Table II

Interatomic Distances, Å

from	to	distance
N(1)	C(2)	1.274 (3)
N(1)	O(1)	1.390 (2)
O(1)	H(2A)	0.928
O(2)	C(3)	1.217 (3)
C(2)	C(3)	1.510 (3)
C(2)	S(1)	1.757 (2)
C(3)	C(4)	1.485 (3)
C(10)	S(1)	1.770 (3)

Bond Angles, deg

from	through	to	angle
C(2)	N(1)	O(1)	113.30 (17)
H(2A)	O(1)	N(1)	103.00
N(1)	C(2)	C(3)	114.60 (18)
N(1)	C(2)	S(1)	122.95 (16)
C(3)	C(2)	S(1)	122.10 (15)
O(2)	C(3)	C(4)	121.92 (21)
O(2)	C(3)	C(2)	119.48 (20)
C(4)	C(3)	C(2)	118.58 (19)
C(2)	S(1)	C(10)	100.78 (10)
O(1)	H(2A)	N(1)	47.53

throughout the data collection. A total of 2263 reflections were obtained over one hemisphere of reciprocal space ($+h,\pm k,\pm l$), of which 1693 with intensities greater than 3σ were retained for structural analysis.

The intensities were derived from an analysis of the scan profiles.[14] The data were corrected for Lorentz and polarization effects. No absorption correction was applied. The initial positions of sulfur and few other non-hydrogen atoms were obtained through direct methods (MULTAN), while the remaining non-hydrogen atoms were obtained through subsequent difference-Fourier maps. This was allowed by several cycles of full-matrix least-squares refinement until convergence. In the final cycles, the calculated hydrogen atom positions were also included but not refined. The final agreement factors with all non-hydrogen atoms as anisotropic are $R = 0.041$ and $R_w = 0.062$.

The final positional parameters are listed in a table in the supplementary material, whereas selected distances and angles can be found in Table II.

Acknowledgment. We thank the National Institutes of Health (Grants GM31349 and GM32279) for financial support.

Registry No. **4a**, 110097-27-3; **4b**, 110097-28-4; **4c**, 110097-29-5; **5a**, 10364-94-0; **5b**, 4122-52-5; **5c**, 2466-76-4; **6**, 20621-03-8; **7a**, 614-21-1; **7c**, 10230-68-9; **14b**, 110097-30-8.

Supplementary Material Available: Tables listing positional parameters and anisotropic temperature factors for **4a** (3 pages); observed and calculated structure factors for **4a** (9 pages). Ordering information is given on any current masthead page.

(H^+–K^+)-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulfinyl]benzimidazoles. 1.[1] Their Reaction with Thiols under Acidic Conditions. Disulfide Containing 2-Pyridiniobenzimidazolides as Mimics for the Inhibited Enzyme

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A model for studying the mechanism of (H^+–K^+)-ATPase inhibition by the sulfoxides **1**, namely, the reaction of **1** with thiols at low pH, is described. These compounds were found to rearrange in acidic media and to incorporate the thiol to give the 2-pyridinio derivatives **3**, containing a disulfide side chain. These can be isolated as the neutral ylides **4**. The structure of **4** is unambiguously supported by detailed spectral data and by an X-ray analysis of **4d**. Cleavage of the disulfide bond of **4** by thiols leads to a second rearrangement, generating the sulfides **6**, which contain the original molecular backbone. Reductive desulfuration of **4c** results in degradation of the disulfide side chain giving 2-(2-methylpyridinio)benzimidazolide **5c**. O-Demethylation of the 4-methoxypyridinio derivative **3f** converts this compound to the pyridone **7f**. The structural prerequisites essential for the reactivity of **1** are discussed.

Introduction

The 2-[(2-pyridylmethyl)sulfinyl]benzimidazoles **1**, belonging to a class of highly potent inhibitors of gastric acid secretion,[2-5] have attracted considerable attention as potential therapeutics for the treatment of peptic ulcer. The antisecretory activity of **1b** in vivo has been ascribed to

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(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.; Zsnolnai, L. *J. Org. Chem.*, following paper in this issue.

0022-3263/87/1952-4573$01.50/0

Table I. Rate Constants[a] of the Reaction of 1c with Thiols 2

pH	thiol[b] 2	mol ratio 1c/2	10^4k, s^{-1}
4.8[c]	GSH	1:4	1.1
3.7[c]	GSH	1:4	3.2
3.0[c]	none		5.8
3.0[c]	GSH	1:4	5.9
3.0[c]	ME	1:1	5.8
3.0[c]	ME	1:10	6.2
3.0[c]	ME	1:100	5.4
1[d]	GSH	1:4	19

[a] Determined by HPLC from the first-order decay of 1 (c_0 = 9.4 × 10^{-4} mol/L) at room temperature. [b] GSH = glutathione, ME = 2-mercaptoethanol. [c] Solvent H_2O (0.01 m KH_2PO_4)/CH_3CN = 3:1 (v/v); pH adjusted with H_3PO_4 and NaOH, respectively. [d] Solvent 0.1 N HCl containing 20% CH_3CN for reason of solubility.

the irreversible inhibition of the gastric (H^+–K^+)-ATPase,[6,7] the proton-pumping enzyme present in the apical membrane of the parietal cell. Previous investigations of the mode of action of **1b** have revealed evidence that, in vitro, the drug is activated and transformed in acidic media into an intermediate which exerts its inhibitory activity by reaction with one or more essential SH groups on the enzyme.[8] Binding studies using [^{14}C]**1b** showed a saturable covalent incorporation of the radiolabel into purified gastric (H^+–K^+)-ATPase preparations.[6] Furthermore, thiols such as 2-mercaptoethanol were found to prevent as well as reverse both the inhibition of the enzyme and, in parallel, the incorporation of the ^{14}C radiolabel. As the major product formed from **1b** in isolated gastric glands was found to be its reduced form,[9] the overall chemical reaction involved in the inhibitory activity of **1b** seemed to be the simple reduction of a sulfoxide to a sulfide.

As a model for the interaction between the sulfoxides **1** and SH groups on the enzyme, we have studied the reaction of **1** with thiols. This reaction has already been the subject of two studies by others[10,11] but with conflicting conclusions concerning the nature of the reactive species and products involved. In a previous paper[12,13] we presented a short summary of our results, which essentially agreed with those reported independently by another group.[14,15]

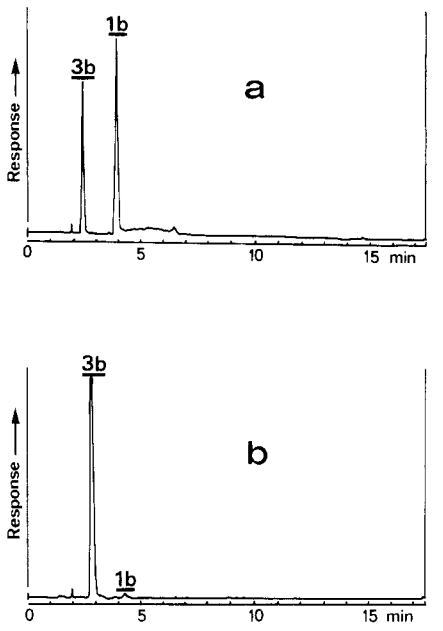

Figure 1. HPLC chromatograms of a 0.35 mM solution of reduced glutathione in 0.1 M HCl: (a) 5 min after the addition of **1b** (initial concentration ca. 0.17 mM); (b) 60 min after the addition of **1b**; column, μBondapak Phenyl; eluent, 20% to 50% acetonitrile gradient over 15 min in 0.15 M potassium phosphate, pH 2.1; column temperature, 40 °C; flow rate, 2 mL min^{-1}; detection, UV at 300 nm.

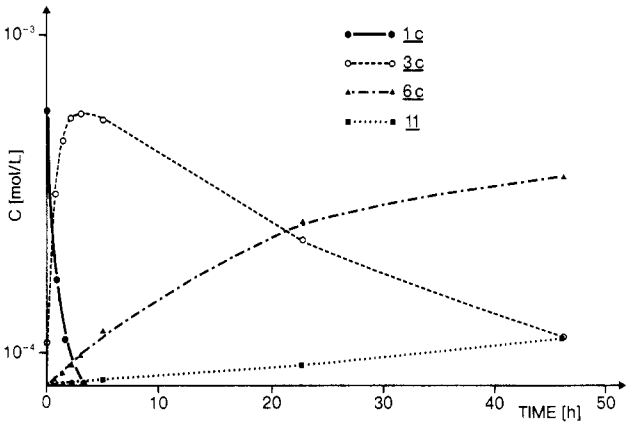

Figure 2. Concentration–time profiles of the reaction of **1c** (9.4 × 10^{-4} mol/L) with 2-mercaptoethanol (10^{-2} mol/L): solvent, CH_3CN/H_2O (0.01 M KH_2PO_4) = 1:3 (v/v); pH 3.85 (H_3PO_4); analysis, HPLC; at the time of the first analysis a small amount of **3c** had already been formed.

This paper presents details of the formation, isolation, and structural characterization of the products obtained from the reaction of **1** with thiols under acidic conditions and the further reaction of these products with thiols. The reactivity of modified derivatives of **1** toward thiols demonstrates the structural prerequisites essential for the unique chemistry involved in the mechanism of action of **1**, the details of which are discussed in Part 2 of this series.[1]

Results

Reaction of 1 with Thiols 2 under Acidic Conditions. The reaction of the sulfoxides **1** with thiols **2** in

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(14) Wallmark, B.; Brändström, A.; Lindberg, P. *Abstract of Papers*, 3rd SCI-RSC Medicinal Chemistry Symposium, Cambridge, UK, September 1985; Churchill College Cambridge: Cambridge, 1985; S. 16. See also: Lambert, R. W., Ed. *Proceedings*, 3rd SCI-RSC Medicinal Chemistry Symposium, Cambridge, England, September 1985; Churchill College Cambridge: Cambridge, 1985; pp 293–311.
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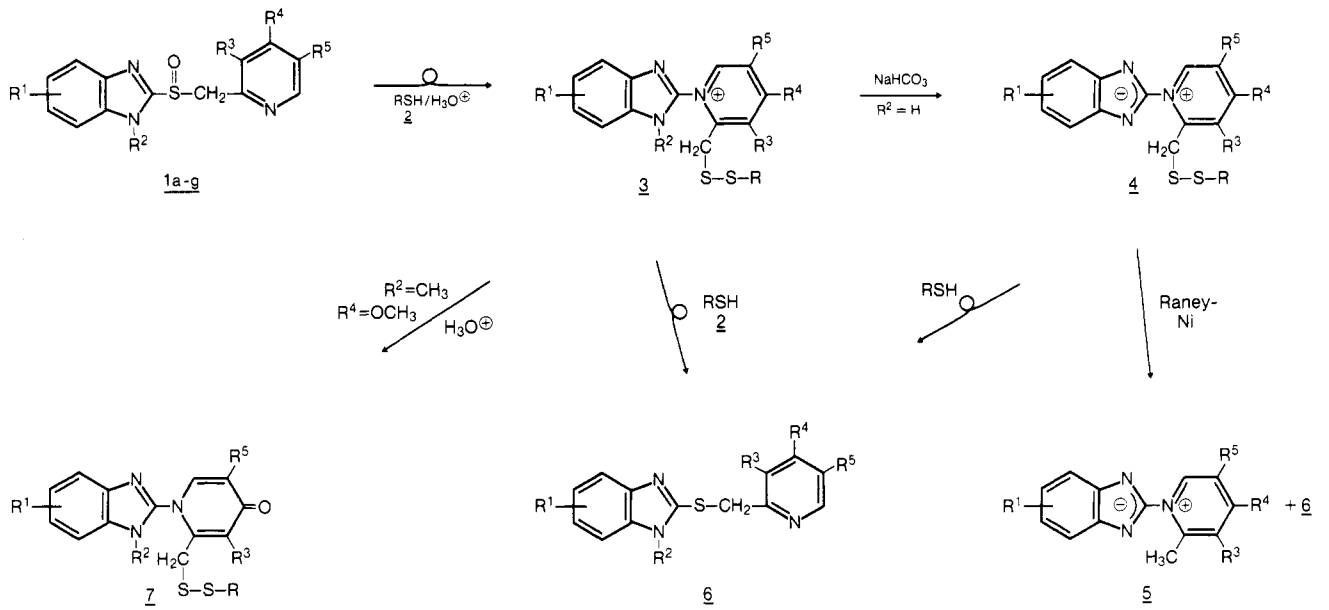
Scheme I



1a: R^1–R^5 = H
1b: R^1 = 5-OCH_3; R^2 = H; R^3 = R^5 = CH_3; R^4 = OCH_3
1c: R^1 = 5-CF_3; R^2 = R^3 = R^5 = H; R^4 = OCH_3
1d: R^1 = 5-CF_3; R^2 = R^5 = H; R^3 = CH_3; R^4 = OCH_3
1e: R^1 = 6-CF_3; R^2 = CH_3; R^3 = R^5 = H; R^4 = OCH_3
1f: R^1 = 6-CF_3; R^2 = R^3 = CH_3; R^4 = OCH_3; R^5 = H
1g: R^1 = 5-OCH_3; R^2 = R^3 = R^5 = H; R^4 = OCH_3
RSH = 2-mercaptoethanol, glutathione, cysteine, or phenylmethanethiol.

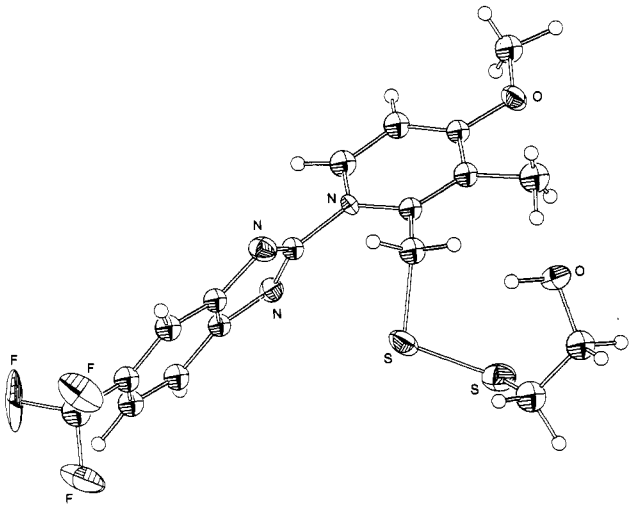


Figure 3. Perspective drawing of **4d** (R = CH_2CH_2OH) derived from the X-ray coordinates.

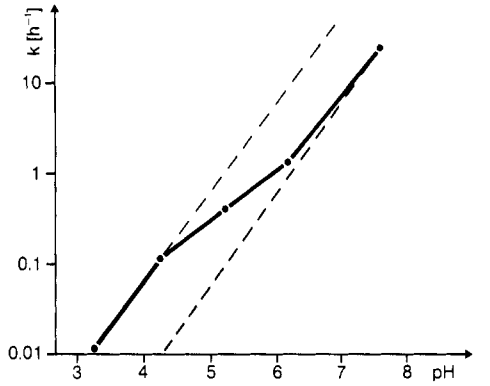


Figure 4. Effect of pH on the pseudo-first-order rate constant of the reaction of **4d** (R = CH_2CH_2OH) (1 mM) with 2-mercaptoethanol (10 mM) in buffer/acetonitrile (3:1). The slope of the broken lines corresponds to the expected effect of the thiolate concentration on the rate constant.

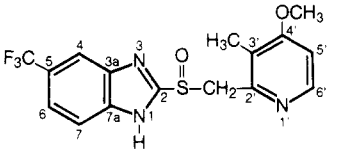
acidic medium was investigated by using glutathione, cysteine, phenylmethanethiol, and 2-mercaptoethanol. The latter was preferred for preparative purposes. Scheme I presents an outline of the course of the reaction and the structures assigned to the products isolated after subsequent transformations.

Treatment of **1** with **2** in aqueous acidic media (e.g., 0.1 N HCl), which resemble the conditions present at the site of action in the parietal cell, results in the formation of a more polar product **3**. The reaction of **1b** with, for example, glutathione was found to be virtually free of byproducts when monitored by HPLC (Figure 1a,b). The acid-catalyzed reaction was found to be kinetically first order and dependent on the pH but not on the concentration or structure of the thiol applied (Table I), suggesting the formation of a reactive intermediate as the rate-limiting step.

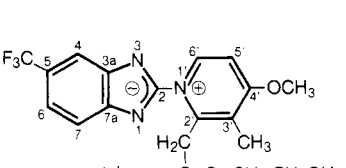
In order to identify the structure of the products, we generated **3** on a preparative scale by dissolving **1** in excess 0.1 N HCl containing an equimolar amount of **2**. After careful neutralization with dilute $NaHCO_3$ solution, the neutral ylides **4** could be isolated. One of these products (**4d**), which is reasonably stable, was recrystallized from acetonitrile without decomposition and subjected to an X-ray analysis (Figure 3).

The further outcome of the reaction of **1** with **2** is highly dependent on the pH of the medium. At low pH, compounds **3** are the final products even in the presence of excess thiol. In less acidic media the formation of **3** is accompanied by the formation of the sulfides **6** (Scheme I, Figure 2). Using isolated **4d**, it could be shown that **6d** is formed from **3d** in a consecutive reaction during the reaction of **1d** with **2**; similar results were obtained with other derivatives **4** (data not included). The cleavage of **3d**/**4d** by thiol is greatly accelerated when the pH of the medium is raised from below 3 to 7.4 (Figure 4). The

Table II. ^{1}H NMR Data of 1d and of 4d (R = CH_2CH_2OH) in CD_3OD



1d

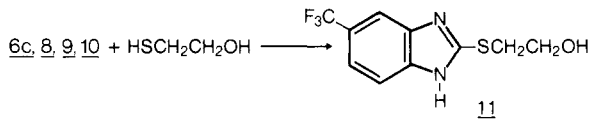


4d

1d		4d			
H	δ	H	δ	mult[a]	J, Hz
H-4	7.99	H-4	7.88	(d)	$J_{4,6}$ = 1
H-6	7.61	H-6	7.39	dd	$J_{6,7}$ = 8.5, $J_{6,4}$ = 1.6
H-7	7.80	H-7	7.71	d	$J_{7,6}$ = 8.4
H-5′	6.94	H-5′	7.64	d	$J_{5',6'}$ = 7.3
H-6′	8.13	H-6′	8.91	d	$J_{6',5'}$ = 7.3
2′-CH_2	4.81 (AB)	2′-CH_2	4.64	s (or AB system)	
3′-CH_3	2.19	3′-CH_3	2.51	s	
4′-OCH_3	3.90	4′-OCH_3	4.26	s	
		SCH_2	2.43	t	J = 6.1
		OCH_2	3.48	t	J = 6.1

[a] s = singlet, d = doublet, t = triplet.

Scheme II

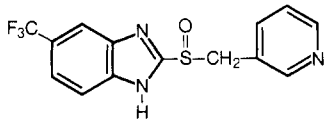


11

maximum accumulating concentration of **3**, during the reaction of **1** with **2**, therefore, decreases with increasing pH due to the inverse pH dependency of the formation of **3** and its reaction to give **6**.

Isolated **4c** (R = CH_2CH_2OH) was desulfurized with Raney Ni to yield a 6:4 mixture of **6c** and **5c**. The latter compound was a key to the structural assignment of **4**.

The N-1 substituted derivatives **1e,f** lead to the unstable compounds **3e,f** (R = CH_2CH_2OH) if the reaction is carried out as described above. **3f** is easily hydrolyzed to the stable 4-pyridone derivative **7f**. To study the scope and the structural requirements for the reaction of **1** with thiols, we synthesized the modified sulfoxides **8** and **9** and the sulfone **10** and examined their ability to interact with thiols. None of these compounds nor the sulfides **6** react



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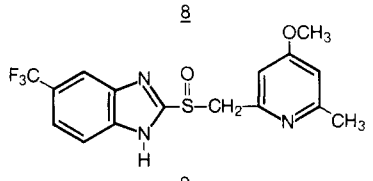

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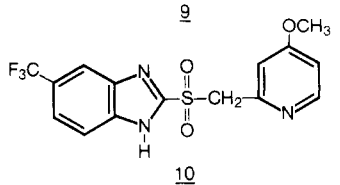
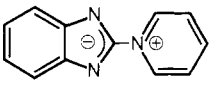


10

with thiols in a fashion comparable to **1**. Instead, the only reaction observed, starting from **8**, **9**, **10**, or **6c**, is the nucleophilic substitution by RSH at the C-2 position of the benzimidazole to give **11** according to Scheme II.

The rate of this reaction is slower, by some orders of magnitude, than the formation of **3** from **1**, as can be seen, for example, in Figure 2. In addition, it can be seen from Figure 2 that the substitution product **11** continues to form even after the concentration of sulfoxide has been reduced to zero. In this experiment **11** presumably arises from a substitution reaction either on the sulfide **6c** or the disulfide **3c**.

Structure Elucidation. The structural assignment of the isolated neutral compounds **4** and the corresponding protonated species **3**, being derivatives of 2-pyridiniobenzimidazolide (**12**) first synthesized by Boyd,[16] is based on the spectral data presented in this section.



12

^{1}H and ^{13}C NMR Spectra. In comparison to the ^{1}H NMR spectra of the original sulfoxides **1**, the spectra of the derived disulfides **4** (R = CH_2CH_2OH) show pronounced deshielding of the pyridine protons and shielding of the protons in the benzimidazole moiety, reflecting the introduction of positive and negative charges in the respective heterocyclic systems. As an example, the ^{1}H NMR data of **1d** and **4d** (R = CH_2CH_2OH) are summarized in Table II. Almost identical chemical shifts are observed for the ^{1}H signals of the sulfur free compound **5c** (Table III). Additionally, both **4** and **5** are similar in their behavior to **12**, with respect to changes in solvent polarity. Thus, a change from chloroform to a more polar solvent such as methanol, leads to upfield shifts for the benzimidazolide proton signals, whereas the pyridinio proton signals—with the exception of the α-proton—are shifted to lower field (Table III).[17]

The protons of the methylene group attached to the pyridine 2-position of **4** are exchangeable in CD_3OD solution (**4d**, 10 mg/mL, signal δ 4.65, $t_{1/2}$ = 19 h). Clearly, the positively charged pyridine ring encourages this exchange, which is not observed for the corresponding pro-

(16) Boyd, G. V. *Tetrahedron Lett.* **1966**, *29*, 3369.

(17) The more polar solvent is expected to favor a more extended separation of the opposite charges in **4**, **5**, and **12** and, therefore, a more twisted arrangement of the two heterocyclic planes. This may explain the observed solvent dependencies, both in the ^{1}H NMR (Table III) and in the UV spectra (see discussion of the UV spectra). UV and X-ray data indicate additional twisting around the central C–N single bond due to steric hindrance by substituents at the pyridine 2-position (Table IV and Figure 3). For a very recent discussion of the geometry of **12** and of the influence of substituents in related structures, see: Alcalde, E.; Dinares, I.; Fayet, J.-P.; Vertut, M.-C.; Elguero, J. *J. Chem. Soc., Chem. Commun.* **1986**, 734.

Table III. Comparison of the Change in ^1H NMR Chemical Shift ($\Delta\delta$) Introduced by Solvent Change from $CDCl_3$ to CD_3OD for 12, 5c, and 4c,d (R = CH_2CH_2OH)

	12			5c			4c			4d		
H[a]	$CDCl_3$	CD_3OD	$\Delta\delta$[b]	$CDCl_3$	CD_3OD	$\Delta\delta$[b]	$CDCl_3$	CD_3OD	$\Delta\delta$[b]	$CDCl_3$	CD_3OD	$\Delta\delta$[b]
H-4	7.69	7.59	0.10	7.99	7.89	0.10	7.99	7.87	0.12	7.99	7.88	0.11
H-5	7.17	7.13	0.04									
H-6	7.17	7.13	0.04	7.40	7.39	0.01	7.42	7.38	0.04	7.42	7.39	0.03
H-7	7.69	7.59	0.10	7.75	7.70	0.05	7.76	7.70	0.06	7.75	7.71	0.04
H-2′	10.07	9.74	0.33									
H-3′	7.88	8.19	−0.31	7.12	7.64	−0.52	7.51	7.75	−0.24			
H-4′	8.25	8.61	−0.36									
H-5′	7.88	8.19	−0.31	7.11	7.51	−0.40	7.23	7.60	−0.37	7.17	7.64	−0.47
H-6′	10.07	9.74	0.33	8.84	8.83	0.01	8.92	8.99	−0.07	8.82	8.91	−0.09
2′-CH_2							4.61	4.56	0.05	4.69	4.64; 4.63	0.05
2′-CH_3				2.90	2.66	0.24						
3′-CH_3										2.48	2.51	0.03
4′-OCH_3				4.14	4.22	−0.08	4.20	4.26	−0.06	4.20	4.26	−0.06
OCH_2							3.72	3.60	0.12	3.54	3.48	0.06
SCH_2							2.67	2.62	0.05	2.49	2.43	0.06

[a] For numbering refer to the structure **4d**, Table II. [b] Positive $\Delta\delta$ values indicate high-field shift in the polar solvent.

Table IV. ^{13}C NMR Data of 4d (R = CH_2CH_2OH) in CD_3OD

C[a]	δ	mult[b]	^1J, Hz	mult[b]	^3J, Hz
C-2	154.96	s		d	3.1
C-3a	142.88	s		d (t)	6.3
C-4	113.74	d	159.4	m (br)	
C-5	121.84	q	(J_{CF} = 31.5)	d (br)	8.6
C-6	116.34	d	160.0	q,d	(J_{CF} = 3.7) 2.6
C-7	116.72	d	162.6	m (br)	
C-7a	145.91	s		d,d,d	9.1, 5.8, 1.2
CF_3	125.09	q	(J_{CF} = 270.5)	t (d)	4.3, 1.2
C-2′	151.40	s		t, d	5.6, 5
C-3′	126.22	s		m (br)	
C-4′	170.27	s		m (br)	
C-5′	106.69	d	174.2	d	4.0
C-6′	145.37	d	192.3	d	1.3
2′-CH_2	35.65	t	146.5		
3′-CH_3	10.43	q	130.7		
4′-OCH_3	57.18	q	148.3		
CH_2O	58.62	t	143.6		
CH_2S	40.31	t	139.6		

[a] For numbering refer to structure **4d**, Table II. [b] q = quartet, m = multiplet, br = broadened; further abbreviations, see Table II.

tons in the sulfoxides **1** and sulfides **6**.

The ^{13}C NMR spectra of **4** and **5** are in complete accordance with the proposed structures (Tables IV and V). A long-range coupling between the benzimidazole 2-carbon atom and the pyridine α-proton also indicates a direct C–N linkage between the two ring systems (Table IV). Furthermore, the reaction between [2-^{13}C,1′-^{15}N]-2-[(2-pyridylmethyl)sulfinyl]benzimidazole (**1g**) and reduced glutathione in aqueous acid produced a product (**3g**, R = glutathionyl) with a spin–spin coupling constant, $J(^{13}C–^{15}N)$, of a magnitude indicating a single bond between the enriched nuclei (24.9 Hz), providing further proof that the products of these reactions are 2-pyridiniobenzimidazolides.

Protonation of the ylides leads to neutralization of the negative charge on the imidazole ring and to a stronger localization of the positive charge in the pyridine part. Consequently, the benzimidazole carbons 2, 3a, and 7a show strong highfield shifts, whereas the carbons 5 and 6, which are in conjugation with the pyridine via the imidazole, show downfield shifts (Table V). The increased positive charge in the pyridine ring is reflected by the predominant downfield shifts of the pyridine carbons; this being most pronounced for position 4.

UV Spectra. Further evidence for the proposed structures **3–5** is derived from their UV spectra. In accordance with the properties reported for **12**, the UV spectra of **4c** and **5c** exhibit comparably pronounced negative solvatochromism (Table VI). The additional hypsochromic shift in the spectra of **4c** and **5c**, relative to **12**, may result mainly from electronic substituent effects.

Table V. ^{13}C Chemical Shift Changes Due to Protonation of the Ylides 5c and 4c,d (R = CH_2CH_2OH). Acidified Solution Contained Excess DCl (per mL of Me_2SO-d_6, 100 mg of Ylide + 100 – 200 μL of 20% DCl)

	5c			4c			4d		
C[a]	Me_2SO	Me_2SO/DCl	$\Delta\delta$[b]	Me_2SO	Me_2SO/DCl	$\Delta\delta$[b]	Me_2SO	Me_2SO/DCl	$\Delta\delta$[b]
C-2	157.57	150.40	7.17	157.18	148.94	8.24	158.07	147.88	10.19
C-3a	144.85	140.43	4.42	144.60	137.59	7.01	144.73	137.79	6.94
C-4	114.55	114.75	−0.20	114.34	115.21	−0.87	114.50	115.60	−1.10
C-5	119.53	122.59	−3.06	119.44	125.04	−5.60	119.53	125.56	−6.03
C-6	115.32	118.56	−3.24	115.22	121.17	−5.95	115.38	121.74	−6.36
C-7	117.72	117.31	0.41	117.51	116.50	1.01	117.68	117.78	−0.10
C-7a	148.16	142.70	5.46	147.84	139.02	8.82	148.01	139.25	8.76
CF_3	126.24	125.36	0.88	125.94	125.04	0.90	126.13	125.41	0.72
C-2′	156.32	157.03	−0.71	153.60	154.95	−1.35	151.38	152.21	−0.83
C-3′	113.78	114.04	−0.26	114.92	117.33	−2.41	125.77	127.95	−2.18
C-4′	170.98	172.40	−1.46	170.44	173.51	−3.07	169.45	172.71	−3.26
C-5′	111.31	111.92	−0.61	111.53	112.79	−1.26	107.95	109.46	−1.51
C-6′	146.74	147.03	−0.29	147.06	145.45	1.61	146.22	146.31	−0.09
C-2′-CH_2				40.33[c]			36.92	36.84	0.08
C-2′-CH_3	21.40	20.71	0.69						
C-3′-CH_3							12.10	12.96	−0.86
C-4′-OCH_3	58.18	58.75	−0.57	58.20	59.20	−1.00	58.57	59.34	−0.77
CH_2O				58.98	59.70	−0.72	59.00	60.32	−1.32
CH_2S				41.14	41.48	−0.34	41.39	41.84	−0.45

[a] For numbering refer to structure **4d**, Table II. [b] Positive $\Delta\delta$ values indicate high-field shifts in the protonated form. [c] Under Me_2SO.

Table VI. Effect of Solvent on the Long Wavelength UV-vis Absorption of 12, 5c, and 4c (R = CH_2CH_2OH)

	λ_{max}, nm					ϵ, L mol^{-1} cm^{-1} (in $MeCl_2$)
	aq buffer (pH 9)	MeOH	$MeCl_2$	1,4-dioxane	benzene	
12	360	385	414	422	445	1.06×10^4
5c	295 sh[a]	300 sh	329	344	354	6.50×10^3
4c	300 sh	308 sh	347	360	358	6.60×10^3

[a] sh = shoulder

The associated decrease in absorptivity (hypochromic effect), however, suggests twisting around the C–N single bond as a consequence of the increased steric hindrance present in **4c** and **5c**;[17] this may also contribute to the hypsochromic shift. Assuming a relationship between the intensity of the long wavelength absorption and the cosine-square of the angle of twist of the two chromophores around the N-1′,C-2 single bond,[18] modulation of the intensity due to steric effects can be easily rationalized.

In contrast to **12**, the shape of the UV spectra of **4c** and **5c** is substantially altered when protonation at one of the imidazole nitrogens occurs. Protonation of the ylides diminishes the tendency for internal charge delocalization and shifts the balance from a predominantly planar conformation, necessary for optimum charge transfer, toward a twisted arrangement necessary for minimum steric hindrance. The effect is greater for **4c** and **5c** than for **12**. Thus, the charge-transfer band, although still present in the spectrum of protonated **12**, vanishes after protonation of the more sterically hindered ylides **4c** and **5c**.[19]

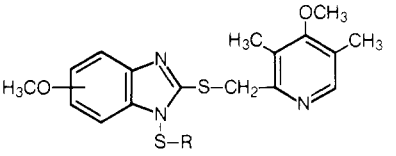
pK_a Values. The UV spectroscopically determined pK_a values of **12**, **4c** (R = CH_2CH_2OH), and **5c** were found to be 5.7, 5.7, and 5.9, respectively, whereas the corresponding values for series **1** are about 8.[4] This enhancement in acidity of the former compounds is in accordance with the proposed ylide structure, in which the directly linked pyridinio moiety exerts a strong acid strengthening effect on the imidazole N–H.

Mass Spectrometry. Analysis of **4b** (R = CH_2CH_2OH) using discharge ionization secondary ion mass spectrometry in the positive ion mode [DISIMS(+)] yielded further confirmation of its proposed structure. The protonated molecular ion species was observed at m/z 406, which confirmed the molecular weight of the compound. Plausible structures can be written for most of the major fragment ions. Substantial spectral differences were observed in a comparison with the DISIMS(+) spectrum of the original sulfoxide **1b**.

X-ray Analysis. An X-ray structure determination obtained from a single crystal of **4d** (R = CH_2CH_2OH) verified the structural assignment (Figure 3). As expected from the NMR and UV spectra, the two heterocyclic planes of the benzimidazole and the pyridine moieties are twisted around the central C–N bond due to the steric repulsion of the pyridinio 2-side chain and subtend an angle of 60° in the solid state.

Discussion

At low pH the sulfoxides **1** react quantitatively with thiols to form the rearranged disulfides **3** which, in the case of R^2 = H, can be isolated as ylides **4**. Spectroscopic data on **4** and on the sulfur free derivative **5** are in complete agreement with the proposed structures and parallel the properties of the previously reported unsubstituted ylide **12**. The disulfide nature of **4** is further supported by its chemical reactivity in the presence of excess thiol. In addition, H/D exchange of the α-methylene protons in **4** and the observed solvent and pH-dependent spectral changes are consistent with the proposed structure. The H/D exchange could not be easily explained by other authors[10] on the basis of the previously proposed *N*-sulfenylated structure **13**.



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Evidence obtained suggests that the acid-catalyzed reaction of **1** to give the disulfides **3** involves the interaction of a thiol with a reactive thiophilic intermediate which is formed from **1** in an initial rate-limiting step. The isolation and characterization of this intermediate together with the details of the mechanism are described in Part 2 of this series.[1] However, the formation of the disulfide structure **3** necessarily requires a preceding rearrangement which must involve, at some stage, attack by the pyridine nitrogen on the benzimidazole 2-position. Structural modifications of **1**, which are realized in the derivatives **8–10**, enable the probing of the prerequisites and limitations of this reaction, irrespective of the exact pathway involved. Thus, the presence of the methyl group in the 6-position of the pyridine ring of **9** completely blocks the formation of the corresponding **3**. This observation is consistent with the "bulky" methyl group hindering the nucleophilic attack of the pyridine at the C-2 position of the benzimidazole moiety. Similarly, the derivative **8**, in which the pyridine is linked in the 3-position, also fails to form the rearranged disulfide analogous to **3**. Again this is consistent, since it is clear from models that it is impossible for this isomer to form the six-membered transition state that would be required in this case. Furthermore, varying the oxidation state of the sulfur from sulfoxide to sulfide or sulfone also prevents the formation of rearranged products. This result can, in part, in the case of the sulfide, be accounted for in terms of the poorer leaving ability of the benzimidazole 2-substituent, but it must also reflect the ability of these compounds to form the postulated thiophilic intermediate.

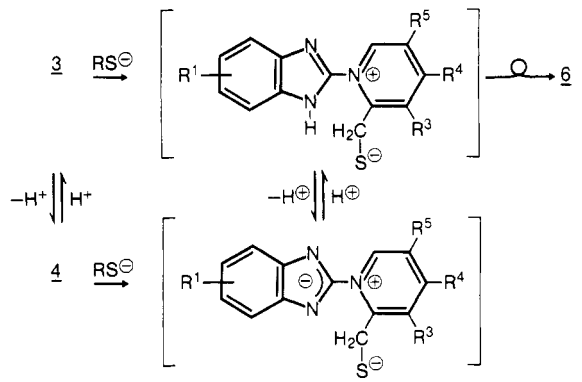
The poor reactivity of these compounds parallels their lack of inhibitory activity against the (H^+–K^+)-ATPase. Effective and long-lasting inhibitory activity is limited to sulfoxides which, after acid-induced transformation, bind covalently to SH groups. However, although this reaction is essential, it is not the sole condition for inhibition of the (H^+–K^+)-ATPase, as will be shown for the benzimidazole N-methylated derivatives in Part 2 of this series.[1]

The formation of the sulfides **6** from the ylides **4** or their salts **3** in the presence of thiols can be accounted for in terms of an initial thiol–disulfide interchange followed by a reverse rearrangement of the displaced thiol to reform the original molecular backbone present in **1** (Scheme III). The pronounced pH dependency of this reaction (Figure 4) suggests that the thiolate anion acts as the initial nucleophile, whereby cleavage of the unsymmetrical disulfide produces a thiolate, in which the negative charge is stabilized by the inductive effect of the pyridinio system (Scheme III). The observed decrease in the reaction rate by a factor of 10 per pH unit outside of the pH range 4–6 corresponds to the change in the thiolate ion concentration. Within the range of pH 4–6, however, an additional effect

(18) Jaffé, H. H.; Orchin, M. *Theory and Applications of Ultraviolet Spectroscopy*; Wiley: New York, 1962; p 384.

(19) In accord with this interpretation, the characteristic long wavelength UV absorption of the almost planar 5*H*-pyrido[1′,2′:4,5][1,2,4]-thiadiazino[2,3-*a*]benzimidazol-13-ium system[1] is very similar to that of protonated **12**.

Scheme III



on the reaction rate is observed which can be rationalized in terms of the protonation equilibrium **3** ⇌ **4** (**4d**, pK_a = 5.7), the protonated form **3**, which offers the better leaving group, being more reactive than the ylide **4**.

Conclusions

In the present study we have shown that, under acidic conditions, sulfoxides of structure **1** generate, in a rate-determining step, a reactive thiophilic intermediate which interacts rapidly with thiols to produce disulfides with the rearranged structure **3**. This reaction is believed to model the mode of action of these compounds, reflecting the unique chemistry that takes place within the acidic compartment of the parietal cell in which the (H^+–K^+)-ATPase is located. Thus, both the peculiar acidity of the parietal cell and the strongly H^+-catalyzed transformation of **1** lead to a targeting of the active form of **1** and, therefore, account for the reported selectivity.

The disulfides **3** and **4** can be cleaved by excess thiol, at a rate directly related to pH of the medium, giving rise to a second rearrangement leading to the sulfides **6**; an overall net reduction of **1**. This reaction accounts for the in vitro reactivation of the enzyme by thiols as well as the observed formation of the sulfide **6b** as the major product when **1b** is incubated with isolated gastric glands.

Experimental Section

General Procedures. Melting points are uncorrected and were determined with a Büchi 510 apparatus (heating rate 3 °C/min). Microanalyses were performed by Dr. W. Rozdzinski, Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität, Pfaffenwaldring 55, D-7000 Stuttgart 80, West Germany. IR spectra were recorded with a Perkin-Elmer 257 grating spectrometer. ^1H and ^{13}C NMR spectra were recorded at 200.13 (or 360.13) MHZ and 50.32 (or 90.56) MHZ, respectively, by using selective 5 mm diameter probes on a Bruker AC 200 (or AM 360) superconducting high-resolution FT-NMR spectrometer. TMS was used as internal standard in organic solvents (TSP in D_2O). Mass spectra were obtained by using a VG MM 7070 F spectrometer interfaced to a VG 2035 data system or on a Finnigan 4610 mass spectrometer interfaced to an Incos 2300 data system. The latter instrument 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 by using a discharge potential of 3.5 kV. UV–vis spectra were recorded on a Perkin-Elmer Model 555 photometer. HPLC analyses were performed on a Gynkotek instrument (250 B gradient former; 600/200 constant flow pump; SP-4 UV-detector) combined with a HP 3357 data system.

Preparation of the Sulfoxides 1, 8, and 9 and of the Sulfides 6. The sulfoxides **1a,b**[20] and **1c,d**[21] and the corresponding sulfides **6a,b**[20] and **6c,d**[21] were prepared according to the literature methods. The synthesis of the dilabeled sulfoxide [2-^{13}C,1′-^{15}N]**1g** will be described in a forthcoming paper.[22]

General Procedure for the Preparation of Sulfoxides 1e,f. The respective sulfoxides **1c,d** (14 mmol) were suspended in saturated aqueous $NaHCO_3$ solution (200 mL) and dichloromethane (200 mL). CH_3I (14 mmol) and a catalytic amount of $(n\text{-}C_4H_9)_4NHSO_4$ were added, and the heterogenous mixture was refluxed for 2 h (good mechanical stirring was required). Another 14 mmol of CH_3I was added, and refluxing was continued for a further 3 h (complete reaction; TLC (CH_2Cl_2/MeOH = 9:1), R_f 0.7 for **1e**). The organic phase was separated and the aqueous extracted twice with dichloromethane (50 mL). The combined dichloromethane solutions were washed with water (25 mL), dried (K_2CO_3), and evaporated to dryness and the residues dissolved hot for crystallization as specified below.

1-Methyl-2-[((4-methoxy-2-pyridyl)methyl)sulfinyl]-6-(trifluoromethyl)-1*H*-benzimidazole (1e).[23] **According to the general procedure from 1c:** 43% yield; mp 141–142 °C after crystallization from 2-propanol, washing twice with diisopropyl ether, and drying in vacuum at 50 °C; ^1H NMR (Me_2SO-d_6) δ 8.29 (d, 1 H, $J_{6',5'}$ = 5.8 Hz, H-6′), 8.22 (s, 1 H, H-7), 7.96 (d, 1 H, $J_{4,5}$ = 8.6 Hz, H-4), 7.64 (dd, 1 H, $J_{5,7}$ = 1.5 Hz, H-5), 7.01 (d, 1 H, $J_{3',5'}$ = 2.5 Hz, H-3′), 6.92 (dd, 1 H, H-5′), 4.85 (AB, 2 H, Δν = 12.37 Hz, J_{AB} = 13.0 Hz, 2′-CH_2), 3.98 (s, 3 H, 1-CH_3), 3.75 (s, 3 H, 4′-OCH_3). Anal. Calcd for $C_{16}H_{14}F_3N_3O_2S$: C, 52.03; H, 3.82; N, 11.38; S, 8.68. Found: C, 52.20; H, 3.96; N, 11.40; S, 8.62.

1-Methyl-2-[((4-methoxy-3-methyl-2-pyridyl)methyl)sulfinyl]-6-(trifluoromethyl)-1*H*-benzimidazole (1f). According to the general procedure from 1d: 43% yield, mp 140–141 °C after crystallization from acetonitrile, washing twice with diisopropyl ether, and drying in vacuum at 50 °C; ^1H NMR (CD_3OD) δ 8.08 (d, 1 H, $J_{6',5'}$ = 5.7 Hz, H-6′), 8.02 (s, 1 H, H-7), 7.87 (d, 1 H, $J_{4,5}$ = 8.6 Hz, H-4), 7.62 (d, 1 H, H-5), 6.89 (d, 1 H, H-5′), 4.99 (s, 2 H, 2′-CH_2), 4.01 (s, 3 H, 1-CH_3), 3.87 (s, 3 H, 4′-OCH_3), 2.19 (s, 3 H, 3′-CH_3). Anal. Calcd for $C_{17}H_{16}F_3N_3O_2S$: C, 53.26; H, 4.20; N, 10.96; S, 8.36. Found: C, 53.29; H, 4.17; N, 11.01; S, 8.56.

2-[(3-Pyridylmethyl)sulfinyl]-5-(trifluoromethyl)-1*H*-benzimidazole (8). A mixture of 2-mercapto-5-(trifluoromethyl)-1*H*-benzimidazole (3.5 g, 0.016 mol), 3-(chloromethyl)-pyridine hydrochloride (2.7 g, 0.016 mol; Aldrich Co.), 2 N NaOH (16 mL), and EtOH (60 mL) was stirred for 5 h at 50 °C. After evaporation of EtOH in vacuum and two extractions of the residue with ethyl acetate (100 mL), the combined extracts were washed with water (20 mL), dried over Na_2SO_4, and evaporated to dryness. Recrystallization of the solid from acetonitrile yielded 3.0 g 2-[(3-pyridylmethyl)thio]-5-(trifluoromethyl)-1*H*-benzimidazole, which was dissolved in dichloromethane (62 mL) and oxidized with 1.9 g 3-chloroperoxybenzoic acid at −30 °C (1 h). Chromatographic purification on silica gel (CH_2Cl_2/MeOH = 96:4) yielded 1.6 g colorless solid, which was recrystallized from acetonitrile: yield, 1.3 g (25%); mp 177–178 °C; ^1H NMR (Me_2SO-d_6) δ 13.7 (s, 1 H, NH), 8.46 (dd, 1 H, $J_{6',5'}$ = 4.8 Hz, $J_{6',4'}$ = 1.5 Hz, H-6′), 8.19 (s, 1 H, H-2′), 8.0 (s, br, 1 H, H-4), 7.78 (s, br, 1 H, H-7), 7.62 (d, 1 H, $J_{6,7}$ = 7.5 Hz, H-6), 7.46 (d, 1 H, $J_{4',5'}$ = 7.9 Hz, H-4′), 7.27 (dd, 1 H, H-5′), 4.67 (AB, 2 H, J_{AB} = 13.3 Hz, Δν = 53.9 Hz, 3′-CH_2). Anal. Calcd for $C_{14}H_{10}F_3N_3OS$: C, 51.69; H, 3.10; N, 12.92; F, 17.52; S, 9.85. Found: C, 51.59; H, 2.96; N, 12.79; F, 17.50; S, 9.82.

2-[((4-Methoxy-6-methyl-2-pyridyl)methyl)sulfinyl]-5-(trifluoromethyl)-1*H*-benzimidazole (9). This sulfoxide was prepared analogously to the synthesis described for **1c,d**[21] as follows.

2-(Acetoxymethyl)-4-methoxy-6-methylpyridine. Acetic

(20) Junggren, U. K.; Sjöstrand, S. E. Eur. Pat. 005 129, 1979.
(21) Senn-Bilfinger, J.; Schaefer, H.; Figala, V.; Klemm, K.; Rainer, G.; Riedel, R.; Schudt, Chr.; Simon, W. Ger. Offen. DE 3 240 248, 1981.
(22) Rawlings, D. A.; Saunders, D., submitted for publication in *J. Labelled Compounds Radiopharm.*
(23) The corresponding 5-trifluoromethyl derivative (mp 128–129 °C; ^1H NMR (Me_2SO-d_6) δ 8.28 (d, 1 H, $J_{6',5'}$ = 5.7 Hz, H6′), 8.16 (s, 1 H, H-4), 7.94 (d, 1 H, $J_{7,6}$ = 8.7 Hz, H-7), 7.73 (d, 1 H, H-6), 7.00 (d, 1 H, $J_{3',5'}$ = 2.3 Hz, H-3′), 6.92 (dd, 1 H, H-5′), 4.84 (AB, 2 H, Δν = 11.4 Hz, J_{AB} = 13.0 Hz, 2′-CH_2), 3.95 (s, 3 H, 1-CH_3), 3.74 (s, 3 H, 4′-OCH_3). Anal. Calcd for $C_{16}H_{14}F_3N_3O_2S$: C, 52.03; H, 3.82; N, 11.38; S, 8.68. Found: C, 51.89; H, 3.80; N, 11.30; S, 8.59) was unambiguously synthesized starting from commercially available 2-fluoro-5-(trifluoromethyl)nitrobenzene and methylamine. The further transformations were performed according to the literature.[21]

anhydride (255 g, 2.5 mol) was heated to 90 °C, and 4-methoxy-2,6-dimethylpyridine *N*-oxide[24] (131 g, 0.86 mol) in dichloromethane (180 mL) was added dropwise. The reaction mixture was kept at 100 °C for 2 h and cooled to room temperature, and the volatiles were removed under vacuum. Distillation of the remaining oil gave 130 g (78%) of product, bp 115 °C (0.05 mm). The compound was found to be pure by TLC (silica gel; toluene/dioxane = 1:1, R_f 0.25): ^{1}H NMR ($CDCl_3$) δ 6.70 (d, 1 H $J_{3,5}$ = 2.3 Hz, H-3 or H-5), 6.60 (d, 1 H, H-5 or H-3), 5.12 (s, 2 H, 2-CH_2), 3.83 (s, 3 H, 4-OCH_3), 2.51 (s, 3 H, 6-CH_3), 2.15 (s, 3 H, CO-CH_3). Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.17. Found: C, 61.44; H, 6.89; N, 7.15.

2-(Hydroxymethyl)-4-methoxy-6-methylpyridine. 2-(Acetoxymethyl)-4-methoxy-6-methylpyridine (149 g, 0.83 mol) was hydrolyzed by stirring with 2 N NaOH (0.5 L) at 80 °C for 2 h. A brown solution was formed which, after cooling to room temperature, was extracted with dichloromethane (5 × 200 mL). The combined organic solutions were washed with 2 N NaOH (2 × 100 mL) and dried over Na_2SO_4. The solvent was removed and the residue recrystallized from ethyl acetate, affording 86.7 g, (68%), mp 79 °C. Purity was proved by TLC (silica gel; toluene/dioxane = 1:5, R_f 0.5); ^{1}H NMR ($CDCl_3$) δ 6.90–6.43 (dd, 2 H, H-3 and H-5), 4.70–4.10 (s, br, 1 H, OH), 4.63 (s, 2 H, 2-CH_2), 3.80 (s, 3 H, 4-OCH_3), 2.47 (s, 3 H, 6-CH_3). Anal. Calcd for $C_8H_{11}NO_2$: C, 67.72; H, 7.24; N, 9.15. Found: C, 62.85; H, 7.11; N, 9.25.

2-(Chloromethyl)-4-methoxy-6-methylpyridine Hydrochloride. To a stirred solution of 2-(hydroxymethyl)-4-methoxy-6-methylpyridine (61.3 g, 0.4 mol) in dichloromethane (500 mL), chilled to 5–10 °C and protected by a drying tube, was added $SOCl_2$ (36.6 mL, 0.5 mol) dropwise over a 1-h period. The reaction mixture was then allowed to warm to room temperature and was stirred for 1 h. Toluene (500 mL) was added, and dichloromethane was evaporated under vacuum. After cooling, the residue was collected by filtration, washed well with toluene/hexane (1:1; 2 × 20 mL), and dried: yield, 83.2 g (100%); mp 98.5–99.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.46–7.11 (dd, 2 H, H-3 and H-5), 5.12 (s, 2 H, 2-CH_2), 4.13 (s, 3 H, 4-OCH_3), 2.88 (s, 3 H, 6-CH_3).

2-[((4-Methoxy-6-methyl-2-pyridyl)methyl)thio]-5-(trifluoromethyl)-1*H*-benzimidazole. A mixture of 5-(trifluoromethyl)-2-mercapto-1*H*-benzimidazole (1.09 g, 5 mmol), 2-(chloromethyl)-4-methoxy-6-methylpyridine hydrochloride (1.05 g, 4.9 mmol), 1 N NaOH (10.5 mL), EtOH (15 mL), and H_2O (40 mL) was stirred for 16 h at room temperature. After dilution with H_2O (30 mL) the solid was filtered, washed with 0.3 N NaOH and water, and dried. Purity was proved by TLC (silica gel; CH_2Cl_2/MeOH = 19:1, R_f 0.6): yield, 1.15 g (64%); mp 105–107 °C; ^{1}H NMR ($CDCl_3$/CD_3OD = 10:1) δ 7.79 (s, 1 H, H-4), 7.59 (d, 1 H, $J_{7,6}$ = 8.4 Hz, H-7), 7.46 (d, 1 H, H-6), 6.83 (d, 1 H, $J_{3',5'}$ = 2.1 Hz, H-3′ or H-5′), 6.67 (d, 1 H, H-5′ or H-3′), 4.39 (s, 2 H, 2′-CH_2), 3.85 (s, 3 H, 4′-OCH_3), 2.58 (s, 3 H, 6′-CH_3). Anal. Calcd for $C_{16}H_{14}F_3N_3OS$: C, 54.38; H, 3.99; N, 11.89. Found: C, 54.29; H, 4.01; N, 11.71.

2-[((4-Methoxy-6-methyl-2-pyridyl)methyl)sulfinyl]-5-(trifluoromethyl)-1*H*-benzimidazole (9). To a stirred solution of 2-[((4-methoxy-6-methyl-2-pyridyl)methyl)thio]-5-(trifluoromethyl)-1*H*-benzimidazole (0.71 g, 2 mmol) in dichloromethane (20 mL) chilled to −50 °C was added 3-chloroperoxybenzoic acid (19 mL of 0.1 m solution in dichloromethane) dropwise over a period of 20 min under N_2, and the resulting solution was kept at −50 °C for 10 min. NEt_3 (0.28 mL) was added, and the cold solution was poured into a stirred mixture of sodium thiosulfate and sodium carbonate (respectively, 20 mL of 2% solution). After warming to room temperature, the layers were separated, and the organic layer was washed with sodium thiosulfate (2% solution, 2 × 10 mL), dried over sodium sulfate, and evaporated to leave the residue, which was crystallized from dichloromethane/diisopropyl ether. Purity was proved by TLC (silica gel; CH_2Cl_2/MeOH = 19:1, R_f 0.5): yield, 0.45 g (61%); mp 165–167 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 8.02 (s, 1 H, H-4), 7.83 (d, 1 H, $J_{7,6}$ = 8.5 Hz, H-7), 7.63 (dd, 1 H, $J_{6,4}$ = 1.4 Hz, H-6), 6.77 (d, 1 H, $J_{3',5'}$ = 2.2 Hz, H-3′ or H-5′), 6.71 (d, 1 H, H-5′ or H-3′), 4.59 (AB, 2 H, $\Delta\nu$ = 22.4 Hz, J_{AB} = 12.9 Hz, 2′-CH_2), 3.71 (s, 3 H, 4′-OCH_3), 2.18 (s, 3 H, 6′-CH_3). Anal. Calcd for $C_{16}H_{14}F_3N_3O_2S$: C, 52.03; H, 3.82; N, 11.38; S, 8.68. Found: C, 51.73; H, 3.93; N, 11.36; S, 8.94.

1-Methyl-2-[((4-methoxy-3-methyl-2-pyridyl)methyl)thio]-6-(trifluoromethyl)-1*H*-benzimidazole (6f). **1f** (2.1 g, 5.4 mmol) was added at room temperature to a well-stirred mixture of 0.84 g (10.8 mmol) 2-mercaptoethanol in 150 mL 0.1 N HCl. After 1 h the pH of the mixture was adjusted to neutral by means of a saturated aqueous Na_2CO_3 solution and extracted twice with ethyl acetate. The combined extracts were dried over Na_2SO_4, evaporated to dryness, and recrystallized from acetonitrile: yield, 1.7 g (86%); mp 159–160 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.27 (d, 1 H, $J_{6',5'}$ = 5.6 Hz, H-6′), 7.92 (s, 1 H, H-7), 7.71 (d, 1 H, $J_{4,5}$ = 8.5 Hz, H-4), 7.53 (d, 1 H, H-5), 6.99 (d, 1 H, H-5′), 4.78 (s, 2 H, 2′-CH_2), 3.87 (s, 3 H, 1-CH_3), 3.82 (s, 3 H, 4′-OCH_3), 2.20 (s, 3 H, 3′-CH_3). Anal. Calcd for $C_{17}H_{16}F_3N_3OS$: C, 55.58; H, 4.39; N, 11.43; S, 8.74. Found: C, 55.64; H, 4.44; N, 11.56; S, 8.70.

General Procedure for the Preparation of Disulfides 4c,d (R = CH_2CH_2OH). Sulfoxides **1c,d** (2.7 mmol) were dissolved at room temperature in a stirred mixture of 0.1 N aqueous HCl (160 mL) and 2.7 mmol of 2-mercaptoethanol. After 30 min the reactions were complete, and the pH of the reaction mixtures was adjusted to 7.0 by means of a saturated $NaHCO_3$ solution in water. Extraction with ethyl acetate and evaporation under reduced pressure yielded yellowish solids, which were dried under vacuum at 50 °C.

2-[2-(5-Hydroxy-2,3-dithiapentyl)-4-methoxy-1-pyridinio]-5-(trifluoromethyl)benzimidazolide (4c, R = CH_2CH_2OH): 32% yield after crystallization from isopropyl alcohol; mp 139.5 °C dec; for ^{1}H NMR and ^{13}C NMR data, see Tables III–V. Anal. Calcd for $C_{17}H_{16}F_3N_3O_2S_2$: C, 49.15; H, 3.88; N, 10.11; S, 15.44. Found: C, 49.32; H, 4.11; N, 10.14; S, 15.04.

2-[2-(5-Hydroxy-2,3-dithiapentyl)-4-methoxy-3-methyl-1-pyridinio]-5-(trifluoromethyl)benzimidazolide (4d, R = CH_2CH_2OH). Recrystallization from acetonitrile gave colorless needles: 85% yield; mp 148 °C; for ^{1}H NMR and ^{13}C NMR data, see Tables II, III, and V. Anal. Calcd for $C_{18}H_{18}F_3N_3O_2S_2$: C, 50.34; H, 4.22; N, 9.78; F, 13.27. Found: C, 50.27; H, 4.32; N, 9.80; F, 13.13.

Single-Crystal X-ray Structure of 4d. Crystals of **4d** suitable for analysis were prepared by slow crystallization from acetonitrile. They were triclinic, space group $P\bar{1}$, with a = 692.7 (4) pm, b = 1153.8 (7) pm, c = 1203.4 (8) pm, α = 98.80 (5)°, β = 101.54 (5)°, γ = 82.21 (5)°, and d_{calcd} = 1.53 g cm^{-3} for Z = 2 ($(C_{18}H_{18}F_3N_3O_2S_2)$, M_r 429.5). Data collection was carried out on a Syntex-Nicolet P3 Diffractometer equipped with a graphite monochromated Mo Kα radiation source. The size of the crystal used for data collection was 0.2 × 0.2 × 0.3 mm. A total of 2583 independent reflections were measured for 1° ≤ 2θ ≤ 46°, of which 2050 were considered to be observed [$I_0 \geq 2\sigma(I_0)$]. The structure was solved by direct methods using the SHELXTL package[25] and refined by least-squares techniques. The positions of the hydrogen atoms were derived from differential Fourier syntheses. The final agreement factors are R = 0.050 and R_w = 0.054.

4-Methoxy-2-[2-(4-phenyl-2,3-dithiabutyl)-1-pyridinio]-5-(trifluoromethyl)benzimidazolide (4c, R = $CH_2C_6H_5$). **1c** (5 g, 14 mmol) was dissolved in a mixture of THF (300 mL) and H_2O (100 mL), and a mixture of benzyl mercaptan (1.65 mL, 14 mmol), THF (250 mL), and 2 N HCl (7 mL) was added dropwise with stirring. After 3 h the mixture was neutralized with $NaHCO_3$ and extracted with ethyl acetate, and the combined extracts were evaporated to dryness: yield, 37%; mp 126–140 °C dec; ^{1}H NMR (CD_3OD) δ 8.94 (d, 1 H, $J_{6',5'}$ = 7.3 Hz, H-6′), 7.88 (s, 1 H, H-4), 7.71 (d, 1 H, $J_{7,6}$ = 8.5 Hz, H-7), 7.56 (dd, 1 H, $J_{5',3'}$ = 2.9 Hz, H-5′), 7.39 (dd, 1 H, $J_{6,4}$ = ca. 1.5 Hz, H-6), 7.34 (d, 1 H, H-3′), 7.16–7.22 (m, 5 H, C_6H_5), 4.91 (s, 6 H, solvent OH + 2′-CH_2), 4.22 (s, 3 H, 4′-OCH_3), 3.74 (s, 2 H, benzylic CH_2). Anal. Calcd for $C_{22}H_{18}F_3N_3OS_2$: C, 57.25; H, 3.93; N, 9.11; S, 13.90; Found: C, 57.46; H, 3.56; N, 9.18; S, 13.80.

Reactions of 3 and 4. 2-(4-Methoxy-2-methyl-1-pyridinio)-5-(trifluoromethyl)benzimidazolide (5c). Addition of excess Raney Ni (Merck-Schuchardt, Darmstadt, FRG) to a

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well-stirred solution of **4c** (R = CH_2CH_2OH) (1 g, 2.4 mmol) in a mixture of acetic acid (10 mL) and MeOH (100 mL) at room temperature and under a N_2 atmosphere gave after 4 h a complete reaction. The Raney Ni was filtered off and washed twice with methanol (100 mL) and the solution evaporated to dryness. The mixture was separated by column chromatography (silica gel; ethyl acetate/CH_2Cl_2/MeOH = 6:4:2) and gave **6c** (480 mg, 58%) and **5c** (300 mg, 40%). **5c**: mp 137–140 °C; For ^1H NMR and ^{13}C NMR data, see Tables III and V. Anal. Calcd for $C_{15}H_{12}F_3N_3O$: C, 58.63; H, 3.94; N, 13.67. Found: C, 58.86; H, 3.99; N, 13.74.

1-Methyl-2-[2-(5-hydroxy-2,3-dithiapentyl)-3-methyl-1,4-dihydro-4-oxopyrid-1-yl]-6-(trifluoromethyl)benzimidazole (7f, R = CH_2CH_2OH). To a well-stirred solution of 0.20 g (2.6 mmol) of 2-mercaptoethanol in 160 mL of 0.1 N HCl was added 1.0 g (2.6 mmol) of **1f** at room temperature. After 30 min the reaction mixture was reduced under vacuum to a small volume, dissolved in 50 mL of dioxane, and refluxed for 1 h. The dioxane was evaporated and the liquid residue purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 98:2): yield, 0.6 g (53.8%); oil which did not crystallize; ^1H NMR ($CDCl_3$) δ 8.10 (s, 1 H, H-7), 7.71 (dd, 1 H, $J_{5,4}$ = 8.7 Hz, $J_{5,7}$ = ca. 1.2 Hz, H-5), 7.55 (d, 1 H, H-4), 7.33 (d, 1 H, $J_{6',5'}$ = 7.7 Hz, H-6'), 6.47 (d, 1 H, H-5'), 4.11 (s, 2 H, 2'-CH_2), 3.73 (s, 3 H, NCH_3), 3.60 (t, 2 H, OCH_2), 2.62 (t, 2 H, SCH_2), 2.61 (s, 3 H, 3'-CH_3). Anal. Calcd for $C_{18}H_{18}F_3N_3O_2S_2$: C, 50.34; H, 4.22; N, 9.78; S, 14.94. Found: C, 50.17; H, 4.51; N, 9.59; S, 14.66.

2-[((4-Methoxy-2-pyridyl)methyl)sulfonyl]-5-(trifluoromethyl)-1*H*-benzimidazole (10). To a stirred suspension of **6c** (300 mg, 0.92 mmol) in dichloromethane (10 mL) at −10 °C was added a solution of 3-chloroperoxybenzoic acid (380 mg of 85%, 1.94 mmol) in dichloromethane (5 mL) during a period of 5 min. After a further 60 min, the temperature was raised to 20 °C (30 min), the solvent was evaporated under reduced pressure, and the solid residue was purified on silica gel (CH_2Cl_2/MeOH = 95:5): 140 mg solid, which was recrystallized from EtOH, yielding 80 mg (34%) of **10**; mp 197–198 °C; ^1H NMR (Me_2SO-d_6) δ 8.17 (d, 1 H, $J_{6',5'}$ = 5.7 Hz, H-6'), 8.12 (s, 1 H, H-4), 7.89 (d, 1 H, $J_{7,6}$ = 8.7 Hz, H-7), 7.70 (dd, 1 H, $J_{6,4}$ = 1.3 Hz, H-6), 7.00 (d, 1 H, $J_{3',5'}$ = 2.4 Hz, H-3' or H-5'), 6.93 (dd, 1 H, H-5' or H-3'), 5.08 (s, 2 H, 2'-CH_2), 3.73 (s, 3 H, 4'-OCH_3). Anal. Calcd for $C_{15}H_{12}F_3N_3O_3S$: C, 48.52; H, 3.26; N, 11.32; S, 8.62. Found: C, 48.47; H, 3.33; N, 10.84; S, 8.72.

2-[(2-Hydroxyethyl)thio]-5-(trifluoromethyl)-1*H*-benzimidazole (11). **11** was formed from **8**, **9**, **10**, and **6c** by substitution, according to Scheme II, and its identity confirmed by independent synthesis. To a mixture of 3 g (14 mmol) of 2-mercapto-5-(trifluoromethyl)-1*H*-benzimidazole in EtOH (120 mL) and 6 N NaOH (2.4 mL) was added 2-chloroethanol (2 mL). Subsequently the mixture was refluxed for 3 h. The solvent was evaporated, the residue dissolved in H_2O and ethyl acetate (1:1, 50 mL) and neutralized by adding 2 N HCl, and the aqueous phase extracted twice with ethyl acetate. The solvent was removed and the residue recrystallized from toluene. For further purification the raw material was dissolved in 1,4-dioxane (1.5 mL) and recrystallized by addition of toluene (70 mL): yield, 2.5 g (70%); mp 134–136 °C. Anal. Calcd for $C_{10}H_9F_3N_2OS$: C, 45.80; H, 3.46; N, 10.68; F, 21.73; S, 12.23. Found: C, 45.90; H, 3.43; N, 10.91; F, 21.57; S, 11.94. ^1H NMR (Me_2SO-d_6) δ 7.76 (s, 1 H, H-4), 7.60 (d, 1 H, $J_{7,6}$ = 8.3 Hz, H-7), 7.44 (d, 1 H, H-6), 3.75 (t, 2 H, OCH_2), 3.42 (t, 2 H, S-CH_2).

Formation of a Protonated 2-Pyridiniobenzimidazolide (3b) from 1b and Reduced Glutathione in Situ in an NMR Tube (R = Glutathionyl). Reduced glutathione (9.22 mg, 0.01 M) was dissolved in phosphoric acid/phosphate buffer (0.20 M) in D_2O (3.0 mL) at pD 3.1 and 37 °C. **1b** (10.36 mg, 0.01 M) was added, and an aliquot (0.5 mL) of the resulting solution was transferred to an NMR tube. The half-life for production of **3b** was about 8.5 min and the yield ca. 90%: ^1H NMR (D_2O) δ 8.76 (s, 1 H, H-6'), 7.71 (d, 1 H, $J_{7,6}$ = 9.2 Hz, H-7), 7.32 (d, 1 H, $J_{4,6}$ = 2.4 Hz, H-4), 7.15 (dd, 1 H, $J_{6,7}$ = 9.0 Hz, $J_{6,4}$ = 2.4 Hz, H-6), 4.50 (m, 1 H, CH (Cys)), 4.44 and 4.36 (AB, 2 H, ^2J = 15 Hz, 2'-CH_2), 4.34 (s, 3 H, 4'-OCH_3), 3.92 (s, 3 H, 5-OCH_3), 3.88 (s, 2 H, NCH_2), 3.80 (m, 1 H, CH (Glu)), 2.70 and 2.66 (m, 2 H, SCH_2), 2.55 (s, 3 H, 3'-CH_3 or 5'-CH_3), 2.52 (s, 3 H, 5'-CH_3 or 3'-CH_3), 2.49 (m, 2 H, CH_2CON), 2.12 (m, 2 H, CCH_2C).

Formation of a Protonated 2-Pyridiniobenzimidazolide (3b) from 1b and Excess Cysteine in Situ in an NMR Tube (R = Cysteinyl). Cysteine hydrochloride (9.46 mg, 0.02 M) was dissolved in phosphoric acid/phosphate buffer (0.20 M) in D_2O (3.0 mL) at pD 2.7 and 20 °C. **1d** (10.36 mg, 0.01 M) was added, and an aliquot (0.5 mL) of the resulting solution was transferred to an NMR tube. The 2-pyridiniobenzimidazolide salt produced reached a maximum concentration after about 19 min but was cleaved by excess cysteine to yield a benzimidazolyl sulfide (**6b**), the reduced form of **1d**, and cystine.

3b (R = cysteinyl): ^1H NMR (D_2O) δ 8.77 (s, 1 H, H-6'), 7.72 (d, 1 H, $J_{7,6}$ = 9.0 Hz, H-7), 7.33 (d, 1 H, $J_{4,6}$ = 2.4 Hz, H-4), 7.16 (dd, $J_{6,7}$ = 9.0 Hz, $J_{6,4}$ = 2.4 Hz, H-6), 4.48 and 4.44 (AB, 2 H, J_{AB} = 14.9 Hz, 2'-CH_2), 4.35 (s, 3 H, 4'-OCH_3), 3.94 (m, 1 H, NCH(C=O)), 3.93 (s, 3 H, 5-OCH_3), 2.85 (m, 2 H, SCH_2), 2.57 (s, 3 H, 5'-CH_3 or 3'-CH_3), 2.55 (s, 3 H, 3'-CH_3 or 5'-CH_3).

6b: ^1H NMR (D_2O) δ 8.28 (s, 1 H, H-6'), 7.59 (d, 1 H, $J_{7,6}$ = 9.2 Hz, H-7), 7.21 (d, 1 H, $J_{4,6}$ = 2.0 Hz, H-4), 7.18 (dd, 1 H, $J_{6,7}$ = 8.9 Hz, $J_{6,4}$ = 2.2 Hz, H-6), 4.64 (s, 2 H, CH_2), 3.95 (s, 3 H, 5-OCH_3 or 4'-OCH_3), 3.89 (s, 3 H, 4'-OCH_3 or 5-OCH_3), 2.37 (s, 3 H, 3'-CH_3 or 5'-CH_3), 2.17 (s, 3 H, 5'-CH_3 or 3'-CH_3).

In Situ Investigation of the Formation of a Protonated [2-^{13}C,1'-^{15}N]-2-Pyridiniobenzimidazolide (3g) by ^1H and ^{13}C NMR Spectroscopy (R = Glutathionyl). [2-^{13}C,1'-^{15}N]-5-Methoxy-2-[((4-methoxy-2-pyridyl)methyl)sulfinyl]-1*H*-benzimidazole (**1g**) (5.26 mg) was added to an equimolar quantity of reduced glutathione (5.06 mg) dissolved in phosphoric acid/phosphate buffer (0.6 mL) to give pD 3 at 25 °C. The condition of the reaction mixture was monitored by proton NMR spectroscopy. After about 20 min the spectrum of the starting material had been replaced by that of a protonated [2-^{13}C,1'-^{15}N]-2-pyridiniobenzimidazolide (**3g**, R = glutathionyl), the carbon-13 spectrum of which was immediately recorded: ^1H NMR (D_2O) δ 8.90 (m, 1 H, H-6'), 7.72 (s, 1 H, H-3'), 7.68 and 7.66 (m, 2 H, H-5' and H-7), 7.29 (s, 1 H, H-4), 7.12 (d, $J_{6,7}$ = 9 Hz, H-6), 4.54 (m, 1 H, C-H (Cys)), 4.29 (s, 3 H, 4'-OCH_3), 3.91 (s, 3 H, 5-OCH_3), 3.83 (m, 2 H, NCH_2), ca. 2.8 (m, 2 H, SCH_2), 2.47 (m, 2 H, CH_2CON)), 2.11 (m, 2 H, CCH_2C) [CH (Glu) and 2'-CH_2 obscured]; ^{13}C NMR (D_2O) δ 142.31 (d, ^1J(^{13}C–^{15}N) = 24.9 Hz; sign not determined, C-2) [reference dioxane, δ 67.4].

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