Probing the Role of Polyphenol Oxidation in Mediating Insect−Pathogen Interactions. Galloyl-Derived Electrophilic Traps for the *Lymantria dispar* Nuclear Polyhedrosis Virus Matrix Protein Polyhedrin

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Galloyl-derived orthoquinone probes have been designed, synthesized, and utilized in an ongoing study of insect−pathogen interactions. A stable galloyl-derived orthoquinone *O*-methyl ether modified with both acidic and fluorescent appendages was successful in trapping the model nucleophile cysteine, a test protein bearing a single cysteine residue, and the viral occlusion body matrix protein polyhedrin from *Lymantria dispar* nuclear polyhedrosis virus (LdNPV), a pathogen of the gypsy moth caterpillar (GMc). This latter observation may be related to the molecular mechanism by which gallotannins decrease LdNPV infectivity in GMc's. Sufficient site isolation was not achieved with a polymer-bound reactive galloyl *hydroxy*orthoquinone electrophile to permit similar nucleophile trapping to compete with oligomerization.

The role of polyphenolic compounds in mediating insect−plant interactions remains an enduring issue in chemical ecology. Numerous studies have demonstrated negative consequences upon polyphenol consumption by insects, including antidigestive/antiassimilatory effects, enzyme inhibition, and feeding deterrence.[1] More recently, a beneficial impact of polyphenol ingestion, stemming from a reduction in pathogen-induced mortality, has been uncovered.[2] This divergence of outcome serves to underscore the complex and multifaceted nature of the molecular-level interactions which might conceivably underlie these phenomena.

Many of the deleterious effects of polyphenol consumption have been attributed to noncovalent protein binding via some favorable combination of hydrogen bonding and hydrophobic association. However, polyphenol prophylaxis in insect−pathogen interactions may arise from alternative modes of chemical interaction. Circumstantial evidence in a few systems implicates covalent bond formation between the phenol ring and a pathogen-derived protein in diminishing mortality. Elegant studies by Felton, Duffey, and co-workers support the hypothesis that the catechol chlorogenic acid, in the presence of the oxidant polyphenol oxidase (PPO), covalently bonds to both the occlusion bodies (OB's) and their constituent protein polyhedrin of the tomato fruitworm virus HzSNPV.[3] The degree of covalent attachment correlates strongly both with a decrease in rate of OB dissolution, as is required for viral infection, and with a decrease in host mortality. The presence of potentially competing foliar proteins did not suppress inhibition of infectivity. Similar observations attend Nakano's work with the catechol caffeic acid and a bacterial pathogen of the silk moth caterpillar.[4] At present, this inferential evidence is consistent with a model in which the covalent modification of a key viral protein(s) with polyphenol thwarts viral infectivity. However, no direct chemical characterization of the molecular-level details of these presumed polyphenol−protein linkages has been forthcoming.

Speculation on the nature of the conjugation process commonly includes combination of a nucleophilic amino acid residue (cysteine, lysine, histidine) with an electrophilic orthoquinone derived from catechol oxidation. Many model studies utilizing simple amino acid derivatives and either in situ-generated or stable orthoquinones undergird this speculation.[5] The case for cysteine is stronger than the nitrogen-based nucleophilic amino acids, but the influence of the protein framework in modulating the reactivity of these nucleophilic species remains unknown.

The dynamics of another pathogen−herbivore system consisting of the gypsy moth caterpillar (GMc) and *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) appears to be mediated by polyphenolic compounds as

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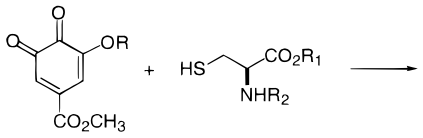
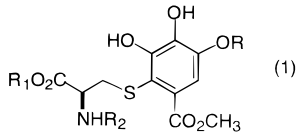
well.[2a,2b,6] Gallotannins (polygalloylated glucose esters) are the presumptive agents which attenuate the severity of LdNPV infection in GMc's. Gallotannins diminish GMc fecundity, but that negative impact is more than compensated by a significantly increased survivorship for GMc's infected with LdNPV.[7] Development of a cellular- or molecular-level rationale for these observations can be guided by the tomato fruitworm study with the similar HzSNPV and by the discovery of GMc midgut conditions (pH = 9−11, redox potential = +47 to +304 mV) conducive to catechol oxidation.[8]

Therefore, it is possible that ingested gallotannins are oxidized to reactive orthoquinones in the GMc midgut and that these electrophiles bind covalently to the LdNPV OB matrix protein polyhedrin (as well as other viral and/or host proteins). This covalent linkage may disrupt any number of recognition/disassembly processes required for viral infection, although lack of any firm evidence on this latter point renders speculation on these downstream events untestable as yet.

A stable galloyl-derived model orthoquinone *O*-methyl ether **1a**, generated by oxidation of methyl 3-*O*-methylgallate, efficiently traps cysteine and protected cysteine derivatives as 1,6 conjugate adducts **3a**/**3b**, eq 1.[5i]



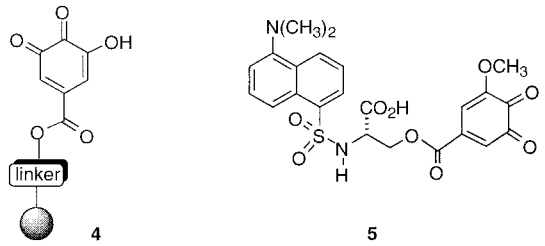
1a R = CH_3 **2a** $R_1 = R_2 = H$
1b R = H **2b** R_1 = Bn, R_2 = CBz



(1)

3a R = CH_3, $R_1 = R_2 = H$
3b R = CH_3, R_1 = Bn, R_2 = CBz

jugate addition of histidine to this electrophile is problematic (~16%), while lysine (or protected versions) is not an effective coupling partner at all. Attempts to prepare and isolate the parent unetherified hydroxyorthoquinone **1b** were frustrated by its ready dimerization (below room temperature) to furnish the dehydrohexahydroxydiphenate substructure characteristic of some ellagitannin natural products.[9] However, this putative oxidation product of methyl gallate could be trapped by thiol nucleophiles under conditions where dimerization is suppressed (vide infra).



4 **5**

Figure 1. Galloyl-derived probe molecules for polyhedrin conjugation.

Acquisition of direct chemical evidence for gallotannin/LdNPV polyhedrin covalent attachment under conditions mimicking the GMc midgut would provide support for this hypothesis. While it is possible that key interactions with other viral (e.g., pp34, p25, pg41) or host proteins play a critical role in reducing infectivity, polyhedrin is a logical first target by analogy to the tomato fruitworm work and by virtue of its overwhelming abundance in the viral package. Two problems attend any attempts to utilize galloyl-derived orthoquinones as protein traps under physiological conditions: (1) The high reactivity of **1b** suggests that it will be difficult to maintain adequate concentrations of a hydroxyorthoquinone during exposure to viral protein, as would be required to obtain sufficient quantities of covalent adduct for characterization. In vivo, this problem may not be so acute, as only a low steady-state concentration of electrophile may be required to disrupt viral chemistry. (2) Reoxidation of the first-formed adduct to furnish a new reactive electrophilic orthoquinone, followed by trapping of this species with a second protein nucleophile,[5a,5b,5l,10] may provide only cross-linked, intractable, uncharacterizable materials. Polyphenol-based cross-linking of biological macromolecules plays a central role in numerous metabolic processes[5a,j,11] and in fact may be important in hampering virion release from the OB's. Nevertheless, these two observations pose great difficulties when the goal is isolation and characterization of galloyl−polyhedrin monoadducts from a physiologically realistic reaction medium.

Two distinct experimental approaches were designed to overcome these problems. The initial approach involved anchoring an unetherified galloyl derivative to a solid support. A reactive hydroxyorthoquinone **4** (Figure 1) derived by oxidation of the triphenolic precursor should not be consumed by dimerization (or polymerization) to the extent that site isolation is achieved. Removal of excess oxidant, application of a basic protein solution to trap the bound electrophile under biologically relevant conditions, and eventual adduct cleavage from the resin

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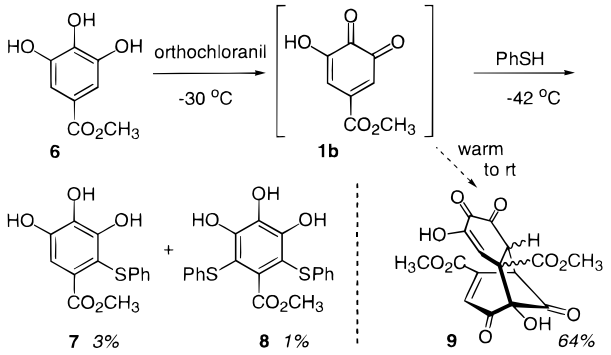
Scheme 1



may provide the desired monoconjugation products. In an alternative strategy, use of a *stable* galloyl-derived orthoquinone monoether **5**, which bears both a base-solubilizing group (CO_2H) and a fluorescent label, may provide access to a polyhedrin monoadduct under conditions similar to those in the GMc midgut. In this instance, adduct overoxidation/cross-linking should be minimized as excess oxidant is not present. The fluorescent tag will facilitate reaction monitoring in these in vitro experiments.

Results and Discussion

Solid Support Studies. The requirement for site isolation of a reactive hydroxyorthoquinone like **1b** was reinforced by the feasibility study shown in Scheme 1. Treatment of methyl gallate (**6**) with orthochloranil at low temperature, followed by cannulation of this hydroxyorthoquinone solution into excess, chilled thiophenol, led to a complex product mixture from which trace but characterizable quantities of the mono- and bis(thiophenol) adducts **7** and **8**, respectively, were isolated. No signals characteristic of the orthoquinone dimer **9** could be identified in the ^{1}H NMR spectrum of the crude product mixture. In fact, the ^{1}H NMR data were not inconsistent with the signature of predominantly oligomeric or polymeric material, perhaps resulting from a nucleophile (PhSH)-initiated oligomerization of the putative intermediate **1b**. Thus, the propensity for both polymolecular condensation and overoxidation (cf. **8**) clearly renders **1b** unsuitable as a solution phase thiol trapping agent in vitro.

The initial attempts to prepare a galloyl hydroxyorthoquinone-capped resin used H_2O-compatible Tentagel-S with a photolabile nitroveratrole linker, Schemes 2 and 3. A preliminary model study (Scheme 2) demonstrated that this photochemical detachment protocol is tolerated by the galloyl moiety. Thus, esterification of the protected gallic acid **11** with the linker unit **12**[12] and desilylation of the derived conjugate **13** afforded the phenolic species **14**. Irradiation of this galloylated linker assembly under standard conditions delivered the gallic acid **10** cleanly and in good yield.

These encouraging results prompted studies with the Tentagel-S system (Scheme 3). Synthesis of the requisite galloyl-functionalized Tentagel resin **21** commenced with the known veratraldehyde derivative **15**[13] and utilized the trisilylated gallic acid **17** as the galloyl donor. Resin loading was accomplished by combining the terminal

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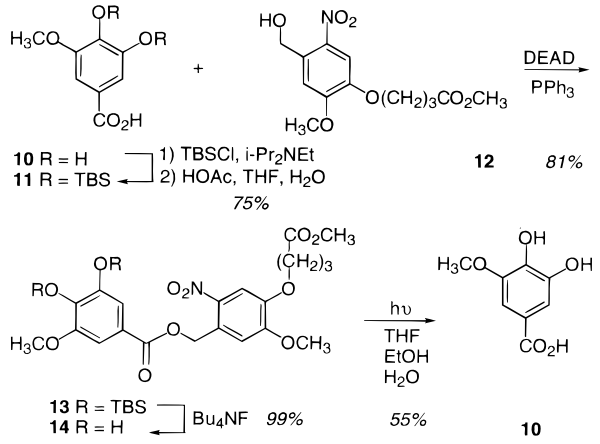
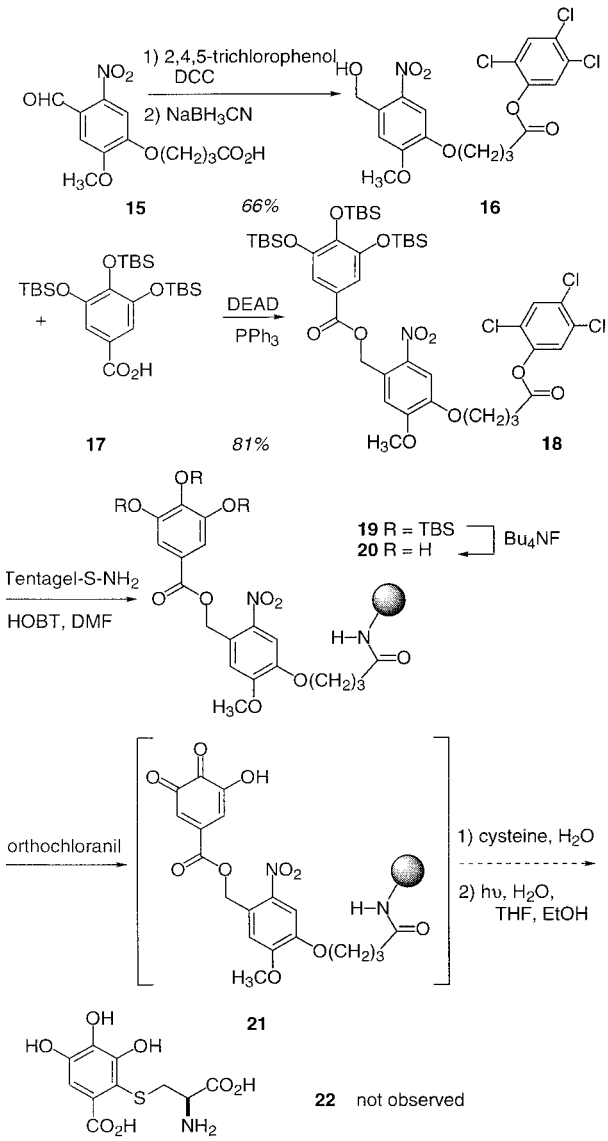
Scheme 2



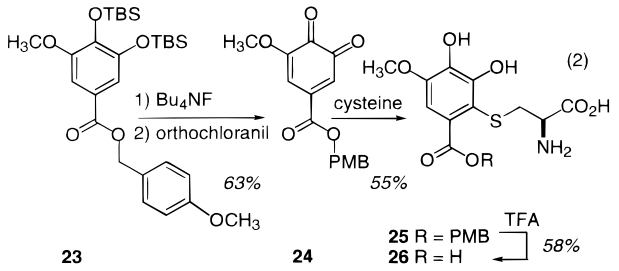
Scheme 3



amine moieties of Tentagel-S with 10 equiv of activated ester **18** in the presence of HOBt. An Ac_2O chaser capped off unreacted amine. The efficiency of galloyl loading was assayed by irradiating a 138 mg sample of **19** for 9 h in aqueous THF−EtOH, which furnished 16.3 mg of **17** following solvent evaporation (77% of the theoretical). Desilylation of the resin-bound galloyl ester **19** and

orthochloranil-mediated oxidation of the resulting triphenol **20** furnished bright red beads after filtering and extensive washing. Addition of an aqueous solution of L-cysteine to these presumably electrophilically activated beads instantaneously discharged the red color. Unfortunately, irradiation of the resultant brown resin under conditions which earlier met with success (e.g., **14** → **10**, **19** → **17**) did not provide any characterizable organic material. It is possible that the long and flexible poly(ethylene glycol) chains which link the functionalized end group to the polystyrene backbone of Tentagel-S could not sustain the required site isolation. In this case, the reactive galloyl hydroxyorthoquinone termini might have been consumed through thiol-initiated oligomerization as suggested by the observations with the model system **1b** (Scheme 1). Release of such a cross-linked oligomer from the Tentagel matrix could be problematic.

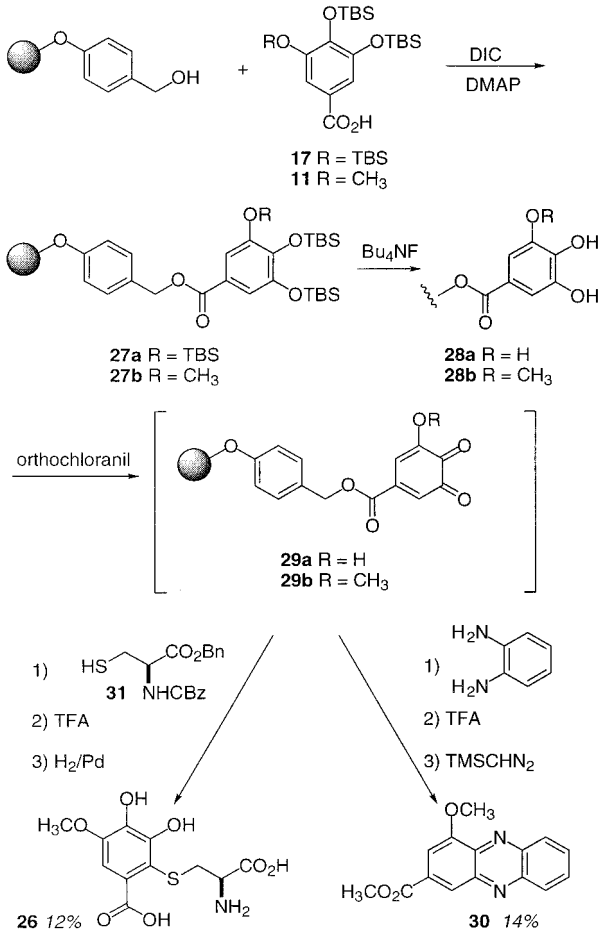
The Wang resin presents an alternative support which does offer better prospects for site isolation but at the expense of H_2O compatibility. Use of this resin requires that the terminal cysteine-galloyl conjugate is stable to the acidic cleavage conditions. To test this point, model system **25** was prepared and subject to typical cleavage conditions (50% TFA in CH_2Cl_2), eq 2. A good yield of



the cysteine conjugate **26**, free of decomposition products, was isolated. With these promising results in hand, synthesis of Wang resins terminated in both galloyl hydroxyorthoquinone and galloyl methoxyorthoquinone, **29a**,**b**, respectively, proceeded as shown in Scheme 4. The hydroxyl functionality of the resin was acylated with either acid **11** or acid **17** in two repetitive cycles of diisopropylcarbodiimide-mediated esterification. Post-esterification treatment of the crude galloylated resins with Ac_2O capped off any unreacted alcohol. Resin loading was assayed, in the case of **28b**, by treatment of 14.7 mg of material with 50% TFA/CH_2Cl_2 for 15 min. Evaporation of the supernatant furnished 2.5 mg of 3-*O*-methyl gallic acid (92% of the theoretical). Oxidation of **28a** with excess orthochloranil provided bright orange beads (**29a**?) following extensive washing. Unfortunately, the thiol trapping/resin release experiments did not proceed any more satisfactorily with the Wang resin than they did with the Tentagel system. Treatment of these orange beads with either L-cysteine, the cysteine derivative **31**, or PhSH all led to immediate color discharge. However, TFA-mediated cleavage of the reaction product(s) did not afford even trace amounts of the expected thiol-gallate adducts whose spectral data were in hand from previous studies.

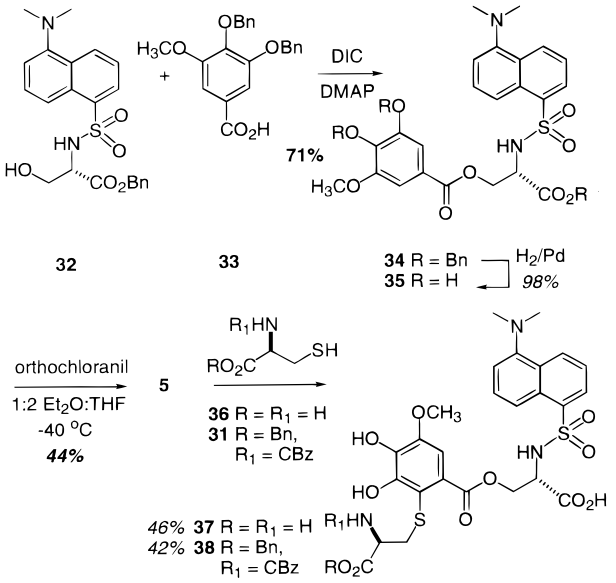
Whether the Wang resin itself contributed to the failure of the desired chemistry was probed by examination of the nucleophile addition/resin release chemistry of the galloyl methoxyorthoquinone **29b**, a stable analogue of the hydroxy species **29a** whose solution counterpart shows no tendency toward polymerization. Synthesis of this deep red resin proceeded in an analogous manner to the hydroxy analogue **29a** (Scheme 4). Exposure of these electrophile-terminated beads to cysteine derivative **31** in THF or, independently, to 1,2-diaminobenzene in HOAc, followed by TFA-mediated adduct release and hydrogenolysis (with the cysteine adduct) or esterification (with the diamine adduct), afforded small quantities of the expected products **26** and **30**, respectively. Cysteine itself did not react with the Wang resin **29b** in aqueous THF, plausibly because of solvent incompatibilities. These results demonstrate that a Wang resin-bound galloyl orthoquinone is a competent partner for combination with certain nucleophiles. Hence, the failure to detect adduct formation with the related hydroxyorthoquinone-capped resin **29a** might speak to the high and indiscriminate reactivity of this latter species, even while presumably site-isolated on the Wang resin. If the more tractable monoetherified galloyl orthoquinone species must be utilized in the planned protein (cysteine) trapping experiments, the requirement for site isolation, and hence solid-phase chemistry, is removed. The documented stability of the monoether galloyl orthoquinones to dimerization/oligomerization and/or subsequent adduct reoxidation suggests that soluble versions of the trapping electrophile, such as **5**, can be explored profitably to probe galloyl−protein interactions relevant to the LdNPV−GMc system.

Scheme 4



Soluble Probe Studies. Synthesis of the base-soluble fluorescently labeled galloyl orthoquinone **5** proceeded as outlined in Scheme 5. The dansyl amide of serine benzyl ester, **32**, was condensed with the *O*-methyl dibenzyl

Scheme 5



gallic acid derivative **33** to furnish ester **34** en route to the unprotected galloyl−serine−dansyl construct **35** (H_2/Pd). Orthochloranil-mediated oxidation of **35** in 1:2 Et_2O−THF then afforded the pure orthoquinone **5** in moderate yield as a pink solid. The solvent composition noted above was critical in preserving solubility on the starting catechol **35** while at the same time promoting precipitation of the pure product **5** from the reaction mixture. Failure to isolate **5** in this manner led to contaminated material whose purification was problematic. Orthoquinone **5** was insoluble in pure H_2O but did dissolve in pH 7.8 phosphate buffer. The electrophilicity of this galloyl orthoquinone was similar to the previously studied **1a**, as indicated by the moderate yields of adduct formation (**37** and **38**) upon reaction with either cysteine (**36**) or the cysteine derivative **31**, respectively (Scheme 5). The regiochemical assignment of these cysteine addition products rested upon comparisons of ^1H and ^{13}C NMR data with those of **3a**/**3b**.

The utility of orthoquinone **5** as a thiol trap within the more demanding context of a protein target was probed with a well-characterized cysteine-containing model protein prior to examining components of the relatively unexplored LdNPV system. The structurally described dihydrofolate reductase (DHFR) mutant N37C/C85A/C152S **39**[14] was chosen for its manageable size (for eventual electrospray MS analysis), availability in serviceable quantities and high purity, and solubility/stability in pH 7.8 phosphate buffer. This particular mutant has a single cysteine residue engineered into a solvent-exposed loop. A C-terminus hexahistidine purification tag raises an issue of competition for electrophile **5** (vide supra), but subsequent studies (vide infra) allay these concerns. A second DHFR double mutant C85A/C152S **40**,[15] which lacks cysteine entirely, was used as a control in the labeling studies with **5**. Neither **39** nor **40** exhibited fluorescence at 560 nm, the wavelength of interest for the dansyl chromophore in **5**. Both proteins were treated with dithioerythritol (DTE) prior to use to ensure that the cysteine thiol of **39** was in the reduced form. The DTE was removed just prior to addition of the orthoquinone **5** through exhaustive dialysis against phosphate buffer.

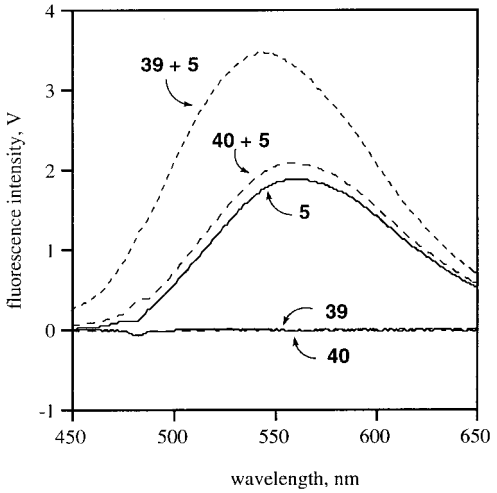
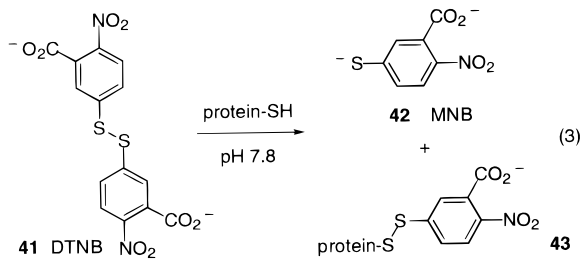


Figure 2. Fluorescence data for the interaction of orthoquinone **5** with **39** and **40**.

Treatment of **40** in pH 7.8 phosphate buffer with a 6.5-fold molar excess of **5** resulted in a modest increase in fluorescence intensity (ca. 10%) and a slight blue shift (∼6 nm), Figure 2. Similar exposure of **39** to 6.5 equiv of **5** in pH 7.8 buffer led to an 80% increase in fluorescence output and a pronounced (19 nm) blue shift, Figure 2. Both observations are consistent with a reaction between **39** and **5** in which the dansyl moiety becomes imbedded in a hydrophobic microenvironment as might accompany attachment to the protein. While the lack of a similar fluorescence response with the thiol-less but otherwise identical protein **40** is suggestive of chemistry occurring at the cysteine residue of **39**, these measurements do not address the issue of covalent vs noncovalent attachment per se.

Evidence which implicates the cysteine thiol of **39** in covalent bond formation can be gleaned from the results of some simple chemical tests. Direct assay of solvent accessible thiol groups in proteins can be accomplished through use of Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid (DTNB, **41**),[16] eq 3. Titration of **39** with



DTNB followed by spectrophotometric quantitation of the generated 5-mercapto-2-nitrobenzoate (MNB, **42**) revealed the expected result: almost all of the single thiol group (0.84 ± 0.04) is modified by reagent (cf. **43**). A similar experiment with the thiol-less protein **40** led to no measurable production of **42**, either before or after treatment with **5**. In the trials of interest, treatment of

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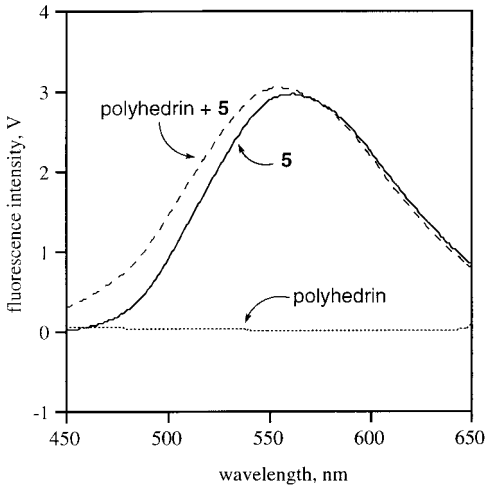

Figure 3. Fluorescence data for the interaction of orthoquinone **5** with polyhedrin.

39 with 1.0 molar equiv of **5** and then DTNB released ca. 0.5 equiv of MNB. Thus, approximately half of the original thiol in **39** is consumed by **5** when admixed in equimolar ratio. When 6.5 molar equiv of **5** to **39** was used, a similar DTNB assay produced no detectable MNB. In this instance, the excess of electrophile **5** was sufficient to modify all of the protein's free thiol.

This circumstantial evidence implicates cysteine as the reactive amino acid with orthoquinone **5**, but it does not address the possibility that other nucleophilic amino acids in **39** (e.g., lysine, histidine) may also trap **5** to a minor extent. However, localization of probe **5** to C37 in **39** can be deduced from comparison of the mass spectral data for **39** before and after treatment with orthoquinone. Electrospray MS was capable of detecting a parent ion for this small and relatively well-behaved protein (obsd *m*/*e* 18 761, calcd *m*/*e* 18 764). Similar analysis of the **5**-labeled version of **39** provided evidence for incorporation of one molecule of **5** only (obsd *m*/*e* 19 266, calcd *m*/*e* 19 266, = **39** + **5**). Since the DTNB studies indicated that cysteine was modified, and the MS data were consistent with attachment of only a single molecule of **5**, it appears that the galloyl-derived orthoquinone remains quite selective for thiol nucleophiles even within the highly complex microenvironment of a protein.

These promising model system results provided encouragement for investigating the interaction of LdNPV polyhedrin with orthoquinone **5**. Polyhedrin[17] in pH 8.5 phosphate buffer was incubated with 6.4 equiv of **5** and then examined by fluorescence spectroscopy, Figure 3. The response was much less pronounced than with **39** and more closely resembled that of the control protein **40**. Only a slight increase in fluorescence intensity (ca. 5%) and a modest blue shift (∼6 nm) accompanied polyhedrin/**5** interaction, which raised concerns about whether any of the protein's three cysteine residues (positions 133, 144, and 178 in the 245 amino acid protein)[17d,e] were bonding to **5**. No structural information on polyhedrin is available, and so the accessibility of **5** to these three nucleophilic residues cannot be ascertained. Similar mobility of native polyhedrin and 2-mercaptoethanol-treated polyhedrin upon SDS−PAGE was observed, a result consistent with an absence of disulfide bridges.

The DTNB assays for cysteine modification in polyhedrin were more encouraging. In the absence of orthoquinone **5**, 2.08 ± 0.10 of polyhedrin's three cysteine residues were modified by DTNB. However, only ca. 1.30 ± 0.06 cysteines, on average, were modified by DTNB after exposure of polyhedrin to a 6.4 molar excess of **5**. Thus, these assays implicate ca. 0.8 cysteine residues per polyhedrin, on average, as forming covalent attachments to **5**.

Companion experiments on a partially denatured sample of polyhedrin[18] engendered similar results. DTNB assays conducted on a sample of polyhedrin in 6 M urea, and on polyhedrin pretreated with a 2.5 molar excess of orthoquinone **5** in 6 M urea, revealed that ∼0.6 equiv of cysteine per protein, on average, were modified by **5**. Unfortunately, further attempts to characterize the putative polyhedrin−**5** adduct via electrospray MS were not fruitful. Neither the native protein nor its **5** conjugate gave interpretable ESMS signals. The higher molecular weight of polyhedrin (∼31 kDa) and its tendency to aggregate compared with **39** may have contributed to the failure of this technique to provide useful information.

In summary, the development of two distinct galloyl orthoquinone-based strategies for protein thiol covalent trapping was pursued. The initial approach, which relied on achieving site-isolation of a very reactive hydroxyorthoquinone gallate on a solid support, did not provide a useful thiol-trapping reagent. It is likely that sufficient site isolation was not achieved. A second approach utilized a soluble, stable methoxyorthoquinone construct which did react productively with cysteine and a small cysteine-containing model protein. Unambiguous evidence for similar reaction with the larger and less stable protein of interest in the LdNPV system, polyhedrin, was not forthcoming. However, the results of DTNB free thiol assays were consistent with covalent modification of one of the three polyhedrin cysteines by orthoquinone reagent **5**. Application of this probe reagent to the GMc/LdNPV system is planned, and results will be reported in due course.

Experimental Section

Fluorescence spectra were obtained on a titrating differential/ratio spectrofluorometer. High-resolution fast atom bombardment mass spectra were run at the University of Texas at Austin. Electrospray mass spectra were run at the Protein Chemistry Laboratory, Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO. Combustion analyses were performed by Midwest Microlabs, Indianapolis, IN, or by Galbraith Laboratories, Knoxville, TN. Liquid (flash) chromatography[19] was carried out using 32−63 mm silica gel and the indicated solvent. Et_2O and THF were purified by distillation from sodium/benzophenone under nitrogen, while CH_2Cl_2 was distilled from CaH_2 under nitrogen. Moisture sensitive reactions

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(18) Circular dichroism measurements of native polyhedrin and polyhedrin in 6 M urea revealed that the native protein possessed a number of elements of secondary structure which were altered upon urea treatment.

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

were carried out in predried glassware under an inert atmosphere of argon (Ar). Copies of ^{13}C NMR spectra are provided in the Supporting Information to establish purity for those compounds that were not subject to combustion analyses.

Oxidation of Methyl 3,4,5-Trihydroxybenzoate (6). Methyl 3,4,5-trihydroxybenzoate (**6**) (0.50 g, 2.7 mmol) in 60 mL of dry Et_2O was added dropwise over 3 h to a stirring solution of orthochloranil (0.74 g, 3.0 mmol) in 12 mL of dry Et_2O at −30 °C. The reaction mixture was transferred to a dry ice−CH_3CN-cooled (−42 °C) addition funnel and was added to a solution of thiophenol (0.34 mL, 3.3 mmol) in 19 mL of dry CH_2Cl_2 over 1 h. The solvents were removed under reduced pressure, and a mixture of the two products **7** and **8** was isolated by silica gel chromatography, using sequential elution with CH_2Cl_2, 2% CH_3OH in CH_2Cl_2, and finally 5% CH_3OH and 0.25% HOAc in CH_2Cl_2. Monoadduct **7** and bisadduct **8** were separated by preparative TLC, using 5% CH_3OH and 0.25% HOAc in CH_2Cl_2, to obtain 33 mg (3%) of **7** and 11 mg (1%) of **8**.

7: IR (CH_2Cl_2) 1725 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 7.23−6.97 (m, 6 H), 3.69 (s, 3 H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 167.7, 148.6, 147.6, 138.9, 136.6, 129.7, 129.5, 127.3, 126.0, 110.8, 107.7, 52.0; FABMS *m/z* (relative intensity) 293 (MH^+, 30); HRMS (FABMS) calcd for $C_{14}H_{12}O_5S_1$ 292.040 545, found 292.040 028.

8: IR (CH_2Cl_2) 1712 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 7.27−7.11 (m, 10 H), 3.66 (s, 3 H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 167.7, 150.3, 141.0, 137.9, 134.9, 129.6, 127.9, 126.5, 106.6, 52.1; FABMS *m/z* (relative intensity) 401 (MH^+, 16); HRMS (FABMS) Calcd for $C_{20}H_{16}O_5S_2$ 400.043 917, found 400.043 872.

3,4-Bis(*tert*-butyldimethylsiloxy)-5-methoxybenzoic Acid (11). Diisopropylethylamine (8.5 mL, 49 mmol) was syringed into a solution of methyl 3-*O*-methyl gallate (**10**)[20] (2.0 g, 11 mmol) and *tert*-butyldimethylsilyl chloride (6.2 g, 41 mmol) in 37 mL of dry DMF under Ar. The reaction mixture was stirred for 16 h at room temperature and was poured into ice-cold 1 M H_3PO_4. The product was extracted into hexanes and then 1:1 Et_2O−hexanes. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated to provide an orange oil. The trisilylated product (and some disilylated material) was treated with 35 mL of 1:1:3 H_2O−THF−AcOH (an additional amount of THF may be required to obtain a homogeneous solution) for 90 min, and the reaction mixture was poured into ice-cold water. The product was extracted into 1:1 Et_2O−hexanes, washed with copious quantities of water and then brine, dried over Na_2SO_4, filtered, and concentrated to yield 3.36 g (75%) of an off-white powder. The acid **11** was used without further purification: IR (CH_2Cl_2) 1718 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.31 (d, J = 2.0 Hz, 1 H), 7.27−7.26 (m, 1 H), 3.83 (s, 3 H), 1.00 (s, 9 H), 0.98 (s, 9 H), 0.23 (s, 6 H), 0.16 (s, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.9, 151.7, 147.4, 142.0, 120.9, 116.3, 106.5, 55.2, 26.0, 25.8, 18.8, 18.6, −3.9, −4.0; FABMS *m/z* (relative intensity) 413 (MH^+, 100). Anal. Calcd for $C_{20}H_{36}O_5Si_2$: C, 58.21; H, 8.79. Found: C, 58.56; H, 8.81.

4-(3-(Carbomethoxy)propoxy)-5-methoxy-2-nitrobenzyl 3,4-Bis(*tert*-butyldimethylsiloxy)-5-methoxybenzoate (13). Nitrobenzyl alcohol **12**[12] (0.16 g, 0.55 mmol), gallic acid derivative **11** (0.15 g, 0.36 mmol), and Ph_3P (0.11 g, 0.40 mmol) were suspended in 5.5 mL of dry Et_2O under Ar. Diethylazodicarboxylate (DEAD) (63 μL, 0.40 mmol) was added dropwise to the reaction mixture via syringe over 20 min. The clear yellow solution obtained turned cloudy on stirring overnight under Ar. The reaction mixture was concentrated to dryness, and the crude product obtained was purified by silica gel chromatography using 20% EtOAc in hexanes as the eluent, to yield a pale yellow oil (0.20 g, 81%): IR (CH_2Cl_2) 1732 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 7.73 (s, 1 H), 7.31 (s, 1 H), 7.30−7.29 (m, 2 H), 5.64 (s, 2 H), 4.19 (t, J = 6.3 Hz, 2 H), 3.98 (s, 3 H), 3.88 (s, 3 H), 3.63 (s, 3 H), 2.55 (t, J = 7.3 Hz, 2 H), 2.17−2.08 (m, 2 H), 1.00 (s, 9 H), 0.99 (s, 9 H), 0.25 (s, 6 H), 0.18 (s, 6 H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 173.7, 166.0, 154.8, 152.8, 148.7, 148.2, 142.1, 141.3, 127.6, 122.8, 116.2, 112.3, 110.4, 107.0, 69.1, 64.3, 56.8, 55.8, 51.8, 30.8, 26.5, 26.4, 25.3, 19.4, 19.2, −3.5, −3.6; FABMS *m/z* (relative intensity) 694 (MH^+, 5).

4-(3-(Carbomethoxy)propoxy)-5-methoxy-2-nitrobenzyl 4,5-Dihydroxy-3-methoxybenzoate (14). A solution of tetrabutylammonium fluoride (TBAF) (1.0 M in THF, 0.63 mL, 0.63 mmol) was syringed into a solution of galloylated nitrobenzyl derivative **13** (0.20 g, 0.29 mmol) in 10 mL of THF under Ar. The reaction proceeded to completion within 5 min (TLC), at which point the reaction mixture was added to ice-cold 1 M H_3PO_4. The product was extracted into Et_2O, washed with brine, dried over Na_2SO_4, filtered, and concentrated, and the residue was purified by silica gel chromatography using 30% EtOAc in hexanes as the eluent. The product was obtained as an off-white solid (0.13 g, 99%): IR (CH_2Cl_2) 1733 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 7.73 (s, 1 H), 7.32 (s, 1 H), 7.27 (d, J = 1.9 Hz, 1 H), 7.22 (d, J = 1.9 Hz, 1 H), 5.62 (s, 2 H), 4.19 (t, J = 6.3 Hz, 2 H), 3.97 (s, 3 H), 3.87 (s, 3 H), 3.63 (s, 3 H), 2.55 (t, J = 7.3 Hz, 2 H), 2.17−2.08 (m, 2 H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 173.7, 166.2, 154.8, 148.64, 148.60, 146.0, 141.1, 140.0, 127.8, 121.2, 112.1, 111.7, 110.4, 105.7, 69.1, 63.9, 56.7, 56.5, 51.7, 30.7, 25.2; FABMS *m/z* (relative intensity) 465 (MH^+, 8).

Alcohol 16. DCC (2.18 g, 10.6 mmol) in 20 mL of dry CH_2Cl_2 was added dropwise over 20 min to a stirring solution of nitrated vanillin derivative **15**[13] (2.0 g, 7.1 mmol) and 2,4,5-trichlorophenol (2.09 g, 10.6 mmol) in 39 mL of dry CH_2Cl_2 under Ar at room temperature. The reaction mixture was filtered at 4 h after the completion of addition to remove dicyclohexylurea, and the filtrate was diluted with CH_2Cl_2 and H_2O. The organic layer was separated out, and the aqueous layer was reextracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated to yield the crude trichlorophenyl ester of **15**. Purification of this ester was achieved via silica gel chromatography, eluting with 40% EtOAc in hexanes, to yield 2.95 g (90%) of the ester as a bright yellow solid: IR (CH_2Cl_2) 1772, 1690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.44 (s, 1 H), 7.62 (s, 1 H), 7.55 (s, 1 H), 7.41 (s, 1 H), 7.30 (s, 1 H), 4.29 (t, J = 6.1 Hz, 2 H), 4.01 (s, 3 H), 2.90 (t, J = 7.1 Hz, 2 H), 2.42−2.32 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.6, 169.8, 153.4, 151.5, 145.6, 143.6, 131.4, 131.0, 130.6, 126.0, 125.6, 125.2, 109.9, 108.2, 68.2, 56.6, 30.1, 24.0; CIMS *m/z* (relative intensity) 462 (MH^+, 8); HRMS (FABMS) calcd for $C_{18}H_{14}Cl_3N_1O_7$ 461.991 410, found 461.991 464. The trichlorophenyl ester (0.1 g, 0.2 mmol) was dissolved in 3.4 mL of 6:1 THF−H_2O at 0 °C. $NaBH_3CN$ (0.16 g, 2.60 mmol) was added in one portion to the reaction mixture, followed by 1 M HCl dropwise, until the pH of the solution ranged between 3 and 4. After 20 min at 0 °C, the reaction mixture was diluted with H_2O, and the product was extracted into EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude benzylic alcohol **16** was chromatographed on silica gel, using 40% EtOAc in hexanes, to yield 74 mg (73%) of a yellow solid. **16:** IR (CH_2Cl_2) 1772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1 H), 7.55 (s, 1 H), 7.30 (s, 1 H), 7.20 (s, 1 H), 4.97 (s, 2 H), 4.20 (t, J = 6.0 Hz, 2 H), 3.99 (s, 3 H), 2.89 (t, J = 7.2 Hz, 2 h), 2.38−2.29 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 154.3, 147.0, 145.7, 139.6, 132.7, 131.4, 131.0, 130.6, 126.0, 125.3, 111.2, 109.7, 68.0, 62.7, 56.4, 30.4, 24.2; CIMS *m/z* (relative intensity) 463 (M^+, 3); HRMS (EIMS) calcd for $C_{18}H_{14}Cl_3N_1O_7$ 462.9992, found 462.9980.

3,4,5-Tris(*tert*-butyldimethylsilyloxy)benzoic Acid (17). Diisopropylethylamine (12.2 mL, 35.3 mmol) was syringed into a suspension of 3,4,5-trihydroxybenzoic acid (2.0 g, 12 mmol) and *tert*-butyldimethylsilyl chloride (8.88 g, 29.4 mmol) in 30 mL of dry DMF under Ar at room temperature. The suspension turned into a clear solution after 30 min and was stirred for an additional 90 min. The reaction mixture was added to cold 1 M H_3PO_4, and the product was extracted into hexanes. The organic extract was washed with a saturated solution of $NaHCO_3$ followed by brine, dried over Na_2SO_4, filtered, and concentrated to a clear oil. The crude tetrasilylated derivative

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was treated with 1:1:3 H_2O−THF−AcOH (35 mL; additional THF may be required to obtain a homogeneous solution) for 1 h at room temperature. The reaction mixture was poured into ice-cold H_2O, and the product was extracted into 1:1 Et_2O−hexanes, washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to a white solid (5 g, 83%), which was used without further purification: IR (CH_2Cl_2) 3000−2400, 1724 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 7.28 (s, 2 H), 1.02 (s, 9 H), 0.96 (s, 18 H), 0.27 (s, 12 H), 0.18 (s, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.7, 148.5, 144.4, 121.0, 116.1, 26.2, 26.1, 18.8, 18.5, −3.6, −3.9; CIMS *m/z* (relative intensity) 513 (MH^+, 100). Anal. Calcd for $C_{25}H_{48}O_5Si_3$: C, 58.54; H, 9.43. Found: C, 58.28; H, 9.29.

Activated Ester 18. DEAD (141 μL, 0.90 mmol) was added dropwise via syringe pump over 25 min to a stirred suspension of activated benzyl alcohol **16** (0.56 g, 1.2 mmol), 3,4,5-tris(*tert*-butyldimethylsiloxy)benzoic acid (**17**) (0.42 g, 0.81 mmol), and Ph_3P (0.23 g, 0.89 mmol) at room temperature under Ar. The reaction mixture was stirred for 16 h and then concentrated to dryness. The crude product was purified by silica gel chromatography, using 20% EtOAc in hexanes as the eluent, to obtain 0.72 g (92%) of **18**: IR (CH_2Cl_2) 1772, 1717 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (s, 1 H), 7.54 (s, 1 H), 7.30 (s, 1 H), 7.27 (s, 2 H), 7.04 (s, 1 H), 5.72 (s, 2 H), 4.21 (t, J = 6.0 Hz, 2 H), 3.88 (s, 3 H), 2.88 (t, J = 7.2 Hz, 2 H), 2.38−2.29 (m, 2 H), 0.99 (s, 9 H), 0.94 (s, 18 H), 0.24 (s, 12 H), 0.14 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 165.7, 153.9, 148.6, 147.2, 145.7, 143.7, 139.8, 131.5, 131.0, 130.6, 128.0, 126.1, 125.3, 121.3, 115.6, 110.5, 109.8, 68.0, 63.4, 56.2, 30.4, 26.12, 26.10, 24.2, 18.8, 18.5, −3.7, −3.9; FABMS *m/z* (relative intensity) 959 (M + 2, 1); HRMS (FABMS) calcd for $C_{43}H_{62}Cl_3N_1O_{11}Si_3$ 957.269 631, found 957.268 942.

Resin Loading with 18. Tentagel-S NH_2 (0.25 g) was allowed to swell in dry DMF under Ar for 10 min. Ester **18** (0.72 g, 0.75 mmol) and HOBt (0.10 g, 0.75 mmol) were added to the swollen resin, and the reaction was allowed to proceed for 12 h. The resin beads were filtered out, washed well with EtOAc and acetone, and dried under vacuum. The unfunctionalized NH_2 termini were capped off by treating the dried resin beads with 0.28 g of DMAP, 2.80 mL of Ac_2O, and 22.5 mL of pyridine for 2 h. The resin beads **19** were washed with EtOAc, acetone, H_2O, and then acetone and then dried under vacuum.

Desilylation of Resin 19. A 100 mg amount of resin **19** was swollen in 1 mL of dry THF for 20 min. A 1.0 M solution of TBAF (0.01 mL, 0.10 mmol) was syringed into the reaction mixture, causing the beads to turn brown in color. The reaction mixture was shaken manually for 5 min, and the beads were then filtered out. The resin **20** was washed well with THF and dried under vacuum.

Orthochloranil Oxidation of Resin 20 and Attempted Cysteine Trapping. The deprotected phenolic resin **20** obtained as described above was allowed to swell for 20 min in 5 mL of dry Et_2O under Ar. The swollen resin suspension in Et_2O was then cooled to −30 °C. A solution of orthochloranil (8 mg, 0.03 mmol) in 1 mL of dry Et_2O was added to the reaction flask via syringe. The reaction flask was maintained at −30 °C for 2 h, and then the deep-red resin beads **21** were filtered out, washed well with Et_2O, and dried under vacuum.

A solution of L-cysteine (**36**) (11 mg, 0.09 mmol) in 1 mL of 2:1 H_2O−THF was added to this deep-red resin suspended in 5 mL of THF under Ar, resulting in a discharge of the deep-red color of the beads. The reaction was allowed to proceed for an additional 2 h, at which point the beads were filtered out and washed sequentially with THF, 1:1 H_2O−THF, and, finally, THF. The brown-colored resin was dried under vacuum, swollen in 4 mL 1:1 THF−EtOH, and then irradiated with a 450 W mercury arc lamp whose output was filtered through a 40% aqueous solution of $CuSO_4$ for 2 days under Ar, while monitoring the supernatant by TLC. No evidence for cysteine−gallic acid adduct **22**, either by TLC or by ^1H NMR, could be discerned.

4-Methoxybenzyl 3,4-Bis(*tert*-butyldimethylsiloxy)-5-methoxybenzoate (23). *p*-Methoxybenzyl alcohol (0.45 mL, 3.6 mmol) was syringed into a suspension of 3,4-bis(*tert*-butyldimethylsiloxy)-5-methoxybenzoic acid (**11**) (1.0 g, 2.4 mmol) and Ph_3P (0.70 g, 2.7 mmol) in 37 mL of dry Et_2O at room temperature under Ar. DEAD (0.42 mL, 2.7 mmol) was added dropwise to the reaction mixture via syringe pump over 1 h, and the resultant clear yellow solution was stirred overnight at room temperature. The reaction mixture was concentrated, and the residue was chromatographed on silica gel, eluting with 20% EtOAc in hexanes, to obtain **23** (0.91 g, 70%) as a pale yellow solid: IR (CH_2Cl_2) 1710 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.38 (d, J = 8.4 Hz, 2 H), 7.27 (d, J = 1.9 Hz, 1 H), 7.21 (d, J = 1.8 Hz, 1 H), 6.91 (d, J = 8.5 Hz, 2 H), 5.26 (s, 2 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 0.98 (s, 9 H), 0.97 (s, 9 H), 0.21 (s, 6 H), 0.20 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3, 159.5, 151.6, 147.3, 141.1, 129.7, 128.5, 122.0, 115.7, 113.9, 106.0, 66.2, 55.2, 26.0, 25.8, 18.7, 18.5, −3.9, −4.1; CIMS *m/z* (relative intensity) 533 (MH^+, 55). Anal. Calcd for $C_{28}H_4O_6Si_2$: C, 63.12; H, 8.32. Found: C, 63.03; H, 8.44.

4-Methoxybenzyl 3-Methoxy-1,2-dioxocyclohexa-3,5-diene-5-carbohydrate (24). A 1.0 M solution of TBAF in THF (3.3 mL, 3.3 mmol) was syringed into a pale yellow solution of **23** (0.80 g, 1.5 mmol) in 53 mL of dry THF under Ar. The reaction mixture turned pale green instantaneously and was poured into ice-cold 1 M H_3PO_4, resulting in a brownish-yellow solution. The product was extracted into Et_2O, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to a brown oil. The crude product was purified by chromatography on silica gel using 50% EtOAc in hexanes as eluent to yield 0.41 g (90%) of the catechol derived from **23** as a reddish-brown oil: IR (CH_2Cl_2) 1710 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 7.40 (d, J = 8.8 Hz, 2 H), 7.28 (d, J = 1.9 Hz, 1 H), 7.20 (d, J = 1.9 Hz, 1 H), 6.92 (d, J = 8.7 Hz, 2 H), 5.24 (s, 2 H), 3.83 (s, 3 H), 3.77 (s, 3 H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 166.6, 160.5, 148.5, 145.7, 139.7, 130.7, 129.5, 121.7, 114.6, 111.6, 105.7, 66.6, 56.5, 55.4; CIMS *m/z* (relative intensity) 304 (M^+, 4); HRMS (EIMS) Calcd for $C_{16}H_{16}O_6$ 304.0947, found 304.0923.

A solution of this catechol (0.12 g, 0.40 mmol) in 6 mL of dry Et_2O was added via syringe pump to a solution of orthochloranil (0.11 g, 0.43 mmol) in 4 mL of dry Et_2O under Ar at −30 °C over 3.5 h. The reaction mixture was stirred for an additional 14 h at −15 °C. The product was obtained as a fine red precipitate, which was filtered out, washed with cold Et_2O, and dried under reduced pressure to yield 84 mg (70%) of orthoquinone **24**: IR (CH_2Cl_2) 1723, 1672, 1626 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 8.3 Hz, 2 H), 6.92 (d, J = 8.0 Hz, 2 H), 6.74 (s, 1 H), 6.48 (s, 1 H), 5.27 (s, 2 H), 3.84 (s. 3 H), 3.83 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.9, 174.2, 164.2, 160.1, 153.2, 141.5, 130.4, 126.6, 124.2, 114.1, 106.4, 68.3, 56.3, 55.3; FABMS *m/z* (relative intensity) 304 (M + 2, 10). Anal. Calcd for $C_{16}H_{14}O_6$: C, 63.35; H, 4.69. Found: C, 63.57; H, 4.67.

4-Methoxybenzyl 5-Methoxy-2-((*S*)-cysteinyl)-3,4-dihydroxybenzoate (25). Orthoquinone **24** (80 mg, 0.26 mmol) in 4.3 mL of THF was added dropwise via syringe pump over 3.5 h to a stirring solution of L-cysteine (**36**) (30 mg, 0.25 mmol) in 2.2 mL of 2:1 H_2O−THF under Ar. The reaction mixture was stirred for an additional 20 min and then diluted with H_2O and EtOAc. The two layers were separated, and the aqueous layer was lyophilized to obtain 61 mg (55%) of the adduct **25** as a pinkish-white solid: IR (CH_2Cl_2) 1718, 1611 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3−0.1 M DCl, 9:1) δ 7.42 (d, J = 8.6 Hz, 2 H), 6.93 (s, 1 H), 6.92 (d, J = 8.4 Hz, 2 H), 5.26 (s, 2 H), 4.09 (dd, J = 8.9 Hz, 4.1 Hz, 1 H), 3.81 (s, 3 H), 3.76 (s, 3 H), 3.60 (dd, J = 14.7 Hz, 4.1 Hz, 1 H), 3.34 (dd, J = 14.7 Hz, 9.0 Hz, 1 H); ^{13}C NMR (75 MHz, CD_3COCD_3: 0.1 M DCl, 9:1) δ 169.3, 168.7, 160.5, 149.4, 148.7, 137.9, 131.0, 128.7, 128.5, 114.6, 109.5, 106.6, 67.6, 56.5, 55.5, 52.8, 35.7; FABMS *m/z* (relative intensity) 424 (MH^+, 100); HRMS (FABMS) calcd for $C_{19}H_{22}NO_8S$ 424.106 614, found 424.107 533.

5-Methoxy-2-((*S*)-cysteinyl)-3,4-dihydroxybenzoic Acid (26). Adduct **25** (0.04 g, 0.09 mmol) was dissolved in 2.3 mL of dry CH_2Cl_2 under Ar, and then 2.3 mL of TFA was added to the solution. The reaction mixture turned pink gradually. The reaction was allowed to proceed for 15 min, and then the solvents were removed under reduced pressure to afford a

brown solid. The crude product was dissolved in H_2O and was washed with EtOAc repeatedly until the wash came out colorless. The aqueous layer was lyophilized to obtain 17 mg (59%) of **26** as a pinkish-white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 6.98 (s, 1 H), 3.88 (s, 3 H), 3.72 (dd, J = 14.5 Hz, 3.2 Hz, 1 H), 3.59−3.55 (m, 1 H), 2.97 (dd, J = 14.4 Hz, 10.4 Hz, 1 H); ^{13}C NMR (75 MHz, CD_3COCD_3−2 M DCl, 9:1) δ 170.6, 169.4, 149.2, 148.7, 137.8, 128.3, 109.3, 106.7, 56.5, 52.8, 34.9; FABMS *m*/*z* (relative intensity) 304 (MH^+, 100).

Synthesis of Wang Resin 29a. Wang resin (2.09 g, ca. 2.09 mmol −OH) in 20 mL of dry CH_2Cl_2 was treated with **17** (1.17 g, 2.30 mmol), diisopropylcarbodiimide (DIC) (0.29 g, 2.3 mmol), and DMAP (51 mg, 0.42 mmol) at room temperature under Ar for 14 h. The beads were filtered out, washed with CH_2Cl_2 and THF, and dried under vacuum. The cycle was repeated once more to obtain resin **27a**. Resin **27a** was treated with Ac_2O (1.9 mL, 20 mmol), pyridine (1.62 mL, 20 mmol), and DMAP (12 mg, 0.1 mmol) to cap off the unreacted −OH groups. The resin was washed well with THF and CH_2Cl_2 and then dried under vacuum. This galloylated resin was then swollen in 18 mL of dry THF for 10 min and treated with a 1.0 M solution of TBAF in THF (4.4 mL, 4.4 mmol). Filtration and washing of the resulting resin with CH_2Cl_2 afforded resin **28a** as pale brown beads. The deprotected resin **28a** (1 g, ca. 1 mmol) in 20 mL of CH_2Cl_2 was then treated with a solution of orthochloranil (0.49 g, 2.0 mmol) in 4 mL of CH_2Cl_2 at −30 °C for 12 h. This orthoquinone-containing resin was resubmitted to a second cycle of oxidant after initial filtration and washing with CH_2Cl_2 to furnish the electrophilic orange colored resin beads **29a**. The resin **29a** obtained was stored at −10 °C.

Synthesis of Wang Resin 29b. Wang resin (4.8 g) in 35 mL of dry CH_2Cl_2 was treated with **11** (1.44 g, 3.85 mmol), DIC (0.60 mL, 3.85 mmol), and DMAP (0.09 g, 0.70 mmol) at room temperature under Ar for 14 h. The beads were filtered out, washed with CH_2Cl_2 and THF, and dried under vacuum. The cycle was repeated once more to obtain resin **27b**. Resin **27b** was treated with 3.3 mL of Ac_2O, 10 mL of pyridine, and 0.02 g of DMAP to cap off the unreacted −OH groups. The resin was washed well with THF and CH_2Cl_2 and then dried under vacuum. This galloylated resin was then swollen in 100 mL of dry THF for 10 min and treated with a 1.0 M solution of TBAF in THF (7.7 mL, 7.7 mmol). Filtration and washing of the resulting resin with THF, 1:1 H_2O−THF, and then THF again afforded resin **28b**. The deprotected resin **28b** in 58 mL of CH_2Cl_2 was then treated with a solution of orthochloranil (0.95 g, 3.85 mmol) in 35 mL of CH_2Cl_2 at −30 °C for 12 h. This orthoquinone-containing resin was resubmitted to oxidant after initial filtration and washing with CH_2Cl_2 to furnish the electrophilic deep-red resin beads **29b**. The resin **29b** obtained was stored at −10 °C.

Cysteine Adduct 26. Wang resin **29b** (0.2 g) in THF was added in small portions to a solution of cysteine derivative **31**[5i] (0.14 g, 0.40 mmol) in 3 mL of THF under Ar over 45 min. The resin beads were allowed to stand in the reaction mixture overnight and were then filtered out and washed well with THF. The cysteine adduct was cleaved from the resin by treating the beads with 2 mL of 1:1 TFA−CH_2Cl_2 for 35 min. The resin beads were filtered off, and the filtrate was concentrated to yield a brown residue. The crude product was purified by silica gel chromatography using 5% CH_3OH and 0.25% acetic acid in CH_2Cl_2 as the eluent. Final purification was effected by preparative TLC, using 5% CH_3OH and 0.25% acetic acid in CH_2Cl_2 as the solvent, to obtain 9 mg (12%, assuming 100% resin loading) of the desired cysteine product. This cysteine derivative was converted into, and characterized as, the known compound **26** by hydrogenolysis over Pd black.

Phenazine 30. Resin **29b** (0.30 g) in 5 mL of dry CH_2Cl_2 under Ar was treated with a solution of 1,2-diaminobenzene (0.12 g, 1.10 mmol, 5 equiv) in 5 mL of glacial HOAc. The beads turned dark brown instantly and were allowed to stand in the reaction mixture for 20 h. The resin was filtered out, washed well with CH_2Cl_2, and dried under vacuum. The brown resin was allowed to swell in 2 mL of CH_2Cl_2, and then 2 mL of TFA was added to the reaction mixture. The beads turned pink upon standing and were filtered off after 30 min. The filtrate was concentrated to yield the crude phenazine derivative. The crude product was purified by preparative TLC, using 10% CH_3OH and 0.25% acetic acid in CH_2Cl_2, to obtain 8 mg (14%, assuming 100% resin loading) of product. A portion of the phenazine product (6 mg, 0.02 mmol) in 1 mL of dry CH_3OH was converted to the corresponding methyl ester **30** by reaction with a 2.0 M solution of trimethylsilyldiazomethane in hexanes (0.11 mL, 0.22 mmol). Removal of solvents afforded pure **30** in quantitative yield. IR ($CDCl_3$) 1711 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.62 (d, J = 1.5 Hz, 1 H), 8.42 (m, 1 H), 8.27 (m, 1 H), 7.90 (m, 2 H), 7.66 (d, J = 1.3 Hz, 1 H), 4.25 (s, 1 H), 4.05 (s, 3 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.5, 155.2, 144.2, 143.4, 143.1, 138.1, 131.7, 131.4, 131.3, 130.2, 129.7, 124.9, 105.4, 56.8, 52.8; EIMS *m*/*z* (relative intensity) 268 (M^+, 19); HRMS (EIMS) calcd for $C_{15}H_{12}N_2O_3$ 268.0844, found 268.0848.

Benzyl (*S*)-2-(5-(Dimethylamino)naphthylene-1-sulfonamidyl)-3-hydroxypropionate (32). A solution of dansyl chloride (0.54 g, 2.0 mmol) in 24 mL of 2:1 acetone−H_2O was added to a stirred solution of L-serine benzyl ester hydrochloride (0.39 g, 2.0 mmol) in 6 mL of saturated aqueous $NaHCO_3$. The reaction mixture was stirred in the dark for 14 h, followed by evaporation of acetone and acidification of the reaction mixture with 1 M H_3PO_4. The product was extracted into EtOAc, and the organic extract was dried over Na_2SO_4, filtered, and concentrated to a fluorescent green oil (0.68 g, 88%). The crude product **32** was used without further purification: IR (CH_2Cl_2) 1743 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 8.3 Hz, 1 H), 8.30 (d, J = 8.6 Hz, 1 H), 8.21 (dd, J = 7.3 Hz, 1.1 Hz, 1 H), 7.56 (t, J = 8.1 Hz, 1 H), 7.47 (t, J = 7.9 Hz, 1 H), 7.29−7.26 (m, 3 H), 7.19 (d, J = 7.6 Hz, 1 H), 7.11−7.08 (m, 2 H), 5.87 (br s, 1 H), 4.89 (s, 1 H), 4.87 (s, 1 H), 4.00 (ddd, J = 7.3 Hz, 4.0 Hz, 4.0 Hz, 1 H), 3.85−3.74 (m, 2 H), 2.88 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 151.5, 134.6, 134.5, 130.5, 129.6, 129.4, 129.3, 128.4, 128.3, 128.1, 127.7, 122.0, 118.8, 115.2, 67.2, 63.4, 57.8, 45.2; FABMS *m*/*z* (relative intensity) 429 (MH^+, 76). Anal. Calcd for $C_{22}H_{24}N_2O_5S$: C, 61.68; H, 5.61; N, 6.54. Found: C, 61.06; H, 5.81; N, 6.33.

Benzyl (*S*)-2-(5-(Dimethylamino)naphthylene-1-sulfonamidyl)-3-((3,4-bis(benzyloxy))-5-methoxybenzoyl)oxy)-propionate (34). Dansyl amide **32** (0.42 g, 0.99 mmol), 3,4-bis(benzyloxy)-5-methoxybenzoic acid (**33**)[21] (0.40 g, 1.1 mmol), and DMAP (0.02 g, 0.2 mmol) were dissolved in 12 mL of dry CH_2Cl_2 under Ar. DIC (0.17 mL, 1.1 mmol) was syringed into the reaction mixture, which was then stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 and poured into 1 M H_3PO_4. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel, eluting with 30% and then 40% EtOAc in hexanes. Compound **34** was obtained as a fluorescent green foam (0.53 g, 70%): IR (CH_2Cl_2) 1723 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, J = 8.5 Hz, 1 H), 8.24−8.18 (m, 2 H), 7.48−7.01 (m, 20 H), 5.72 (d, J = 8.0 Hz, 1 H), 5.12 (s, 2 H), 5.04 (s, 1 H), 5.03 (s, 1 H), 4.92 (s, 2 H), 4.46−4.37 (m, 2 H), 4.28−4.25 (m, 1 H), 3.79 (s, 3 H), 2.83 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 164.9, 153.0, 151.8, 151.6, 141.6, 137.2, 136.4, 134.6, 134.3, 130.6, 129.5, 129.2, 129.0, 128.3, 128.24, 128.21, 128.0, 127.83, 127.80, 127.7, 127.3, 123.7, 122.8, 118.4, 114.9, 108.4, 106.9, 74.7, 70.7, 67.5, 64.3, 55.9, 55.0, 45.1; FABMS *m*/*z* (relative intensity) 775 (MH^+, 50). Anal. Calcd for $C_{44}H_{42}N_2O_9S$: C, 68.22; H, 5.43; N, 3.62. Found: C, 68.05; H, 5.60; N, 3.65.

(*S*)-2-(5-(Dimethylamino)naphthylene-1-sulfonamidyl)-3-((3,4-dihydroxy-5-methoxybenzoyl)oxy)propionic Acid (35). Debenzylation of **34** (0.53 g, 0.69 mmol) was carried out in the presence of 10% Pd/C (250 mg) in 50 mL of dry THF. The reaction mixture was stirred for 14 h at room temperature under 1 atm of H_2. The Pd/C was removed by filtration through Celite, and the THF was evaporated under reduced pressure to afford crude **35**. Silica gel chromatography, using 10% methanol in CH_2Cl_2, and then 100:1 EtOAc−HOAc, yielded

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35 as a fluorescent green foam (0.34 g, 98%): IR (CH_2Cl_2) 1716 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 8.50 (dd, J = 8.5 Hz, 0.9 Hz, 1 H), 8.39 (d, J = 8.7 Hz, 1 H), 8.27 (dd, J = 7.3 Hz, 1.3 Hz, 1 H), 7.17 (dd, J = 7.5 Hz, 0.6 Hz, 1 H), 7.08 (d, J = 1.9 Hz, 1 H), 7.05 (d, J = 1.9 Hz, 1 H), 4.45−4.32 (m, 3 H), 3.81 (s, 3 H), 2.83 (s, 6 H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 170.5, 166.0, 152.6, 148.3, 137.4, 130.9, 130.6, 130.5, 129.4, 128.7, 124.0, 120.8, 120.3, 115.9, 111.8, 106.0, 65.3, 56.5, 56.0, 45.5; FABMS m/z (relative intensity) 505 (MH^+, 82); HRMS (FABMS) calcd for $C_{23}H_{24}N_2O_9S$ 504.120 253, found 504.120 037.

Orthoquinone 5. A solution of **35** (200 mg, 0.40 mmol) in 9 mL of 2:1 THF−Et_2O (sufficient THF for dissolving **35**, making up the remaining volume with Et_2O) was added dropwise via an addition funnel to a solution of orthochloranil (0.11 g, 0.44 mmol) in 2 mL of dry Et_2O at −42 °C over 30 min. The deep red solution was stirred for an additional 30 min at −42 °C and then stored in a −20 °C freezer for 12 h. The pale pink precipitate obtained was filtered out, washed with copious volumes of cold Et_2O, and dried under reduced pressure to yield 88 mg (44%) of orthoquinone **5**. Orthoquinone **5** was characterized as the corresponding phenazine derivative, prepared in a 26% yield as described previously for **30**: IR (CH_2Cl_2) 1724 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.36−8.20 (m, 5 H), 8.11 9d, J = 1.5 Hz, 1 H), 8.02−7.97 (m, 2 H), 7.47 (dd, J = 8.6 Hz, 7.3 Hz, 1 H), 7.41−7.33 (m, 1 H), 7.39 (d, J = 1.4 Hz, 1 H), 6.76 (d, J = 7.4 Hz, 1 H), 4.50−4.47 (m, 2 H), 4.19−4.15 (m, 1 H), 4.15 (s, 3 H), 2.51 (s, 6 H); ^{13}C NMR (90 MHz, CD_3OD) δ 166.1, 155.8, 152.7, 145.0, 143.9, 143.8, 138.5, 137.5, 133.1, 132.9, 132.3, 131.2, 130.81, 130.80, 130.4, 130.3, 129.8, 129.0, 125.0, 124.1, 120.4, 115.6, 106.5, 66.2, 57.0, 45.4; FABMS m/z (relative intensity) 575 (MH^+, 3); HRMS (FABMS) calcd for $C_{29}H_{27}N_4O_7S$ 575.160 046, found 575.159 712.

Cysteine Adduct 37. Dropwise addition of a solution of orthoquinone **5** (0.03 g, 0.07 mmol) in 2 mL of THF containing 100 μL of water to a stirred solution of L-cysteine in 1 mL of 2:1 H_2O−THF at room temperature over 30 min afforded a clear pale yellow solution. The reaction mixture was diluted with H_2O and EtOAc, and the aqueous layer was lyophilized to yield crude **37** as a white solid. The white solid was then dissolved in CH_3OH and filtered through a membrane filter. The filtrate was collected and dried under vacuum to afford **37** (17 mg, 46%) as a white solid: IR (KBr) 1718 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.46 (d, J = 8.7 Hz, 1 H), 8.32 (d, J = 8.7 Hz, 1 H), 7.55−7.45 (m, 2 H), 7.18 (d, J = 7.4 Hz, 1 H), 6.95 (s, 1 H), 4.57−4.53 (m, 1 H), 4.49−4.32 (m, 2 H), 4.09 (t, J = 5.1 Hz, 2 H), 3.84 (s, 1 H), 3.80−3.73 (m, 1 H), 3.66−3.58 (m, 1 H), 3.07−3.05 (m, 1 H), 2.83 (s, 6 H); ^{13}C NMR (90 MHz, CD_3COCD_3−2 M DCl, 9:1) δ 170.6, 169.3, 167.4, 149.1, 148.4, 139.7, 138.1, 130.6, 129.9, 128.3, 128.0, 127.7, 127.3, 126.4, 120.7, 107.5, 65.0, 56.7, 55.9, 52.9, 47.7, 35.6; FABMS m/z (relative intensity) 624 (MH^+, 75); HRMS (FABMS) calcd for $C_{26}H_{30}N_3O_{11}S_2$ 624.132 178, found 624.132 041.

Cysteine Adduct 38. Orthoquinone **5** (48 mg, 0.10 mmol) in 2.2 mL of THF containing 100 μL of water was added dropwise via an addition funnel to a solution of cysteine derivative **31**[5i] (31 mg, 0.09 mmol) at room temperature over 10 min. Removal of solvents under reduced pressure afforded crude **38**, which was purified by preparative TLC using 5% CH_3OH and 0.25% HOAc in CH_2Cl_2 as the eluent. Adduct **38** was obtained as a yellow solid (30 mg, 42%): IR (CH_2Cl_2) 1714 cm^{-1}; ^1H NMR (360 MHz, CD_3COCD_3) δ 8.46 (d, J = 8.6 Hz, 1 H), 8.38 (d, J = 8.7 Hz, 1 H), 8.25(d, J = 7.3 Hz, 1 H), 7.52−7.46 (m 2 H), 7.33−7.30 (m, 10 H), 7.15 (d, J = 7.6 Hz, 1 H), 5.11−4.99 (m, 4 H), 4.45−4.37 (m, 4 H), 3.77 (s, 3 H), 3.34−3.24 (m, 2 H), 2.81 (s, 6 H); ^{13}C NMR (90 MHz, CD_3COCD_3) δ 171.3, 166.7, 157.0, 152.6, 148.6, 147.8, 138.0, 137.5, 136.8, 130.8, 130.7, 130.6, 129.26, 129.20, 128.86, 128.81, 128.7, 128.5, 127.1, 124.0, 120.4, 115.9, 112.6, 107.7, 67.4, 67.0, 65.8, 56.6, 55.25, 55.20, 45.6, 38.6; FABMS m/z (relative intensity) 848 (MH^+, 15); HRMS (FABMS) calcd for $C_{41}H_{42}N_3O_{13}S_2$ 848.215 908, found 848.216 732.

Protein Conjugation Studies. DHFR mutants **39** and **40** were provided by Dr. C. R. Matthews, Department of Chemistry and Center for Biomolecular Structure and Function, The Pennsylvania State University, University Park, PA 16802.[14,15] Polyhedrin was isolated from LdNPV-infected *Lymantria dispar* larvae.[17f] The purity of polyhedrin was judged by SDS−polyacrylamide gel electrophoresis. The alkaline isolate of polyhedrin in 0.1 M Na_2CO_3 and 0.17 M NaCl (pH 10.6) was dialyzed into 50 mM and then 10 mM phosphate buffer (pH 8.5), stored at −20 °C, and used without further purification.

DHFR mutants **39** and **40** were dialyzed into 4 L of fresh, degassed 10 mM phosphate buffer (pH 7.8), while polyhedrin was dialyzed into degassed 10 mM phosphate buffer (pH 8.5) prior to use. Both buffers contain 1 mmol DTE in the first 1 L volume; subsequent volumes do not contain the reducing agent. The concentrations of **39** and **40** were determined spectrophotometrically by the absorbance at 280 nm using the extinction coefficient for wild-type DHFR ($\epsilon_{280} = 3.11 \times 10^4$ M^{-1} cm^{-1}). Polyhedrin concentrations were determined by the absorbance at 276 nm, using the extinction coefficient determined by the method of Gill and von Hippel[22] ($\epsilon_{276} = 4.28 \times 10^4$ M^{-1} cm^{-1}).

DTNB assays were performed by incubating orthoquinone reagent **5** with protein for 1 h at 4 °C, followed by the addition of DTNB **41** and a second period of incubation at 4 °C for 1 h. MNB **43** concentrations were measured at 412 nm, using the molar extinction coefficient $\epsilon_{412} = 1.37 \times 10^4$ M^{-1} cm^{-1}. All spectroscopic determinations were carried out at 25 °C.

Fluorescence assays were carried out by incubating the protein of interest and orthoquinone reagent **5** in the indicated buffer for 1 h at 4 °C prior to spectrofluorometric analysis. Fluorescence spectra were recorded at 15 °C for **39** and **40** and at 25 °C for polyhedrin.

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Supporting Information Available: Copies of ^{13}C NMR spectra for compounds **7**, **8**, **13**, **14**, **16**, **18**, **25**, **26**, **30**, **32**, **35**, **37**, and **38**. This material is available free of charge via the Internet at http://pubs.acs.org.

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