(H^+-K^+)-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulfinyl]benzimidazoles. 3.[1] Evidence for the Involvement of a Sulfenic Acid in Their Reactions

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The (H^+-K^+)-ATPase inhibiting sulfoxides **1a,b** undergo acid-induced transformations into the cyclic sulfenamides **2a,b** via the putative sulfenic acids **4a,b**. At high concentrations mixtures of **2a,b** and thiosulfinates **5a,b** were obtained. In contrast, **1c**, which cannot form **2**, is completely transformed under similar conditions into **5c**, the anhydride of **4c**. The temperature-dependent equilibrium between **2a** and **5a** and the kinetics of the ring opening of **2a** by water provide evidence for the sulfenic acid **4** as an intermediate involved in the pH-dependent transformations of **1**. The thiosulfinates **5** decompose mainly to the disulfides **6** at a rate depending on the solvent and conditions and are also reduced to **6** by thiols. The dimeric ylide **7a** could be isolated from **6a** (prepared in situ from **1a** as well as from **2a**). The symmetrical disulfides **6** and **7** are cleaved by thiols to yield the mixed disulfides **3** and the thiols **8**. Similarly, cleavage of **3** liberates an additional equivalent of **8**. Finally, the unstable thiols **8** are removed from the thiol–disulfide interchange equilibrium by irreversible rearrangement to the sulfides **9**.

Introduction

Recently, considerable attention has been focused on the synthesis of 2-[(2-pyridylmethyl)sulfinyl]benzimidazoles and their use as potential therapeutics for the treatment of peptic ulcer. We[1-3] and others[4,5] have studied their mode of action and ascribed their antisecretory activity to the irreversible inhibition of the gastric (H^+-K^+)-ATPase, the proton pumping enzyme present in the apical membrane of the parietal cell. It has been shown that the inhibited state of the enzyme can be represented by the disulfide **3** (Scheme I, R = enzyme).[2]

In the preceding paper[1] we discussed the acid-induced transformation of **1a,b** into the sensitive sulfenamides **2a,b** via the unstable sulfenic acids **4a,b**. Using MeOH as solvent and HBF_4 as the acid, we were able to isolate the cyclic compounds, the structures of which were confirmed by an X-ray analysis of **2**. We also described the reactions of **2** with thiols giving rise to the formation of the disulfides **3**. The structural prerequisites for **1** giving **2** have also been reported. During these studies, we found the formation of **2** from **1**, and its stability, to be extremely dependent on the concentration of the solutions studied.

This paper presents details of the further reactions of **2** that limit the lifetime of the cyclic sulfenamide and further implicates the involvement of the sulfenic acid **4** in the formation of **5** and **6/7**. Finally, we report on the reactions of these dimerization products with thiols under different conditions.

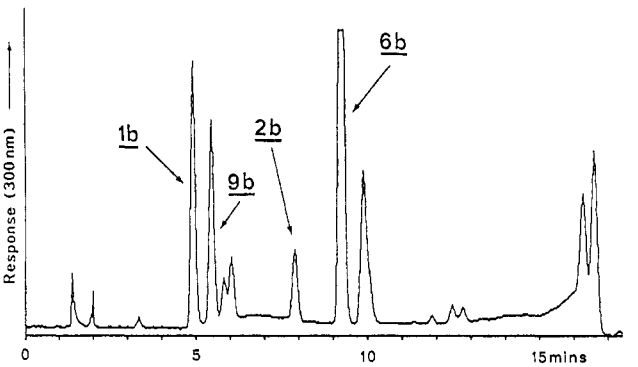

Figure 1. A typical HPLC chromatogram following partial reaction of **1b** in 0.1 M HCl.

Results and Discussion

I. Formation and Structure of 6a,b/7a,b. In the preceding paper[1] we described the formation of **2a,b** by the treatment of **1a,b** with acid in both methanolic and aqueous solution. The formation of **2a,b** can easily be followed by ^1H NMR spectroscopy since proton H-1 in **2a,b**, is extremly deshielded ($\delta > 9.5$ ppm), relative to the corresponding former pyridine proton H-6′ in **1a,b** ($\delta \sim 8.1$ ppm).

Using a methanolic solution of **1a,b** and 50% aqueous HBF_4, we were able to obtain analytically pure sulfenamides **2a,b** on a preparative scale since **2a,b**·BF_4 precipitated readily. During an attempt to obtain a ^{13}C NMR spectrum from a more highly concentrated solution of isolated **2a** in acidified CD_3OD at room temperature, we observed the formation of a new compound, **6a**. Similarly, following the reaction of **1b** in D_2O (pH = 3.1) by ^1H NMR, the new product **6b** was also observed.

By following the acid-catalyzed reactions of **1b** in aqueous solution by HPLC (Figure 1), the formation of the new compound **6b** could also be demonstrated. Furthermore, we found that the formation of **6b** was highly dependent on the initial concentration of **1b** used. Thus, whereas at low concentration the formation of the sulfenamide **2b** was observed, with increasing concentration

(1) Part 2: Senn-Bilfinger, J.; Krüger, U.; Sturm, E.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J.; Huttner, G.; Zsolnai, L. *J. Org. Chem.* **1987**, *52*, 4582.
(2) Part 1: Sturm, E.; Krüger, U.; Senn-Bilfinger, J.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J.; Huttner, G.; Zsolnai, L. *J. Org. Chem.* **1987**, *52*, 4573.
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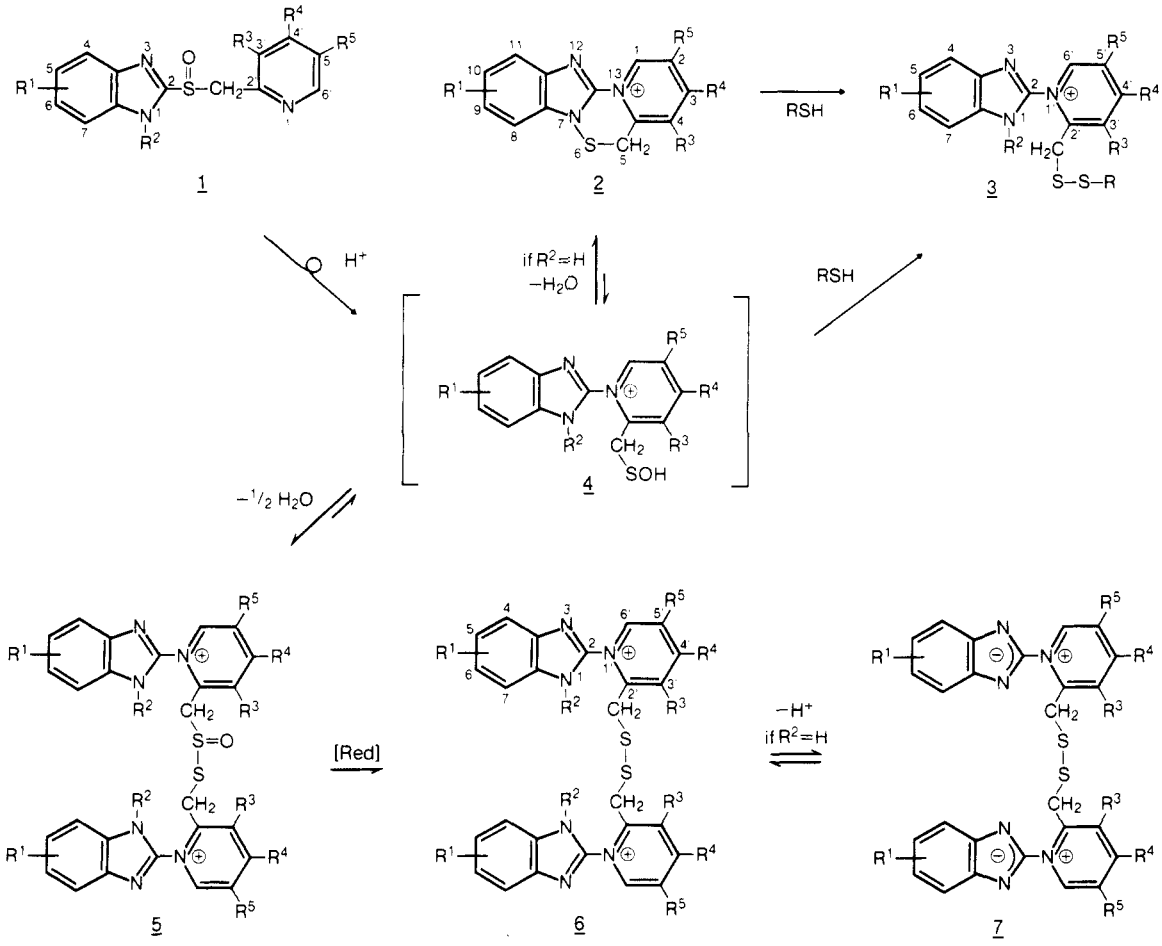
(4) Lindberg, P.; Nordberg, P.; Alminger, T.; Brändström, A.; Wallmark, B. *J. Med. Chem.* **1986**, *29*, 1327-1329.
(5) Nagaya, H.; Satoh, H.; Kubo, K.; Maki, Y. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 799.

Scheme I[a]



[a] **1a**, R^1 = 5-CF_3, R^2 = R^5 = H, R^3 = CH_3, R^4 = OCH_3; **1b**, R^1 = 5-OCH_3, R^2 = H, R^3 = R^5 = CH_3, R^4 = OCH_3; **1c**, R^1 = 6-CF_3, R^2 = R^3 = CH_3, R^4 = OCH_3, R^5 = H.

the formation of **6b** became dominant (see Experimental Section). **6b** was isolated by preparative HPLC and shown to be identical with the product observed in the NMR experiments.

6a was similarly formed on a preparative scale. In this case, careful neutralization of the acidic reaction mixture with aqueous $NaHCO_3$ gave a precipitate of a new compound to which we assigned the dimeric ylide structure **7a**.

II. Elucidation of the Structure of 6/7. The assignment of the symmetrical disulfide structure to **6/7** is based largely on the spectral similarities between **6/7** and the corresponding mercaptoethanol adduct **3** (R = CH_2CH_2OH), the structure of which has been unambiguously determined.[2] From a comparison of the ^1H and ^{13}C NMR spectra of **3a**[2] and **6a/7a**, it is initially apparent that **6a/7a** contains the pyridiniobenzimidazole backbone present in **3**. Analysis of **7a** and **7b** using discharge ionization secondary ion mass spectrometry in the positive ion mode [DISIMS (+)] yielded the molecular weights of *m/z* 705 and *m/z* 657, respectively, clearly indicating the dimeric nature of these compounds. Since only one set of signals is observed in the NMR spectrum and mass spectroscopy suggests a dimer, only a symmetrical structure is possible.

The ease of deprotonation of **6** to give **7** is explained by the acidifying influence of the positively charged pyridine moiety attached to the benzimidazole 2-position and adds further support for the proposed close relationship of **6/7** to **3** and the deprotonated form of **3**,[2] respectively. In both sets of compounds similar chemical shift differences are involved in the deprotonation reaction.

III. Reactions Involved in the Formation of 6. The overall reaction of **1a/b** to **6a/b** in acidified concentrated aqueous solutions or in acidified MeOH at room temperature is accompanied by the formation of a variety of byproducts (up to 30%) that have not been identified. These byproducts must account for the loss of the oxidation equivalents during the transformation of **1a/b** to **6a/b**. The same holds true for this reaction in Me_2SO-d_6, although the reaction is cleaner (**6a**, 85% yield). As expected for a dimerization reaction, the reaction of **1a/b** to **6a/b** is forced toward **6a/b** by high concentrations of **1a/b**. Detailed ^1H NMR spectroscopic studies varying the concentration and the reaction temperature were performed with **2a**, generated in situ from **1a**, to gain more insight into this complicated reaction, which consists of both a dimerization and a reduction.

During ^1H NMR studies using highly concentrated solutions of **1a** in CD_3OD acidified by DCl, we were able to detect the intermediate **5a**. The time courses, the composition, and the ratio **5a/2a** of these mixtures are given in Table I. As can be concluded from Table I there is a temperature-dependent equilibrium between **2a** and **5a** favoring **5a** at low temperature.

From the ^1H NMR spectrum of the cooled solution, we assigned the thiosulfinate structure to the intermediate **5a** (Scheme I). The spectrum of **5a** shows two sets of signals for both the benzimidazole and the pyridine part. The shifts of one set are virtually identical with those of

Scheme II

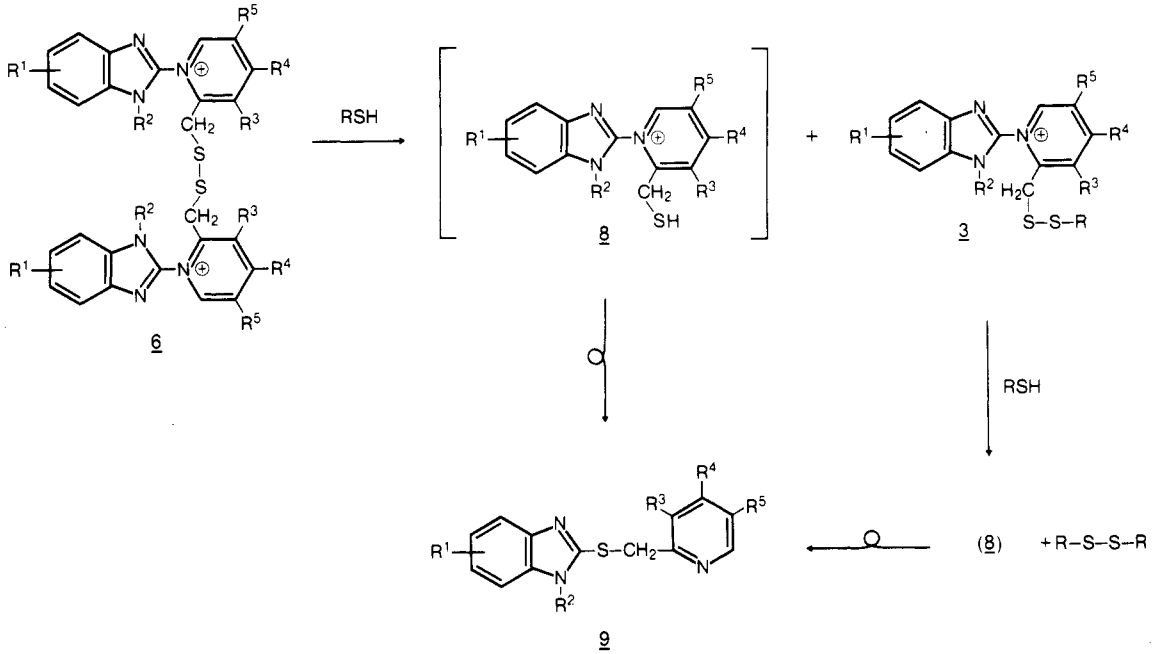


Table I. Distribution of Products[a] Generated in Situ from 1a in CD_3OD/DCl

temp, °C	time, min	mol % of products 2a	5a	6a	other	ratio 5a/2a
20[b]	3	46.8	34.5	11.2	7.7	0.73
	6	46.1	34.2	11.6	8.0	0.74
	10	41.6	28.5	17.9	12.1	0.69
	20	34.6	22.6	26.5	16.3	0.65
	40	24.3	12.9	37.5	25.3	0.53
-45[c,d]	2700	28.4	65.9		5.7	2.32
20[e]	2	41.9	34.3	13.8	10.0	0.82
	6	38.7	30.2	18.6	12.5	0.78
	10	36.0	26.6	21.1	16.3	0.74
	20	27.5	16.9	31.2	24.4	0.61
	40	18.7	10.3	38.5	32.5	0.55

[a] Calculated from the integrals of the well-separated ^1H NMR signals for the pyridinium 3-methyl group at δ 2.56, 2.43, and 2.36 in **2a**, **5a**, and **6a**, respectively. [b] Solution of **1** prepared at 20 °C. [c] Solution of **1a** prepared at -60 °C and measured at -45 °C after storage between -60 °C and -45 °C for 45 h. [d] Separation of signals at -45 °C was not as good as at 20 °C; for this reason the value for **5a** may include a small amount of **6a**. [e] Measured at 20 °C after warming up the solution previously equilibrated at -45 °C.

6a, whereas the shifts of the second set are slightly different. Furthermore, an AB system due to diastereotopic splitting of the 2′-methylene protons adjacent to the sulfoxide in **5a** was observed in addition to a singlet assigned to the protons of the 2′-CH_2S group.

Acid treatment of the N-methylated sulfoxide **1c**, which cannot form the cyclic sulfenamide **2**, in CD_3OD acidified with DCl gives exclusively and rapidly **5c**. Due to the sensitive nature of this compound, we were unable to isolate it. In a consecutive reduction, the corresponding symmetrical disulfide **6c** is formed as the main product.

According to Scheme I, the presence of water is a prerequisite for the equilibrium between **2** and **5** via the putative sulfenic acid **4**, which is formed by nucleophilic attack of water on the sulfur atom and cleavage of the weak S-6-N-7 bond. Therefore, we performed ^1H NMR investigations at room temperature, using isolated **2a** in solutions of acetonitrile-d_3 containing a small amount of water. Since **5a** undergoes further reactions at room temperature, we could only observe the water-induced decay of **2a**. This decay obeys a rate law that is second order in **2a** and first order in water[6] (Table II).

The kinetic data observed for the initial period of the reaction are in accord with a pre-equilibrium followed by a rate-determining coupling. They suggest that the sulfenic acid **4** present at low steady-state concentration adds to residual sulfenamide **2** to give **5**. Consistent with this

$$\mathbf{2a,b} + H_2O \rightleftharpoons \mathbf{4a,b}$$

$$\mathbf{4a,b} + \mathbf{2a,b} \rightarrow \mathbf{5a,b}$$

complex reaction a (pseudo) second-order decay of **2a** was observed in aqueous solution at low pH by monitoring the time-dependent changes in the UV spectra.[7] The reaction rate increases by approximately a factor of 10 when the pH is raised from 2 to 3 and, at pH 4, **2a** disappeared immediately during preparation of the solution. This pH dependency may be explained in terms of a deprotonation equilibrium generating the ylide form of **4**[9] as an intermediate involved in the coupling reaction.[10]

The pH and concentration dependent decay of **2** provides a clear answer to why, even in acidic aqueous solution, at concentrations below 10^{-6} mol/L, the elusive compounds **2** are apparently stable. For these reasons it was possible to isolate **2b** by HPLC techniques using an acidic eluent (pH 2.1). Once isolated in acidic solution, **2b** showed no significant decomposition at low concentration over a period of 10 h. The relative stability of the sulfenamide under these conditions is of some relevance, since this species has previously been identified[1,4] as the

(6) The corresponding rate expressions for reactions second order in **2a** and zero or second order in water do not fit the data.

(7) Under aqueous conditions the alternative condensation of two sulfenic acids **4** as the usual route for their stabilization[8] cannot be ruled out.

(8) Davis, F. A.; Jenkins, L. A.; Billmers, R. L. *J. Org. Chem.* **1986**, *51*, 1033.

(9) The corresponding pK_a of disulfide **3** (R = CH_2CH_2OH, $R^1 = CF_3$, $R^2 = R^3 = R^5 = H$, $R^4 = OCH_3$) was found to be 5.7.[2] A similar value is expected for **4**.

(10) The addition of the neutral ylide form of **4** to positively charged **2** is expected to be more favorable than the reaction between the two cations **2** and **4**.

Table II. Kinetics of the Decomposition of 2a in Acetonitrile-d_3 Containing 0.044 mol/L of H_2O

time, h	2a, mol %	5a,[a] mol %	[2a]	k,[b] L^2 mol^{-2} h^{-1}
0	96.4	0.9	0.220	
0.5	95.6	1.8	0.218	(0.192)
17	86.2	8.9	0.197	0.83
24	82.7	9.3	0.189	0.90
48	76.5	15.3	0.174	0.83
72	73.2	19.3	0.167	0.73
146	64.7	24.7	0.148	0.80

[a] Expressed in mole percentages of 2a incorporated in 5a. [b] Linear regression: $k = 0.78$ L^2 mol^{-2} h^{-1}; $n = 6$; $r^2 = 0.996$.

principal biologically active component of the acid-activated sulfoxides.

IV. Reactions of 5 and 6/7 with Thiols. In the preceding paper,[1] we discussed the reaction of thiols with the sulfenamides **2** and the putative sulfenic acids **4** in the context of the covalent inhibition of the thiol-bearing gastric (H^+–K^+)-ATPase. Similarly, we were interested in the thiol reactions of **5** and **6** that could also, in principle, give compounds **3** (Schemes I and II). The reaction of the thiosulfinates **5** with 2-mercaptoethanol and DL-dithiothreitol (DTT) was studied using the N-methylated derivative **5c** since it is not involved in an equilibrium with the cyclic form **2** and it can be obtained easily in situ by simple treatment of **1c** with acids.

In acidic solution we found that, with both thiol reagents, **5c** is reduced to the symmetrical disulfide **6c** without formation of the unsymmetrical disulfides **3c**. The formation of **6c** is in contrast to the formation of **3c** previously discussed, when **1c** was treated with acid in presence of thiols. In the latter case, the initially generated sulfenic acid **4c** is presumably quenched by thiols to give **3c** more rapidly than dimerization to **5c** can occur.

The reaction of the symmetrical disulfides **6/7** with thiols resembles the cleavage of the unsymmetrical disulfides **3** (R = CH_2CH_2OH).[2] This reaction proved to be base catalyzed (deprotonation of RSH), yielding, after nucleophilic attack by thiolate anion on one sulfur of the disulfide bridge, the putative thiol **8**, which immediately rearranged to give the sulfide **9** (Scheme II). Under strongly acidic conditions in CD_3OD/DCl (pH = 1.0) the disulfides **6** were stable for more than 46 h in the presence of thiols, such as 2-mercaptoethanol, glutathione, or DTT, as seen in ^1H NMR experiments. At pH = 3 or above, the disulfides **6** react with an excess of thiols to form the sulfides **9** and the oxidation product of the thiol. Under these conditions, only trace amounts of the intermediate **3** were detected. The overall reaction, described by the equation **6** + $2R'S^- \rightarrow R'SSR' + 2$ **9**, is surely more complicated as can be seen from experiments using less than stoichiometrically equivalent amounts of thiol. In this case, increasing amounts of unsymmetrical disulfides **3**, in addition to the final products **9**, appear in the reaction mixtures (Table III). Theoretically, one would expect a rapid thiol–disulfide interchange equilibrium. However, the reaction is driven completely to the right by irreversible rearrangement of the thiol intermediate **8** to the sulfide **9**. The reduction of the isolated derivative **7a** with DTT was monitored quantitatively by ^1H NMR and the stoichiometry was found to be in the range of 1:1 for the reaction components.

Conclusion

In the preceding paper we concluded that the sulfenamide **2** is the long-lived "active principle" of the highly gastric (H^+–K^+)-ATPase inhibitory precursor sulfoxides **1a,b**. In this paper we have presented evidence that the "dimeric" products **5** and **6** are generated only at high initial concentration of **1** and, therefore, are not likely to be formed under physiological conditions occuring in the acidic lumenal part of mammalian parietal cells (pH 0.8). The N-1-methylated sulfoxide **1c** is easily transformed to **5c** and **6c** under acidic condition but is unable to form **2**. This compound was found to be almost inactive in in vivo models; these new results support the conclusion published in our previous paper.[1]

Table III. Product Composition from the Cleavage Reaction of 6a[a] with 2-Mercaptoethanol[b]

	composition of reaction mixtures,[c] mol %		
time, min	6a	3a	9a
3	62.4	3.5	34.2
10	22.0	29.5	48.4
30	7.5	31.0	61.6

[a] 1.5×10^{-5} mol of **7a** dissolved in 1 mL of CD_3OD containing 80 μL of D_3PO_4/Na_2DPO_4-buffer (pH 5.7). [b] Molar ratio **7a**/2-mercaptoethanol 1:1.3. [c] Data extracted from ^1H NMR spectra.

Experimental Section

Melting points are uncorrected and were determined with a Büchi 510 apparatus (heating rate 3 °C/min). ^1H and ^{13}C NMR spectra were recorded at 200.13 MHz and 50.32 MHz, respectively. A Finnigan 4610 mass spectrometer interfaced to an Incos 2300 data system was used to obtain positive ion DISIMS (discharge ionization secondary ion mass spectrometry) spectra. The sample matrix employed was 3-nitrobenzyl alcohol. The bombarding gas was xenon, and the system was operated using a discharge potential of 3.5 kV. The sulfoxides **1a**[11] and **1b**[12] were prepared according to literature methods. The synthesis of the sulfoxide **1c** and of the disulfides **3** has been reported in ref 2 and the synthesis of **2a,b** in ref 1.

HPLC Monitoring of the Acid-Induced Decomposition of 1b. Solutions of **1b** were prepared in 10-mL amber volumetric flasks. T_0 was taken as the time of addition of acid or buffer solution. The contents of the flask were shaken and then sonicated for 2 min. All decomposition studies were performed at room temperature. At time intervals, 20-μL aliquots were subjected to high performance liquid chromatography (HPLC) using the following conditions: column, μ-Bondapak phenyl; eluent A, acetonitrile (HPLC grade); eluent B, 0.15 M aqueous H_3PO_4 adjusted to pH 2.1 with 10 M KOH; gradient 20 → 50% A over 15 min, held at 50% A for 2.5 min; equilibration time, 5 min; column temperature, 40 °C; flow rate, 2 mL min^{-1}; detection, UV at 300 nm and also by multiwavelength diode array detector (DAD); integration, Perkin-Elmer LIMS/CLAS 2000 instrument.

With use of [^{14}C]-**1b**, radiolabeled at either the benzimidazole C-2 position or at the methylene carbon,[13] response factors at 300 nm could be obtained for each of the peaks in the chromatogram. Normalized to the sulfide **9b**, these were **1b**, 0.61; **2b**, 1.55; and **6b**, 0.71. Peak areas observed after 10-min degradations of **1b** in 0.1 M HCl were multiplied by these factors to obtain the ratios of **2b**/**6b** which are given for the following initial concentrations of **1b**: 0.02 mM, 1/0.017; 0.2 mM, 1/0.29; 2 mM, 1/4.3; and 20 mM, 1/11.

Isolation of 2b and 6b by HPLC; MS Characterization of 6b. **6b** was isolated by semipreparative HPLC from a solution of **1b** (10 mg mL^{-1}) in pH 2.1 buffer at room temperature, which had been allowed to react for at least 30 min; 50-μL aliquots of this solution were chromatographed using the instrumental conditions described above. The peak identified as **6b** by its retention time and its DAD-UV spectrum was collected and pooled with subsequent isolations. This pooled solution was freeze-dried

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(12) Junggren, U. K.; Sjöstrand, S. E. Eur. Pat. 005129, 1979.
(13) Crowe, A. M.; Ife, R. J.; Mitchell, M. B.; Saunders, D. *J. Labelled Compd. Radiopharm.* **1986**, *23*, 21.

to remove water and acetonitrile. For mass spectrometry isolated **6b** was rechromatographed with dilute hydrochloric acid (pH 2.1) replacing the aqueous phosphoric acid in the semipreparative HPLC system. **6b** was again collected as it eluted from the column and freeze-dried to remove water and acetonitrile. MS [DISIMS (+)]: m/z 657 (6, MH^+), 361 (12), 330 (100), 329 (32), 328 (47), 298 (91), 297 (40), 296 (32), 282 (28), 273 (63), 192 (29).

2b was isolated from 0.2 mg mL^{-1} solutions of **1b** using an identical procedure to that described above for **6b**. The spectroscopic data are identical with those reported in the preceding paper.[1]

Decomposition of 3-Methoxy-4-methyl-9(10)-(trifluoromethyl)-5*H*-pyrido[1′,2′:4,5][1,2,4]thiadiazino[2,3-*a*]benzimidazol-13-ium Tetrafluoroborate (2a·BF_4^-).[14] (a) ^{1}H NMR Monitored Decay of 2a in Acetonitrile. **2a** dissolved in acetonitrile-d_3 (0.22 mol/L) decomposes in a slow and unclean reaction into a mixture of products consisting mainly of **5a**. Residual water in the initial solution was determined by ^{1}H NMR to be 0.044 mol/L. The decrease of **2a** and the associated increase of **5a** was easily observed from the shifts of the well-separated NMR signals of the pyridinium part of the molecule (9.40 (H-1) and 2.45 ppm (4-CH_3) in **2a**; 8.65 (H-6′) and 2.30 ppm (3′-CH_3) in **5a**, respectively). The composition of the mixture consisting of **2a**, **5a**, and unknown byproducts is given as a function of time in mol % units of compound **2a** in Table II. The decrease of **2** can be described as a reaction that is second order in **2a** and first order in H_2O by the equation

$$\frac{2}{(2[H_2O]_0 - [2a]_0)}\left[\frac{1}{[2a]} - \frac{1}{[2a]_0} + \frac{1}{(2[H_2O]_0 - [2a]_0)} \ln \frac{2[H_2O]_0[2a]}{[2a]_0(2[H_2O]_0 - [2a]_0 + [2a])}\right] = kt$$

(b) Decay of 2a in Aqueous Medium Monitored by UV. The decay of **2a** in aqueous acidic medium (10^{-2} M KH_2PO_4 acidified with H_3PO_4 to pH 2 or 3) is associated with pronounced UV spectral changes. A long wavelength band at 319 nm, characteristic of **2a**, disappears and a new band at 280 nm appears. The concentration of **2a** was estimated from the absorbance values at 319 nm and 280 nm using the extinction coefficients of **2a** (ϵ_{319} = 16 700, ϵ_{280} = 7600) determined from the initial spectrum in methanol/HCl and of the ring-opened products (ϵ_{319} = 4000, ϵ_{280} = 16 000) taken from the final spectrum. A straight line is obtained by plotting $1/[2a]$ versus t where the slope represents the rate constant k' of the decay rate expression $-d[2a]/dt = k'[2a]$.[2] The following k' values are obtained: at pH 2.0, $[2a]_0$ = 3, 10, 30, 60 μmol L^{-1}, 17, 21, 23, 21 L mol^{-1} s^{-1}; at pH 3.0, $[2a]_0$ = 3 μmol L^{-1}, 280 L mol^{-1} s^{-1}.

1,4-Bis[1-[5-(trifluoromethyl)benzimidazol-2-yl]-4-methoxy-3-methylpyridinium-2-yl]-2,3-dithiabutane 2-Oxide (5a). ^{1}H NMR Study of the Equilibrium between 2a and 5a. Dissolution of **1a** (100 mg) in CD_3OD (0.8 mL) and 20% DCl/D_2O (0.2 mL) at room temperature gave the mixture of compounds listed in Table I. In a similar set of experiments, the solution of **1a** was cooled immediately to −60 °C and kept for 45 h between −60 °C and −45 °C. Subsequently the solution was rewarmed to room temperature. ^{1}H NMR of **5a** (CD_3OD/DCl, from 10 mg of **1a** per mL of CD_3OD containing 10 μL of 20% DCl/D_2O per mL of CD_3OD, only the related signals for both pyridine rings are listed): δ 9.19 and 9.10 (2 H, H-6′), 7.80 (2 H, H-5′), 5.15 and 4.85 (AB and s, respectively, 4 H, 2′-CH_2), 4.39 (6 H, 4′-OCH_3), 2.44 and 2.42 (6 H, 3′-CH_3).

Generation of 1,4-Bis[1-[1-methyl-6-(trifluoromethyl)-benzimidazol-2-yl]-4-methoxy-3-methylpyridinium-2-yl]-2,3-dithiabutane 2-Oxide (5c) in the NMR Tube. **1c** (20 mg) was dissolved in CD_3OD (0.98 mL) and 20% DCl/D_2O (20 μL) at room temperature. After 2.6 min, about half of the initial **1c** was converted to **5c**. **5c** decays in the methanolic solution with $t_{1/2}$ approximately 40 min mainly into **6c** and some unknown products. ^{1}H NMR (CD_3OD/DCl, from 10 mg of **1c** per mL of CD_3OD containing 10 μL of 20% DCl/D_2O per mL of CD_3OD, taken 8 min after dissolution, only the related signals for both pyridine rings are listed): δ 9.20 and 9.13 (2 H, H-6′), 7.92 and 7.89 (2 H, H-5′), 5.08 and 4.69 (AB and s, respectively, 4 H, 2′-CH_2), 4.39 and 4.38 (6 H, 4′-OCH_3), 2.45 (6 H, 3′-CH_3).

Reaction of 5c with 2-Mercaptoethanol and DL-DTT in the NMR Tube. To a solution of **5c** in acidic CD_3OD, generated as described above, was added an excess of 2-mercaptoethanol (12.2 mg/mL, molar ratio 1:3) or of DL-DTT (8 mg/mL, molar ratio 1:2). **5c** was reduced by the thiols to the symmetrical disulfide **6c**, which could be followed by characteristic changes in the ^{1}H NMR spectrum ($t_{1/2}$ approximately 3 min for 2-mercaptoethanol; DL-DTT showed a comparable reaction rate). Under these conditions **6c** was stable for more than 48 h. The sulfide **9c**, which is the final product under less acidic conditions (pH > 3.0), was not detected.

1,4-Bis[1-[5-(trifluoromethyl)benzimidazol-2-yl]-4-methoxy-3-methylpyridinium-2-yl]-2,3-dithiabutane (7a). (a) From 1a in MeOH/HCl Solution. **1a** (1.0 g, 2.7 mmol) was dissolved in a mixture of MeOH (10 mL) and 20% aqueous HCl (1 mL) and kept for 2 h at room temperature. After evaporation of the solvents under reduced pressure (water bath 30 °C), the resulting semisolid residue was dissolved in ice water (200 mL), the pH of the solution was adjusted to 6.5 by addition of a saturated aqueous $NaHCO_3$ solution, and the precipitate was filtered off and dried in vacuo (30 h, 35 °C). Yield, 0.3 g of **7a** (30%); mp 139 °C dec. IR (KBr): 3420, 1620 cm^{-1}. ^{1}H NMR (CD_3CN): δ 8.92 (d, 1 H, $J_{6',5'}$ = 7.4 Hz, H-6′), 7.86 (d, 1 H, $J_{4,6}$ = 1.0 Hz, H-4), 7.62 (d, 1 H, $J_{7,6}$ = 8.2 Hz, H-7), 7.32 (dd, 1 H, H-6), 7.28 (d, 1 H, H-5′), 4.62 (s, 2 H, 2′-CH_2), 4.02 (s, 3 H, 4′-OCH_3), 2.21 (s, 3 H, 3′-CH_3). ^{1}H NMR (CD_3OD): δ 8.88 (1 H, H-6′), 7.88 (1 H, H-4), 7.69 (1 H, H-7), 7.61 (1 H, H-5′), 7.45 (1 H, H-6), 4.33 (2 H, 2′-CH_2), 4.21 (3 H, 4′-OCH_3), 2.19 (3 H, 3′-CH_3). ^{1}H NMR (CD_3OD/DCl, 10 mg of **7a** dissolved in 1 mL of CD_3OD containing 20 μL of 20% DCl/D_2O): δ 9.02 (1 H, H-6′), 8.01 (1 H, H-4), 7.86 (1 H, H-7), 7.81 (1 H, H-5′), 7.71 (1 H, H-6), 4.46 (2 H, 2′-CH_2), 4.32 (3 H, 4′-OCH_3), 2.37 (3 H, 3′-CH_3). ^{13}C NMR (CD_3OD/DCl, 50 mg of **7a** dissolved in 0.5 mL of CD_3OD containing 25 μL of 20% DCl/D_2O): δ 172.16 (C-4′), 150.87 (C-2′), 145.89 (C-2), 144.56 (C-6′), 137.88 (C-7a), 136.63 (C-3a), 127.20 (C-3′), 125.77 (C-5), 123.94 (CF_3), 120.60 (C-6), 115.94 (C-7), 114.11 (C-4), 107.94 (C-5′), 58.21 (4′-OCH_3), 37.26 (2′-CH_2) 10.70 (3′-CH_3). MS [DISIMS (+)]: m/z 705 (5, MH^+), 503 (5), 354 (56), 352 (100), 350 (22), 321 (40), 320 (42). Anal. Calcd for $C_{32}H_{26}F_6N_6O_2S_2 \cdot 1.5H_2O$: C, 52.52; H, 3.99; N, 11.49; S, 8.76. Found: C, 52.47; H, 3.84; N, 11.54; S, 8.61.

(b) From 2a in Neutral MeOH Solution. **2a** (0.5 g) was dissolved in MeOH (250 mL). After 2 h of standing at room temperature, the solvent was evaporated under reduced pressure and the resulting semisolid treated as described above; yield 0.2 g of **7a**; mp 139 °C dec.

Reaction of 6a/7a with 2-Mercaptoethanol. ^{1}H NMR Monitored Reactions. (a) With a Surplus of 2-Mercaptoethanol. A ^{1}H NMR spectrum of a solution of **7a** (10 mg/mL) and of 2-mercaptoethanol (6.7 mg/mL, molar ratio **7a**/2-mercaptoethanol 1:6) in CD_3OD/DCl, pH < 1.0 (40 μL of 20% DCl per mL of CD_3OD), was recorded immediately and again after 47 h. During this time period there was no reaction of **6a** (protonated form of **7a**) with the thiol.

Similarly, a less acidic solution of **7a** (10 mg/mL) and 2-mercaptoethanol (6.7 mg/mL) in CD_3OD (0.9 mL) and aqueous phosphate buffer (0.1 mL, pH 5.3) was monitored at 3, 10, and 30 min after preparing the solution. The reduction of **6a** to **9a**, which could be followed by the decrease of the H-6′, 4′-OCH_3, and 3′-CH_3 signals of **6a** at 8.84 ppm, 4.24 ppm, and 2.18 ppm, respectively, and the appearance of the corresponding signals of **9a** at 8.31 ppm, 3.98 ppm, and 2.26 ppm, respectively, was nearly complete after 10 min. After half an hour the sample contained only a trace of **3a** together with the main product **9a**.

(b) With Less Than Stoichiometrically Equivalent Amounts of 2-Mercaptoethanol. A solution of **7a** (10.4 mg/mL) in CD_3OD containing 2-mercaptoethanol (1.5 mg/mL, molar ratio **7a**/mercaptoethanol 1:1.3) at pH = 5.7 (80 μL of D_3PO_4/Na_2DPO_4-buffer in D_2O/mL of CD_3OD) was monitored after 3, 10, and 30 min by ^{1}H NMR. Signals selected for monitoring: **7a**, δ 8.84 (H-6′), 2.16 (3′-CH_3); **3a**, δ 8.93 (H-6′), 2.54 (3′-CH_3); **9a**, δ 8.26 (H-6′), 2.26 (3′-CH_3); 2-mercaptoethanol, δ 3.64 (CH_2O); dimeric oxidation product of 2-mercaptoethanol, δ 3.80 (CH_2O); **3a**: δ 3.50 (CH_2O). From the integrals of these signals the amount

(14) Mixture of regioisomers.[1]

of products given in Table III were calculated.

Reaction of 6a/7a with DL-Dithiothreitol. (a) On a Preparative Scale. **7a** (704 mg, 1 mmol) was dissolved in a stirred mixture of 1,4-dioxane (25 mL) and water (30 mL), DL-DTT (154 mg, 1 mmol) was added, and the colorless precipitate was filtered off after 30 min. Recrystallization from acetonitrile gave 140 mg (48.1%) of pure **9a**, mp 148–150 °C, identical in every respect with **9a** previously described.[2]

(b) ^{1}H NMR Monitored Reactions. A solution of **6a** was prepared by dissolving **7a** (10 mg, 14 μmol) in CD_3OD (1 mL), pH <1.0 (20 μL of 20% DCl per mL of CD_3OD) and DL-DTT (5.2 mg, 34 μmol) was added. **6a** did not react with the excess of DL-DTT (molar ratio 1:2.4) during 46 h. For the reaction with the thiol to start, the pH had to be increased to 3.0.

Under neutral conditions, **7a** (12–13 mg/mL) in CD_3CN was reduced with increasing amounts of DL-DTT (1.18, 2.35, 5.80 mg/mL) to the corresponding sulfide **9a**. This reaction could be easily followed in a quantitative manner by NMR as there were some well-separated characteristic signals for the starting materials as well as for the products. Selected signals: **7a**, δ 4.02 (4′-OCH_3), 7.28 (H-5′), 8.92 (H-6′); **9a**, δ 3.88 (4′-OCH_3), 6.91 (H-5′), 8.38 (H-6′); DL-DTT, δ 2.62 (CH_2S), 3.60 (CHO); cyclic oxidation product of DTT, δ 2.84–3.06 (CH_2S), 3.44–3.48 (CHO). From the integrals of these signals, the amount of DL-DTT consumed for the reduction of 1 mol of **7a** to **9a** was calculated for different DL-DTT/**7a** ratios: These values are 0.78, 0.82, and 0.88 mol for the initial mole ratios DL-DTT/**7a** = 0.41, 0.83, and 2.21, respectively.

Registry No. **1a**, 86604-68-4; **1b**, 73590-58-6; **1c**, 110374-18-0; **2a**, 102804-86-4; **2b**, 102353-88-8; **3a**, 126543-60-0; **4a**, 126543-62-2; **4b**, 126543-64-4; **4c**, 126543-66-6; **5a**, 126543-68-8; **5c**, 126543-70-2; **6a**, 126543-72-4; **6b**, 126578-23-2; **6c**, 126543-74-6; **7a**, 126543-75-7; **7b**, 126578-24-3; **9a**, 86604-69-5; **9b**, 73590-85-9; DTT, 27565-41-9; 2-mercaptoethanol, 60-24-2.

Enantioselective Conjugate Addition of Grignard Reagents to Enones Catalyzed by Chiral Zinc(II) Complexes

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Various chiral zinc(II) complexes catalyze the asymmetric 1,4-addition of Grignard reagents to α,β-unsaturated ketones with high chemoselectivities (yields of 1,4-adducts, 83–99%), high regioselectivities (1,4/1,2 ratios up to 499) and modest enantioselectivities (ee up to 33%). A study of several factors, i.e. ligand, solvent, counterions, order and rate of additions, temperature, and the nature of Grignard reagents, that influence the regio- and enantioselectivities is given. Based on the addition of isopropylmagnesium halides to 2-cyclohexenone as a model reaction, it was established that the highest enantioselectivities are reached with in situ prepared zinc complexes derived from optically active diamino alcohol ligands using lithium bases in tetrahydrofuran as the solvent. A mechanistic rationalization is given.

Conjugate addition reactions are among the most important methods for carbon–carbon bond formation with a central role for organocopper reagents.[1] Much effort has been devoted to chemo- and stereoselective additions of organocuprates, and considerable progress has been made using so-called second generation organocopper reagents,[2] by the use of organocopper catalysts[3] and in asymmetric conjugate addition via organocuprates. Following the early work of Kretchmer[4] on asymmetric induction in conjugate addition via organocopper(I) reagents in the presence of (−)-sparteine, numerous approaches to achieve asymmetric conjugate addition have been described.[5] High diastereoselectivities have been achieved using chiral enones and chiral enonates[6] and cuprates with chiral transferable ligands.[7] Impressive results were obtained by several groups on cuprates with chiral nontransferable ligands. Leyendecker and co-workers[8] reported the addition of Me_2CuLi to chalcone, using 4(*S*)-(*tert*-butylthio)-(*S*)-prolinol as tridentate chiral ligand with ee's as high as 94%. Optical yields ranging from 41 to 83% were reported by Dieter and Tokles[9] in a systematic investigation of conjugate additions to enones employing chiral organo(hetero)cuprates based on (*S*)-proline-derived chiral nontransferable ligands. Up to 50% ee was reached in asymmetric additions of chiral amidocuprates.[10] Corey and co-workers[11] reported the enantioselective addition of chiral cuprate reagents to 2-cycloalkenones (ee 75–95%) using (+)- and (−)-ephedrine derived chiral ligands. Lippard[12] recently described the first catalytic conjugate addition of Grignard reagents to 2-cyclohexenone (ee 4–14%) in the presence of a chiral copper(I) catalyst employing chiral *N,N′*-dialkyl-substituted aminotroponeimines as ligands. In recent years a parallel development on conjugate addition by organozinc reagents is seen, initiated by the discovery of Isobe and co-workers[13] of the facile

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