The deuteride **12**-d was prepared from a $CH_2Cl_2$ solution of **12** under 1 atm of $D_2$: IR ($CH_2Cl_2$) 1955 $cm^{-1}$.

**(T-BDCP)Rh(CO)$_2$H (14).** A solution of **12** (6 mg, 1:2 mixture of **12**/P($C_6H_4$-*p*-$CH_3$)$_3$) in $CD_2Cl_2$ was prepared under 0.56 atm of 1:1 $CO/H_2$ and immediately placed in an NMR probe at 258 K. The $^1H$ NMR spectrum of the solution exhibited a multiplet at $\delta$ -10.50 (q, $J_{PH}$ = 16 Hz) due to **12** and a multiplet at $\delta$ -9.87 (td, $J_{PH}$ = 16 Hz, $J_{RhH}$ = 6 Hz) assigned to the dicarbonyl hydride **14**. Integration of the hydride resonances indicated a 1.6:1 ratio of **12** and **14**. The $^{31}P\{^1H\}$ NMR spectrum exhibited an ABCRh pattern centered at $\delta$ 37.5 due to compound **12** and a doublet at $\delta$ 32.3 (d, $J_{RhP}$ = 130 Hz) assigned to **14**. A singlet due to free P($C_6H_4$-*p*-$CH_3$)$_3$ was observed at $\delta$ -7.5.

**Catalytic Hydroformylation of 1-Hexene.** Hydroformylation reactions were performed in a 90-mL Fischer-Porter bottle equipped with a gas inlet valve, liquid sampling valve, and star-head magnetic stir bar. The pressure apparatus was immersed in a constant-temperature bath maintained at 33.6 ± 0.5 °C in a well-ventilated fume hood. A magnetic stirrer placed below the bath provided efficient stirring.

(acac)Rh(CO)$_2$[25] (7.9 mg, 0.031 mmol) and a chelating diphosphine (0.031 mmol) were placed in the pressure apparatus under nitrogen. The system was flushed with 70 psig of $CO/H_2$ three times and then pressurized to 70 psig with analyzed $CO/H_2$ (50.02% CO, 49.98% $H_2$). Benzene (6.0 mL) and toluene (internal GC standard, 0.20 mL, 1.9 mmol) were added by gastight syringe to the pressurized system. After 1 h of stirring, 1-hexene (2.50 mL, 0.020 mmol) was added. The pressure of the system was maintained throughout the reaction by adding additional $CO/H_2$ periodically. Samples were removed via the liquid sampling valve for analysis. Heptanal and 2-methylhexanal were analyzed by temperature-programmed gas chromatography on an HP5890A chromatograph interfaced to a HP3390A integrator using a 10 m × 0.53 mm methyl silicone capillary column.

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**Registry No. 1**, 141434-93-7; (±)-**2**, 141434-94-8; **3**, 125282-09-9; **4**, 17185-29-4; **7**, 141376-55-8; **7**·$CH_2Cl_2$, 141396-43-2; **7**-$^{13}$CO, 141376-64-9; **7**-*d*, 141376-60-5; **9**-ea, 141376-56-9; **9**-ea-$^{13}$CO, 141376-63-8; **9**-ea-*d*, 141376-61-6; **10**, 141376-57-0; **11**, 141376-58-1; **11**·$^1/_2$O($CHMe_2$)$_2$, 141376-62-7; **12**, 141396-44-3; **12**-*d*, 141396-45-4; **14**, 141376-59-2; (+)-DIOP, 37002-48-5; DIPHOS, 1663-45-2; ($Ph_3P$)$_3$Rh(CO)H, 17185-29-4; ($Ph_3P$)$_3$Rh($^{13}$CO)H, 141376-65-0; ($Ph_3P$)$_3$Ir(CO)H, 33541-67-2; Rh[P($C_6H_4$-*p*-$CH_3$)$_3$]$_3$(CO)H, 27709-98-4; (acac)Rh(CO)$_2$, 14874-82-9; 1-hexene, 592-41-6.

**Supplementary Material Available:** Tables of crystal data and collection parameters, atomic coordinates, bond lengths, bond angles, thermal parameters, and H-atom coordinates (19 pages); listings of observed and calculated structure factors for (BISBI)Rh($PPh_3$)(CO)H·$CH_2Cl_2$ (**7**·$CH_2Cl_2$) and (BISBI)Ir(CO)$_2$H (**11**·$^1/_2$O($CHMe_2$)$_2$) (38 pages). Ordering information is given on any current masthead page.

# A Receptor-Mediated Immune Response Using Synthetic Glycoconjugates

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**Abstract:** Antibody recognition of bacterial pathogens is important for activating complement and macrophage-mediated processes. Many bacterial antigens, however, undergo genetic variation to avoid antibody recognition. A synthetic glycoconjugate can direct antibodies to *E. coli* cells via their type 1 pili mannose-specific receptors. The receptor targeted antibodies activate both complement and macrophage-mediated processes that result in cell death. Bacterial cell-surface receptors can therefore be exploited to confer antigenicity onto the organism.

## Introduction

Antibody-mediated pathways in humoral and cellular immunity include complement activation and receptor-mediated phagocytosis.[1-4] These processes rely upon the coating of the pathogen with antibodies and recognition of the Fc region of the antibodies by effector molecules such as complement factor C1q and macrophage Fc receptors.[5,6] Several strains of enterobacteria express proteinaceous appendages called pili that present antigenic recognition sites for the host's immune system.[4,7] Type 1 pili also contain receptors specific for terminal $\alpha$-linked mannosides which mediate the adhesion of bacteria to host cells, a process that is essential for infectivity.[8-14] We report herein that antibodies directed to the bacterial cells by a synthetic mannosyl glycoconjugate activate both complement and macrophage-mediated processes that result in cell killing. The conserved binding domain of cell-surface receptors can therefore be utilized to direct antibodies to pathogens and prime them for killing by host defense mechanisms.

Early work toward the introduction of antigenic components onto otherwise non-immunogenic cells focused on the covalent modification of target cells in vitro with immunogenic small molecules such as trinitrophenol (TNP).[15,16] These covalently altered model systems were particularly useful for studying the

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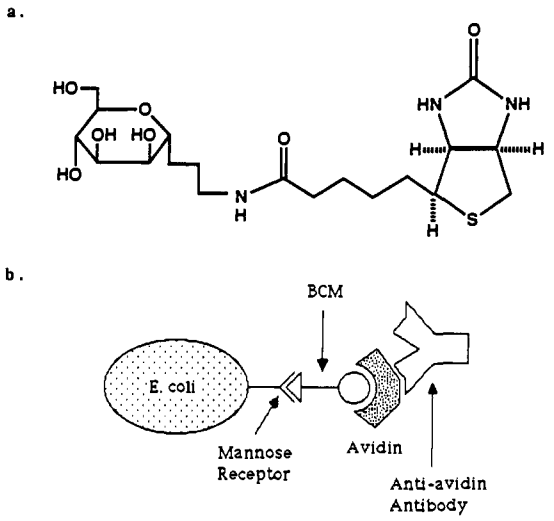


**Figure 1.** (a) A biotinylated $\alpha$-*C*-glycoside of mannose (BCM). The synthesis of BCM is described in ref 22. (b) Schematic representation of antibody targeting to the bacterial receptors. The synthetic glycoconjugate binds to mannose receptors on the pili, bringing anti-avidin antibodies to the bacterial cell surface and priming the cell for killing by complement proteins or macrophage cells. The BCM–avidin conjugate is depicted as monomeric for illustrative purposes.

events of immune recognition and cell lysis. The idea that synthetic substrates can function as antibody recognition sites and induce an immune response in the same way as natural antigens led to the development of liposome model systems for studying these events.[17,18] However, covalent modification is limited to in vitro applications and cannot serve as a strategy for antibody targeting to kill pathogenic organisms.

In recent landmark studies, Capon et al.[19] and Traunecker et al.[20] have shown that the Fc region of IgG or IgM molecules can be targeted to HIV-infected cells using genetically engineered antibodies in which the Fab domain has been replaced with the gp120 binding element of CD4. Also, Shokat and Schultz have shown that 2,4-dinitrophenol conjugates of CD4 can direct antibodies to purified gp120 in vitro.[21] These systems capitalize on the natural cell-surface receptor of the virus, without need for modification of target cells. However, their ability to induce both complement and macrophage-mediated cell lysis was not extensively investigated.

Recently, we described the design and synthesis of a high-affinity glycoconjugate ligand that recognizes type 1 pili mannose receptors on bacterial cells and contains an antibody binding domain.[22,23] The glycoconjugate consists of the complex of avidin with a biotinylated *C*-glycoside of mannose (BCM, Figure 1a) and is capable of targeting anti-avidin antibodies to the surface of the bacterial cell. We reasoned that the *E. coli* mannose receptors would provide a good model system to test the ability of ligand-directed antibodies to induce an immune response. Here we report that the BCM–avidin conjugate can direct anti-avidin antibodies to *E. coli* cells resulting in specific killing by both complement and macrophage-mediated pathways.

## Results and Discussion

The capability of the BCM–avidin–antibody complex to activate complement was assayed using *E. coli* K-12 HB101 cells containing a plasmid (pSH2) that confers type 1 piliation.[24] The cells were incubated with the BCM–avidin–antibody complex and treated with guinea pig complement; the results are summarized in Figure 2. The surviving cells were counted as colonies on solid media. We found that the BCM–avidin–antibody complex activates complement at the surface of the bacterial cells resulting in cell death (Figure 2a). Increasing the concentration of the BCM–avidin–antibody complex also led to increased cell killing. Control experiments in which either the BCM–avidin conjugate or the anti-avidin antibodies were omitted show no significant cell death. When the same experiments were performed on nonpiliated *E. coli* K-12 HB101 cells, no significant cell death was observed (data not shown). Furthermore, the bactericidal effect of the BCM–avidin–antibody complex was reversed in the presence of increasing concentrations of $\alpha$-methylmannopyranoside (Figure 2b).[25] This compound binds to the bacterial receptors and prevents an otherwise lethal amount of the BCM–avidin–antibody complex from coating the cells. The same protective effect was not observed in the presence of $\alpha$-methylglucopyranoside, which does not bind to the receptors. The BCM–avidin–antibody complex can therefore specifically bind to the cell-surface receptors and activate complement for cell killing.[26]

We also investigated the capability of the BCM–avidin–antibody complex to stimulate macrophage-mediated endocytosis. Macrophage-mediated killing of the BCM–avidin–antibody coated bacterial cells was assayed using a macrophage-monocyte related cell line (J774) that kills antibody-coated cells predominantly by Fc receptor-mediated endocytosis.[27] *E. coli* cells were incubated with the BCM–avidin–antibody complex and treated with macrophage cells in an effector–target ratio of 1:2. Bacterial cell killing was determined by counting survivors on solid media, and the results are summarized in Figure 3. We found that the BCM–avidin–antibody complex stimulates macrophage-mediated killing when bound to the bacterial cell surface (Figure 3a). Increasing concentrations of the BCM–avidin–antibody complex resulted in a significant increase in cell death. Both the BCM–avidin conjugate and the anti-avidin antibodies had no bactericidal activity alone. Furthermore, macrophage-mediated killing was inhibited in the presence of $\alpha$-methylmannopyranoside and not by $\alpha$-methylglucopyranoside (Figure 3b) similar to the results obtained using complement proteins.

It is interesting to note that, unlike the complement experiments (Figure 2, a and b), total cell killing by macrophage cells was not obtainable even at concentrations of the BCM–avidin–antibody complex up to 1 $\mu$M (about 75% killing was observed at this concentration). It is possible that at higher concentrations of BCM–avidin–antibody complex the antibody clusters in solution effectively compete for Fc receptor sites thus preventing the Fc receptors from binding to antibodies localized on the bacterial cell. A similar result has been observed with antibody coated erythrocytes.[27]

These results demonstrate that antibodies directed to the surface of the bacterial cells through the interaction of the BCM–avidin conjugate with the mannose receptors are capable of activating both complement proteins and macrophages for cell killing. Similar bifunctional glycoconjugates may be used as a general strategy to target antibodies to pathogens that would otherwise not recognize the organism.

## Experimental Section

**Complement Assays.** *E. coli* K-12 HB101 (pSH2) were grown for 24 h in static LB broth supplemented with chloramphenicol and washed once with PBS before use. The cells were tested for piliation by agglutination

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(25) The bactericidal effect of complement is inhibited by ca. 80% at a concentration of 200 mM $\alpha$-methylmannopyranoside. Full inhibition can be obtained at higher concentrations (ca. 0.5 M), but these concentrations have unfavorable effects on cell viability.
(26) We were also interested in the ability of the BCM–avidin conjugate to target antibodies to bacteria in the presence of agglutinated yeast cells. While cell killing experiments have not been performed, we have found that agglutinated bacterial cells can be released from yeast cells by the glycoconjugate. Therefore, these cells should be susceptible to antibody targeting even when they are adhering to host cells in a colonizing fashion.
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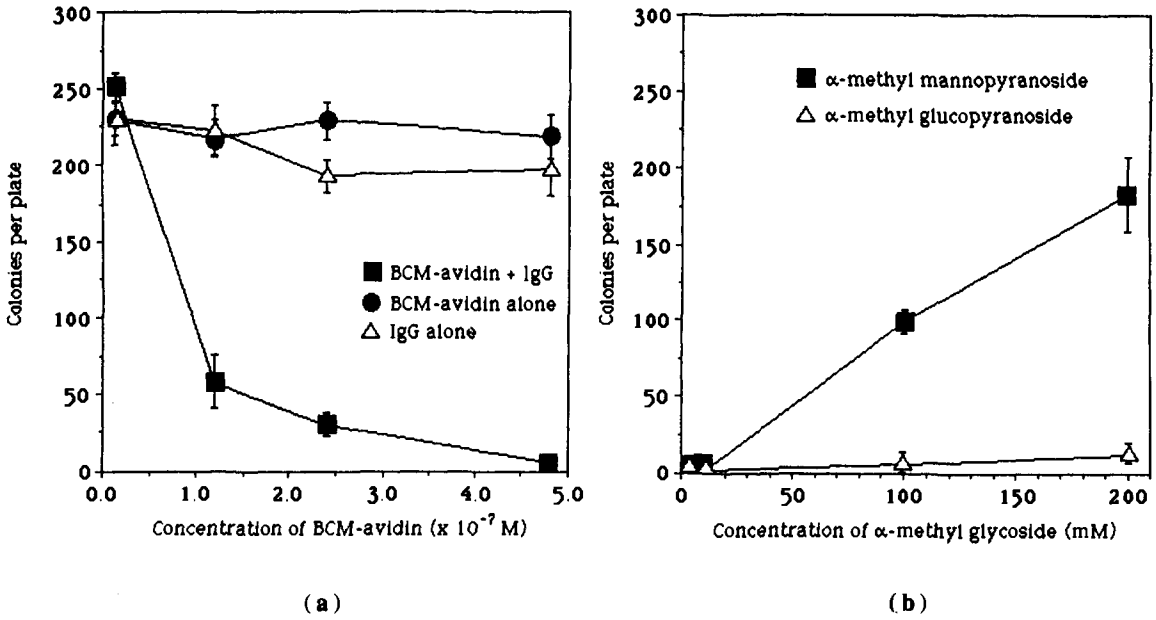


**Figure 2.** Complement-mediated killing of *E. coli* K-12 HB101 (pSH2). (a) Bacteria were incubated with the BCM–avidin–antibody complex (■) followed by 10% guinea pig complement. Increased amounts of the BCM–avidin–antibody complex resulted in increased cell death. Control experiments containing only anti-avidin antibodies (no BMC–avidin) (△) or BCM–avidin (no anti-avidin antibodies) (●) showed no significant cell death. The concentration of antibodies is four times that of BCM–avidin for each data point. (b) Bacteria were incubated with the BCM–avidin–antibody complex ($6.0 \times 10^{-7}$ M in BCM–avidin and $24 \times 10^{-7}$ M in IgG) in the presence of increasing amounts of α-methylmannopyranoside (■) or α-methylglucopyranoside (△) and treated with 10% guinea pig complement. Mannose inhibits cell killing (■) while glucose has no protective effect (△). Data points represent average values for three plates and error bars represent the standard deviation of the mean. Similar results were obtained in duplicate experiments.

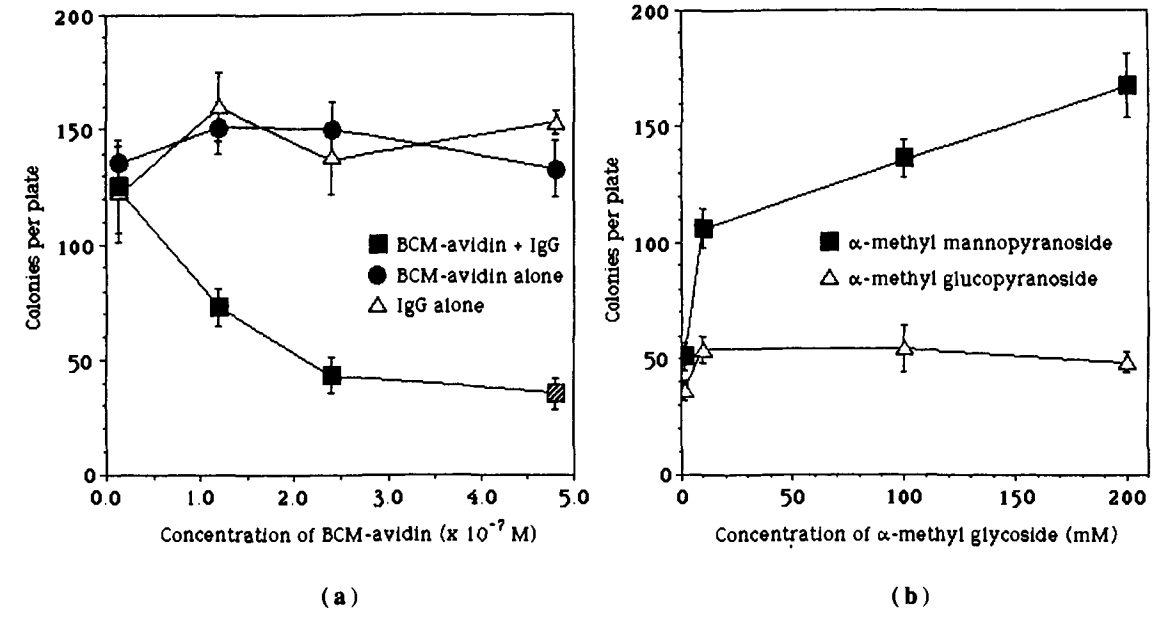


**Figure 3.** Macrophage-mediated killing of *E. coli* K-12 HB101 (pSH2) cells. (a) Bacterial cells were incubated with the BCM–avidin–antibody complex (■) followed by macrophage cells. Increased cell death is observed with increased concentrations of the BCM–avidin–antibody complex. Control experiments in which either anti-avidin antibodies (△) or BCM–avidin (●) were incubated with bacterial cells and macrophages show no change in the amount of cell death with increased concentration. The concentration of antibodies is four times that of BCM–avidin for each data point. (b) α-Methylmannopyranoside (■) inhibits the bactericidal activity of the BCM–avidin–antibody complex ($6.0 \times 10^{-7}$ M in BCM–avidin and $24 \times 10^{-7}$ M in IgG). Unlike α-methylmannopyranoside, the α-methylglycoside of glucose (△) has no inhibitory effect. Data points represent average values for three plates and error bars represent the standard deviation of the mean. Similar results were obtained in duplicate experiments.

experiments with yeast cells[7-13] and by colony morphology.[24] The conjugate of BCM with avidin was formed by incubating a 10-fold excess of BCM with egg-white avidin (Sigma) overnight at 0 °C. The solution was then dialyzed against PBS (3 × 400 mL, 6000–8000 MW cutoff) and the total protein concentration determined by BCA protein assay (Pierce) and $E^{1\%}_{280}$. The BCM–avidin–antibody complex was formed by incubating BCM–avidin with a 4-fold excess of monoclonal anti-avidin IgG1k (Sigma) overnight at 0 °C. In a typical experiment, $10^7$ *E. coli* cells were incubated with the BCM–avidin–antibody complex and guinea pig complement (10% v/v, Calbiochem) for 2 h at 37 °C. The suspension was diluted 1000-fold with PBS, and 25 μL of the resulting suspension was plated in triplicate on solid LB media supplemented with chloramphenicol. The plates were developed overnight at 37 °C, and the surviving cells were counted as colonies.

**Macrophage Assays.** Macrophage J774 cells (ATCC) were grown in DME media supplemented with 10% heat inactivated fetal calf serum. Cells were harvested, washed twice with 45 mL of PBS, and resuspended to a final dilution of $5 \times 10^7$ cells/mL. *E. coli* cells were prepared as previously described. In a typical experiment, $10^6$ bacterial cells were treated with the BCM–avidin–antibody complex followed by $5 \times 10^5$ macrophage cells. The suspension was incubated at 37 °C for 3 h. After a 100-fold dilution with PBS, 25 μL of the resulting suspension was

plated in triplicate. Survivors were counted as previously described.

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# Reduction of Daunomycin and 11-Deoxydaunomycin with Sodium Dithionite in DMSO. Formation of Quinone Methide Sulfite Adducts and the First NMR Characterization of an Anthracycline Quinone Methide

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**Abstract:** Daunomycin (**1**) yields 7-deoxydaunomycinon-7-yl sulfonates (**6** and **7**) upon anaerobic reaction with 6 mol equiv of dithionite in 5% $H_2O$–95% DMSO followed by oxidation with molecular oxygen. The reaction is proposed to occur via reductive glycosidic cleavage to 7-deoxydaunomycinone quinone methide (**4**) followed by reversible dithionite addition to form adduct hydroquinone **17** and subsequent molecular oxygen oxidation at the hydroquinone and at the sulfur functional groups to yield **6** and **7**. Byproducts 7-deoxydaunomycinone (**2**) and bi(7-deoxydaunomycinon-7-yl) (**3**), epidaunomycinone (**8**), 7-deoxy-7-ketodaunomycinone (**9**), 7-deoxy-7,13-epidioxydaunomycinol (**10**), and daunomycinone (**11**) result from protonation of **4** and molecular oxygen oxidation of **4**, respectively. Sulfonate adducts **6** and **7** are relatively stable even in semiquinone and hydroquinone redox states. 11-Deoxydaunomycin (**12**) yields 7,11-dideoxydaunomycinon-7-yl sulfonates (**14** and **15**) upon similar reduction with even 1 mol equiv of dithionite. Sulfonates **14** and **15** are proposed to form by both dithionite and hydrogen sulfite addition to intermediate 7,11-dideoxydaunomycinone quinone methide (**16**), with hydrogen sulfite being a byproduct of quinone reduction by dithionite. With these reaction conditions, quinone methide **16** is long-lived and is characterized by $^1H$ NMR spectroscopy; the spectrum suggests a B-ring quinone methide structure.

## Introduction

Anthracyclines such as daunomycin (**1**) and 11-deoxydaunomycin (**12**) are potentially bioreductively activated to quinone methide transients[1,2] via sequential semiquinone and hydroquinone redox states.[3,4] In vitro reductive activation with chemical,[5,6] electrochemical,[7] and enzymatic[8-10] reducing agents has been observed. Reaction of quinone methides with nucleophilic sites in critical biological molecules to yield adducts is possibly a source of cytotoxicity, and reaction with a proton source such as water to yield the 7-deoxyaglycons is possibly a detoxification pathway. Reactive free radical transients from reaction of the quinone methide with molecular oxygen have also been reported.[11] Consequently, the chemistry of quinone methides, generally,[12] and quinone methides from anthracyclines, specifically, is being actively studied.

Anaerobic reactivity is substituent-dependent especially with regard to the 11-position. The 11-deoxyanthracycline quinone methides, such as 7,11-dideoxydaunomycinone quinone methide (**16**), are less reactive with protic solvents to form 7-deoxyaglycons than 7-deoxydaunomycinone quinone methide (**4**). Consequently, they have longer lifetimes. Formation of aglycon dimers is sometimes the primary reaction pathway.[13-16] Dimerization occurs with one quinone methide serving as a nucleophile and the other as an electrophile with formation of the aglycon dimer initially in a half quinone, half hydroquinone state.[14] In aqueous media, both **16** and **4** also react with divalent sulfur nucleophiles such as *N*-acetylcysteine to give adducts[10,17] initially in hydroquinone states; subsequent oxidation yields stable cysteinyl adducts in quinone states. The adduct isolated yield is higher from 11-

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