Thiol-Selective Fluorogenic Probes for Labeling and Release

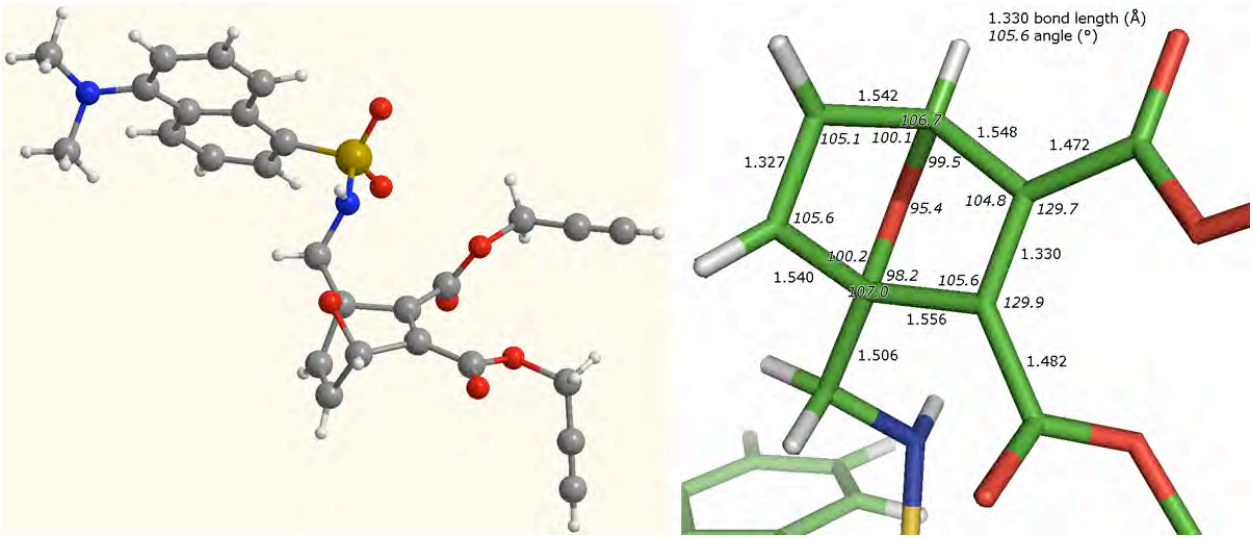
*Vu Hong, Alexander Kislukhin, M.G. Finn**

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Supporting Information

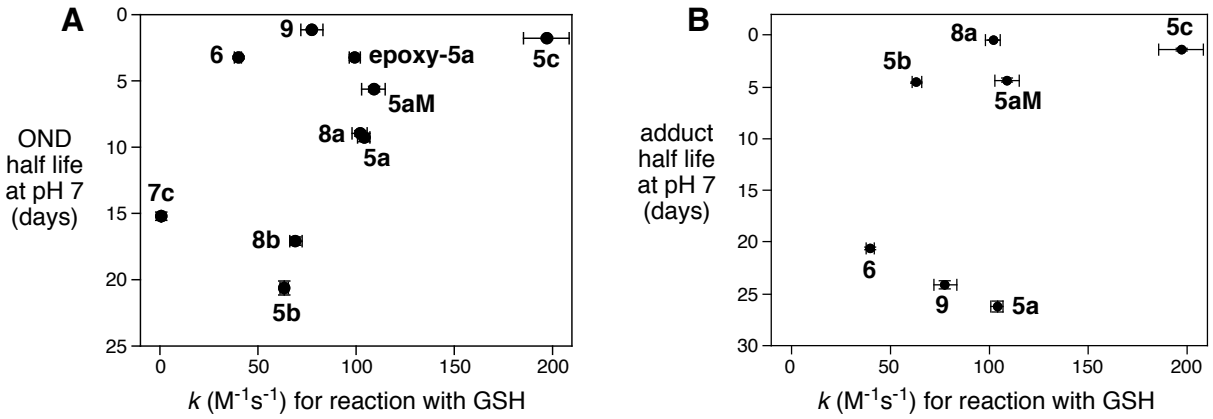
Structure

Figure S1. (Left) X-ray crystal structure of **5c**, and (right) structural features of the OND ring (CIF file available separately).



Reactivity

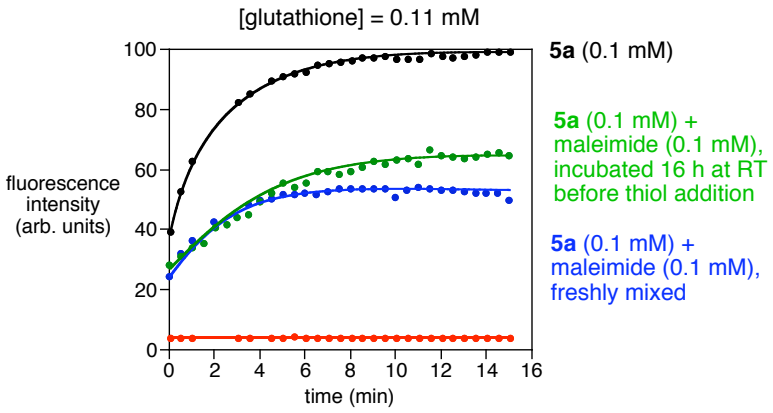
Figure S2. Plots of data in Table 1, relating OND thiol reactivity to (A) half life of the electrophile, and (B) half life of the β-mercaptoethanol adduct with respect to retro-Diels-Alder reaction, each at pH 7 and room temperature. **Epoxy-5a** does not appear on this plot because it does not undergo cleavage.



Relative Reactivity and Stability of Representative OND and Maleimide Electrophiles

The results of reactions using competing electrophiles for a limiting amount of glutathione are shown in Figure S3. When mixed with an equimolar amount of *N*-ethylmaleimide, the amount of fluorogenic adduct formed from **5a** decreased by about 50%, showing that **5a** and the maleimide are approximately equally reactive. When the mixture of two electrophiles was allowed to stand at room temperature in buffer for 16 hours before introduction of the nucleophile, the amount of reaction due to **5a** was found to increase by approximately 12%. Approximately 5% of **5a** is consumed by standing in buffer over 16 hours, as calculated from the half life of **5a** reported in Table 1 (9.3 days). The increase in signal observed after incubation of the electrophiles shows that *N*-ethylmaleimide undergoes faster decomposition, in agreement with the literature.

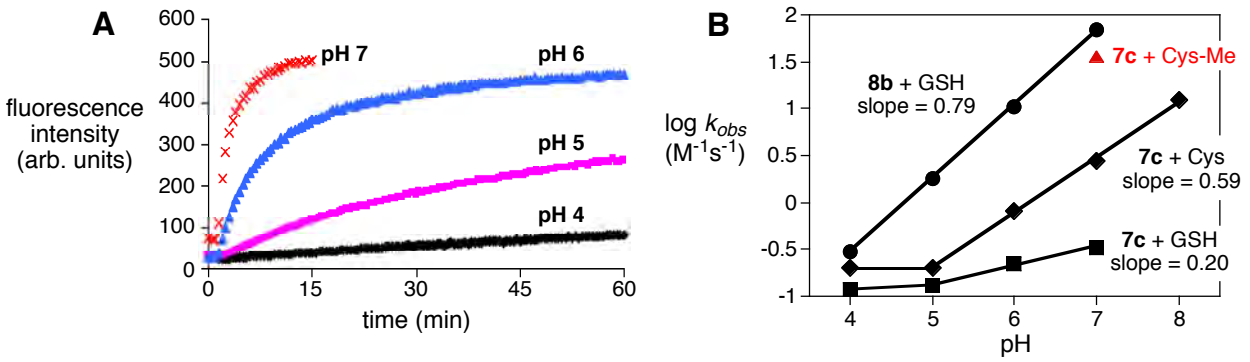
Figure S3. Reaction of fluorogenic OND compound **5a** with glutathione, in competition with *N*-ethylmaleimide at room temperature. The starting concentrations are indicated; solvent = 10% DMSO in 0.1M phosphate buffer, pH 7. The fluorescence observed in the reaction of **5a** alone was set to 100%, with the assumption that this represents complete conversion to 0.1 mM of the adduct.



pH Dependence

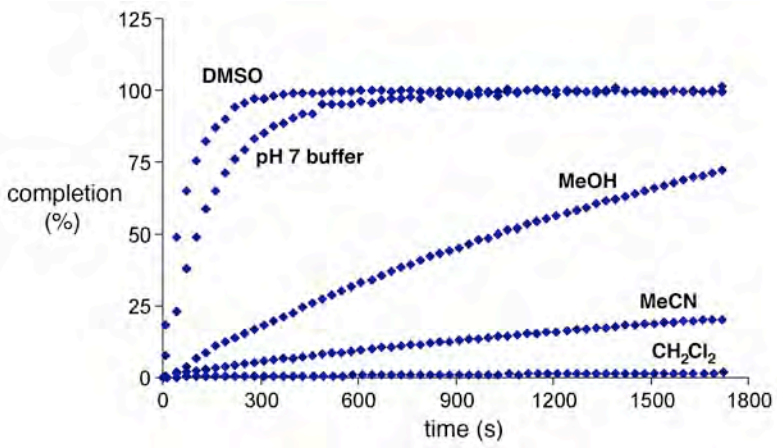
The reactions of **7c** with glutathione and cysteine were found to proceed at the same rates at pH 4 and 5 (Figure S4), reflecting a trade-off between accelerating increases in thiolate concentration and deactivating increases in the concentration of OND carboxylate anions. At values greater than 5, the reactivity of **7c** increased with increasing pH, but with significantly less pH sensitivity for cysteine, which contains a carboxylic acid group, than for glutathione, which does not (Figure S4). Cysteine methyl ester was substantially more reactive than the unprotected amino acid at pH 7. In contrast to **7c**, amide electrophile **8b** showed a linear relationship of pH *vs*. log(rate) through the entire pH 4–7 range, with the most sensitive dependence on pH. These observations demonstrate that the reactivity of OND electrophiles may be tuned by several factors.

Figure S4. (A) Reaction of **8b** as a representative example (0.1 mM) and glutathione (0.11 mM) at various pH values monitored at 550 nm (λ_{ex} = 332 nm). These types of data were used to derive values of k_{obs} in Table 1. (B) Dependence of thiol addition rate constant on pH for the reaction between electrophiles **7c** and **8b** (0.10 mM) with thiol nucleophiles (0.11 mM) at 24 °C (GSH = glutathione; Cys-Me = cysteine methyl ester; 0.25 M citrate at pH 4, 5, or 6, 0.1 M phosphate at pH 7, 0.1 M HEPES at pH 8.



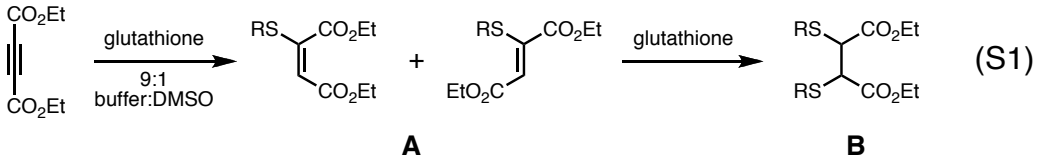
Solvent Polarity

Figure S5. Time course of the reactions of 5a (0.1 mM) with β-mercaptoethanol (1 mM) in various solvents, measured by corrected fluorescence intensity, normalized to that of the pure adduct in each solvent. Similar results were obtained with ethyl-3-mercaptopropionate (data not shown).



Thiomaleate Reactivity

The retro-Diels-Alder reaction from OND-thiol adducts releases thiomaleate or thiomaleimide adducts (Figures 4 and 6, structures **11**, **14**, **17**) that are also electrophilic. This reactivity was illustrated in the reaction of glutathione (0.2 mM) with diethylacetylene dicarboxylate (0.1 mM), as shown in Eq. S1. Electrospray ionization mass spectrometry revealed the formation of thiomaleates **A** within several minutes, followed by the formation of **B** more slowly (approximately 30 minutes). This process and others of the same type involving thiomaleimides will be explored in more detail and discussed elsewhere.



(S1)

Peptide Labeling

Figure S6: MS analysis of peptide modification. MALDI MS spectra for a) unmodified peptide, b) peptide modified with **5a,** c) peptide modified with **8a**, d) peptide modified with **8b**.

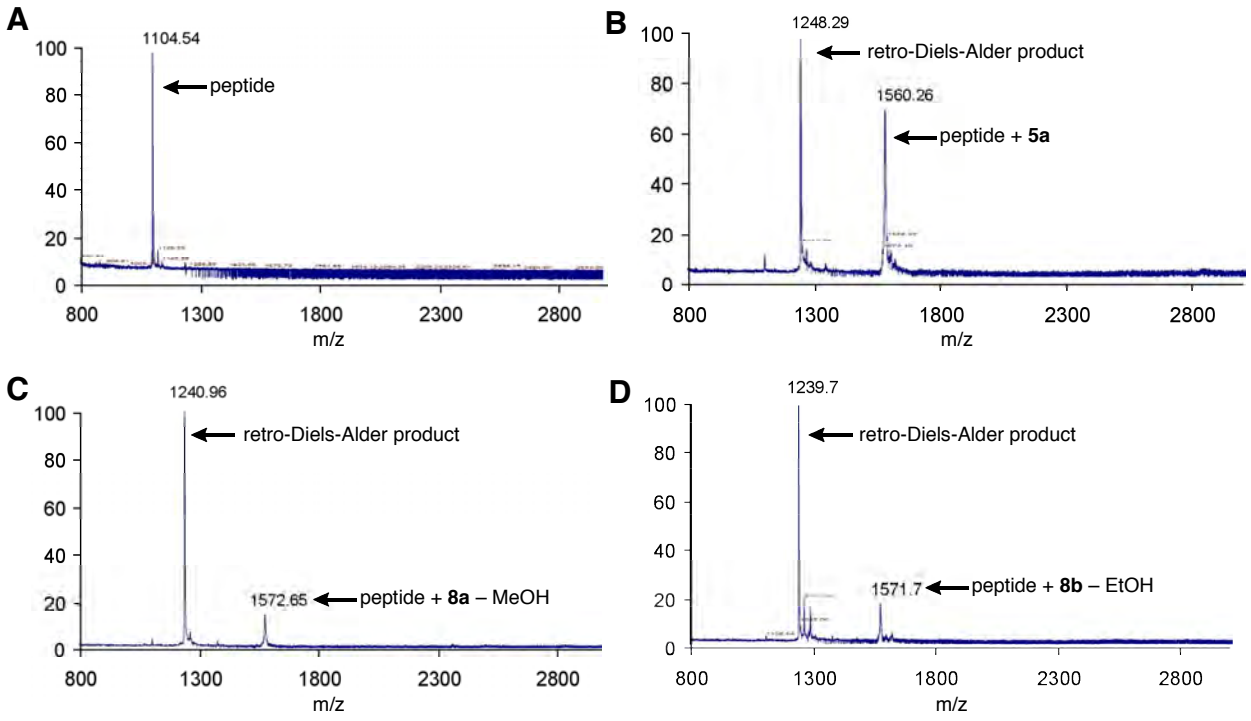
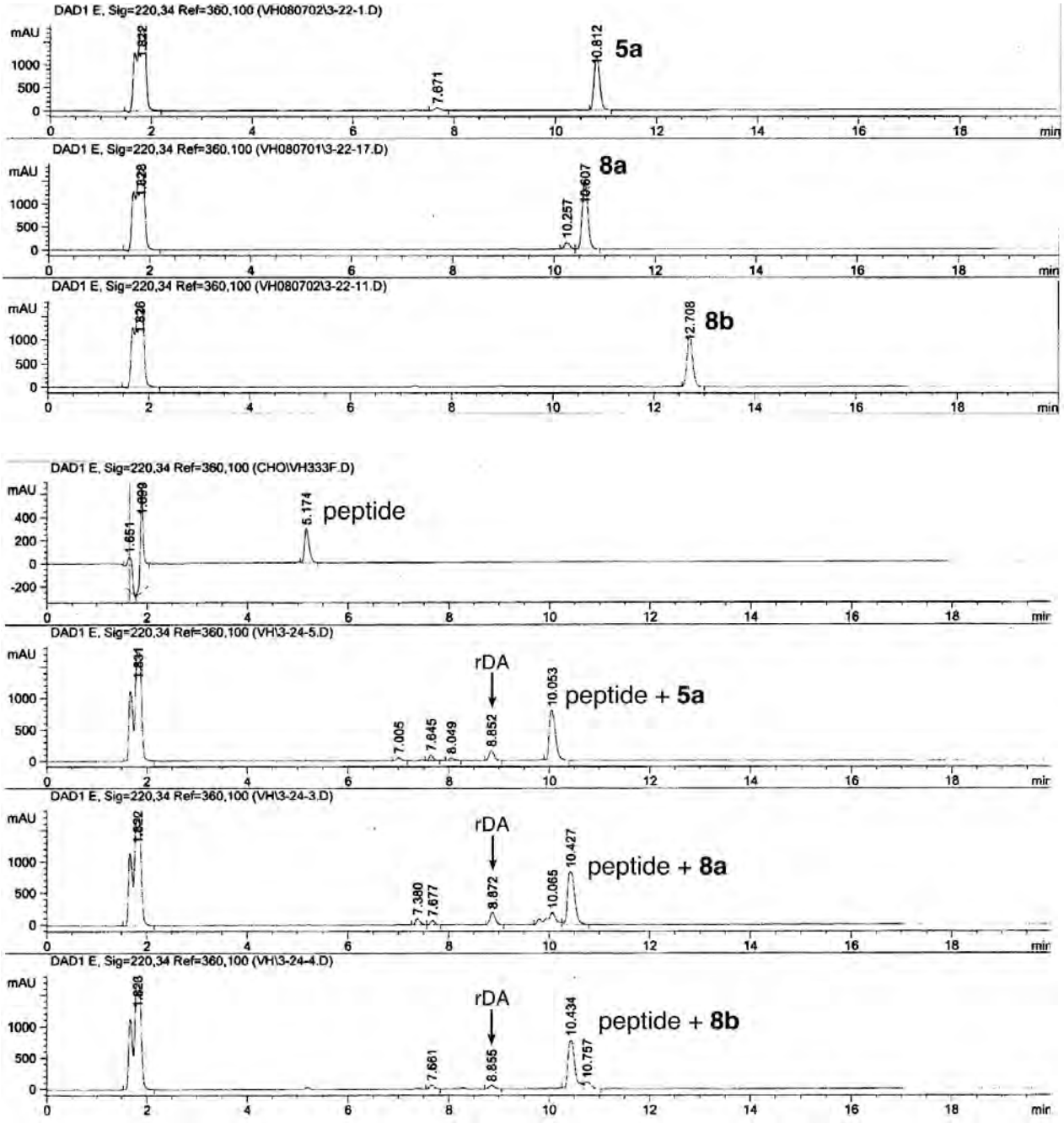
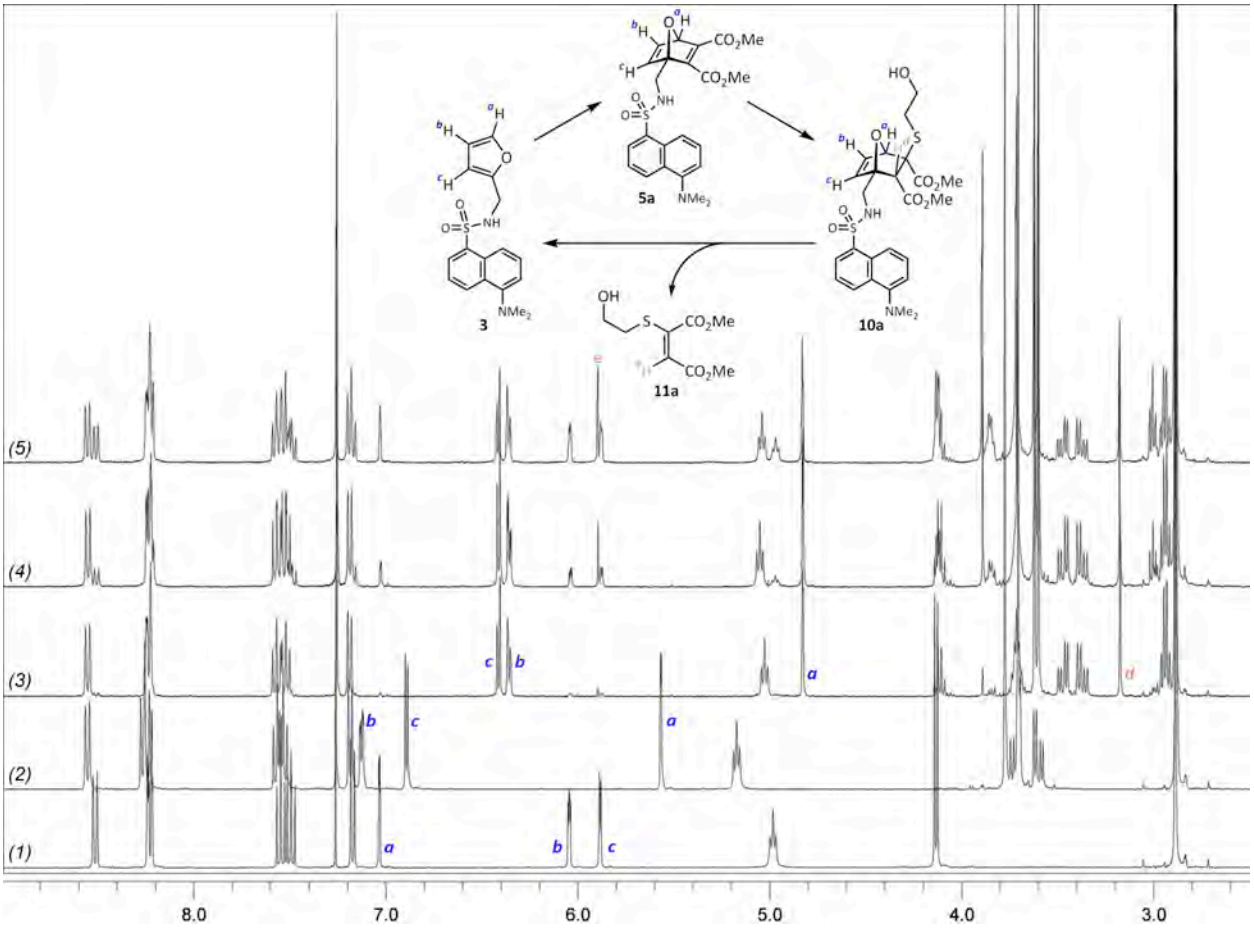


Figure S7. HPLC traces of the components and the reactions of peptide, **5a**, **8a**, and **8b** in 0.1 M phosphate buffer, pH 7.



Retro-Diels-Alder fragmentation

Figure S8. ^{1}H NMR spectra ($CDCl_3$, 400 MHz) of (1) **3**; (2) **5a**; (3) freshly purified **10a**; (4) **10a**, same sample after 5 days; (5) **10a**, same sample after 14 days.



Structural assignment for thiol adducts:

The formation of a single isomer in thiol additions to OND electrophiles was shown by the observation of clean NMR spectra reflecting a single species in high yield. The structure of that isomer was inferred from the following information. (1) The preference for *exo* attack of thiol is reported in references 23 and 24 (and is consistent with other addition reactions to oxanorbornadiene species). (2) The proposed regiochemistry (thiol attack away from the sulfonamidomethyl substituent) is supported by the lack of coupling between the proton a to the ester group and the bridgehead proton in structures such as **10a**, and with the sensitivity of addition rate to the installation of a bridgehead methyl group. (3) The *syn* nature of thiol addition has also been previously described (references 23 and 24) and is consistent with ring closure to imides such as **13**.