7-Deoxydaunomycinone Quinone Methide Reactivity with Thiol Nucleophiles

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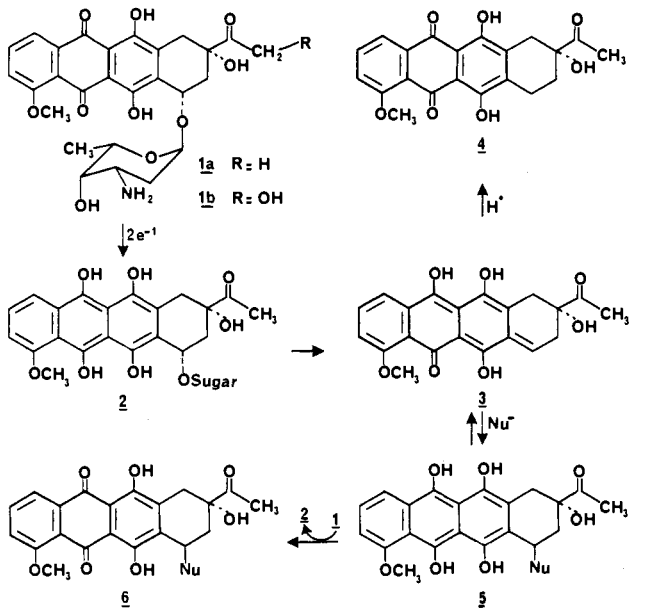
Under anaerobic conditions and with NADPH as a reducing agent, daunomycin is reduced in the presence of spinach ferredoxin: $NADP^+$ oxidoreductase as the enzyme catalyst to its hydroquinone, from which intramolecular elimination of the C-7 glycoside proceeds to provide a quinone methide intermediate. This quinone methide is capable of bimolecular reaction with the thiolate nucleophiles *N*-acetyl-L-cysteine, *N*-(*tert*-butoxycarbonyl)-L-cysteine, and 1-thio-β-D-glucose, providing a pair of C-7 diastereomers, when the reaction is carried out under the autocatalytic conditions offered by substoichiometric quantities of NADPH. With 0.4 equiv of NADPH, optimal yields of the adducts are obtained of approximately 65%. In each case, the 7*S* adduct is the major product, with the observed stereoselectivities (7*S* to 7*R*) ranging from 2.6 to 1 for *N*-acetyl-L-cysteine to 4 to 1 for both the *N*-(*tert*-butoxycarbonyl)-L-cysteine and 1-thio-β-D-glucose as nucleophiles. By standard blocking and deblocking procedures, the complete set of complementary functionalized (7*S*)- and (7*R*)-*N*-acetyl and *O*-methyl 7-L-cysteinyl-7-deoxydaunomycinones is prepared. All efforts to extend this quinone methide trapping reaction to additional nucleophiles (such as I^- or N_3^-), including the use of Fe(III) chelation, are unsuccessful. The Fe(III) chelate of daunomycin is however reduced by ferredoxin reductase and NADPH to the Fe(III) chelate of 7-deoxydaunomycinone, suggesting that quinone reduction of the chelate to the quinone methide has occurred. Of the new compounds prepared, only (7*R*)-7-*S*-(β-D-glucopyranosyl)-7-thio-7-deoxydaunomycinone has biological activity. As an in vitro inhibitor of P_{388} cell growth, it has a 50% inhibitory concentration 25 times greater than that of daunomycin.

Daunomycin (**1a**) and adriamycin (**1b**) are among the most effective antitumor antibiotics.[1] As there exists considerable uncertainty as to the precise mechanism that expresses their antitumor activity, the chemical scrutiny of these two quinones continues in several disparate areas.[2] One particular focus concerns the role redox chemistry may have in the activation of these anthracyclines to potentially reactive quinone methide intermediates.[3] In particular, a possibility exists that enzyme-catalyzed reduction of the quinone moiety to the hydroquinone,[2] which is known to provide the quinone methide[3] by intramolecular expulsion of the C-7 glycoside,[4] may proceed at nearby critical cellular targets (such as DNA). Covalent attachment may then occur, with the quinone methide acting either as a nucleophile[5] or as an electrophile.[6] An attractive feature of the quinone methide as an in vivo intermediate is its accounting for the appearance of 7-deoxyanthracyclinone metabolites,[7] which arises from interception of the quinone methide by solvent (H^+ acting as an electrophile).[8] For this reason considerable effort has been placed toward the chemical characterization of these quinone methides, and certain of their properties are now known. The quinone methide derived from the 11-deoxyanthracyclines (exemplified by aclacinomycin) displays modest bimolecular nucleophilic and electrophilic character;[4b,5b,6] whereas the 11-hydroxy-substituted anthracyclines (exemplified by daunomycin and adriamycin) have thus far only been seen to display modest bimolecular nucleophilic character.[4b,5a] We now complete this initial characterization of the quinone methide reactivity by the description of the circumstances under which the 7-deoxydaunomycinone quinone methide behaves as an electrophile.

The essential requirement for quinone methide interception by nucleophiles is a recognition of the instability of the resulting hydroquinone adducts **5** (Scheme I). These retain the propensity to eliminate the C-7 substituent, and hence a requirement exists for an oxidant to transform these adducts to the stable quinone state **6**.[6] Aware of these experimental limitations, an extensive examination of the 7-deoxydaunomycinone quinone methide was undertaken in our laboratories using ethyl xanthate ($EtOCS_2^-$) as the nucleophile. The choice of xanthate anions appeared apt, given their demonstrated success with

Scheme I



not only 11-deoxyanthracyclinone quinone methides[6] but mitosene quinone methides as well.[9] To our disap-

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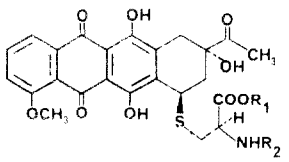
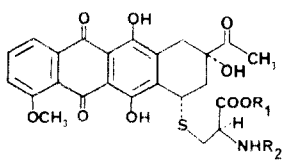
pointment, however, no reaction circumstances were found where adduct formation was a detectable occurrence. Consequently an initial conclusion was made that the 7-deoxydaunomycinone quinone methide was unreactive as an electrophile.[6] This conclusion is now found to be incorrect. Rather, this quinone methide appears generally capable of bimolecular reaction with several simple thiolate nucleophiles. Reaction circumstances leading to adduct formation exist for two such nucleophiles (*N*-acylcysteines and 1-thio-β-D-glucose), and they are described. These observations not only suggest that the general elaboration of new C-7 sulfur-functionalized anthracyclines within the most active anthracycline subfamily will be straightforward but also provide the long sought evidence for the electrophilic nature of these particular quinone methides, as first anticipated by Moore.[3]

Results

The minimal requirements for quinone methide formation are anaerobicity (otherwise the hydroquinone glycoside is returned to the quinone oxidation level at the expense of O_2 reduction), an enzyme catalyst to mediate anthracycline reduction, and a suitable reducing agent (compatible with the enzyme). In addition, to observe nucleophile trapping one requires not just a suitable nucleophile, but also an oxidant to transform the hydroquinone–nucleophile adduct. The choice of spinach ferredoxin:$NADP^+$ oxidoreductase (EC 1.6.7.1; ferredoxin reductase) as the enzyme catalyst is made with consideration to its ready availability and excellent capability for anthracycline reduction.[4b] Although both reduced pyridine nucleotides (NADH and NADPH) may be used as reducing agents with this enzyme, NADPH (with its higher catalytic velocity) proves the better choice. No better oxidant is available than the anthracycline glycosides themselves; hence, the NADPH is used in substoichiometric quantities, and the overall reaction assumes autocatalytic character.[6] Last, as a choice for a nucleophile to succeed where xanthate anions fail, the *N*-acyl-L-cysteines are appropriate since the conjugate base of these, the thiolate anion, possesses excellent nucleophilic character and since an amino acid nucleophile preserves some biological relevance.

For the initial evaluations, a concentration of 20 mM *N*-acetylcysteine is arbitrarily chosen. Under this circumstance the following procedure describes the optimal conditions for the conversion of daunomycin to the diastereomeric 7-(*N*-acetyl-L-cysteinyl)-7-deoxydaunomycinones **7a** and **8a**. To an anaerobic solution of daunomycin (0.3 mM) in pH 7 buffer, containing 0.4 mol equiv of NADPH, is added sufficient ferredoxin reductase to sustain an initial zero-order velocity for quinone methide





7a: R_1 = H, R_2 = $COCH_3$
b: R_1 = CH_3, R_2 = $COCH_3$
c: R_1 = H, R_2 = $CO_2C(CH_3)_3$
d: R_1 = CH_3, R_2 = $CO_2C(CH_3)_3$
e: R_1 = H, R_2 = H
f: R_1 = CH_3, R_2 = H

8a: R_1 = H, R_2 = $COCH_3$
b: R_1 = CH_3, R_2 = $COCH_3$
c: R_1 = H, R_2 = $CO_2C(CH_3)_3$
d: R_1 = CH_3, R_2 = $CO_2C(CH_3)_3$
e: R_1 = H, R_2 = H
f: R_1 = CH_3, R_2 = H

generation of approximately 15 nM s^{-1}. After 6 h at ambient temperature, the daunomycin is converted to the adducts (combined yield 65%) and 7-deoxydaunomycinone (4) (approximately 35% yield); only very small quantities of unreacted daunomycin remain. Altering these conditions has the following effects: Increasing the daunomycin concentration beyond 0.3 mM significantly lowers the adduct yield, as does replacement of NADPH by NADH. (It is probable that increasing the enzyme concentration with NADH to give an identical initial zero-order velocity would correct this latter effect.) A stoichiometry of 0.3–0.4 equiv of NADPH is optimal; decreasing this stoichiometry results in incomplete conversion, while increasing this stoichiometry enriches the conversion to 7-deoxydaunomycinone at the expense of the adduct yield. The enzyme concentration may be adjusted several-fold greater or less with little effect on yield, this adjustment merely serving to somehat decrease or increase, respectively, the overall reaction time. Separation of the adduct mixture from the other products is easily accomplished by selective extraction. The isolation of the individual two isomers is more difficult and is most straightforwardly done for small-scale separations through the use of reversed-phase liquid chromatography. Quantitation of the two separate peaks eluting during purification indicates that the diastereomers **8a** and **7a** are present in a relative ratio of 2.6 to 1, respectively.

The proof of structure for the adducts and the assignment of configuration for each are undertaken on the respective methyl esters **7b** and **8b**, obtained in quantitative yield with diazomethane as the esterification reagent. The constitution of each diastereomer as the *N*-acetylcysteine adduct is established by high-resolution FAB mass spectrometry and NMR spectroscopy. The ^1H NMR of the major adduct indicates the C-7 hydrogen to have vicinal coupling constants to the respective C-8 hydrogens of approximately 4.5 and 0 Hz, which suggest a 7*S* configuration. This suggestion follows from the general preference of the C-9 hydroxyl in C-6-functionalized anthracyclinones to occupy a pseudoaxial position ,[10] which in turn places the C-7 hydrogen of a 7*S* adduct in the pseudoequatorial position. Confirmation of this assignment for the major isomer is obtained from the CD difference spectrum between the adduct and 7-deoxydaunomycinone, which exhibits the required positive band near 365 nm for the assignment of a 7*S* configuration.[11] Likewise, the minor adduct possesses couplings between the C-7 and C-8 hydrogens indicative of a pseudoaxial C-7 hydrogen (J = 7.1, 4.3 Hz) and a mirror-image CD difference spectrum to its diastereomer. Hence, the major adduct is the 7*S* diastereomer **8b** and the minor adduct is the 7*R* diastereomer **7b**.

In order to establish the generality of this procedure, and also to generate a complementary set of blocked and un-

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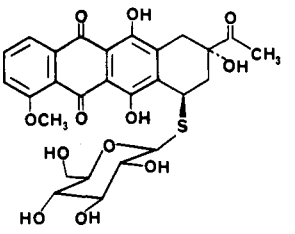
blocked 7-cysteinyl-7-deoxydaunomycinone adducts, the efficacy of L-cysteine itself in this reaction was determined. Under otherwise identical conditions, the use of L-cysteine as a nucleophile results in poor yields of adducts (no better than 5% total yield) compared to *N*-acetyl-L-cysteine. Direct trapping is therefore not a feasible pathway to the 7-cysteinyl derivatives. The required circumstances for chemical hydrolysis of the *N*-acetyl group of **7a** and **8a** are too demanding with respect to the anthracycline moiety; furthermore, both are resistant to enzymatic hydrolysis of the *N*-acetyl group (acylase I, carboxypeptidase A, and chymotrypsin are ineffective). Thus, the *N*-acetyl derivatives are unsuitable precursors to the C-7 cysteinyl structure. An alternative method that proved itself successful is the use of a cysteine derivative with an easily removable protecting group on the amine. Accordingly *N*-(*tert*-butoxycarbonyl)-L-cysteine was examined for its nucleophilic competence and found to provide the C-7 adducts **7c** and **8c** in an identical combined yield (65%) as with *N*-acetylcysteine but with an increased stereoselectivity (a ratio of 7*S* to 7*R* substitution of 4 to 1). The two diastereomers are readily separated by reversed-phase liquid chromatography (although care is necessary to avoid loss of the protecting group, by limiting contact of the adducts to the minimum necessary with the CF_3CO_2H present in the mobile phase) and are characterized as the methyl esters **7d** and **8d**. The *N*-*tert*-butoxycarbonyl group is removed from the isolated diastereomers by a higher CF_3CO_2H concentration. (Methanolic HCl both deblocks and forms measurable quantities of the methyl ester.) This sequence provides the individual L-cysteinyl derivatives **7e** and **8e**. As must be anticipated, treatment with acetic anhydride in pyridine yields the *N*-acetyl species **7a** and **8a**, identical with those obtained directly with *N*-acetyl-L-cysteine as the nucleophile. Likewise, reaction of either **7e** or **8e** with CH_2N_2 gives the respective methyl esters **7f** and **8f**. Thus, these reactions provide not only the parent L-cysteinyl diastereomers, but the complementary set of amide- and ester-blocked C-7 L-cysteinyl-7-deoxydaunomycinone derivatives.

In order to determine the possible generality of thiolate addition to this quinone methide, an alternative nucleophile to L-cysteine was desired. Given that a C-7 glycoside is present in daunomycin and that synthetic manipulation of this glycoside is perhaps the most promising approach to the development of more effective anthracyclines,[1b,12-15] a 1-thio derivative appeared a reasonable choice. The particular selection of 1-thio-β-D-glucose[16] is made with consideration to its commercial availability. At 20 mM concentration, under conditions identical with those found successful for *N*-acetyl-L-cysteine, no adducts are formed with this nucleophile. When however the pH of the reaction is increased to pH 8.1 from pH 7.0, the trapping of the 7-deoxydaunomycinone quinone methide proceeds well and two diastereomers, present in a ratio of 4 to 1, are formed in a combined yield of 65%. This observation suggests that under autocatalytic conditions formation of stable C-7 adducts from thiolate nucleophiles may be a general occurrence.

The assignment of constitution and configuration of the two products as **9** and **10** follows from the spectroscopic



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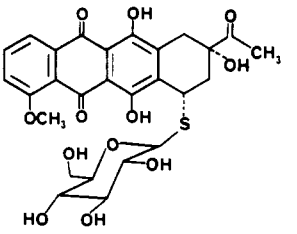

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data. In particular, the ^1H NMR spectrum of the major adduct **10** shows an AA'X pattern for the C-7 and C-8 hydrogens having the average of $(^3J_{7,8(ax)} + {}^3J_{7,8(eq)})$ equal to 3.6 Hz, suggesting a pseudoaxial C-7 substituent cis to the C-9 hydroxyl. The certainty of this 7*S* assignment is established from the CD difference spectrum, which likewise has the positive band near 365 nm. A β-linkage at the glycosidic carbon of this adduct is demanded by the appearance of its hydrogen as a doublet at δ4.83, having $J_{1',2'}$ = 9.7 Hz. Likewise the assignment of the minor isomer as the C-7 epimer **9** follows from its own spectroscopic data ($J_{1',2'}$ = 9.5; $J_{7,8(ax)}$ = 6.9 Hz, $J_{7,8(eq)}$ = 3.6 Hz; difference CD, positive band at 415 nm and negative at 360 nm). Once again, the minor adduct is that which derives from *re* approach to the quinone methide.[6]

Given the ability to generate the 7-deoxydaunomycinone quinone methide and to observe its trapping by thiolate nucleophiles under autocatalytic conditions, a reasonable inquiry is whether this procedure may be used to assess the competence of other possible nucleophiles. Unfortunately, the answer is equivocal. In the presence of *N*-acetylhistidine, histidine, NC^-, N_3^-, I^-, 5,5-dimethyl-1,3-cyclohexanedione, 5'-GMP, and pyridine aldoxime (all at 20 mM) and substoichiometric quantities of NADPH, quinone methide generation proceeds but fails to produce detectable quantities of adduct. Rather, 1 equiv of 7-deoxydaunomycinone is obtained for each 1 equiv of NADPH present. Hence, under these conditions only thiolates are competent nucleophiles.

An area of anthracycline chemistry that is growing in its importance is the role of metal chelation in modulating the anthracycline redox activity,[17] particularly with regard to lipid peroxidation in cardiac tissue.[18] The best studied anthracycline–metal chelate is that of Fe(III), where complex formation requires three molecules of the anthracycline and involves the C-11 and C-12 oxygens as chelating atoms.[17] This neutral, and presumed octahedral, complex has a characteristic dark purple color. Its redox chemistry is being examined in some detail, and it is now known that mild reducing agents (such as sulfhydryls) will pass electrons through the metal to O_2, thus initiating peroxidation pathways.[17,18] Quinone methide formation from the chelate is not previously described. It seemed reasonable however to believe that in the presence of a suitably strong reducing

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agent (such as ferredoxin reductase) electron input into the anthracycline followed by glycosidic elimination to the quinone methide might occur and that *if* this quinone methide retained metal chelation, its electrophilic potency might be enhanced. Accordingly the competence of the Fe(III)–daunomycin chelate was examined as a *quinone* oxidant, by examining the anaerobic conversion of this compex, with excess NADPH and ferredoxin reductase as a catalyst, to 7-deoxydaunomycinone. Under this circumstance reductive deglycosylation proceeds smoothly, leading to the eventual deposition of a precipitate retaining the dark purple color of the chelate. Exposure of this suspension to the atmosphere, and addition of either acid (to approximately pH 4) or excess EDTA, permits 7-deoxydaunomycinone to be isolated in quantitative yield. Although more detailed kinetic study is necessary to determine the sequence of the redox transformations in this reaction, the ultimate product is unquestionably a 7-deoxydaunomycinone–iron chelate. Further redox transformations under these conditions of this chelate are precluded by its aqueous insolubility.

Nevertheless, the above transformation offers presumptive evidence that quinone methide formation has proceeded from the metal chelate. Accordingly, the outcome of this reaction was examined with substoichiometric NADPH (0.5 equiv relative to daunomycin) and *N*-acetyl-L-cysteine (20 mM) as a nucleophile. At the conclusion of the reaction, the solution retains the dark purple color of the chelate, but upon acidification and $CHCl_3$ extraction the orange color of the free anthracycline appears within the organic layer. Chromatographic analysis indicates the presence of both *N*-acetyl-L-cysteinyl adducts **7a** and **8a**, as well as substantial quantities of 7-deoxydaunomycinone. Further examination indicates the optimal stoichiometry for NADPH to be 1.0 equiv, giving a combined yield for the two diastereomers of 70% and a ratio of the 7*S* to 7*R* diastereomer of 1.1 to 1.0. The analogous experiment with thioglucose likewise shows adduct formation from the chelate, although with a poor overall yield (30%) and with a substantial change in stereoselectivity (ratio of **9** to **10** of 1 to 9), that is opposite to the change seen with *N*-acetylcysteine. It does not appear that the stereoselectivity of nucleophile addition to the chelate will be predictable. These observations indicate that the iron chelation preserves the fundamental elements of the autocatalytic nucleophile trapping. An explanation for the increased quantity of NADPH necessary must await a detailed examination of the kinetics and intermediates of this pathway; it is probable however that preferential reduction of the metal relative to the quinone, or nonenzymic NADPH oxidation, may account for part of this quantity. To our disappointment, examination of the chelate's behavior with N_3^- and *N*-acetyl-L-histidine as alternate nucleophiles shows no evidence for adduct formation; only 7-deoxydaunomycinone is obtained as a product. L-Cysteine—a poor nucleophile in the absence of the metal—cannot be assessed as a nucleophile in its presence as it rapidly dechelates the complex, as evidenced by the prompt restoration of the orange color of daunomycin upon its addition to the chelate. In brief, the iron–daunomycin chelate does not appear to provide an increased electrophilicity to the quinone methide derived from it. The yield of the thiol adducts is essentially unchanged, and nucleophiles that are unreactive in the absence of the metal remain so in its presence.

An evaluation of the biological activity of the new compounds was undertaken. Using an in vitro P_{388} assay with daunomycin as a standard, no activity is observed for the (7*R*)- and (7*S*)-cysteinyl derivatives **7a**, **7e**, **7f** and **8a**, **8e**, **8f** nor for the (7*S*)-7-*S*-(β-D-glucopyranosyl)-7-thio-7-deoxydaunomycinone. (The highest concentration examined is 10 μM.) To our surprise, the 7*R* epimer **9** of this latter compound does possess activity, having a 50% inhibitory concentration that is approximately 25 times larger than that of daunomycin (duplicate determinations). It is unlikely that this activity arises from contamination of **9** by daunomycin. The ^1H NMR spectrum shows no indication of anthracycline contamination, with the strongly deshielded region for the hydrogen-bonded hydroxyls possessing only the two resonances anticipated for **9**. More telling is the presence of a single peak in the liquid chromatographic analysis. Under the conditions used for the purification of (7*R*)-1-thio-β-D-glucosyl-7-deoxydaunomycinone, it elutes with a retention time of 14.5 min. Its epimer **10** elutes with a retention time of 15.7 min, while daunomycin elutes much later at 38.5 min. Given the sensitivity of the chromatographic analysis a 4% contamination by daunomycin is detectable. The conclusion is made that the observed activity—although weak—is properly attributable to (7*R*)-1-thio-β-D-glucosyl-7-deoxydaunomycinone.

Discussion

Although the precise biological locus for the expression of the antineoplastic activity of the anthracyclines remains unknown, an attractive hypothesis (for at least some of the biological activity) is metabolic activation to the quinone methide, as a potentially reactive intermediate. As formulated by Moore,[3] the quinone methide is seen as an unstable alkylating agent capable of covalent modification of critical cellular loci. While subsequent experimentation supports quinone methide like intermediates in the biological activity of other antitumor antibiotics—notably with the mitomycin c[19] and benzyl-substituted quinones[20] (where the quinone methide is obtained by reductive metabolism) and with the ellipticines[21] (where it is obtained oxidatively)—little evidence has been forthcoming for the anthracyclines. When previous data are compared with that from the present study, the basic reactivity of both the 11-unsubstituted and 11-hydroxyanthracyclinone quinone methides is however established. In both cases the quinone methide is surprisingly stable and possesses modest *bimolecular* reactivity toward both electrophiles and nucleophiles. Indeed, the 7-deoxydaunomycinone quinone methide appears limited to thiolate nucleophile trapping under these autocatalytic conditions. In light of these facts the potential competence of this quinone methide as an in vivo alkylating agent may be called into question.

A careful consideration of this point does not lead to a definite answer. Although several studies have proven consistent with covalent bond formation between reduc-

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tively activated anthracylines and both DNA and protein,[22] none satisfy even the most rudimentary requirements for proof of covalent structure. Thus, observation as yet fails to answer, and interpretation likewise is found wanting for the following reasons. Given the extraordinary potency of the anthracyclines, it is reasonable to presume that they have a specific in vivo target. Should reductive conversion to the quinone methide occur from the complex of the anthracycline and its target, any suitably positioned functional group will have an advantage with respect to covalent bond formation. Since this reaction occurs from within the complex, it may be quite facile, and certainly its reactivity is not constrained by whatever limitations exist for bimolecular nucleophilic trapping. Indeed it is possible to argue that a low bimolecular reactivity is a desirable property for a potent antibiotic. Many of the coenzymes (such as glutathione and NADH) retain low bimolecular activity to minimize undesirable chemical reactivity while in transit within the cell but express strong chemical reactivity within the proper confines of the appropriate enzyme. By this circumstance specificity is achieved. Likewise, the anthracyclines may be specifically reactive only at the susceptible locus. Without knowledge of the biological locus for anthracycline activity, and the assurance that redox chemistry transpires at this locus, no chemical study may pass judgement on the mechanism of the in vivo activity of these antibiotics.

Likewise with respect to the chemistry observed in this study, the one conclusion concerning the electrophilic character of the 7-deoxydaunomycinone quinone methide is that it is likely to be generally susceptible to thiolate nucleophile addition and subsequent stabilization by comproportionation. The failure of other nucleophiles to react is disappointing, but it must be remembered that the reaction conditions for the isolation of a stable adduct are demanding. Nucleophile addition must be competitive with solvent protonation of the quinone methide (k = 0.046 s^{-1}, pH 8)[4b] *and* quinone methide re-formation must be slower than bimolecular comproportionation. Thiolate nucleophiles themselves barely qualify. Given the optimal NADPH stoichiometry of 0.4 equiv and the final product distribution (65% adducts and 35% 7-deoxydaunomycinone) the average length of the autocatalytic chain is only three. Under these constraints it is easily imagined that small differences in bimolecular reactivity may have a much larger effect on the overall adduct yield. For example, the most probable explanation for the failure to obtain xanthate adducts is not that the xanthate fails to add to the quinone methide but that it is too easily lost as a leaving group in the re-formation of the quinone methide. If circumstances allowing a more facile oxidation of the initial hydroquinone adduct might be devised, an entirely new spectrum of quinone methide reactivity might appear.

At the very least, the elaboration of daunomycin to C-7 thiol-functionalized derivatives is suggested to be a straightforward process. It is probable that reaction circumstances exist that allow this reaction to proceed for daunomycinone as well, in addition to daunomycin congeners (carminomycin, adriamycin, etc.). At present the limitations of this procedure are its poor stereoselectivity and consequent requirement for a chromatographic separation of diastereomers. The value of these derivatives is an open question. Thiol-substituted anthracyclines are uncommon, limited to a weakly active 7-(methylthio)-7-deoxynogarol derivative prepared by Wiley and colleagues[10a,23b] by CF_3CO_2H-dependent exchange of the C-7 glycoside and to the inactive 7-[(2-aminoethyl)thio]-7-deoxydaunomycinone prepared by Acton and colleagues[23a] under very similar conditions. In this latter case the 7*S* to 7*R* stereoselectivity of 2.5 to 1 and overall yield (77%) are comparable to the quinone methide trapping procedure. Of the present series of compounds prepared, the one of foremost interest is the (7*R*)-thioglucosyl derivative **9** by virtue of its biological activity. Anthracyclines having a neutral C-7 glycoside that retain biological activity are also uncommon, limited to the nogalamycin family (although of course an amino glycoside is present in the D ring of these) and to the *arabino*- and *lyxo*-7-*O*-(2,6-dideoxy-β-L-hexopyranosyl)adriamycinones recently prepared by Horton and colleagues.[15] These latter two compounds are however L-glycosides having a 7*S* configuration, as has daunomycin itself. There are several reports of (7*S*)-D-glycosides and (7*R*)-L-glycosides (all without activity), but to our knowledge **9** is the first reported (7*R*)-D-glycoside daunomycin derivative. Whether its rather modest biological activity is exceptional, or identifies a new approach in anthracycline design, must await further evaluation.

Last and perhaps in some senses most importantly, the electrophilic capability of the 7-deoxydaunomycinone quinone methide has been proven. While this knowledge alone does not directly bear on the biological activity of these compounds, it may prove of value toward the understanding of the biological properties of this antibiotic class.

Experimental Section

Materials. *N*-Acetyl-L-cysteine and 1-thio-β-D-glucose, sodium salt, are obtained from Sigma and used as received. *N,N*-Bis(*tert*-butoxycarbonyl)cystine is reduced with tributylphosphine[24] to provide *N*-(*tert*-butoxycarbonyl)cysteine. Daunomycin is the generous gift of the Drug Synthesis and Chemistry Branch of the National Cancer Institute. Ferredoxin reductase is isolated from spinach and assayed with NADPH and ferricyanide anion, as previously described.[4b]

Instrumentation. Infrared spectra are obtained on a Beckman 4240 spectrometer. The NMR spectra are obtained on a Nicolet NT300 spectrometer, using the solvent as the internal standard referenced to $Si(CH_3)_4$. All coupling constants (Hz) given are those observed (uncorrected for non-first-order behavior). Fast atom bombardment mass spectra are obtained on a Kratos MS 50 spectrometer, at the Midwest Center for Mass Spectrometry, University of Nebraska. The CD spectra are obtained on a Jasco 41C spectropolarimeter equipped with a data processor, scanning from 550 to 325 nm. All CD spectra are of 0.12 mM solutions in CH_3OH, in a 2.0-cm path-length quartz cell, with the final spectrum representing an average of four successive scans. Difference CD spectra are obtained by substracting the absolute spectrum of 7-deoxydaunomycinone from that of the sample. The molar ellipticities (deg M^{-1} cm^{-1}) are given by $[\theta] = 100\theta/cl$).

Methods. The testing for cell inhibitory activity is done by serial dilution of the samples into tubes inoculated with 240,000 P_{388} mouse lymphoid leukemia cells, in a solution consisting of 4 mL of Fischer's medium and 10% horse serum.[25] The tubes are plugged with silicone stoppers and incubated, without agitation, at a 30° angle and at 37 °C. After 72 h the cell count is determined by a hemocytometer, and the relative inhibition is calculated with reference to controls. In this assay daunomycin

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inhibits cell growth by 50% at a concentration of 20 nM.

7-(*N*-Acetyl-L-cysteinyl)-7-deoxydaunomycinones 7a and 8a. Daunomycin hydrochloride (15.0 mg, 28.3 μmol) is dissolved in 37 mL of 8 mM sodium phosphate pH 7.0 buffer, containing 0.5 mM EDTA. To this solution are added 9.5 mg of NADPH (11.6 μmol, equal to 0.4 mol equiv) and 368 mg of *N*-acetyl-L-cysteine (2.07 mmol, for a final concentration of 20 mM), and the total volume is made to 100 mL with the buffer. The solution is made anaerobic by repeated evacuation and flushing with N_2. Ferredoxin reductase (0.33 nmol, total activity of 2.3 μmol of NADPH oxidized/min under the standard assay conditions) is then added to initiate the reaction. After 6 h at ambient temperature, the reaction is exposed to oxygen and extracted with $CHCl_3$ (2 × 75 mL) to remove 7-deoxydaunomycinone and traces of unreacted daunomycin. The aqueous layer is acidified with dilute HCl to pH 3.5 and further extracted with $CHCl_3$ (3 × 50 mL). The solution is dried (Na_2SO_4) and the $CHCl_3$ evaporated to provide 9.8 mg (65%) of the diastereomeric adducts **7a** and **8a**, in a ratio of 2.6 to 1. These are separated by reversed-phase chromatography on a 2.1 × 25 cm Zorbax C_{18} column, using a mobile phase of 3/1 CH_3CN/H_2O containing 0.2% (w/v) CF_3CO_2H, at a flow rate of 3.75 mL min^{-1}.

The individual diastereomers are isolated from the respective fractions by concentration on the rotary evaporator, to remove most of the CH_3CN, followed by partitioning between water and chloroform. The resulting $CHCl_3$ solution is dried and evaporated and the residue esterified with CH_2N_2. The methyl esters are obtained in quantitative yield. Chromatographic analysis of the samples submitted for high-resolution mass spectral confirmation of structure indicates that each has a minimum purity of 95% and is free to within limits of detection (approximately 1%) of the other diastereomer.

(7*R*)-(*N*-Acetyl-L-cysteinyl)-7-deoxydaunomycinone Methyl Ester (7b). This is obtained by diazomethane reaction of the isolated diastereomer. ^1H NMR ($CDCl_3$, 300 MHz): δ 14.15 (s, 1 H, 6-OH), 13.28 (s, 1 H, 11-OH), 8.00 (d, 1 H, 3-H, *J* = 7.7), 7.77 (app t, 1 H, 2-H, *J* = 8.1), 7.38 (d, 1 H, 1-H, *J* = 8.5), 6.90 (d, 1 H, NH, *J* = 8.2), 4.96 (m, 1 H, C*H*(CO_2R)NHAc), 4.63 (dd, 1 H, 7-H, *J* = 7.3, 4.3), 4.09 (s, 3 H, 4-OCH_3), 3.72 (s, 3 H, CO_2CH_3), 3.29 (dd, 1 H, SCH_2, *J* = 9.8, 4.5), 3.17 (d, 1 H, 10-H_{ax}, *J* = 16.8), 3.10 (dd, 1 H, 10-H_{eq}, *J* = 16.8, 0.7), 3.04 (dd, 1 H, SCH_2, *J* = 9.8, 4.5), 2.55 (dd, 1 H, 8-H_{ax}, *J* = 14.9, 4.3), 2.44 (s, 3 H, $NHCOCH_3$), 2.34 (dd, 1 H, 8-H_{eq}, *J* = 14.9, 6.9 with broadening due to *W* coupling with 10-H_{eq}), 2.09 (s, 3 H, 14-CH_3). MS: (FAB, dithiothreitol/dithioerythritol matrix, low resolution, positive ion) *m/z* (%) 558 (M + H, 6), 382 (MH^+ - $SCH_2CH(CO_2CH_3)NHCOCH_3$, 18), 363 (28), 339 (13), 337 (12), 321 (aglycon - $COCH_3$ - H_2O, 100), 306 (7), 178 ($HSCH_2CH(CO_2CH_3)NHCOCH_3$ + H, 6), 135 (22); (FAB, high resolution) 558.1434 (M + H; calcd for $C_{27}H_{28}NO_{10}S$ 558.1433). CD: 435 ([θ] = −13 300), 365 nm (4650); (difference CD) 415 (8640), 355 nm (−9630).

(7*S*)-(*N*-Acetyl-L-cysteinyl)-7-deoxydaunomycinone Methyl Ester (8b). 1-Methyl-3-nitro-1-nitrosoguanidine (66 mg, 0.5 mmol) and H_2O (0.5 mL) are added to the inner tube of a diazomethane apparatus, and 7*S*-(*N*-acetyl-L-cysteinyl)-7-deoxydaunomycinone (15 mg, 0.028 mmol) is added as a suspension in 5 mL of 4/1 $CHCl_3/Et_2O$ to the outer tube. The entire apparatus is briefly warmed in a 60 °C water bath to expel the air, sealed with a septum, and cooled to 0 °C. An NaOH solution (10%, 0.5 mL) is added dropwise through the septum, and afterward the reaction is kept at 0 °C for 15 min and ambient temperature for 2 h. The solvent is evaporated and the residue dissolved in $CHCl_3$. This solution is extracted with dilute phosphate pH 7 buffer and dried and the solvent evaporated to provide a quantitative yield of the ester. ^1H NMR: ($CDCl_3$, 300 Mz) δ 14.12 (s, 1 H, 6-OH), 13.22 (s, 1 H, 11-OH), 8.00 (d, 1 H, 3-H, *J* = 7.8), 7.76 (app t, 1 H, 2-H, *J* = 8.0), 7.37 (d, 1 H, 1-H, *J* = 8.3), 6.55 (d, 1 H, NH, *J* = 7.6), 4.94 (m, 1 H, C*H*-(CO_2CH_3)NHAc), 4.49 (1/2/1 t of AA′*X*, 1 H, 7-H, $(J_{AX} + J_{A'X})/2$ = 3.1), 4.07 (s, 3 H, 4-OCH_3), 3.80 (s, 3 H, CO_2CH_3), 3.41 (dd, 1 H, SCH_2, *J* = 13.9, 4.7), 3.25 (dd, 1 H, SCH_2, *J* = 13.9, 6.2), 3.14 (d, 1 H, 10-H, *J* = 18.8), 2.98 (d, 1 H, 10-H, *J* = 18.8), 2.38 (s, 3 H, 14-CH_3), 2.38 (m, 2 H, 8-CH_2, unresolved under the CH_3 s), 2.08 (s, 3 H, $NHCOCH_3$); (C_6D_6, 300 MHz) inter alia δ 4.35 (d, 1 H, 7-H, *J* = 4.5), 2.28 (d, 1 H, 8-H_{eq}, *J* = 14.8), 2.05 (dd, 1 H, 8-H_{ax}, *J* = 14.8, 5.2). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 211.6 (C-13), 187.0 and 186.4 (C-5, C-12), 171.3 (CO_2CH_3), 170.2 ($NHCOCH_3$), 161.0 (C-4), 155.6 (C-6, C-11), 136.1, 135.7, and 135.5 (C-12a, C-1a, C-6a), 133.9 (C-2), 120.8 (C-4a), 119.7 (C-1), 118.3 (C-3), 111.2 and 110.5 (C-5a, C-11a), 76.7 (C-9), 56.7 (4-OCH_3), 53.0 (C-7), 51.8 (CO_2CH_3), 37.2 (*C*H(CO_2CH_3)NHAc), 36.2 (SCH_2), 34.8 and 32.7 (C-10, C-8), 24.6 (C-14), 23.3 ($NHCOCH_3$). MS: (FAB, dithiothreitol/dithioerythritol matrix, low resolution, positive ion) *m/z* (rel intens) 558 (M + H, 78), 539 (M - H_2O, 6), 514 (M - $COCH_3$, 16), 382 (M + H - $SCH_2CH(CO_2CH_3)NHAc$, 18), 363 (29), 339 (17), 337 (18), 321 (aglycon - $COCH_3$ - H_2O, 100), 306 (10), 178 (*N*-Ac-Cys + H, 6), 135 (19); (FAB, high resolution) *m/z* 558.1434 (M + H; calcd for $C_{27}H_{28}NO_{10}S$ 558.1434). CD: 425 ([θ] = 5250), 365 nm (−11 630); (difference CD) 415 (−4000), 365 nm (8650).

7-[*N*-(*tert*-Butoxycarbonyl)-L-cysteinyl]-7-deoxydaunomycinones 7c and 8c. These are obtained from the 7-deoxydaunomycinone quinone methide by a procedure identical with that described for *N*-acetyl-L-cysteine as a nucleophile. The products are also isolated in a similar fashion, with the exception that a cold 5% citric acid solution is used for the acidification. Separation of the diastereomers is accomplished by reversed-phase liquid chromatography on a 2.1 × 25 cm Zorbax C_{18} column, using a mobile phase of 3/1 CH_3CN/H_2O containing 0.2% (w/v) CF_3CO_2H, at a flow rate of 3.75 mL min^{-1}. The retention time of the minor 7*R* isomer is 28.6 min, and that of the major 7*S* isomer is 35.4 min; these are present in a ratio of 1 to 4, respectively. The individual fractions are concentrated on the rotary evaporator, and the residue is diluted with water and extracted with $CHCl_3$. The organic solvent is dried and then evaporated to provide the products, which are characterized as the methyl esters. Chromatographic analysis of the samples submitted for high-resolution mass spectral confirmation of structure indicates that each has a minimum purity of 90% and is free to within limits of detection (approximately 1%) of the other diastereomer.

(7*R*)-[*N*-(*tert*-Butoxycarbonyl)-L-cysteinyl]-7-deoxydaunomycinone Methyl Ester (7d). ^1H NMR ($CDCl_3$, 300 Mz): δ 14.21 (s, 1 H, 6-OH), 13.31 (s, 1 H, 11-OH), 8.03 (d, 1 H, 3-H, *J* = 7.7), 7.78 (app t, 1 H, 2-H, *J* = 8.3), 7.39 (d, 1 H, *J* = 8.5), 5.67 (d, 1 H, NH, *J* = 8.7), 4.67 (dd, 1 H, 7-H, *J* = 7.4, 4.8), 4.59 (m, 1 H, C*H*(CO_2R)NHAc), 4.09 (s, 3 H, 4-OCH_3), 3.73 (s, 3 H, CO_2CH_3), 3.71 (s, 1 H, 9-OH, D_2O exchangeable), 3.27 (dd, 1 H, SCH_2, *J* = 14.1, 5.3), 3.14 (s, 2 H, fortuitous equivalence of 10-CH_2 in both $CDCl_3$ and C_6D_6), 3.06 (dd, 1 H, SCH_2, *J* = 14.1, 4.7), 2.52 (dd, 1 H, 8-H_{eq}, *J* = 14.7, 4.4), 2.45 (s, 3 H, 14-CH_3), 2.33 (dd, 1 H, 8-H_{ax}, *J* = 15.0, 7.5), 1.45 (s, 9 H, $C(CH_3)_3$). MS: (FAB, positive ion, dithiothreitol/dithioerythritol matrix) *m/z* (rel intens) 516 (MH_2^+ - $(CH_3)_3COCO$, 78), 382 (16), 363 (28) 337 (20), 321 (100), 152 (25), 135 (58); (FAB, high resolution) *m/z* 516.1311 (MH_2^+ - $(CH_3)_3COCO$; calcd for $C_{25}H_{26}NO_9S$ 516.1327). CD: 425 ([θ] = −4480) 365 nm (11 760); (difference CD) 415 (4660), 360 nm (−11 200).

(7*S*)-[*N*-(*tert*-Butoxycarbonyl)-L-cysteinyl]-7-deoxydaunomycinone Methyl Ester (8d). IR (KBr, cm^{-1}): 1725, 1630, 1600, 1295, 1220 cm^{-1}. ^1H NMR: ($CDCl_3$, 300 MHz) δ 14.17 (s, 1 H, 6-OH), 13.34 (s, 1 H, 11-OH), 8.02 (dd, 1 H, 3-H, *J* = 8.6, 0.9), 7.77 (app t, 1 H, 2-H, *J* = 8.3), 7.38 (dd, 1 H, 1-H, *J* = 8.5, 0.7), 5.46 (d, 1 H, NH, *J* = 8.2), 4.67 (m, 1 H, C*H*(CO_2R)NHAc), 4.54 (d, 1 H, 7-H, *J* = 3.6), 4.33 (s, 1 H, 10-OH, D_2O exchangeable), 4.08 (s, 3 H, 4-OCH_3), 3.79 (s, 3 H, CO_2CH_3), 3.42 (dd, 1 H, SCH_2, *J* = 14.1, 4.5), 3.16 (dd, 1 H, SCH_2, only partly resolved from 10-H resonance), 3.16 (d, 1 H, 10-H, *J* = 19.7), 3.02 (d, 1 H, 10-H, *J* = 18.9), 2.40 (m, 2 H, 8-CH_2), 2.39 (s, 3 H, 14-CH_3), 1.46 (s, 9 H, $C(CH_3)_3$); (C_6D_6, 300 MHz) inter alia δ 4.35 (d, 1 H, 7-H, *J* = 4.8), 2.30 (d, 1 H, 8-H_{eq}, *J* = 15.3), 2.05 (dd, 1 H, 8-H_{ax}, *J* = 14.6, 5.0). MS: (FAB, positive ion, dithiothreitol/dithioerythritol matrix) *m/z* (rel intens) 516 (MH_2^+ - $(CH_3)_3COCO$, 18), 382 (10), 363 (25), 337 (20), 321 (aglycon - $COCH_3$ - H_2O, 100), 135 (4); (FAB, high resolution) *m/z* 516.1327 (MH_2^+ - $(CH_3)_3COCO$; calcd for $C_{25}H_{26}NO_9S$ 516.1327). CD: 415 ([θ] = 2800), 365 nm (−6340); (difference CD) 415 (−6530), 365 nm (12 500).

L-Cysteinyl-7-deoxydaunomycinones 7e and 8e. Removal of the *N*-(*tert*-butoxycarbonyl)amine protecting group is easily accomplished; a representative procedure is given. A mixture of the 7-[*N*-(*tert*-butoxycarbonyl)amino]-7-deoxydaunomycinones (10 mg) is dissolved in 10 mL of CH_3CN/H_2O (3/1) containing 4% (w/v) trifluoroacetic acid. After 30 min at ambient temperature, the solvents are evaporated, providing the L-cystei-

nyl-7-deoxydaunomycinone diastereomers in quantitative yield. These are separated by liquid chromatography (2.1 × 25 cm Zorbax C_{18} using a CH_3CN/H_2O (3/1) mobile phase containing 0.2% (w/v) CF_3CO_2H at a flow rate of 3.75 mL min^{-1}). The 7*S* isomer has a retention time of 22.3 min and the 7*R* a retention time of 24.9 min. The separate fractions are concentrated on the rotary evaporator to an oil, and the remaining solvent is removed by successive evaporation with anhydrous CH_3CN. The resulting powder is dried (at 65 °C, 0.1 mm vacuum in the drying pistol) to provide the isolated products. These are characterized by successive *N*-acetylation and esterification with CH_2N_2, followed by direct comparison with the authentic materials described above. Chromatographic analysis of **8e** and **8f** indicates a purity of at least 90% and the presence of no more than 5% of the minor diastereomer.

(7*S*)-L-Cysteinyl-7-deoxydaunomycinone (8e). MS: (FAB, positive ion, dithiothreitol/dithioerythritol matrix) *m/z* (rel intens) 502 (M + H^+, 18), 484 (14), 466 (21), 433 (23), 401 (38), 382 (8), 363 (24), 337 (18), 321 (70) 122 ($HSCH_2CH(NH_2)CO_2H$, 100); (FAB, high resolution) 502.1162 (M + H^+; calcd for $C_{24}H_{24}NO_9S$ 502.1172).

(7*S*)-L-Cysteinyl-7-deoxydaunomycinone Methyl Ester (8f). MS: FAB, positive ion, dithiothreitol/dithioerythritol matrix) *m/z* (rel intens) 516 (M + H^+, 100), 455 (14), 431 (31), 411 (26), 382 (3), 363 (8), 321 (26); (FAB, high resolution) 516.1335 (M + H^+; calcd for $C_{25}H_{26}NO_9S$ 516.1328).

N-Acetylation of the L-Cysteinyl-7-deoxydaunomycinones. A mixture of the L-cysteinyl-7-deoxydaunomycinones is dissolved in pyridine (0.1 mL) and acetic anhydride (0.2 mL). After 2 min, water (10 mL) is added and the reaction allowed to stand for 30 min. At this time the solution is adjusted to pH 3.5 and extracted with $CHCl_3$. The solution is dried and the solvent evaporated. Chromatographic analysis indicates that the yield of *N*-acetylation is 70% and that the two adducts coelute with the authentic material prepared directly from daunomycin and *N*-acetyl-L-cysteine.

(7*S*)-(β-D-Glucopyranosyl)-7-thio-7-deoxydaunomycinones 9 and 10. Daunomycin hydrochloride (35 mg, 66 μmol) is dissolved in 50 mL of Tris-HCl pH 8.0 buffer. A solution of the sodium salt of the 1-thio-β-D-glucose in 20 mL of water is adjusted to pH 8.1 with HCl and added to the daunomycin solution. NADPH (9.2 mg, 0.15 mol equiv) is added, and the reaction is made anaerobic. Ferredoxin reductase (4.2 μmol NADPH oxidized min^{-1}) is added to initiate the reaction, and after 6 h the reaction is exposed to air and extracted with $CHCl_3$ (2 × 50 mL) to remove 7-deoxydaunomycinone. The aqueous solution is then passed through a short 1.5 × 2.0 cm 40-μm C_{18} silica reversed-phase column to absorb the thioglucose adducts. The column is thoroughly washed with water, and the adducts are removed with CH_3CN/H_2O (3/1) containing 0.2% (w/v) CF_3CO_2H. Final purification is accomplished by preparative reversed-phase liquid chromatography. Chromatographic analysis of the samples submitted for high-resolution mass spectral confirmation of structure, and for biological testing, indicates that each has a minimum purity of 95% and is free within limits of detection (approximately 1%) of the other diastereomer.

(7*R*,9*S*)-9-Acetyl-7-[(β-D-glucopyranosyl)thio]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (9). ^1H NMR (300 MHz, $(CD_3)_2SO$, D_2O exchanged): δ 7.89 (m, 2 H, 2-H and 3-H), 7.63 (dd, 1 H, 1-H, *J* = 5.9, 3.9), 4.73 (dd, 1 H, 7-H, *J* = 6.9, 3.6), 4.43 (d, 1 H, 1′-H, *J* = 9.5), 3.98 (s, 3 H, 4-OCH_3), 3.46 (s, 2 H, 10-CH_2), 3.6 (dd, 1 H, 6′-H, *J* = 11.1, 2.2), 3.35 (dd, 1 H, 6′-H, *J* = 11.7, 3.6), 3.06 (complex m, 4 H, 2′-H, 3′-H, 4′-H, 5′-H), 2.58 (dd, 1 H, H-8_{eq}, *J* = 14.8, 3.8), 2.29 (s, 3 H, 14-CH_3), 2.27 (m, 1 H, H-8_{ax}, poorly resolved underneath the methyl resonance). MS: (FAB, positive ion, dithiothreitol/dithioerythritol matrix) *m/z* (rel intens) 599 (M + Na^+, 13), 501 (7), 481 (8), 461 (15), 443 (12), 427 (8), 403 (8), 329 (16), 313 (8), 193 (12); (FAB, high resolution) *m/z* 599.1169 (M + Na^+; calcd for $C_{27}H_{28}NaO_{12}S$ 599.1199). CD: 410 ([θ] = −1860), 360 nm (8400); (difference CD) 415 (2240), 360 nm (−8400).

(7*S*,9*S*)-9-Acetyl-7-[(β-D-glucopyranosyl)thio]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (10). ^1H NMR (300 MHz, $(CD_3)_2SO$): δ 14.14 (s, 1 H, 6-OH), 13.03 (s, 1 H, 11-OH), 7.85 (m, 2 H, 2-H and 3-H), 7.61 (dd, 1 H, 1-H, *J* = 5.2, 2.2), 5.67 (br s, 1 H, 9-OH), 4.83 (d, 1 H, 1′-H, *J* = 9.7), 4.64 (1/2/1 t of AA′*X*, 1 H, 7-H, $(J_{AX} + J_{A'X})/2$ = 4.6), 3.96 (s, 3 H, 4-OCH_3), 3.72 (dd, 1 H, 6′-H, *J* = 11.8, 1.2), 3.47 (dd, 1 H, 6′-H, *J* = 11.8, 5.8), 3.14 (m, 3 H, 3′-H, 4′-H, 5′-H), 2.94 (dd, 1 H, 2′-H, *J* = 9.7, 8.4), 2.92 (d, 1 H, 10-H, *J* = 18.1), 2.85 (d, 1 H, 10-H, *J* = 18.1), 2.28 (app d, Δν = 4.5 Hz, two strong lines of *AA′X*, 2 H, 8-H; collapses to a s upon irradiation of the 7-H). ^{13}C NMR ($(CD_3)_2SO$): δ 212.56 (C-13), 186.18 and 186.01 (C-5, C-12), 160.7 (C-4), 155.2 and 154.7 (C-6, C-11), 139.7, 136.1, and 134.7 (C-12a, C-10a, and C-6a), 133.1 (C-2), 119.9, 119.5, and 118.8 (C-4a, C-1, and C-3), 110.5 and 109.5 (C-5a and C-11a), 86.4 (C-1′), 80.9 (C-2′), 78.4 and 75.5 (C-5′ and C-3′), 73.56 (C-9), 70.2 (C-4′), 61.3 (C-6′), 56.6 (4-OCH_3), 37.2 (C-7), 33.6 and 31.7 (C-8 and C-10), 24.5 (C-14). MS: (FAB, positive ion, dithiothreitol/dithioerythritol matrix) *m/x* (rel intens) 577 (M + H^+, 100), 461 (50), 459 (60), 439 (22), 415 (56), 382 (9), 363 (13), 321 (45), 309 (84), 275 (34); (FAB, high resolution) *m/z* 577.1354 (M + H^+; calcd for $C_{27}H_{29}SO_{12}$ 577.1379). CD: 415 ([θ] = 4100), 365 nm (−8960); (difference CD) 415 (−4650), 365 nm (9330).

Preparation of the Fe(III)–Daunomycin Chelate. A procedure similar to that of Myers et al.[17a] is followed. Daunomycin (10.5 mg, 20 μmol) is dissolved in 10 mL of water and the pH adjusted to 3.0 with dilute HCl. Ferric chloride (1.6 mg, 10 μmol) in 10 mL of water, also adjusted to pH 3.0, is added to the above solution. The combined solution is adjusted to pH 7.4 with a small volume of 50 mM NaOH to give a stock solution of the chelate. The buffer used for most reactions of this chelate is 8 mM sodium phosphate pH 7.1 buffer, containing 8 mM NaCl.

Chelate Reduction to the 7-Deoxydaunomycinone Chelate. An anaerobic solution of the chelate (0.33 mM based upon a composition of $Fe(daunomycin)_3$) containing NADPH (2.8 mM) is treated with ferredoxin reductase (3.7 μmol of NADPH oxidized min^{-1} by $Fe(CN)_6^{3-}$ under standard assay conditions) for a period of 4 h. At this time a purple precipitate appeared. To the solution is added excess EDTA and the solution extracted with $CHCl_3$. Spectral and chromatographic analysis of the $CHCl_3$ layer indicates the quantitative presence of 7-deoxydaunomycin.

***N*-Acetylcysteine Interception of the Chelate-Derived Quinone Methide.** These reactions are done on an analytical scale; a representative procedure is given. A 5-mL solution of the iron–daunomycin chelate (0.38 mM), NADPH (1.23 mM), and *N*-acetylcysteine (20 mM) in phosphate buffer is made anaerobic by repeated evacuation and flushing with N_2. Ferredoxin reductase (0.17 μmol of NADPH oxidized min^{-1}) is added to initiate the reaction. After 4 h, the reaction is acidified, extracted with $CHCl_3$, and analyzed by reversed-phase chromatography. This shows the formation of **7a** and **8a** in a relative ratio of 1 to 1.1, in a combined yield of 70%. The conditions for thioglucose reaction with the chelate are similar, but with phosphate pH 7.85 buffer. Liquid chromatographic analysis shows the formation of **9** and **10** in a relative ratio of 1 to 9, with an overall yield of 30%.

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