Supporting Information

Experimental Section.

General Procedures. Mitomycin C was received from Bristol-Mayer and mitomycin A was received from Kyowa Hokko Kogyo Co., Ltd., Tokyo, Japan. N-Ac-Cys, MER and DTT were from Sigma. LCMS was performed with a Hewlett Packard Series 1100 diode array HPLC system connected to a Hewlett Packard Series 1100 MSD mass spectrometer. HPLC was performed in a Beckman System Gold 125 instrument, equipped with a diode array detector System Gold 168, fitted with a Rainin C-18, 5 x 250 mm analytical column. Unless noted otherwise, elution system: 30% to 90% B in 30 min, 1 mL/min flowrate (A = 0.03 M K-phosphate, pH 5.5; B = 30% acetonitrile in A). A Dinamax semipreparative column was also used, employing analogous elution system.

2-amino-7-methoxymitosene (5b):

A solution of mitomycin A (1 mM) in 10 mL of 0,10 M NaH_2PO_4-Na_2HPO_4 buffer of pH 6.0 was admixed with 0.25 mL of a solution of DTT (50 mM) in 0,10 M NaH_2PO_4-Na_2HPO_4 buffer. The reaction was stirred for 24 h, and purified by HPLC. Buffer system: solvent A was 10 mM TEAA pH 6.8, solvent B was a mixture containing 60% CH_3CN and 40% buffer A. Linear gradient from 10%B to 50%B in 25 minutes (retention time 24 min).

UV (λmax) 228, 289, 355 nm. ESIMS (m/z): 259 (M—$^-OCONH_2$); 342 (M + Na^+); 358 (M + K^+); 421 (M + NEt_3H^+). ^{1}HNMR ($CDCl_3$): 1.95 (s, 3 H); 2.68 (dd, 1 H J = 4.40, 16.5 Hz); 3.22 (dd, 1 H, J = 6.9, 16.6 Hz); 3.93 (dd, 1 H, J = 4.5, 12.8 Hz); 4.00 (m, 1 H); 4.23-4.28 m, 3 H); 4.39 (dd, 1 H, J = 12.8, 6.6 Hz); 4.70 (brs, 2 H); 5.21 (s, 2 H).

2,7-diaminomitosene (5a) from 2-amino-7-methoxymitosene (5b)

A solution of **5a** (approx. 1 mM, 0.5 mL) was admixed with aqueous NH_3 (150 μL) and incubated for 2 h. at room temperature. After this period the reaction mixture was lyophylized, dissolved in 0.5 mL of tris buffer pH 7.5. and analyzed by HPLC, using the same buffer system described above (retention time 17 min). An authentic sample of 2,7-diaminomitosene was prepared as reported.[3]

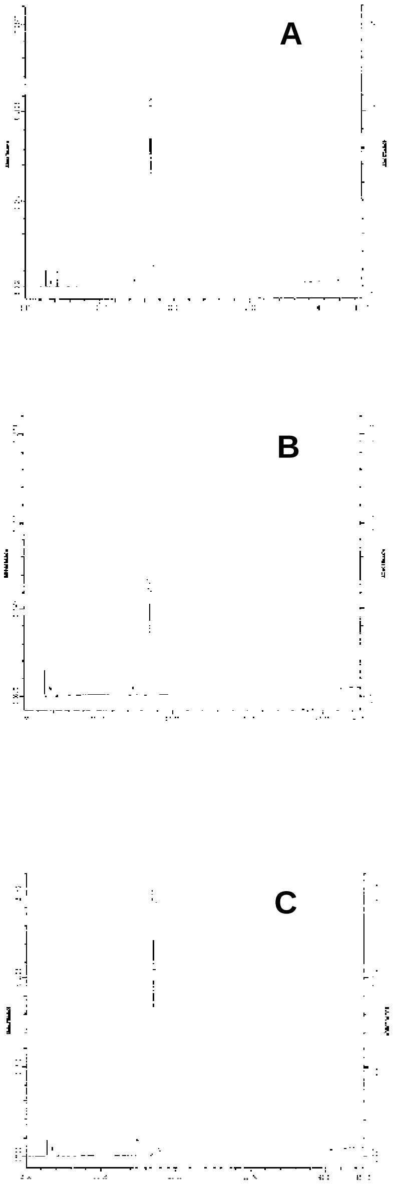
Authentic 1-α and 1-β-hydroxy-2-amino-7-methoxymitosene (6c) by the procedure of Taylor and Remers (Ref. 9).

A solution of 30 microgram of MA in 1 mL 0.05 M HCl was allowed to stand at room temperature for 18 h, then neutralized by dilution with 1 mL of 0.1 M tris buffer, pH 7.5. HPLC of the solution showed only two products (**6c** isomers) which were collected and their UV spectra were determined (λ_{max} (MeOH) 232, 285, 346 nm for both isomers). HPLC was performed as described in General Procedures. Retention times: 18.86 and 20.5 min. The particular 1-α or 1-β isomers were not specified.

Identity of the putative 6c formed in the reaction of MA with MER.

In a separate run using the same HPLC system two substances in the reaction mixture of 1 mM MA and 10 mM MER, with their MW corresponding to **6c**, had retention times 18.6 and 20.2 min, corresponding to the retention times of authentic **6c** within experimental error.

HPLC chromatograms showing the conversion of **5b** to **5a**:

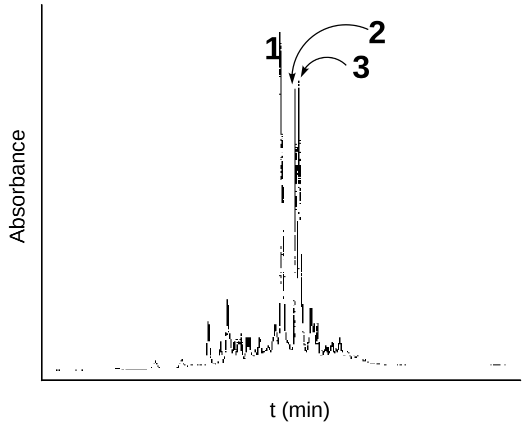


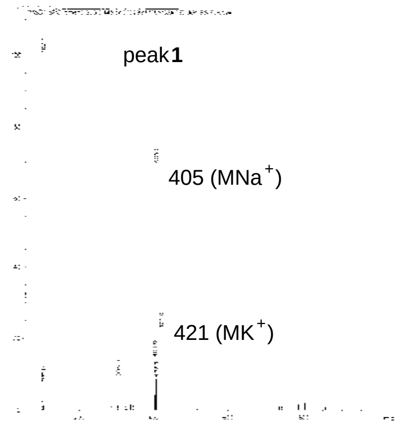
(A) Reaction of **5b** with aqueous ammonia.

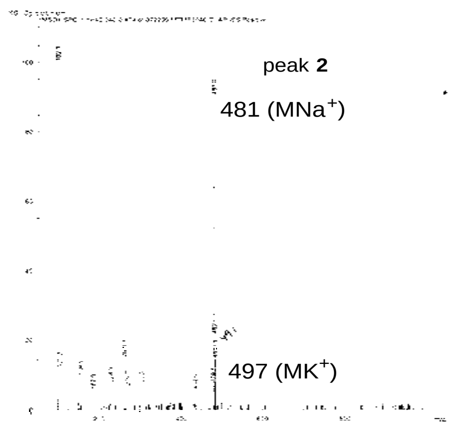
(B) Authentic sample of **5a** prepared by hydrogenation of MC.

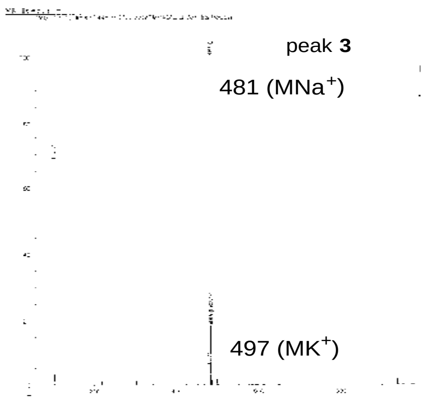
(C) Mixture of (A) and (B).

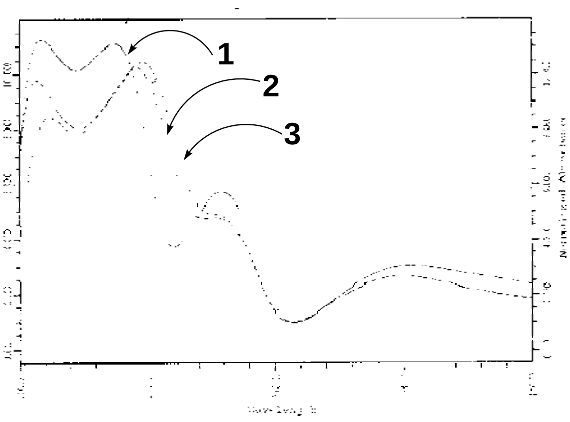
HPLC chromatogram showing the products of a reaction mixture containing 5 mM MA, 500 mM MER in 0.8 M fosfate buffer at pH 7.5, after 48 h of reaction. The ESIMS and UV of the three major products are also shown.

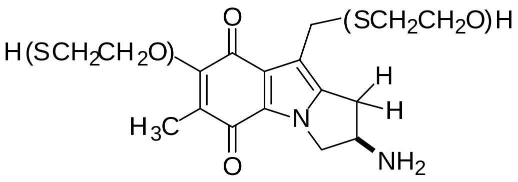




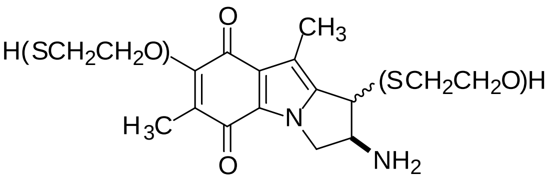




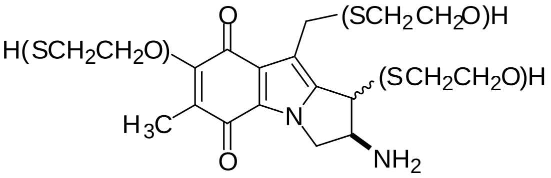




or

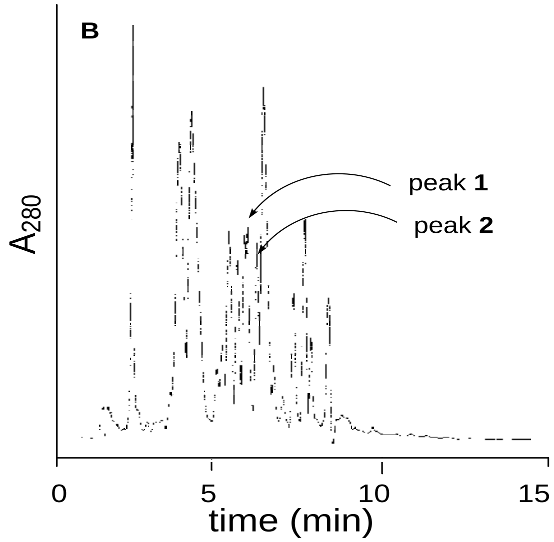


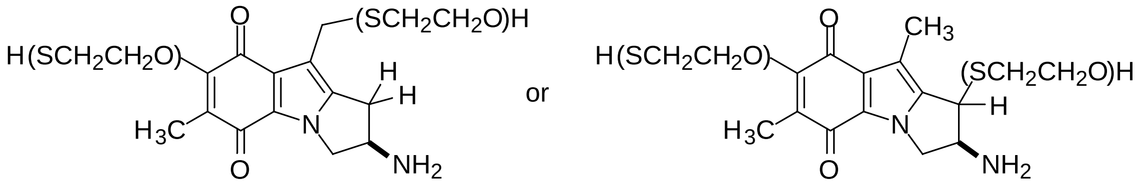
peak **1** (MW 382)



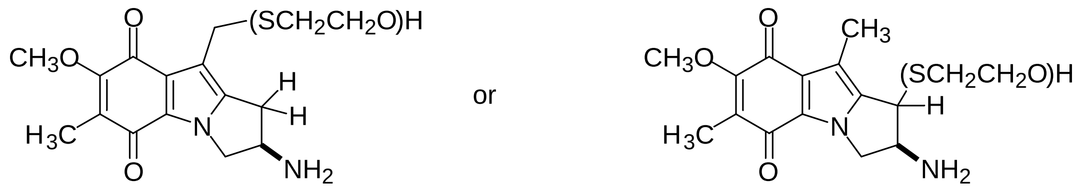
peaks **2,3** (MW 458)

ESIMS of two additional peaks from figure 2b.

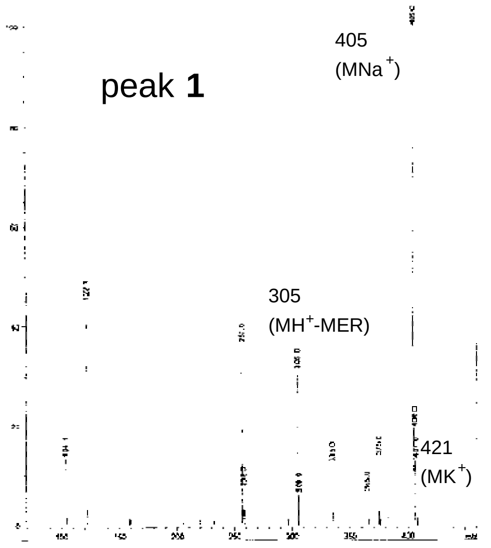


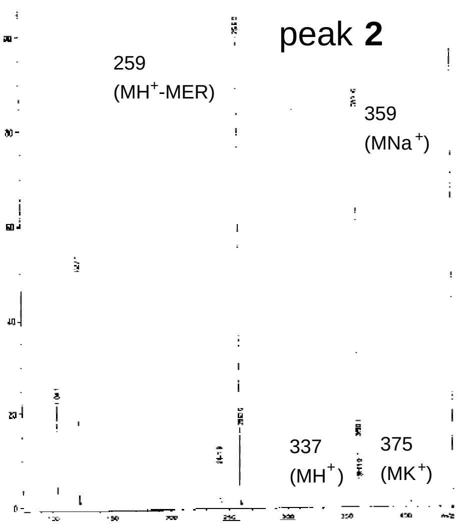


peak **1** (MW 382)



peak **2** (MW 336)





Experimental procedure for kinetics measurements of the reactions between MA and thiols. Determination of k_{obs}

A solution of mitomycin A (20 µM) in 0.95 mL of 0,20 M NaH_2PO_4-Na_2HPO_4 buffer of the desired pH was placed in a UV cubet and the UV absorbance was recorded. The solution was admixed with 50 µL of a solution of thiol (20 times the desired final concentration) in 0,20 M NaH_2PO_4-$Na_2HPO_{4\ o}$ of the desired pH and the absorbance at 317 nm was recorded at 5-second intervals. The molar fraction of mitomycin A at time t ($\chi_{MA(t)}$)was calculated from the absorbance readings using the following equation:

$$\chi_{MA(t)} = 1 - \left(\frac{A_0 - A_t}{A_0 - A_\infty}\right)$$

Where A_0 is the absorbance of the solution at 317 nm before the addition of thiol (corrected for the final volume). A_t is the absorbance of the solution at a time t after the addition of thiol. A_∞ is the absorbance of the solution after all the mitomycin A is consumed. The resulting data was fitted to a exponential curve, and the value of A_∞ was floated for best fitting. k_{obs} is the constant obtained from the fitting. ($\chi_{MA(t)} = e^{-k_{obs}\,t}$)

Postulated mechanism: The rate limiting step is intramolecular redox reaction of **9** (see text):

$$MA\text{-}RS^- \xrightarrow{k} MA_{red} + RS_{ox}$$

9

(MA-thiolate adduct as intermadiate)

and the corresponding rate-law should be

$$-\frac{d[MA]}{dt} = k[MA\text{-}RS^-]$$

Calculation of k from the experimental kinetic data provides a test for the postulated mechanism. If the experimental data fit this rate law, it supports the proposed mechanism. The calculations are as follows.

Derivation of k and k' based on the postulated mechanism:

$$RSH \overset{Ka}{\Leftrightarrow} MA\text{-}RS^- + H^+ \qquad K_a = \frac{[RS^-][H^+]}{[RSH]} \qquad \text{(eq. 1)}$$

$$RS^- + MA \overset{Keq}{\Leftrightarrow} MA\text{-}RS^- \qquad K_{eq} = \frac{[MA\text{-}RS^-]}{[MA][RS^-]} \qquad \text{(eq. 2)}$$

$$-\frac{d[\mathrm{MA}]}{dt} = k_{\mathrm{obs}}[\mathrm{MA}]$$ (eq. 3; observed)

$$-\ln \chi_{\mathrm{MA(t)}} = k_{\mathrm{obs}} t$$ (eq. 4)

$$-\frac{d[\mathrm{MA}]}{dt} = k[\mathrm{MA\text{-}RS^-}]$$ (eq. 5; postulated)

From eq. 2:

$$[\mathrm{MA\text{-}RS^-}] = K_{\mathrm{eq}}[\mathrm{MA}][\mathrm{RS^-}]$$ (eq. 6)

From eq. 5 and eq. 6:

$$-\frac{d[\mathrm{MA}]}{dt} = k\,K_{\mathrm{eq}}[\mathrm{MA}]\,[\mathrm{RS^-}]$$

$$-\int_{t=0}^{t} \frac{d[\mathrm{MA}]}{[\mathrm{MA}]} = k\,K_{\mathrm{eq}}\,[\mathrm{RS^-}]\,dt$$

Under the reaction conditions (buffered solution, large excess of thiol) the concentration of thiolate will remain constant, therefore:

$$-\ln \frac{[\mathrm{MA}]_t}{[\mathrm{MA}]_o} = k\,K_{\mathrm{eq}}[\mathrm{RS^-}]\,t$$

<u>Define k'</u>: k' = k K_{eq}, where K_{eq} is unknown, but assumed to be similar for all three thiols

$$-\ln \chi_{\mathrm{MA(t)}} = k'\,[\mathrm{RS^-}]\,t$$ (eq. 7)

From eq. 4 and eq. 7:

$$k_{\mathrm{obs}} t = k'\,[\mathrm{RS^-}]\,t$$

and

$$k' = \frac{k_{\mathrm{obs}}}{[\mathrm{RS^-}]}$$ (eq. 8)

From eq. 1:

$$[\mathrm{RS^-}] = \frac{K_a[\mathrm{RSH}]}{[\mathrm{H^+}]}$$

Furthermore:

$$[\mathrm{RSH}] = [\mathrm{RSH}]_0 - [\mathrm{RS^-}],$$

were $[\mathrm{RSH}]_0$ is the total concentration of thiol

Solving for [RS$^-$]:

$$[\mathrm{RS^-}] = \frac{K_a[\mathrm{RSH}]_0}{[\mathrm{H^+}] + K_a} \qquad \text{(eq. 9)}$$

Therefore the final expression for k' is (from eq. 8 and 9):

$$k' = \frac{k_{obs}([\mathrm{H^+}] + K_a)}{K_a[\mathrm{RSH}]_0} \qquad \text{(eq 10)}$$

Results of calculation of k' using the experimental parameters (Table 1): See Table 1.

Conclusion from the results (Table 1):

k' (and therefore k=k' K_{eq}) is constant under varying pH and thiol ($[\mathrm{RSH}]_0$) concentration conditions, consistent with the postulated mechanism (Schemes 3, 4).

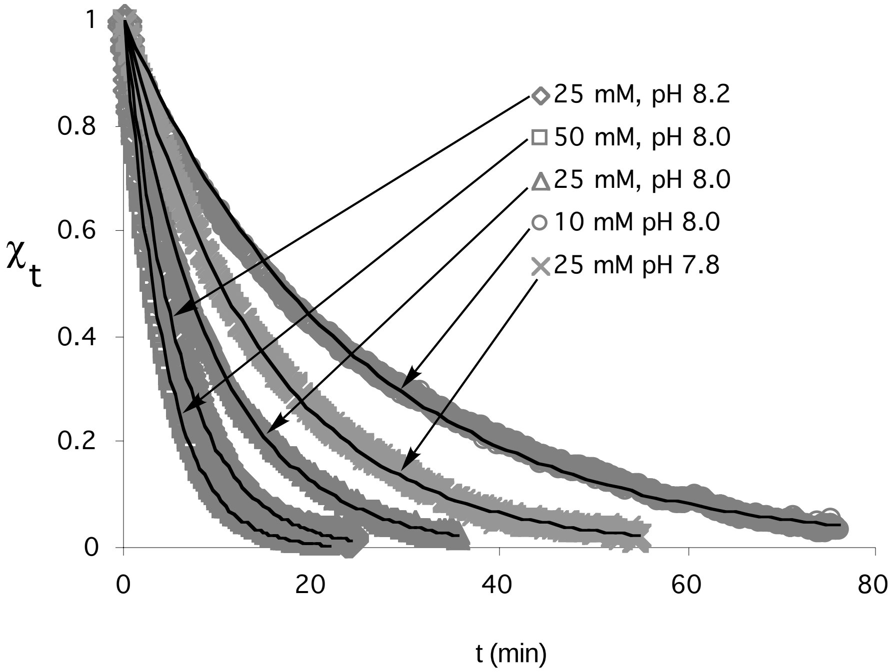
The pKa values used in the calculations were 9.72 for MER[a], 9.52 for N-acetylcysteine[a], and 9.2 for DTT.[b]

(a)Friedman, Mendel. The Chemistry and Biochemistry of de Sufhydryl Group in Amino Acids, Peptides and Proteins. 1st eddition. Pergamon Press, NY, 1973.

(b)Szajewski, R. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *102,* 2011-2016.

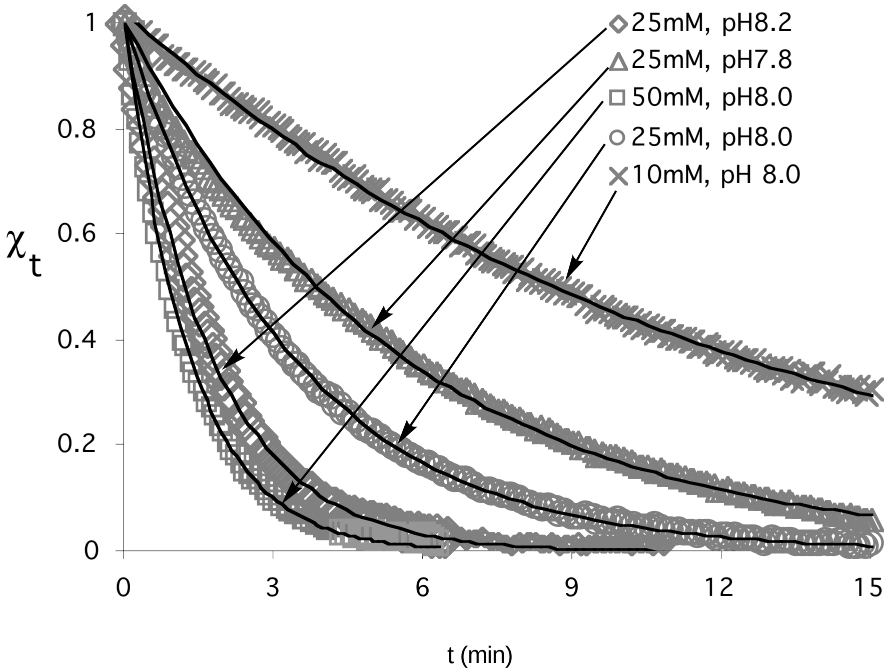
.

ACTIVATION WITH N-ACETYL CYSTEINE



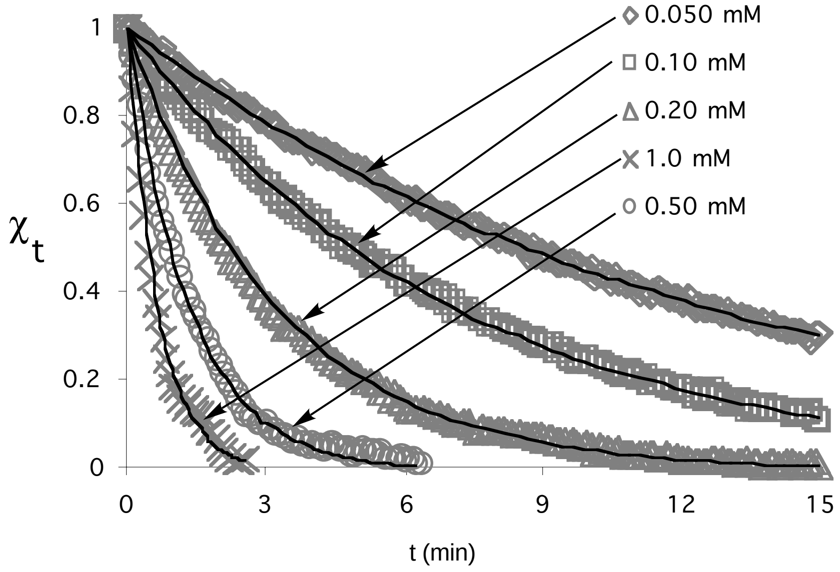
pH	Conc. RSH	R^2	k_{obs}	k'
7.8	25 mM	0.994	0.0675	144
8.0	10 mM	0.992	0.0412	140
8.0	25 mM	0.994	0.1309	178
8.0	50 mM	0.994	0.234	160
8.2	25 mM	0.994	0.1708	142

ACTIVATION WITH MERCAPTOETHANOL

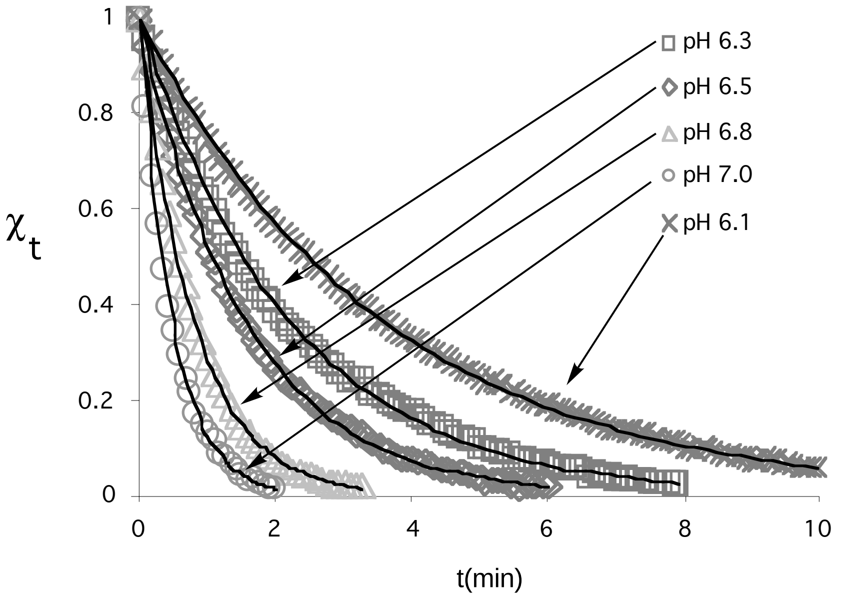


pH	Conc. RSH	R^2	k_{obs}	k'
7.8	25 mM	0.997	0.1785	600
8	10 mM	0.998	0.0804	429
8	25 mM	0.995	0.296	633
8	50 mM	0.998	0.764	817
8.2	25 mM	0.997	0.569	776

ACTIVATION WITH DITHIOTHREITOL



pH	Conc. RSH	R^2	k_{obs}	k'
6.1	0.05 mM	0.999	0.0803	$2.02{\bullet}10^{6}$
6.1	0.10 mM	0.999	0.1428	$1.80{\bullet}10^{6}$
6.1	0.20 mM	0.980	0.3111	$1.96{\bullet}10^{6}$
6.1	0.50 mM	0.997	0.7507	$1.89{\bullet}10^{6}$
6.1	1.00 mM	0.972	1.5376	$1.93{\bullet}10^{6}$



pH	Conc. RSH	R^2	k_{obs}	k'
6.1	0.2 mM	0.999	0.2804	$1.76{\bullet}10^{6}$
6.3	0.2 mM	0.998	0.4545	$1.80{\bullet}10^{6}$
6.5	0.2 mM	0.997	0.6487	$1.62{\bullet}10^{6}$
6.8	0.2 mM	0.998	1.2492	$1.56{\bullet}10^{6}$
7	0.2 mM	0.993	2.0758	$1.65{\bullet}10^{6}$