$H_{48}N_7O_3$ (req m/z = 506.3819). Anal. Calcd for $C_{26}H_{48}N_7O_3\cdot5HCl\cdot2H_2O$: C, 43.07; H, 7.92; N, 13.52; Cl, 24.44. Found C, 44.22; H, 7.57; N, 13.40; Cl, 24.32.

21-Amino-2,2-dimethyl-4-oxo-3-oxa-5,9,14,18-tetraazaheneicosane-9,14-dicarboxylic Acid Bis(1,1-dimethylethyl) Ester (28). Nitrile **11** (1.15 g, 2.07 mmol) was hydrogenated as described above for **9** to give 1.10 g (95%) of crude amine **28**: ^{1}H NMR ($CDCl_3$) 1.29–1.64 (m, 40 H), 2.48–2.71 (m, 6 H), 3.01–3.15 (m, 10 H), 5.24 (s, 1 H); ^{13}C NMR ($CDCl_3$) 25.85, 28.14, 28.43, 28.73, 29.01, 33.93, 37.89, 40.55, 44.17, 44.96, 46.74, 47.26, 47.87, 79.16, 155.59, 155.98; HR FABMS observed (M + H) m/z = 560.4373. $C_{28}H_{58}N_5O_6$ (req 560.4387).

24-[1-[(1,1-Dimethylethoxy)carbonyl]-4-(methoxymethoxy)-1*H*-indol-3-yl]-18-hydroxy-2,2-dimethyl-4,23-dioxo-3-oxa-5,9,14,18,22-pentaazatetracosane-9,14-dicarboxylic Acid Bis(1,1-dimethylethyl) Ester (29). Condensation of amine **28** (0.380 g, 0.0068 mol) with a dichloromethane solution (15 mL) containing acid **16** (0.228 g, 0.0068 mol), *N*-hydroxysuccinimide (0.078 g, 0.0068 mol), and DCC (0.140 g, 0.0068 mol) as described in the preparation of **18** afforded 0.407 g (68%) of desired amide intermediate after silica gel chromatography (9:1 CH_2Cl_2/MeOH followed by 9:1:0.5 CH_2Cl_2/MeOH/diisopropylamine): ^{1}H NMR ($CDCl_3$) δ 1.28–1.71 (m, 47 H), 2.33–2.52 (m, 4 H), 3.06–3.27 (m, 12 H), 3.47 (s, 3 H), 3.73 (s, 2 H), 5.24 (s, 2 H), 6.40 (br s, 1 H), 6.84 (d, J = 8 Hz, 1 H), 7.19 (dd, J = 8 Hz, J = 8 Hz, 1 H), 7.39 (s, 1 H), 7.78 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.95, 28.20, 28.48, 29.39, 35.09, 37.56, 38.17, 44.09, 46.75, 47.52, 56.35, 79.34, 79.59, 83.94, 94.61, 106.78, 109.46, 113.70, 119.80, 124.22, 125.78, 137.44, 149.44, 151.36, 156.06, 171.21; HR FABMS observed (M + H) m/z = 877.5626, $C_{45}H_{77}N_6O_{11}$ (req 877.5650). Treatment of this intermediate (0.350 g, 0.0004 mol) with 2-(phenylsulfonyl)-3-phenyloxaziridine (0.230 g, 0.00088 mol) as described in the preparation of hydroxylamine **20** gave 0.274 g (77%) of desired hydroxylamine **29** following silica gel chromatography (50:50 acetone/hexane). This sample contained minor amounts (<5%) of phenyl sulfonamide which was further purified on silica gel (ethyl acetate followed by 50:50 acetone/hexane): ^{1}H NMR ($CDCl_3$) δ 1.17–1.72 (m, 47 H), 2.41 (t, J = 6.7 Hz, 2 H), 2.48 (t, J = 6.3 Hz, 2 H), 3.05–3.29 (m, 12 H), 3.46 (s, 3 H), 3.73 (s, 2 H), 5.25 (s, 2 H), 5.30 (br s, 1 H), 6.29 (br s, 1 H), 6.85 (d, J = 8 Hz, 1 H), 7.20 (dd, J = 8 Hz, 8 Hz, 1 H), 7.39 (s, 1 H), 7.79 (d, J = 8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.90, 26.30, 26.95, 28.19, 28.47, 28.77, 29.32, 35.07, 38.23, 44.25, 45.01, 46.96, 54.12, 56.36, 58.04, 58.39, 79.25, 79.57, 83.96, 94.84, 106.97, 109.56, 113.82, 124.24, 125.77, 137.49, 149.40, 151.42, 155.61, 156.04, 171.05; HR FABMS observed (M + H) m/z = 893.5618, $C_{45}H_{77}N_6O_{12}$ (req m/z = 893.5599).

***N*-(16-Amino-4-hydroxy-4,8,13-triazahexadec-1-yl)-4-hydroxy-1*H*-indole-3-acetamide (5).** Treatment of hydroxylamine **29** (166 mg, 0.186 mmol) with a saturated dioxane/HCl solution as described in the preparation of **27** provided 130 mg of crude acid. Crude acid (114 mg) was dissolved in water under an argon purge for 8 h and then freeze-dried as described above for the preparation of **2** to give 77 mg (73%) of Agel 448 (**5**) as its hydrochloride salt: ^{1}H NMR (D_2O) of 1.72–2.09 (m, 10 H), 2.93–3.28 (m, 16 H), 3.72 (q, J_{AB} = 4 Hz, 2 H), 6.51 (m, 1 H), 7.03 (m, 2 H), 7.11 (s, 1 H); ^{13}C NMR (D_2O) δ 21.18, 23.54, 24.05, 24.53, 34.55, 36.53, 37.32, 45.22, 45.32, 47.77, 56.49, 57.08, 104.77, 105.46, 107.64, 116.98, 123.98, 125.00, 139.50, 150.71, 177.72; HR FABMS observed (M + H) m/z = 449.3252, $C_{23}H_{41}N_6O_3$ (req m/z = 449.3240).

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Intramolecular Trapping of the Quinone Methide from Reductive Cleavage of Daunomycin with Oxygen and Nitrogen Nucleophiles

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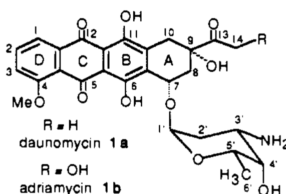
Abstract: Intramolecular trapping with oxygen and nitrogen nucleophilic sites of the quinone methide from reductive cleavage of daunomycin (**1a**) is described. The oxygen and nitrogen nucleophilic sites were located at the 13-position of daunomycin in oxime, semicarbazone, and benzoylhydrazone derivatives. Reduction of daunomycin oxime (**2a**) and semicarbazone (**2b**) in methanol and water with the one-electron reducing agents bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer, **3a**) and bi[3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl] (DHM-3 dimer, **3b**) yielded cyclooxime **8a** and cyclosemicarbazone **8b** as well as 7-deoxydaunomycinone oxime (**6a**) and 7-deoxydaunomycinone semicarbazone (**6b**), respectively. Product ratios were pH dependent. Cyclooxime but not cyclosemicarbazone was reductively cleaved to the respective 7-deoxy aglycon. Reduction of daunomycin benzoylhydrazone (**2c**) yielded only 7-deoxydaunomycinone benzoylhydrazone (**6c**). Quinone methide intermediates, **5b** and **5c**, were observed by UV–visible spectroscopy. Cyclomer formation is discussed in terms of intramolecular nucleophilic attack at the 7-position of the quinone methide. The lack of cyclomer formation during the reduction of **2c** resulted from the configuration of the benzoylhydrazone functionality, syn to the methyl at the 14-position.

Introduction

Daunomycin (**1a**) and adriamycin (**1b**) are antitumor antibiotics of the anthracycline class,[1] which have been proposed to be bioreductively activated.[2,3] Under aerobic conditions, chemical or enzymatic reduction leads to the catalytic production of toxic reactive oxygen species.[4] Under anaerobic conditions, reduction in one-electron steps leads to formation of a quinone methide via semiquinone and hydroquinone states.[5,6] The quinone methide



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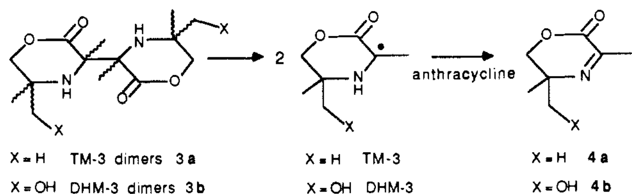
has been proposed as a reactive intermediate for covalently binding the aglycon portion of the drug to nucleophilic sites in biological macromolecules. In vitro, the quinone methide from elimination of daunosamine from daunomycin hydroquinone has been shown to react with protons from solvent to give 7-deoxydaunomycinone[5,7] or with thiol-type nucleophiles to give adducts.[8] Trapping of the quinone methide with oxygen or nitrogen nucleophiles has not been previously successful. The primary factor that limits the reactivity of the quinone methide with nucleophiles is the competitive process of protonation. The half-life at 25 °C with respect to the pseudo-first-order formation of 7-deoxydaunomycinone is only 15 s in water[7] and 55 s in methanol.[5] The quinone methide from reductive cleavage of adriamycin similarly reacts with protic solvents, only a little more slowly, to yield the 7-deoxy aglycon.[6] Reaction with oxygen or nitrogen nucleophilic sites is of interest because of the potential for binding the aglycon to nucleic acids or proteins. When so bound, the aglycon might then serve as a site-specific catalyst for the production of reactive oxygen species, which can react to cut the nucleic acid or protein.[4,9] Earlier efforts to trap the quinone methide from reductive cleavage of daunomycin have employed an intermolecular reaction.[7,8] In vivo, the quinone methide might be generated in association with a nucleic acid, protein, or other biologically important molecule. To mimic the attack of a nucleophilic site close to the reactive center of the quinone methide, we have now explored the intramolecular trapping with oxygen and nitrogen nucleophiles created by derivatization of the acetyl group at the 9-position of daunomycin (**1a**).

Results

An oxygen nucleophile was appropriately located with respect to the 7-position of the quinone methide by transformation of the acetyl group of daunomycin to the oxime. Daunomycin oxime (**2a**) was synthesized in 72% yield from daunomycin (**1a**) and hydroxylamine hydrochloride by a slightly modified literature procedure.[10,11] Nitrogen nucleophiles were similarly located by transformation of the acetyl group to semicarbazone and *N*-benzoylhydrazone derivatives. Daunomycin semicarbazone (**2b**) was prepared as described in the literature by reaction of daunomycin with semicarbazide hydrochloride[11] and daunomycin *N*-benzoylhydrazone (**2c**) was obtained from Rhône Poulenc as Zorubicin. Proton NMR spectroscopy indicated that all three derivatives were formed predominantly as single stereoisomers with respect to the carbon–nitrogen double bond; the configurations are discussed below.

Reduction was effected with the one-electron reducing agents bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer, **3a**)[12] in



methanol solvent and bi[3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl] (DHM-3 dimer, **3b**)[13] in water solvent. Both reducing agents react via bond homolysis of the weak 3,3′-bond to form 3,5,5-trimethyl-2-oxomorpholin-3-yl (TM-3) and 3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl (DHM-3), respectively. TM-3 and DHM-3 function as effective one-electron reducing agents for the anthracyclines to form semiquinone and hydroquinone states.[5,6,14] The byproducts of reduction are 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**4a**)[5,12] and 5,6-dihydro-3,5-dimethyl-5-(hydroxymethyl)-1,4-oxazin-2-one (**4b**),[13] respectively.

Anaerobic reduction of daunomycin oxime (**2a**) with an excess of DHM-3 dimer in water at pH 9.0 yielded, as determined by HPLC, approximately 10% 7-deoxydaunomycinone oxime (**6a**) and 90% 7,13-dideoxy-7,13-(epoxynitrilo)daunomycinone (cyclooxime **8a**) upon exposure to air. 7-Deoxydaunomycinone oxime was identified by spectral and chromatographic comparison with an authentic sample prepared by reaction of 7-deoxydaunomycinone (**9**) with hydroxylamine hydrochloride. The cyclooxime **8a** was characterized from spectroscopic data as reported in the Experimental Section. In particular, the IR spectrum showed an oxime ether stretching band at 1678 cm^{-1}; the UV–visible spectrum showed the daunomycinone chromophore; and the high-field ^1H NMR spectrum showed the absence of the daunosamine substituent of daunomycin and consistent chemical shifts and splitting patterns. A long-range coupling between one of the protons at C10 and one of the protons at C8 was observed; such long-range coupling commonly occurs in rigid bicyclic systems. The proton at C7 appeared as a doublet at δ 5.61 with coupling to only one of the protons at C8. The FAB mass spectrum showed an M + 2 peak characteristic of the daunomycin quinone functionality without the amino sugar substituent.[5] The molecular formula was established from exact mass measurement of the M − CH_3 peak of the tris(trimethylsilyl) ether derivative.

Reduction of **2a** in methanol solvent with TM-3 dimer (**3a**) also yielded a mixture of cyclooxime **8a** and 7-deoxydanomycinone oxime (**6a**). The product ratio, **8a**/**6a**, was pH dependent: 9/1 at apparent pH 9.3, 4/1 at pH 8.2, and 1/10 at pH 7.0. A control experiment showed that **2a** in methanol at pH 9.3 is unreactive in the absence of the reducing agent.

Anaerobic reduction of cyclooxime **8a** with an excess of TM-3 dimer in methanol solvent yielded 7-deoxydaunomycinone oxime (**6a**). HPLC yields of 60 and 96% of **6a** were obtained when the reaction mixture was exposed to oxygen after 12 and 90 min, respectively. Spectroscopic monitoring showed the formation and disappearance of a hydroquinone band at 430 nm and the disappearance and formation of a quinone band at 480–490 nm.

Anaerobic reduction of daunomycin semicarbazone (**2b**) at pH 11 with TM-3 dimer in aqueous medium containing 10% methanol yielded, as determined by HPLC, approximately 50% 7,13-dideoxy-7,13-(iminocarbonyliminonitrilo)daunomycinone (cyclosemicarbazone **8b**), and 20% 7-deoxydaunomycinone semicarbazone (**6b**). A third product could not be obtained pure in sufficient quantities for identification. The ratio of 7-deoxydaunomycinone semicarbazone to cyclosemicarbazone (**6b**/**8b**) was pH dependent: 5/1 at pH 8.2, 2/1 at pH 9.0, and 1/3 at pH 11. At lower pH the unidentified product accounted for less than 10% of the reaction mixture. Cyclosemicarbazone **8b** was identified from spectroscopic data reported in the Experimental Section, and the characteristic features were analogous to those mentioned above for the cyclooxime. The cyclized structure proposed for the cyclosemicarbazone could in principle have had a three-atom bridge. The five-atom bridge was established from the ^1H NMR spectrum in deuteriodimethyl sulfoxide, which showed two NH absorptions, one a singlet at 9.0 ppm and the other a broad signal at 6.4 ppm; the corresponding NH and NH_2 groups of **6b** had similar shape and chemical shift, 9.0 and 6.1 ppm, respectively. The resonance for the proton at the 7-position appeared characteristically at 4.5 ppm, and coupling to both protons at the 8-position was evident in the COSY spectrum. The NH resonance at 6.4 ppm was too broad to establish connectivity to

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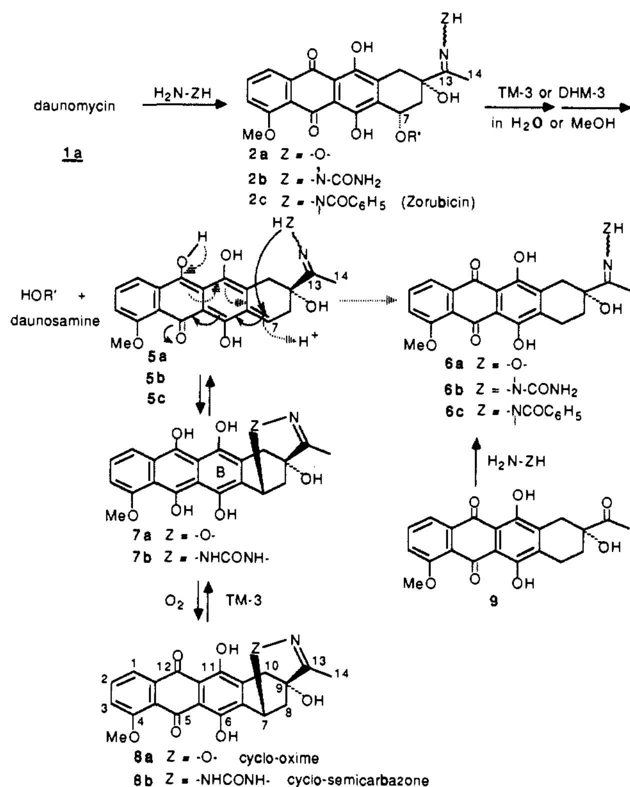
Scheme I



the 7-position from the COSY spectrum. 7-Deoxydaunomycinone semicarbazone (**6b**) was identified by spectroscopic and chromatographic comparison with an authentic sample prepared by reaction of 7-deoxydaunomycinone (**9**) with semicarbazide hydrochloride.

In separate experiments, reductions of daunomycin semicarbazone in basic methanolic and aqueous media were monitored by UV-visible spectroscopy, which revealed the formation and reaction of the quinone methide intermediate **5b**. In basic methanolic medium, 10^{-4} M daunomycin semicarbazone showed absorbance bands at 556 (A = 1.2) and 596 nm (A = 1.4) characteristic of the daunomycin chromophore in an anionic state.[15] Addition of 3 equiv of TM-3 dimer resulted in a decrease in absorbance of the quinone bands coupled with the rise of two bands at 370 (A_{60s} = 0.8) and 605 nm (A_{60s} = 0.65), indicative of formation of a quinone methide transient (see structure **5b**, Scheme I).[5,16] No appreciable amount of the intermediate hydroquinone was observed during this time. In the 60–900-s period of time, the bands at 370 and 605 nm decreased to almost disappear while an intense band at 435 nm (shoulder at 495 nm) rose and reached its maximum (A = 1.31) through two sharp isosbestic points at 405 and 520 nm, indicative of destruction of quinone methide and formation of hydroquinone species (and possibly quinones). In basic aqueous medium, similar spectral changes were observed, except maximum absorbance for the quinone methide bands occurred in less than 10 s.

Anaerobic reduction of the cyclosemicarbazone in methanolic medium at an apparent pH of 8 was also monitored spectroscopically. The spectra as a function of time showed a decrease in the quinone band at 503 nm coupled with a rise of a hydroquinone band at 425 nm during the first 30 min. During a subsequent 65-h time period the 425-nm band slowly decreased with a rise of the quinone absorption at 503 nm together with a rise of new bands at 420 and 445 nm characteristic of formation of leuco isomers of the hydroquinone. Leuco isomers of molecules with the daunomycin hydroquinone chromophore are commonly B-ring tautomers.[17] Upon exposure to air the residual hydroquinone band disappeared immediately and the leuco bands disappeared slowly, both with growth of the 503-nm quinone band. The only anthracycline material present after complete air oxidation was the starting cyclosemicarbazone **8b**.

Anaerobic reduction of daunomycin benzoylhydrazone (Zorubicin, **2c**) at pH 9 in aqueous medium containing 10% methanol with DHM-3 dimer yielded predominantly the benzoylhydrazone of 7-deoxydaunomycinone (**6c**) and a small amount of unreacted **2c**. 7-Deoxydaunomycinone benzoylhydrazone was identified by spectral comparison with an authentic sample prepared by reaction of 7-deoxydaunomycinone (**9**) with benzoylhydrazine. Spectroscopic monitoring of the reduction of **2c** revealed the formation of a quinone methide intermediate **5c** with bands at 370 and 600 nm.

Discussion

The reactions of the daunomycin derivatives **2a**, **2b**, and **2c** are summarized in Scheme I together with a mechanism based upon the observations described above and earlier mechanistic investigations of the reductive glycosidic cleavage of the anthracyclines.[5-7] TM-3 and DHM-3 radicals react with the quinones to produce in succession semiquinone and hydroquinone states. The hydroquinone states then eliminate daunosamine to yield the quinone methide transients **5a**, **5b**, and **5c**. The quinone methides react either as bases with solvent to yield the 7-deoxy aglycons **6a**, **6b**, and **6c** or as electrophiles with oxygen or nitrogen nucleophilic sites off the 13-position to yield cyclomers **8a** and **8b**. Competition between 7-deoxy aglycon formation and cyclomer formation is pH dependent, with pH affecting mostly the rate of the former reaction. Reversibility of the cyclization in the case of reduction of **2a** was established by reduction of cyclooxime **8a** at slightly lower pH, promoting irreversible protonation of quinone methide **5a**. Cyclosemicarbazone **8b** does not reductively cleave, possibly, because the five-atom bridge of **7b** creates less ring strain than the three-atom bridge of **7a** and because the semicarbazone is a poorer leaving group than the oxime. For the former reason, quinone methide **5b** originally formed the five-atom bridge. Observation of leuco bands upon reduction of **8b** is also consistent with lack of ring opening of **7b**. Leuco isomers commonly form from anthracycline hydroquinones that do not have other reaction pathways such as the hydroquinone of daunomycin in aprotic solvent or in protic solvent at low pH[18] and the hydroquinone of 7-deoxydaunomycinone.[17] Leuco isomers are also known to isomerize slowly back to the hydroquinone state, which rapidly reacts with molecular oxygen to restore the quinone state, accounting for the observed quantitative recovery of cyclosemicarbazone **8b**. Observation of some **8b** after complete reduction of **2b** and **8b** before exposure to air is consistent with the known oxidation of long-lived hydroquinone states such as **7b** by oxazinone **4a** with formation of 3,5,5-trimethyl-2-oxomorpholine.[5,19] Oxazinone **4a** is the byproduct of initial reduction of **2b** and **8b**. Similar oxidation of the hydroquinone state of **6a** by oxazinone **4a** was observed during reduction of cyclooxime **8a** with an excess of TM-3 dimer (see Experimental Section).

The lack of cyclomer formation upon reduction of the benzoylhydrazone derivative was inconsistent with the cyclomer formation upon reduction of the semicarbazone derivative. An explanation was apparent from the configuration of the hydrazone substituent. An NOE difference spectroscopic measurement as described in the Experimental Section established that the potentially nucleophilic nitrogen of the hydrazone **2c** was syn to the C14 methyl and, consequently, not properly located for reaction with the 7-position of the quinone methide intermediate **5c**. The quinone methide then could only react via the competitive route of protonation to give 7-deoxydaunomycinone benzoylhydrazone (**6c**). Cyclomer formation upon reduction of oxime **2a** and semicarbazone **2b** is clearly consistent with configurations of the

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respective functional groups anti to the C14 methyl. The oxime functional group of 7-deoxydaunomycinone oxime (**6a**) must also be anti with respect to the C14 methyl because it was formed from reductive ring opening of cyclooxime **8a**. Correspondingly, the benzoylhydrazone functional group of **6c** must be syn to the C14 methyl.

The results described here represent the first observation of reactivity of the quinone methide transient from reductive glycosidic cleavage of daunomycin with an oxygen or nitrogen nucleophilic site. Previously, only intermolecular coupling with sulfur nucleophilic sites has been achieved.[8] Recently we have reported intermolecular trapping of the quinone methide from reductive cleavage of the 11-deoxyanthracycline, menogaril, with nitrogen nucleophilic sites in imidazole[20] and in guanosine.[21] However, reduction of menogaril yields a long-lived quinone methide state because of the absence of the electron-donating 11-hydroxy group.[22]

The importance of the reactivity of the quinone methide state with nucleophilic sites in nucleic acids remains obscure.[23] Although daunomycin intercalates in duplex DNA,[24] once intercalated, reduction is sterically inhibited.[9,25] Recent experiments of Ferradine and co-workers[26] indicate that intercalated daunomycin can be anaerobically reduced by the small molecule reducing agent carbon dioxide radical anion. 7-Deoxydaunomycinone was formed without covalent binding to the DNA. This experiment indicates that reaction of nitrogen or oxygen nucleophilic sites in DNA, which were present during formation and reaction of the intercalated quinone methide, was not competitive with protonation. Possibly, reversible nucleophilic addition occurred; however, in the absence of an oxidizing agent, irreversible protonation drove the reaction to the 7-deoxy aglycon. In vivo, the small molecule reducing agent carbon dioxide radical anion has been observed but is not abundant.[27] A reasonable oxidizing agent for an adduct hydroquinone is molecular oxygen; however, molecular oxygen is rapidly reduced to superoxide by carbon dioxide radical anion.[28] Possibly, covalent binding of the daunomycin electrophore to oxygen and/or nitrogen nucleophilic sites in DNA occurs through a complex interaction of enzyme reducing agent, daunomycin, an oxygen or nitrogen nucleophilic site at a groove of DNA and, subsequently, molecular oxygen. Nucleophilic sites in proteins in addition to the thiol group of cysteine, which might similarly react, include the phenolic OH of tyrosine, the amido groups of asparagine and glutamine, the guanidino group of arginine, and the imidazole group of histidine. Attachment of the daunomycin electrophore at a groove of DNA or to a protein might permit subsequent catalytic activity for the production of DNA or protein cutting, reactive oxygen species.

Experimental Section

General Remarks. UV-visible spectra were recorded with a Hewlett-Packard 8452A diode array spectrometer. ^{1}H NMR spectra were obtained with a Bruker CXP300, Bruker AC 250, or Varian VXR-300S spectrometer. Chemical shifts are reported (in ppm) on the δ scale from internal tetramethylsilane, and coupling constants are given in hertz. COSY experiments were performed with a Varian VXR-300S or Varian VXR-500S spectrometer. IR spectra were recorded with samples as Nujol mulls with a Perkin-Elmer 1600 series FTIR spectrometer; only the most intense bands are reported. Mass spectra were obtained with a VG ZAB 2F or VG 7070 EQ-HF high-resolution mass spectrometer. FAB spectra were obtained by bombarding solutions of samples in either dithiothreitol (DTT), 3-nitrobenzyl alcohol (NBA), or thioglycerol (TG) with 8-keV xenon or argon atoms. Microanalyses were obtained from Istituto di Chimica Organica, Università di Milano. HPLC analyses were performed with a 600E Waters chromatograph equipped with a Model 481 UV-visible detector set a 480 nm. A 4 × 125 mm Lichrosphere 100 RP-18, 5-μm column (Merck LicroCart) was employed, eluting at 1 mL/min with 70% v/v methanol and 30% aqueous pH 4 buffer (3% ammonium formate adjusted to pH 4.0 with formic acid). The percentages of anthracyclines reported from HPLC analyses are the area percentages of the peaks resulting from compounds absorbing in the 450–500-nm region. TLC was performed with Merck precoated silica gel 60 F-254 sheets. Flash chromatographies were performed according to the literature,[29] using Merck 0.040–0.063-mm silica gel. Freeze–pump–thaw degassing was performed at liquid nitrogen temperature at 1×10^{-5} Torr through three cycles, followed by sealing under vacuum. Cells, degassed via the freeze–thaw method, were fabricated from Pyrex or quartz and constructed as described previously.[30] Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (**3a**, TM-3 dimer) and bi[3,5-dimethyl-5-(hydroxymethyl)-2-oxomorpholin-3-yl] (**3b**, DHM-3 dimer) dissolved in dichloromethane and acetonitrile, respectively, were loaded into the cuvette compartment of the cell. The solvent was then removed with a nitrogen stream. Trizma buffers were prepared from tris(hydroxymethyl)aminomethane (Tris) and its hydrochloride (Tris-HCl). TM-3 and DHM-3 dimers (**3a** and **3b**), as mixtures of diastereoisomers, were prepared by photoreductive dimerization of the corresponding 5,6-dihydro-1,4-oxazin-2-ones.[13,31] The diastereomers of TM-3 dimer were separated by low-temperature alumina flash chromatography.[31] Daunomycin hydrochloride (**1a**) was a gift from Farmitalia Carlo-Erba, Milan, Italy, and 7-deoxydaunomycinone (**9**) was prepared according to the literature.[32] Daunomycin semicarbazone (**2b**) was prepared from daunomycin hydrochloride and semicarbazide hydrochloride as described in the literature.[11] Daunomycin hydrochloride *N*-benzoyl hydrazone was obtained as Zorubicin from Rhône Poulenc, Neuilly sur Seine Cedex, France.

Synthesis of Daunomycin Oxime (2a). A slight modification of the literature procedures was employed.[10,11] Daunomycin hydrochloride (56 mg, 0.10 mmol) and hydroxylamine hydrochloride (117 mg, 1.7 mmol) were dissolved in 5 mL of water, and the pH was adjusted to 7.4 with 1 mL of 1 M sodium hydroxide. The solution was kept at 50 °C for 15 min. After cooling, the pH was brought to 8.7 with 1 M sodium hydroxide whereby a dark red precipitate of the oxime, free base, separated. The precipitate was collected, washed with water, and dried to give 42 mg (72%) of **2a**, 97% pure by HPLC (R_t 3.3 min). TLC (10/2/0.2 v/v dichloromethane–methanol–water) gave only one spot, R_f 0.22.

Reaction of Daunomycin Oxime (2a) with DHM-3 Dimer (3b) and with TM-3 Dimer (3a): Synthesis of 7,13-Dideoxy-7,13-(epoxynitrilo)-daunomycinone (Cyclooxime 8a). (A) Daunomycin oxime (**2a**; 20 mg, 3.5×10^{-2} mmol) was dissolved in 1.0 mL of dimethylformamide and mixed with 10 mL of water buffered at pH 9.0 with 5×10^{-2} M Trizma (88/12 mol/mol Tris–Tris-HCl). The solution was deareated by bubbling with argon. DHM-3 dimer (**3b**; 22 mg, 7.0×10^{-2} mmol) was dissolved in 5 mL of the same buffer, previously deareated with nitrogen, and added to the stirred solution of **2a**. A precipitate separated from the dark solution. The mixture was kept under argon and stirred at ambient temperature for 1 h. The reaction flask was then opened to the air: the precipitate was collected by centrifugation and washed with water. HPLC showed only two peaks with R_t 3.0 (**8a**) and 6.7 min [7-deoxydaunomycinone oxime (**6a**)] in a 10/1 ratio. TLC (95/5 v/v chloroform–methanol) showed a major spot, R_f 0.3 (**8a**), and a minor spot, R_f 0.4 (**6a**). The mixture was resolved by flash chromatography; **6a** was eluted first and identified by HPLC and TLC comparison with an authentic sample (see below). Pure **8a** (7.2 mg, 52% yield) was then eluted with 9/1 v/v chloroform–methanol. Recrystallization from hot methanol (1 mL/mg) gave red crystals, mp 270 °C, with the following spectral properties: IR (Nujol) 3436, 1678, 1617, 1582, 1277, 1184, 1131, 1074, 996, 803, 721 cm^{-1}; UV (methanol) λ_{max}, nm (ϵ, M^{-1} cm^{-1}) 232 (3.5×10^4), 249 (2.3×10^4), 288 (6.7×10^3), 478 (1.1×10^4), 496 (sh, 1.0×10^4); ^{1}H NMR (CDCl$_3$) δ 13.47 (s, phenolic OH), 13.08 (s, phenolic OH), 8.03 (d, $J_{3,2}$ = 8, H3), 7.78 (t, $J_{2,3} = J_{2,1}$ = 8, H2), 7.40 (d, $J_{1,2}$ = 8, H1), 5.61 (d, $J_{7,8a}$ = 5.5, H7), 4.08 (s, CH$_3$O), 3.50 (s, C9-OH),

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3.19 [A part of ABX pattern, $J_{10a,10b}$ = 18, $J_{10a,8a}$ = 1.5, H10a(b)], 3.00 [B part of ABX pattern, $J_{10b,10a}$ = 18, H10b(a)], 2.61 [m, $J_{8a,8b}$ = 10, $J_{8a,10a}$ = 1.5, $J_{8a,7}$ = 5.5, H8a(b)], 2.30 [d, $J_{8b,8a}$ = 10.2, H8b(a)], 2.03 (s, CH_3C=N): ^{1}H NMR (DMSO-d_6) δ 13.62 (s, phenolic OH), 12.91 (s, phenolic OH), 7.87 (m, 2 aromatic H), 7.65 (m, 1 aromatic H), 6.34 (s, C9-OH, disappears with D_2O), 5.26 (d, $J_{7,8}$ = 5.4, H7), 3.99 (s, CH_3O), 3.02 [A part of AB pattern, $J_{10a,10b}$ = 18.4, H10a(b)], 2.84 [B part of AB pattern, $J_{10b,10a}$ = 18.6, H10b(a)], 2.48 (m, partially overlapped with the solvent peak, $J_{7,8}$ = 5.7, H8a(b)], 2.19 [B part of AB pattern, $J_{8b,8a}$ = 10.8, H8b(a)], 1.82 (s, CH_3C=N); MS (FAB, positive ion, NBA matrix) m/z 397 (M + 2). The tris(trimethylsilyl) ether of **8a**, obtained by a known procedure for silylation of anthracyclines,[33] showed EI/MS peaks (rel intensity) above m/z 500 as expected based upon literature precedent:[34] 611 (M, 28), 596.1952 (M − 15, 100; calcd for $C_{29}H_{38}NO_7Si_3$, 596.1956), 581 (67), 565 (61), 553 (39), 539 (25), 524 (48), 508 (83); in addition peaks at m/z 683 (23), 668 (32), and 653 (43) were observed, suggesting, along with simple silylation, occurrence of elimination at C7–C8 with oxazine ring opening and formation of the oxime trimethylsilyl ether.

(B) Daunomycin oxime (**2a**: 58 mg, 0.10 mmol) was dissolved in 100 mL of methanol. Sodium hydroxide (1.5 mL of a 1 M aqueous solution) was added to give an apparent pH of 9.3, and the purple solution was deareated with argon. TM-3 dimer (**3a**; meso isomer, 60 mg, 0.21 mmol) was added and the mixture was stirred under argon at 30 °C. After an initial darkening, the solution became green-yellow in a few minutes. After 30 min, air was let into the reaction flask. HPLC analysis of the solution showed **8a** and **6a** in a 9/1 ratio. After 1 day in the refrigerator, a dark red precipitate formed and 21 mg (52% yield) of **8a** (97% pure by HPLC) was collected by suction filtration. A small-scale experiment similar to (B) in methanol, apparent pH 7.0 (Trizma buffer), gave **8a** and **6a** in a 1/10 ratio. At pH 8.2 (Trizma buffer, DHM-3 dimer as reducing agent) in water, the **8a** and **6a** were formed in a 4/1 ratio. A control experiment at pH 9.3 showed that no cyclooxime **8a** resulted from heating oxime **2a** at 30 °C for 30 min in methanol solvent; HPLC analysis showed only unreacted **2a**.

Synthesis of 7-Deoxydaunomycinone Oxime (6a). 7-Deoxydaunomycinone (**9**: 38 mg, 0.10 mmol) was dissolved in 40 mL of hot methanol, and the resulting solution was mixed with a solution of hydroxylamine hydrochloride (70 mg, 1.0 mmol) in 4 mL of aqueous 0.1 M sodium hydroxide. After 30 min of refluxing, a HPLC analysis showed quantitative conversion of **9**, R_t 10 min, into **6a**, R_t 7 min. The hot solution was filtered and the filtrate kept overnight in the refrigerator to give 15 mg of 100% pure **6a**, mp 265 °C. A second crop of pure oxime was obtained upon concentration of the mother liquors. The total yield was 29 mg (73%), and the product had the following spectroscopic properties: IR (Nujol) 3287, 1607, 1571, 1258, 1213, 1170, 1106, 1079, 971, 837, 796, 769, 731 cm^{-1}; UV–vis (MeOH) λ_{max} 494 nm (ϵ = 1.2 × 10^4 cm^{-1} M^{-1}); ^{1}H NMR (DMSO-d_6) δ 13.88 (s, phenolic OH), 13.36 (s, phenolic OH), 10.62 (s, =NOH), 7.88 (m, 2 aromatic H), 7.62 (m, 1 aromatic H), 5.28 (s, C9-OH), 3.98 (s, CH_3O), 3.3–3.6 [m, H7(a+b)], 3.14 [A part of AB pattern, $J_{10a,10b}$ = 19, H10a(b)], 2.75 [B part of AB pattern, $J_{10b,10a}$ = 18, H10b(a)], 1.8–2.1 (m, H8), 1.86 (s, CH_3C=N): MS (FAB, positive ion, DTT matrix) m/z 399 (M + 2). Anal. Calcd for $C_{21}H_{19}NO_7$: C, 63.47; H, 4.82; N, 3.52. Found: C, 63.2; H, 4.8; N, 3.8.

Conversion of Cyclooxime 8a into 7-Deoxydaunomycinone Oxime (6a) by Reductive Ring Opening with TM-3 Dimer (3a). TM-3 dimer (**3a**, *d,l* isomer; 0.113 mg, 4.0 × 10^{-4} mmol) was deposited into the cuvette portion of each of three reaction cells (here referred to as A, B, and C) as described under General Remarks. A 1.0 × 10^{-4} M solution of **8a** was made in pH 8.2, (2 + 2) × 10^{-4} M methanolic Trizma buffer. Aliquots (2.0 mL, 2.0 × 10^{-4} mmol) of the solution were introduced into the degassing chamber of the three cells. After freeze–thaw degassing and sealing, the cells were brought to 25 °C in a water bath. The solutions in each cell were poured at the same time into the cuvette portion and the cells vigorously shaken for a quick dissolution of the solid TM-3 dimer. Cell A was immediately transferred to the cell holder of the UV-visible spectrometer thermostated at 25.0 ± 0.1 °C, while cells B and C were kept in the water bath at 25 °C. The spectral changes in cell A at 300–800 nm were monitored, taking spectra every 15 s for 3 min. During the initial part of the reaction (0–240 s), the absorbance at 480 nm decreased due to the destruction of the starting quinone **8a**: this was coupled with a rise of a band at 430 nm, indicative of formation of hydroquinones (sharp isosbestic point at 452 nm). After 240 s the trend was reversed: the hydroquinone band at 430 nm slowly decreased to be replaced by a quinone band at ca. 490 nm. After a total of 1300 s the ratio between the bands was 1/1. However, it took several hours for the hydroquinone band to disappear. No meaningful absorption of quinone methide was observed during the experiment. Cell B was opened 12 min after mixing: HPLC analysis showed only two peaks corresponding to **8a** and **6a** in a 4/6 ratio, which did not change with time. Cell C was opened 90 min after mixing: HPLC analysis showed 4% **8a** and 96% **6a**.

Reaction of Daunomycin Semicarbazone (2b) with TM-3 Dimer (3a) in Aqueous Basic Medium: Synthesis of 7,13-Dideoxy-7,13-(iminocarbonyliminonitrilo)daunomycinone, Cyclosemicarbazone 8b. Daunomycin semicarbazone (**2b**; 170 mg, 0.29 mmol) was dissolved in 50 mL of a pH 11, 0.1 M, sodium carbonate solution. The solution was deareated by bubbling with nitrogen. TM-3 dimer (**3a**, *d,l* isomer; 160 mg, 0.56 mmol) in 5 mL of deareated methanol was added to the stirred solution of **2b**. The reaction mixture was kept sitrring under a nitrogen atmosphere. In a few seconds the color of the solution changed from purple to brown and then, in a few minutes, to green-yellow. After 15 min the flask was opened to the air. A fine dark precipitate, which separated, was collected. HPLC analysis of the precipitate showed both **8b**, R_t 2.4 min, and the semicarbazone of 7-deoxydaunomycinone **6b**, R_t 4.6 min, in a 2.5/1 ratio (ca. 70% of the product mixture). A third compound, R_t 7.5 min (25% peak area), was also present. No unreacted **2b**, R_t 2.2 min, was detected in the mother liquors. The solid mixture was thoroughly washed with chloroform, which dissolved preferentially **6b**. The undissolved residue (60 mg) showed three spots on TLC (9.5/1 v/v chloroform–methanol) with R_f 0.35 (residual **6b**), 0.30 (**8b**), and 0.26 (compound with HPLC R_t 7.5 min). Product **6b** was obtained 95% pure by flash chromatography: first **6b** was eluted with 9.5/1 v/v chloroform–methanol and then **8b** with a 9.5/1.5 mixture. Cyclosemicarbazone **8b** was then recrystallized from hot ethanol. The resulting material had the following properties: mp 270 °C dec: IR (Nujol) 3452 (br), 3358 (v br), 1695, 1682, 1613, 1575, 1283, 1247, 1215, 1074, 1043, 984 cm^{-1}; UV-vis (MeOH) λ_{max}, nm (ϵ, M^{-1} cm^{-1}) 255 (2.1 × 10^4), 289 (6.2 × 10^3), 484 (1.1 × 10^4), 498 (1.1 × 10^4), 534 (7.2 × 10^3); ^{1}H NMR (DMSO-d_6) δ 14.55 (s, phenolic OH), 13.44 (s, phenolic OH), 8.98 (s, CONHN), 7.94 (m, 2 aromatic H), 7.68 (m, 1 aromatic H), 6.1–6.4 (br, CONH-C7), 5.37 (s, C9-OH), 4.55 (m, H7, coupling to H8a and H8b from the COSY spectrum), 4.01 (s, CH_3O), 3.51 [A part of AB pattern, $J_{10a,10b}$ = 16, H10a(b)], 2.47 [B part of AB pattern, partially overlapped with the solvent signal, H10b(a)], 1.74 [m, partially overlapped with the solvent signal, H8a(b), coupling to H7 from the COSY spectrum], 1.65 (s, CH_3C=N), 1.40 [m, H8b(a), coupling to H7 from the COSY spectrum]: peaks at 14.55, 13.49, 8.98, 6.1–6.4, 5.37 exchanged with D_2O; MS (FAB, positive ion, DTT matrix) m/z 439 (M + 2). The mass spectral peaks for **8b** or for its derivative from trimethylsilation were not sufficiently intense for exact mass measurement. Because of instability, **8b** could not be sufficiently purified for combustion analysis. Compound **6b** was identified by HPLC and TLC comparison with a sample obtained by treatment of 7-deoxydaunomycinone with semicarbazide hydrochloride in methanol and with a sample obtained from reduction of **2b**, both as described below.

Reaction of Daunomycin Semicarbazone (2b) with TM-3 Dimer (3a) in Basic Methanol: Synthesis of 7-Deoxydaunomycinone Semicarbazone (6b). Daunomycin semicarbazone (**2b**; 44 mg, 7.5 × 10^{-2} mmol) was dissolved in 50 mL of methanol containing 0.9 mL of aqueous 1 M sodium hydroxide. The solution was deaereated by bubbling with nitrogen. TM-3 dimer (**3a**, meso isomer; 45 mg, 0.16 mmol) was added and the solution kept stirring under a nitrogen atmosphere at ambient temperature. The color of the solution changed from purple to brownish and then yellow-green. After 20 min, HPLC analysis of a small sample showed the presence of **8b** (ca. 10%) and **6b** (ca. 65%). After a total of 75 min, air was let into the reaction mixture. HPLC analysis showed 80% **6b**, 9% **8b**, and minor side products. After one night in the refrigerator, a fine precipitate was obtained (13 mg), which upon recrystallization from 2-propanol gave 97% pure (HPLC) **6b**: mp 275 °C dec; IR (Nujol) 3474, 3280, 3200, 1673, 1613, 1575, 1248, 1211, 1060, 988, 819, 768, 724 cm^{-1}; UV-vis (MeOH) λ_{max}, nm (ϵ, M^{-1} cm^{-1}) 253 (2.5 × 10^4), 290 (6.9 × 10^3), 476 (sh, 9.2 × 10^3), 492 (1.0 × 10^4), 528 (6.1 × 10^3); ^{1}H NMR (DMSO-d_6) δ 13.8 (s, phenolic OH), 13.4 (s, phenolic OH), 9.03 (s, NH), 7.90 (m, 2 aromatic H), 7.64 (m, 1 aromatic H), 6.14 (br s, NH_2), 5.21 (s, C9-OH), 3.99 (s, CH_3O), 3.3–3.55 (m, H7), 3.11 [d, $J_{10a,10b}$ = 18.5, H10a(b)], 2.65–2.8 [m, H10b(a)], 2.2–2.7 (m, H8), 1.90 (s, CH_3C=N); MS (FAB, positive ion, DTT matrix) m/z 441 (M + 2), (FAB, positive ion, NBA matrix) 439 (M), (FAB, negative ion, DTT matrix) 439 (M).

The semicarbazone **6b** was also prepared by reaction of 7-deoxydaunomycinone (**9**) with semicarbazide hydrochloride. A solution of 30 mg of **9** in 30 mL of methanol was mixed with a 1-mL solution of water containing a 10-fold excess of semicarbazide hydrochloride, and the pH was adjusted to 5 with 0.6 mL of a 1 M aqueous sodium hydroxide solution. The resulting solution was refluxed for 30 min. After being filtered, the reaction mixture was kept overnight at ambient temperature.

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The semicarbazone **6b** (22 mg), collected by filtration, was shown to be 99% pure by reverse-phase HPLC analysis. The material was spectroscopically and chromatographically identical with the **6b** isolated as described above. Anal. Calcd for $C_{22}H_{21}N_3O_7$: C, 60.13; H, 4.82; N, 9.56. Found: C, 60.0; H, 4.8; N, 9.5.

Effect of pH on the Reaction of Daunomycin Semicarbazone (2b) with DHM-3 Dimer (3b). Three separate small-scale experiments were run at different pH in water: pH 8.2 (Trizma buffer), pH 9.0 (Trizma buffer), pH 11 (0.1 M sodium carbonate). In each experiment daunomycin semicarbazone (**2b**) was dissolved in the basic solution and an excess of DHM-3 dimer (**3b**) was added under nitrogen. After 10 min, air was let in and the reaction mixture analyzed by HPLC. The following **6b**/**8b** ratios were obtained: pH 8.2, 5/1; pH 9.0, 2/1; pH 11, 1/3. Total yields of **6b** + **8b** were 90% at pH 8.2 and pH 9.0, 70% at pH 11.

Evidence for Quinone Methide Formation in the Reaction of Daunomycin Semicarbazone (2b) with TM-3 Dimer (3a). A 1.0×10^{-4} M solution of **2b** was prepared in methanol containing 3.6% by volume of 1 M aqueous sodium hydroxide. A 2.5-mL aliquot of the solution was introduced into a UV cuvette and deareated by bubbling with nitrogen passed through a serum stopper. The purple solution showed two maxima at 556 (A = 1.2) and 596 nm (A = 1.4). The temperature was maintained at 25 °C. A 0.2-mL aliquot of a deareated methanolic solution of TM-3 dimer (**3a**, *d,l* isomer; 3 mol equiv) was introduced into the cell. Spectra were then recorded every 10 s for a total of 900 s. The spectral changes are described under both Results and Discussion. After quenching with air, HPLC analysis showed **6b** (80%), **8b** (<10%), and minor amounts of side products. Similar behavior was observed when the reaction was run using a solution of **2b** in aqueous 0.1 M sodium carbonate. The only significant difference was the quicker disappearance of the quinone chromophore, which was replaced by the quinone methide chromophore in less than 10 s. HPLC analysis showed 50% **8b** and 20% **6b**.

Attempted Reductive Ring Opening of Cyclosemicarbazone (8b) with TM-3 Dimer (3a). The *d,l* isomer of TM-3 dimer (**3a**; 0.028 mg, 1.0×10^{-4} mmol) was deposited into the cuvette portion of a reaction cell as described under General Remarks. A 2.0-mL aliquot of a 5.0×10^{-5} M solution of **8b** in pH 8.2, $(1 + 1) \times 10^{-3}$ M methanolic Trizma buffer 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 solution was then poured into the cuvette portion of the cell; the cell was shaken and quickly transferred into the cell holder of the UV–visible spectrometer thermostated at 25.0 ± 0.1 °C. The spectral changes from 340 to 800 nm were monitored by taking spectra every 60 s for the first 30 min. A decrease of the quinone absorbance at 503 nm was observed coupled with a rise of a hydroquinone band at 425 nm (sharp isosbestic point at 452 nm). During the following 65 h, the hydroquinone absorption at 425 nm decreased very slowly to about half of its maximum value coupled with a rise of the quinone absorption at 503 nm together with a rise of new bands at 420 and 445 nm characteristic of a leuco isomer.[17,18] The cell was opened to the air; whereby, the residual hydroquinone absorption quickly disappeared and the quinone band increased in intensity. In a slower process requiring hours, the leuco isomer bands at 420 and 445 nm were replaced by the quinone band at 503 nm. At this point in time, HPLC analysis revealed the presence of only the starting cyclosemicarbazone **8b**.

NOE Experiment on the Benzoylhydrazone of Daunomycin (2c). An ^{1}H NMR spectrum of **2c** taken in DMSO-d_6 at 300 MHz showed the NHCO at δ 10.25, the two ortho protons of the benzoyl group at 7.75 (d, J = 7.2) and the $CH_3C{=}N$ at 2.06 ppm. In an NOE difference spectroscopy experiment, saturation of the $CH_3C{=}N$ group at 2.06 ppm induced a 7% enhancement of the NHCO signal at 10.25 ppm. Conversely irradiation of the NHCO group induced a 5.2% increase of the $CH_3C{=}N$ signal and a 4% enhancement of the two ortho proton signals of the benzoyl group at 7.75 ppm.

Reaction of Daunomycin Benzoylhydrazone (2c) with DHM-3 Dimer (3b): Detection of an Intermediate Quinone Methide and Formation of 7-Deoxydaunomycinone Benzoylhydrazone (6c). A 2×10^{-5} M solution of Zorubicin (**2c**) was made by mixing a concentrated solution of **2c** in methanol in ca. 10 volumes of pH 9.0 Trizma-buffered water. The solution was deareated in a UV cuvette, and a 5 molar excess of DHM-3 dimer (**3b**) was added at 20 °C, keeping the solution protected from air. UV monitoring showed the quick disappearance of the quinone band at 500 nm, coupled with a simultaneous rise of a quinone methide spectrum characterized by two maxima at 370 and 600 nm. After ca. 1 min the quinone methide spectrum disappeared, while a hydroquinone band appeared at 420 nm. Air was let into the cell. HPLC analysis showed a small peak, R_t 4.2 min, corresponding to unreacted **2c**, as shown by coinjection, and a large peak, R_t 9.3 min, due to the benzoylhydrazone of 7-deoxydaunomycinone (**6c**), as shown by coinjection with an authentic sample (see below). TLC (95/5 v/v chloroform–methanol) showed only two spots, R_f 0 (**2c**) and R_f 0.7 (**6c**, as indicated by comparison with an authentic sample). When a similar experiment was run at pH 11 (0.1 M sodium carbonate), the main reaction product (ca. 40%) was **5c**, HPLC R_t 9.2 min, accompanied by at least eight byproducts with R_t <9 min.

7-Deoxydaunomycinone Benzoylhydrazone (6c). A solution of benzoylhydrazine (230 mg, 1.74 mmol) in 5 mL of water was added to a suspension of 7-deoxydaunomycinone (**9**; 50 mg, 0.131 mmol) in methanol. Hydrochloric acid (5.5 mL of a 0.1 N solution) was added and the mixture was kept at ambient temperature for 3 days until substantial conversion was observed by TLC (95/5 v/v chloroform–methanol; R_f 0.7 for **9**, 0.3 for **6c**). The methanol was rotary evaporated, 20 mL of water was added, and the solid was collected by centrifugation. Pure **6c** was obtained by flash chromatography using a 95/5 v/v mixture of chloroform and methanol followed by crystallization from methanol. HPLC showed a retention time of 9.3 min. The pure **6c** had the following spectroscopic properties: ^{1}H NMR ($DCCl_3$) δ 13.86 (s, phenolic OH), 13.47 (s, phenolic OH), 8.80 (br, NH), 8.02 (d, J = 8, 1 aromatic H), 7.66–7.86 (m, 3 aromatic H), 7.26–7.6 (m, 4 aromatic H), 4.07 (s, CH_3O), 2.8–3.2 (m, H7 and H10), 2.08, (s, $CH_3C{=}N$), 1.99 (m, partially overlapped with the 2.08 signal, H8); MS (FAB, positive ion, DTT or TG matrix) m/z 501 (M + 1). Anal. Calcd for $C_{28}H_{24}N_2O_7$: C, 67.2; H, 4.8; N, 5.6. Found: C, 66.6; H, 5.0; N, 5.4.

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