 60 F-250 sheets. Flash chromatographies were performed according to the literature, using Merck 0.040–0.063-mm silica gel.[34] Freeze–pump–thaw–sonicate degassing was performed at liquid nitrogen temperature at 5 × 10^-6 Torr through four cycles, followed by sealing under vacuum. Cells, degassed via the freeze–thaw method, were fabricated from Pyrex glass and constructed as described previously.[14,15] TM-3 and DHM-3 dimers dissolved in dichloromethane and acetonitrile, respectively, were loaded into the cuvette compartment of the cells. The solvent was then removed with a nitrogen stream. No metal needles were used in any of the syringe transfers. Trizma buffers were prepared from tris(hydroxymethyl)aminomethane (Tris) and its hydrochloride (Tris·HCl). All the experiments with menogaril were run protected from direct light. Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*d,l*- and *meso*-TM-3 dimers) and bi[3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl] (DHM-3 dimers, mixture of diastereoisomers) were

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(34) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

prepared by photoreductive dimerization of the corresponding 5,6-dihydro-1,4-oxazin-2-ones.[24,35] 7-Deoxydaunomycinone was prepared according to the literature.[36] Menogaril, 7-deoxynogarol, and nogarol were gifts of the Upjohn Co., Kalamazoo, MI. *N*-Acetyl-L-cysteine, alizarin, and 1,4-benzoquinone were obtained from Aldrich Chemical Co., Milwaukee, WI. EDTA, Tris, and Tris·HCl were purchased from Sigma Chemical Co., St. Louis, MO. Desferal (desferamine mesylate) was obtained from Ciba, Basel, Switzerland. Reagent-grade, spectrograde, and HPLC-grade solvents were purchased from Fisher Scientific Co., Fair Lawn, NJ.

Reaction of Menogaril (1) with 2–10-Fold Molar Excess of Morpholinyl Radical Dimers (2–4) in Methanol. The desired amount of radical dimer was deposited into the cuvette portion of the reaction cell as described under General Remarks. Menogaril solutions, at concentrations as reported in Table I, were made in pH 8.2 (1 + 1) × 10^{-3} M Trizma buffer. An aliquot (2.5 mL) of the appropriate solution was introduced into the degassing chamber of the cell. After freeze–thaw degassing and sealing, the cell was brought to 25 °C in a water bath. The menogaril solution was poured into the cuvette and the cuvette vigorously shaken for a quick dissolution of the radical dimer. The cell was immediately transferred into the cell holder of the UV–visible spectrometer thermostated at 25.0 ± 0.1 °C. The spectral changes at 350–800 nm were then monitored at appropriate time intervals (see for instance, Figure 1). During the initial part of the reaction (100–200 s) the absorbance at 475 nm decreased, due to destruction of the starting quinone **1**; this was coupled with a rise of a band at 424 nm, characteristic of formation of hydroquinones, and a rise of a band at 604 nm, indicative of formation of quinone methide **8**. During the following 50–210 s the hydroquinone was destroyed as shown by the fall of the 424-nm band, while the absorbance due to the quinone methide continued to rise reaching a maximum at 60–210 s, depending on the reactant concentrations. The decay of the quinone methide at 604 nm then followed, coupled with an increase of a band at 420–424 nm due to the hydroquinones of **5** and **6**. In an experiment where **1** (c = 1.0 × 10^{-4} M) was reacted with **2** (c = 2.0 × 10^{-4} M) in the presence of added oxazinone **7** (c = 2.8 × 10^{-4} M), the final spectrum after the disappearance of the quinone methide appeared as an overlap of hydroquinone and quinone spectra due to partial oxidation of the hydroquinones of **5** and **6** to the corresponding quinones by **7**.[13-15] For kinetic calculations the decay of the absorbance of **8** at 646–650 nm, a region where only **8** absorbed significantly, was measured as a function of time. The decay of the absorbance over a range from 80% to 15% of its maximum intensity was fit to the rate law expressed by eq 1, using a nonlinear least-squares fitting procedure. The results are reported in Table I. A reasonable UV–visible spectrum of the quinone methide **8** was obtained by reacting a 2.4 × 10^{-5} M solution of **1** with a 10-fold excess of **2**. The spectrum of **8** reached its maximum intensity after 190 s. The spectrum showed that no more **1** was present. About 6–7% of hydroquinone species appeared to be present as shown from the low-intensity maximum at 422 nm. The ϵ_{422} for the hydroquinone species was calculated to be 2.0 × 10^4 from the spectrum obtained after all the absorbance due to **8** had virtually disappeared. The ϵ values for **8** calculated from these observations were 1.26 × 10^4 at 368, 1.13 × 10^4 (sh) at 574, 1.38 × 10^4 at 604, and 1.02 × 10^4 (sh) at 648 nm. In all the experiments reported in Table I, when the reaction was over (<3% of residual **8**) the cell was opened. In a few minutes, after the excess of the radical dimers and the hydroquinones had been oxidized by air oxygen, the spectrum showed the characteristic quinone chromophore with λ_{max} = 472–476 nm. If the cell was opened several hours after the reaction was over, broadening of the absorption spectrum due to the presence of leuco[31] forms absorbing in the 310–410-nm region was observed. HPLC analysis of the solutions showed the presence of 7-deoxynogarol (**5**; R_t 9 min, 80–90%) and bi(7-deoxynogarol-7-yl) (**6**; R_t 12 min, 5–20%), accounting for ca. 95% of the starting menogaril. The identity of **5** and **6** was established by coinjections with authentic samples (**5** from Upjohn Co. and **6** from a sample isolated as described below). When the reaction had been run for several hours after the disappearance of the quinone methide, HPLC analyses showed, along with **5** and **6**, their leuco forms (leuco **5**: λ_{max} = 310 and 404 nm, R_t 7.6 min; leuco **6**: λ_{max} = 430 and 470 nm, R_t 10.5 min). The nature of these leuco forms is suggested from their spectroscopic characteristics.[31] Moreover, both "leucos" from **5** and **6** were slowly oxidized back to **5** and **6**, respectively, when kept several hours in the presence of air, as shown by HPLC analysis.

Reaction of Menogaril (1) with Excess *d,l*-TM-3 Dimer (2) in the Presence of EDTA or Desferal. A cell was set up as described in the preceding paragraph. A sample equal to 0.14 mg of **2** (2 mol/mol of **1**) was introduced into the cuvette compartment of the cell, and 2.5 mL of a 1.0 × 10^{-4} M buffered methanolic solution of **1** (1.1 × 10^{-4} M in EDTA) was introduced into the degassing chamber of the cell. After the freeze–thaw degassing procedure the reaction was run as reported in the previous paragraph. In a similar experiment EDTA was replaced by 1.0 × 10^{-4} M Desferal. Kinetic data for the decay of the quinone methide absorbance at 646–650 nm are reported in Table I. HPLC analyses of the reaction mixtures after exposure to air only showed **5** and **6** and minor amounts of their leuco forms.

Reaction of Menogaril (1) with Excess *d,l*-TM-3 Dimer (2) in the Presence of Quinones Other Than Menogaril. The reactions were run as described above, in the second paragraph of the Experimental Section. The only difference was that the solutions of **1** to be reacted had different amounts of the following species: 7-deoxynogarol (**5**), bi(7-deoxynogarol-7-yl) (**6**), 7-deoxydaunomycinone, alizarin, and 1,4-benzoquinone, as reported in Table I. The qualitative course of the reaction, as shown by spectral changes, was the same as without the added quinones. However, the presence of the added quinone, with the only exception of 1,4-benzoquinone, caused an increased rate of fall of the quinone methide absorbance at 646–650 nm. Kinetic data are reported in Table I. HPLC analyses of the reaction mixtures after exposure to air showed **5** and **6**, along with the starting added quinones. No significant amounts of other products were observed.

Reaction of 7-Deoxynogarol (5) with *d,l*-TM-3 Dimer (2). The reaction was run as described above, in the second paragraph of the Experimental Section, using 7-deoxynogarol at c = 1.0 × 10^{-4} M instead of menogaril, and an equimolar amount of **2**. Under these conditions the quinone absorption band of **5**, at 480 nm, almost completely disappeared in 700 s to be replaced by a hydroquinone band at 426 nm. The half-life for the bond homolysis of **2** with the same experimental conditions is 170 s (see below). The cell was opened after a total of 210 min; whereby, the original quinone spectrum was restored, slightly broadened. HPLC analysis showed 90–95% of **5**, R_t 9 min, and ca. 5% of the corresponding leuco form, R_t 7.6 min. After 2 days at ambient temperature, the leuco species had disappeared and only the peak corresponding to **5** was present in the chromatogram.

Reaction of Bi(7-deoxynogarol-7-yl) (6) with *d,l*-TM-3 Dimer (2). The reaction was run as described above, in the second paragraph of the Experimental Section, using bi(7-deoxynogarol-7-yl) at c = 3.9 × 10^{-5} M instead of menogaril, and 4 mol of **2**/mol of **6**. With these conditions, the quinone absorption band of **6** at 480 nm disappeared in 190 s to be replaced by a hydroquinone band at 424 nm. The cell was opened after 160 min; whereby, the original quinone spectrum was restored, overlapped with absorption from a leuco species (a shoulder was present in the spectrum at 410 nm). HPLC analysis showed 60% of **6**, R_t 12.5 min, and 40% of its leuco form, R_t 10.5 min. No 7-deoxynogarol was detected. After 3 days at ambient temperature the leuco product decreased to less than 10%. Accordingly, the 410-nm shoulder in the spectrum disappeared and the 480-nm band increased.

Effect of pH on the Rate of Decay of the Quinone Methide 8 from Menogaril (1). Three separate experiments were run as described above, in the second paragraph of the Experimental Section. The concentration of menogaril in each experiment was 6.0 × 10^{-5} M. The solvent for the three experiments was a methanolic solution of buffer at three different pHs: (a) pH 8.2 [Tris + Tris·HCl at c = (1 + 1) × 10^{-3} M]; (b) pH 7.2 [Tris + Tris·HCl at c = (0.2 + 1.8) × 10^{-3} M]; (c) pH 6.0 [phthalic acid + potassium hydrogen phthalate at c = (0.07 + 1.0) × 10^{-3} M]. Five moles of **2** was used per mole of menogaril. The course of the reaction was analogous to that described previously at pH 8.2 in the second paragraph of the Experimental Section. However, the rate of decay of the quinone methide was strongly pH dependent, increasing with decreasing pH. The decay was fit to the rate law of eq 1. The results are reported in Table I.

Rate of Bond Homolysis of *d,l*- and *meso*-TM-3 Dimers (2, 3) in Methanolic Buffers at Different pHs. The rates of bond homolysis were measured by the method described in the literature with diphenylpicrylhydrazyl as a trapping agent.[23,37] The temperature was 25.0 ± 0.1 °C. Trizma-buffered solutions of total buffer concentration equal to 2 × 10^{-3} M were obtained by dissolving Tris and Tris·HCl in methanol according to the following molar ratios: 0.12 (pH 7.2); 1.0 (pH 8.2); 8.4 (pH 9.0). The rate constants, (4.2 ± 0.1) × 10^{-3} s^{-1} for **2** and (2.3 ± 0.1) × 10^{-3} s^{-1} for **3**, were virtually independent of the pH.

Reaction of Menogaril (1) with 0.5 Mole Equivalent of *d,l*-TM-3 Dimer (2). The experiments were run as described above in the second paragraph of the Experimental Section using 0.5 mol equiv of **2**/mol of **1**, at 25 °C. An initial 1.0 × 10^{-4} M concentration of **1** was used. Soon

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after **1** and **2** were mixed in the cell, the quinone absorbance at 475 nm began to slowly decrease as a band at 604 nm, indicative of quinone methide formation, developed, reaching a maximum absorbance of 0.35 at 1000 s. A small inflection at ca. 420 nm was also observed, indicative of formation of hydroquinone species. After 1000 s the absorbance at 604 nm slowly decreased as the absorbance at 476 was restored. At 8 h the absorbance of quinone methide, measured at 648 nm, was ca. 15% of its maximum value reached at 1000 s. The fall in the absorbance at 648 nm, over an 80–15% range of decay, which did not fit eq 1, gave a good fit by nonlinear least-squares analysis to a second-order rate law giving, in three separate experiments, $k_2 = 5.2 \pm 0.2\ M^{-1}\ s^{-1}$. After 24 h from the beginning of the experiment the cell was opened. HPLC analysis showed unreacted menogaril (**1**), 7-deoxynogarol (**5**), and bi(7-deoxynogarol-7-yl) (**6**) in a 1:1:1 ratio; 10–15% of minor products were also detected. In an experiment run at pH 6 [buffered with phthalic acid–potassium hydrogen phthalate = $(0.07/1.0) \times 10^{-3}$ M, 6.0×10^{-5} M menogaril], a value of $4.8 \pm 0.1\ M^{-1}\ s^{-1}$ for the second-order rate constant for the decay of **8** was found. Some experiments were run to show the dependence of the amount of the reaction products on the reaction conditions. (a) A 19-h experiment was run starting with an initial 2.2×10^{-4} M concentration of menogaril; the HPLC analysis showed 15% of unreacted **1**, 20% of **5**, and 60% of **6**. (b) Three separate experiments, starting with 1.0×10^{-4} M menogaril (**1**), were run as described above. The cells were opened at different times, 4.2, 17, and 26 h. Unreacted **1** was found by HPLC to be present in different amounts, namely 44%, 31%, and 27%, respectively.

Isolation and Characterization of Bi(7-deoxynogarol-7-yl) (6) from Reduction of Menogaril (1) with Substoichiometric Amounts of *meso*-TM-3 Dimer (3). A reaction cell was constructed as a two-compartment Pyrex apparatus consisting of a thick-wall 50-mL tube (A) fused in the upper part to a 3-mL Pyrex tube (B). The angle between the tubes was 90°. The top of tube A was also coaxially fused to an open tube for attachment to a vacuum line. *meso*-TM-3 dimer (**3**; 5.7 mg, 0.02 mmol) was dissolved in ca. 0.5 mL of dichloromethane. The solution was quantitatively transferred into tube B and the solvent evaporated at ambient temperature with a stream of nitrogen. A mixture of menogaril (**1**; 21.7 mg, 0.04 mmol), Tris (54 mg, 0.45 mmol) and Tris·HCl (98 mg, 0.62 mmol) was introduced into tube A. A tiny magnetic bar and 40 mL of methanol were also added, and the mixture was sonicated for ca. 10 min to ensure the maximum possible dissolution of the suspended material. After a 3-cycle freeze–thaw degassing procedure the cell was sealed under vacuum. The solution was then mixed with **3** and the mixture was sonicated for ca. 15 min. The red-brown solution was kept in the dark with stirring for 40 h. During this time a flocky precipitate had separated while the intentiy of the color of the solution had decreased. The cell was opened to air and the solid was separated from the supernatant and washed twice by centrifugation using 7 mL of methanol for each washing. The residual solid (5 mg) appeared 95% pure by HPLC (R_t 12 min) and had the following spectral properties: UV (pH 8.2 Trizma buffer in methanol) λ_{max} 474 nm ($\epsilon = 2.5 \times 10^4$); ^{1}H NMR (DMSO-$d_6$) δ 12.78 (s, phenolic OH), 12.18 (s, phenolic OH), 7.40 (s, C11 H), 7.17 (s, C3 H), 6.68 (d, J = 3 Hz, C2′ OH), 6.18 (br s, C4′ OH), 5.71 (d, J = 3 Hz, C1′ H), 4.80 (s, C9 OH), 4.56 (m, C7 H), 4.19 (dd with D_2O added, J = 3, 11 Hz, C2′ H), 3.99 (d with D_2O added, J = 11 Hz, C4′ H), 2.70–2.98 (m, C10 H and C3′ H), 2.81 (br s, N-$(CH_3)_2$), 1.66 (s, C5′ CH_3), 1.33 (m, C8 H), 0.85 (s, C9 CH_3); coupling between protons at C7 and C8 was established by double resonance; MS (FAB, positive ion, racemic mixture of $HSCH_2CHOHCHOHCH_2SH$ matrix) m/z (rel intensity) 1022 (M + 2, protonated semiquinone, 75), 511 (100, protonated monomer). TLC (2:1 v/v chloroform–methanol) gave only one spot, $R_f = 0.05$; **1** gave $R_f = 0.40$; and **5** gave $R_f = 0.35$.

Reduction of Bi(7-deoxynogarol-7-yl) (6) with an Equimolecular Amount of *d,l*-TM-3 Dimer (2) Followed by Reaction with Menogaril (1). A three-compartment Pyrex cell was prepared consisting of a 1-cm cuvette fused at 90° to two parallel degassing chambers both 1.6 cm o.d. × 4.1 cm long, and a 9 mm o.d. tube, coaxial to one of the two chambers, for attachment to the vacuum line. A dichloromethane solution of **2** (1.0 mL containing 0.03 mg, 1×10^{-4} mmol) was introduced into the cuvette, and the solvent was evaporated with a stream of nitrogen. A 0.14-mg sample (2.6×10^{-4} mmol) of **1** in a tiny glass open-mouth container was gently slid down into the degassing chamber coaxial to the 9-mm tube. A methanol solution of **6** (3.0 mL, 3.4×10^{-5} M, 1.0×10^{-4} mmol), buffered at pH 8.2 with $(1 + 1) \times 10^{-3}$ M Trizma, was introduced into the other degassing chamber. After the freeze–thaw degassing, the cell was sealed under vacuum and brought to 25 °C. The solution of **6** was then carefully poured into the cuvette while keeping **1** in the degassing chamber. UV spectra taken at 25 °C showed a decrease of the quinone band at 480 nm as a hydroquinone band at 424 nm developed. The maximum intensity of the 424-nm band was reached after 17 min. After a few more minutes the solution from the cuvette was poured into the chamber containing **1**, shortly sonicated for a quick dissolution of **1**, and poured back into the cuvette. UV–visible spectra were taken at 25 °C at 30-s intervals. Soon the band of the quinone methide of **1** at 604 nm began to appear as the hydroquinone band at 424 nm began to decrease. The 604-nm band reached its maximum after 250 s, to then slowly disappear (ca. 15 h) as a quinone band at 480 nm increased.

Stepwise Reduction of Menogaril (1) with *d,l*-TM-3 Dimer (2). A three-compartment cell was made as described in the previous paragraph. The cuvette portion of the cell was loaded with **2** (0.035 mg, 1.25×10^{-4} mmol). One of the degassing chambers was also loaded with **2** (0.14 mg, 5.0×10^{-4} mmol). A 2.5-mL solution of 1.0×10^{-3} M **1** (2.5×10^{-4} mmol) in methanol buffered at pH 8.2 with $(1 + 1) \times 10^{-3}$ M Trizma was introduced into the second degassing chamber. After freeze–thaw degassing, the cell was sealed under vacuum and brought to 25 °C. The menogaril solution was gently transferred into the cuvette, and UV–visible spectra were taken at 25 °C. A band with a maximum at 604 nm appeared, indicative of quinone methide **8** formation, whose maximum ($A_{604} = 0.64$) was reached after 950 s. At this point the solution from the cuvette was poured into the chamber containing the 0.14 mg of **2**, quickly shaken, and put back into the cuvette, which in turn was set back in the thermostated cell holder of the spectrometer, at 25.1 ± 0.1 °C. In ca. 2 min the absorbance of **8** at 604 nm reached a new maximum value of 1.05, to then quickly decay to almost zero in less than 1 h. The decay of the absorbance of **8** at 646–650 nm was measured as a function of time, and the data were fit to the rate law of eq 1 by a nonlinear least-squares fitting procedure. With k_2 set at 11 $M^{-1}\ s^{-1}$, k_1 was found to be $25.0 \pm 0.2\ M^{-1}\ s^{-1}$.

Effect of the Addition of Excess Menogaril (1) to Quinone Methide 8 on the Rate of Decay of 8. A three-compartment cell was made as described in the two previous experiments. The cuvette portion of the cell was loaded with 0.063 mg (2.2×10^{-4} mmol) of *d,l*-TM-3 dimer (**2**). Solid **1** (0.12 mg, 2.2×10^{-4} mmol) was introduced into one of the two degassing chambers as described previously. A 2.2-mL volume of a 5×10^{-5} M solution of **1** (1.1×10^{-4} mmol) in methanol, buffered at pH 8.2 with $(1 + 1) \times 10^{-3}$ M Trizma, was introduced into the second chamber. After the freeze–thaw degassing procedure, the cell was sealed under vacuum and brought to 25 °C. The solution of **1** was then carefully poured into the cuvette while keeping the solid **1** in the second chamber. UV spectra, taken at 50-s intervals at 25 ± 0.1 °C, showed a fall of the 480-nm quinone band as the quinone methide band at 604 nm increased to reach a maximum (0.7) in 250 s. At this time the solution from the cuvette was poured into the degassing chamber containing the solid **1**, shortly sonicated for a quick dissolution of **1**, and poured back into the cuvette. A further increase of the absorbance at 604 nm up to 1.2 was observed during a period of time of ca. 600 s. The UV spectra taken at 25.0 ± 0.1 °C then showed a decay of the 604-nm band much slower than in a similar experiment run with no addition of extra **1**, as reported in the second paragraph of the Experimental Section and Table I. When the absorbance of quinone methide **8** was measured at 646–650 nm as a function of time, the data gave a good fit to a second-order rate law by nonlinear least-squares analysis to give a rate constant $k_2 = 5.4 \pm 0.1\ M^{-1}\ s^{-1}$.

EPR Detection of the Semiquinone 9 of Menogaril (1). A three-compartment cell was made consisting of a 1.0-mm quartz UV cuvette fused at 120° with a Pyrex degassing chamber 1.6 cm o.d. × 4.5 cm long. A quartz EPR tube (1.8 mm o.d.) was also fused at 60° to the degassing chamber, in the same plane but opposite direction with respect to the cuvette. Finally, a 0.9 cm o.d. tube for attachment to the vacuum line was fused, again in the same plane, opposite to the degassing chamber. The cuvette was loaded with 0.28 mg (1.0×10^{-3} mmol) of *d,l*-TM-3 dimer (**2**) dissolved in 0.3 mL of dichloromethane. The solvent was removed with a stream of nitrogen. The degassing chamber was loaded with 1.0 mL of a 1.0×10^{-3} M solution of menogaril (**1**) made by dissolving 0.54 mg (1.0×10^{-3} mmol) of **1** in 0.1 mL of dimethylformamide and mixing with 0.9 mL of methanol-*d* buffered with $(1 + 1) \times 10^{-2}$ M Trizma. After freeze–thaw degassing the cell was sealed under vacuum, brought to 5 °C, and shaken to bring **2** into solution. The cell was then transferred to the cell holder of the UV spectrometer, thermostated at 5 °C, and spectra were taken at 10–15-min intervals to monitor the rise of the quinone methide **8** band at 604 nm. The band reached its maximum intensity (0.2) after ca. 1.5 h, to then slowly decay. In the meantime, alternating with the UV spectra, EPR spectra were also recorded at ca. 5 °C. A weak signal, consisting of a broad doublet of triplets was seen, whose intensity reached its maximum after ca. 1.5 h, to then slowly decay. Microwave power was 1 mW and modulation amplitude was 0.5 G. EPR signal showed $g = 2.0037$; hyperfine splittings were 3.7 (1:1), 1.6 G (1:2:1). A similar experiment run with aclacinomycin A at the same concentration gave a significantly stronger signal.[14]

Trapping of Quinone Methide 8 by *N*-Acetyl-L-cysteine. Two-compartment cells were used for the experiments, as described in the General

Remarks paragraph. Three different experiments were run using the same concentration of menogaril (**1**; 1.0×10^{-4} M) and *N*-acetyl-L-cysteine (2.5×10^{-2} M) but different concentrations of *d,l*-TM-3 (**2**; 4.2, 2.1 and 1.1×10^{-5} M). For each experiment the cuvette portion of the cell was loaded with the appropriate amount of **2** following the already described procedure. The degassing chamber of the cell was loaded with 2.0 mL of a methanolic solution 1.0×10^{-4} M in **1**, 2.5×10^{-2} M in *N*-acetyl-L-cysteine, 5.2×10^{-2} M in Tris, and 2.5×10^{-4} M in Tris·HCl. After the freeze–thaw degassing procedure and sealing under vacuum, the cell was brought to 25 °C, the reactants were mixed, and the cell was placed in the cell holder of the UV–visible spectrometer thermostated at 25.0 ± 0.1 °C. Spectra were taken every 20 s for a total of 2000 s. The reaction was manifested by the appearance of the quinone methide **8** band at 604 nm with a simultaneous decrease of the menogaril band at 475 nm. The quinone methide band at 604 nm reached its maximum after 10–20 min, depending on the amount of **2**, the lower the amount the longer the time. After 24 h the cell was opened and the solution analyzed by HPLC, monitoring at $\lambda > 480$ nm. The chromatogram showed peaks corresponding to menogaril (**1**; R_t 8 min), 7-deoxynogarol (**5**; R_t 9 min), bi(7-deoxynogarol-7-yl) (**6**; R_t 11 min), plus two partially overlapped peaks, R_t 1.5 and 1.9 min (relative areas ca. 2/1), corresponding to diastereoisomeric quinone methide–*N*-acetyl-L-cysteine adducts (**10**) (see next paragraph for identification). Minor peaks corresponding to side products accounts for ca. 5–10% of the total park area. Relative amounts of **1**, **5**, **6**, and **10** are reported in Table III and compared with amounts formed in a blank experiment run in the absence of *N*-acetyl-L-cysteine. An experiment similar to the ones reported above, but in the absence of TM-3 dimer (**2**), was run using the following concentrations: 1.0×10^{-4} M **1**, 2.5×10^{-2} M *N*-acetyl-L-cysteine, $(1 + 1) \times 10^{-3}$ M Trizma. The reacting cell was opened after 64 h at 25 °C. HPLC analysis showed 90% of unreacted **1** and 10% of **10**.

Reaction of *N*-Acetyl-L-cysteine Methyl Ester with Quinone Methide 8. A 50-mL preparative-scale cell was used as reported in the paragraph describing the isolation of **6**. *d,l*-TM-3 dimer (**2**; 1.1 mg, 3.9×10^{-3} mmol) was introduced into the small compartment of the cell. Menogaril (**1**; 10.9 mg, 2.0×10^{-2} mmol), Tris (122 mg, 1.0 mmol), Tris·HCl (167 mg, 1.1 mmol), and *N*-acetyl-L-cysteine methyl ester (95 mg, 0.49 mmol) were mixed with 10 mL of methanol in the large compartment of the cell. A small magnetic spin bar was also added. After freeze–thaw degassing and sealing, the reactants were mixed, sonicated, and then stirred at ambient temperature. Within 20 min all of **1** dissolved to form a dark orange solution. The reaction mixture was left in the dark for 44 h. The cell was then opened and analyzed by HPLC to show a major product with R_t 5 min, with only traces of **1** and minor amounts of **5**. The methanol was evaporated under reduced pressure at ambient temperature, and the residue was partitioned between water (10 mL, pH 7) and chloroform. The chloroform solution was evaporated to 2 mL and applied to a precoated TLC silica gel 60 F-254 plate and eluted with a 3/2 v/v chloroform–methanol mixture. The major middle band containing the adduct was removed. Further purification was achieved by preparative HPLC. The resulting product was a 3:1 mixture of diastereomeric 7-(*N*-acetyl-L-cysteinyl)-7-deoxynogarol methyl esters (**11**). Attempts to separate those diastereoisomers were fruitless. The mixture gave the following spectral absorptions: ^{1}H NMR ($CDCl_3$) for the major diastereoisomer δ 12.63 (br s, phenolic OH), 12.05 (s, phenolic OH), 7.21 (s, C11 H), 6.76 (br d, J = 6 Hz, NHCO), 6.67 (s, C3 H), 5.87 (d, J = 3 Hz, C1′ H), 5.04–4.95 (X part of ABX with further coupling to NH, $CHCO_2$), 4.35 (d, J = 4 Hz, C7 H), 4.17 (br d, J = 9 Hz, C2′ H), 3.79 (s, OCH_3), 3.61 (d, J = 10 Hz, C4′ H), 3.40–3.25 (AB part of ABX pattern, J_{AB} = 14 Hz, CH_2S), 2.95 (m, C3′ H), 2.87 (d, J = 18 Hz, C10 H), 2.70 (d, J = 18 Hz, C10 H), 2.57 (slightly br s, $N(CH_3)_2$), 2.40 (br d, J = 15 Hz, C8 H), 2.25 (dd, J = 4, 15 Hz, C8 H), 2.12 (s, $NCOCH_3$), 1.72 (s, CH_3), 1.46 (s, CH_3); MS (FAB, positive ion, 2,4-di-*tert*-pentylphenol matrix) m/z (rel intensity) 687 (M + 1, 12), 511 (M + 1 − RS, 25), 466 [511 − $HN(CH_3)_2$, 100].

A reaction of **1** with *N*-acetyl-L-cysteine and **2** under the above conditions gave a water-soluble zwitterionic product which could not be purified for NMR spectroscopy and proved by HPLC to be the same as the diastereoisomeric mixture of **10** obtained as reported in the previous paragraph: MS (FAB, positive ion, 2,4-di-*tert*-pentylphenol matrix) m/z (rel intensity) 673 (M + 1, 10), 511 (M + 1 − RS, 25), 437 (100). Treatment of this product with excess methanol (25 mL) and 2 drops of concentrated sulfuric acid converted most of it to the corresponding methyl ester, as shown by HPLC.

Attempted Reaction of *N*-Acetyl-L-cysteine with Bi(7-deoxynogarol-7-yl) (6) upon Reduction with *d,l*-TM-3 Dimer (2). The cuvette portion of a two-compartment cell was loaded with **2** (0.012 mg, 4.3×10^{-5} mmol) as described in the previous paragraphs. A 2.5-mL methanolic solution 3.5×10^{-5} M in **6** (8.6×10^{-5} mmol), 1.7×10^{-2} M in *N*-acetyl-L-cysteine (4.2×10^{-2} mmol), and $(1 + 1) \times 10^{-3}$ M in Trizma was introduced into the degassing chamber of the cell. After the freeze–thaw degassing and sealing procedure, the cell was brought to 25 °C, the reactants were mixed, and UV–visible spectra were taken periodically at 25 °C. A partial decrease of the quinone band at 475 nm was observed, coupled with the appearance of a shoulder at ca. 420 nm, indicative of hydroquinone species. No quinone methide band at 604 nm appeared in the spectra. After 24 h the cell was opened. HPLC analysis monitoring at $\lambda > 400$ nm only showed one peak corresponding to the starting material **6**.

Reaction of Menogaril (1) with Excess DHM-3 Dimer (4) in Water. The reactions were run by using the same technique as reported for the reactions run in methanol with **2** except for a few minor differences: (a) because of its low solubility in halocarbons, DHM-3 was dissolved in acetonitrile rather than in dichloromethane before loading (half-life for bond homolysis of 4 in acetonitrile at 25 °C is ca. 13 h[24]); (b) due to the very low solubility of **1** in water, the proper amount of **1** was dissolved in a small amount of dimethylformamide and the solution was properly diluted with a $(1 + 1) \times 10^{-3}$ M aqueous Trizma buffer to reach a 5% v/v concentration of dimethylformamide. Even so, after several hours sometimes precipitation of **1** was observed. Concentrations of **1** from 0.9×10^{-4} to 3.1×10^{-4} M were tested for the reaction with **4**; 10 mol of 4/mol of **1** was used. After the reagents in the reacting cell were mixed, the quinone band of **1** at 475 nm disappeared in a few seconds to be replaced by a hydroquinone band at 420 nm. Only a low-intensity absorption band (ca. 5% of the original absorption at 475 nm) with a maximum at 580 nm then appeared, which reached its maximum after ca. 50–100 s, depending on the concentration of **1**, to then slowly fade. When the cell was opened, after about 1 h the HPLC analysis showed >90% of 7-deoxynogarol (**5**). When the initial menogaril concentration was higher than 1.5×10^{-4} M, trace amounts (1–2%) of **6** were also detected.

Formation of Quinone Methide 8 from Menogaril (1) and DHM-3 Dimer (4) in Methanol Followed by Its Destruction upon Addition of Water. A solution of DHM-3 dimer (**4**; 1.14 mg, 3.6×10^{-3} mmol) in 0.5 mL of acetonitrile was introduced into a 1-cm UV cuvette provided with a serum stopper. The solvent was evaporated with a stream of nitrogen. The cell was placed in the cell holder of the spectrometer kept at 25 °C. A 3.6×10^{-4} M methanolic solution of **1**, $(1 + 1) \times 10^{-3}$ M in Trizma, containing 5% v/v of dimethylformamide was purged with argon. A 1.0-mL volume (3.6×10^{-4} mmol of **1**) of the solution was introduced via syringe into the cuvette filled with argon. Spectra were then taken at 30-s intervals. Soon the 604-nm quinone methide band appeared and reached its maximum (A_{648} = 2.7) in 200 s. At this time a 4.0-mL volume of a deoxygenated aqueous $(1 + 1) \times 10^{-3}$ M Trizma buffer was quickly injected into the cell while spectra were taken every 2 s. The absorbance at 648 nm, which in 4 s had dropped to 0.5, partially because of the dilution, kept falling very quickly to 0.21 during the next 4 s, to 0.14 during the following 4 s, and to 0.02 in the next 60 s. A blank experiment had shown that 5% v/v of dimethylformamide in methanol does not affect appreciably the kinetics or the chemistry of the reaction of **1** with TM-3 dimer (**2**) when compared with the experiments run in pure methanol. The cell was opened after 0.5 h. HPLC analysis showed the presence of 7-deoxynogarol (**5**; 70%) and bi(7-deoxynogarol-7-yl) (**6**; 20%), along with minor peaks. The Lambert–Beer law for both methanolic and aqueous solutions of menogaril, buffered at pH 8.2 with Trizma, was found to be obeyed at least for concentrations between 1.5×10^{-4} and 1.5×10^{-5} M. Molar extinction coefficients were $\epsilon_{474} = (1.15 \pm 0.03) \times 10^4$ in water and $\epsilon_{475} = (1.45 \pm 0.05) \times 10^4$ in methanol. A slight blue-shift from 476 to 472 nm in the absorption maximum was observed in a 1.5×10^{-4} M pH 8.2 aqueous solution of **1** upon a 1 to 10 dilution. No such effect was observed in methanol.

Reaction of Menogaril (1) with 0.5 Mole Equivalent of DHM-3 Dimer (4) in Water. The reaction was run under the same conditions as described for the reaction of **1** with excess **4**. A 2.2-mL volume of 1.7×10^{-4} M menogaril (**1**, 3.7×10^{-4} mmol) and 0.06 mg of DHM-3 dimer (**4**; 1.9×10^{-4} mmol) were used. During the first 550 s after mixing the reagents, the 476-nm quinone band of **1** decreased as a hydroquinone band at ca. 420 nm developed. A low-intensity band in the 600-nm region also appeared whose maximum intensity was ca. 5% of the initial quinone absorbance at 476 nm. After 69 h at 25 °C the cell was opened. HPLC analysis showed, along with some unreacted menogaril, both 7-deoxynogarol (**5**) and bi(7-deoxynogarol-7-yl) (**6**) in a 1:1 ratio.

Reaction of Menogaril (1) with Excess *d,l*-TM-3 Dimer (2) in Methanol–Water Solutions. Three separate experiments were run in two-compartment cells as described above for the reactions between **1** and excess **2** run in methanol, the only difference being the use of 95/5, 90/10 and 80/20 v/v mixtures of buffered methanol–water instead of buffered pure methanol. The buffer was $(5 + 5) \times 10^{-3}$ M Trizma. The initial concentration of menogaril was 1.0×10^{-4} M. A fourth experiment was run in 95/5 methanol–water using an initial 1.5×10^{-4} M concentration

of **1**; 15 mol of **2**/mol of **1** was used. After the reagents were mixed, spectral changes were observed similar to those seen in the reaction run in pure methanol, the main difference being a faster decay of the quinone methide absorbance. The rate of decay of the absorbance at 648 nm, over a range from 60% to 20% of its maximum intensity, was fitted to the rate law expressed by eq 1, by a nonlinear least-squares fitting procedure. The results are reported in Table II. In each experiment, when the reaction was over, the cell was opened and the solution analyzed by HPLC. 7-Deoxynogarol (**5**) and bi(7-deoxynogarol-7-yl) (**6**) in ca. 3.5/1 ratio accounted for about 90% of the total peak area. Part of **5** and **6** appeared in their leuco[31] forms, especially when the cell had been opened several hours after the disappearance of the quinone methide absorbance.

Reaction of Menogaril (1) with 0.33 Mole Equivalent of DHM-3 Dimer (4) in the Presence of *N*-Acetyl-L-cysteine in Water. DHM-3 dimer (**4**; 0.036 mg, 1.15×10^{-4} mmol) was loaded into the cuvette portion of a two-compartment cell as described in the General Remarks paragraph. The degassing chamber was loaded with 2.5 mL of a solution 1.38×10^{-4} M in **1**, 3.75×10^{-2} M in *N*-acetyl-L-cysteine, 8.0×10^{-2} M in Tris, and 1.4×10^{-3} M in Tris·HCl. The solvent was 5% v/v dimethylformamide in water, and the pH of the solution was 8.2. After freeze–thaw degassing and sealing under vacuum, the cell was brought to 25 °C. The reactants were mixed, the cell was placed in the cell holder of the UV–vis spectrometer thermostated at 25.0 ± 0.1 °C, and spectra were taken periodically. A partial decrease of the quinone band at 472 nm coupled with the appearance of a hydroquinone absorption band at 426 nm was observed. The quinone band reached a minimum (1.3 from the original 1.7 absorbance) after ca. 400 s. After ca. 6000 s the quinone band began to increase very slowly at the expense of the hydroquinone band, which had reached its maximum intensity (absorbance 1.6). After an additional 70 h, the absorption maximum of the quinone had reached a value of 1.4 while the hydroquinone band appeared as a shoulder with an intensity of 1.2 absorption units. During the course of the reaction no absorption band for quinone methide, expected at ca. 600 nm, was observed. After a total of 72 h the cell was opened and the solution was analyzed by HPLC. The chromatogram showed the same two peaks, at 1.5 and 1.9 min, observed for the menogaril–cysteine diastereoisomeric adducts (**10**) found from the reaction of **1**, **2**, and *N*-acetyl-L-cysteine in methanol, as reported previously. These two peaks accounted for 80% of the total area. A minor peak corresponding to **5** was also present accounting for 9% of the total area. No unreacted menogaril (**1**) was detected.

Magnetic Exchange Interactions in Semiquinone Complexes of Iron. Structural and Magnetic Properties of Tris(3,5-di-*tert*-butylsemiquinonato)iron(III) and Tetrakis(3,5-di-*tert*-butylsemiquinonato)tetrakis(3,5-di-*tert*-butylcatecholato)tetrairon(III)

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Abstract: Iron–semiquinone magnetic interactions have been studied in complexes obtained from the reaction between $Fe(CO)_5$ and 3,5-di-*tert*-butyl-1,2-benzoquinone (DBBQ). The green product formed when DBBQ is used in excess is $Fe(DBSQ)_3$; when $Fe(CO)_5$ is used in excess, blue $Fe_4(DBSQ)_4(DBCat)_4$ is obtained. Mössbauer spectra for both complexes indicate that they contain high-spin Fe(III) ions. The X-ray structure of $Fe(DBSQ)_3$ shows that the molecule has C_3 symmetry with metrical values consistent with the tris(semiquinonate)iron(III) charge distribution. The structure of centrosymmetric $Fe_4(DBSQ)_4(DBCat)_4$ consists of a planar arrangement of iron atoms, resembling two triangles sharing a common edge. Semiquinone and catecholate ligands are clearly distinguishable by their structural features. Oxygen atoms of the ligands bridge edges of the triangles, and one catecholate oxygen is located atop each triangle, bridging three metal ions. The magnetic moment of $Fe(DBSQ)_3$ is 2.9 μ_B consistent with the $S = 1$ ground state that arises from strong coupling between the $S = {}^5/_2$ metal ion and the three $S = {}^1/_2$ ligands. The tetranuclear complex shows temperature-dependent magnetic behavior with an effective moment of 5.94 μ_B per molecule at 321 K which decreases to 0.72 μ_B at 5 K. This arises both from iron–semiquinone coupling and from antiferromagnetic coupling between Fe(DBSQ)(DBCat) monomeric units of the molecule.

Metal complexes containing paramagnetic organic ligands have been confined primarily to nitroxyl ligands[3] and unsaturated imines including porphyrins[4] and bipyridine.[5] Over the past several years we have been able to show that the *o*-semiquinones have a rich coordination chemistry. Complexes consisting of both paramagnetic metal ions and radical semiquinone ligands have been of particular interest in these studies. Reactions carried out between the neutral metal carbonyl complexes $Cr(CO)_6$ and $Fe(CO)_5$ and the *o*-benzoquinones 3,5-di-*tert*-butyl-1,2-benzoquinone, tetrachloro-1,2-benzoquinone, and 9,10-phenanthrenequinone gave neutral tris(quinone) products.[6,7] At the time that these compounds were synthesized, the intramolecular distribution of charge remained unclear even though structural information was available.[8] Subsequent results of characterization using

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