Structure−Activity Relationship of 2-[[(2-Pyridyl)methyl]thio]-1*H*-benzimidazoles as Anti *Helicobacter pylori* Agents in Vitro and Evaluation of their in Vivo Efficacy

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A relationship between the structure of 21 2-[[(2-pyridyl)methyl]thio]-1*H*-benzimidazoles (**6**) and their anti *Helicobacter pylori* activity expressed as minimum bactericidal concentration (MBC) values is described. Observed MBCs ranged from 256 to 1 μg/mL. The structure−activity relationship (SAR) showed that larger and more lipophilic compounds, especially compounds with such substituents in the 4-position of the pyridyl moiety, generally had lower MBC values. Four new compounds that were predicted to be potent by the established SAR model were synthesized and tested. One such compound, i.e., 2-[[(4-[(cyclopropylmethyl)oxy]-3-methyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (**18**), was tested for in vivo efficacy in a mouse *Helicobacter felis* model (125 μmol/kg bid given orally for 4 days, $n = 4$). Unfortunately, antibacterial activity could not be clearly demonstrated in this model. Instead a potent acid secretion inhibition was observed. This finding was attributed to the methylthio compound being oxidized to the corresponding methyl sulfinyl derivative, i.e., a proton pump inhibitor, in vivo. Although the antibacterial activity had the potential of decreasing *H. felis* cell counts in vivo the proton pump inhibitory effect became dominant and actually promoted *H. felis* cell growth. Hence, we conclude that the antibacterial utility of the 2-[[(2-pyridyl)methyl]thio]-1*H*-benzimidazoles (**6**) as a compound class is compromised by their propensity to become proton pump inhibitors upon metabolic oxidation in vivo.

Introduction

The proton pump inhibitor omeprazole (**1**) has become an integral part of contemporary antimicrobial combination therapies directed against the gastric pathogen *Helicobacter pylori*.[1,2] The beneficial interaction between omeprazole and the antimicrobial(s) has been suggested to be secondary to potent acid inhibition following a regimen of omeprazole, rather than being a result of an additional antibacterial activity.[3−5] Nevertheless, the in vitro antibacterial activity of omeprazole and other proton pump inhibitors (**2**) has attracted considerable interest.[6−8] Indeed, we ourselves recently elaborated on this mechanism and demonstrated that, in addition to omeprazole, certain break-down products are responsible for the observed in vitro antibacterial activity. The formation of each of these depends on the experimental conditions employed, including the pH, the incubation time, and the composition of the growth medium used.[9]

In the course of this and earlier work it became evident that the immediate synthetic precursor of omeprazole, the sulfide **3**, also exhibits antibacterial activity. From a chemical point of view the sulfide is less complex than omeprazole. Omeprazole is a prodrug and the precursor of the sulfenamide **4** which covalently modifies cysteine residues **5** on the luminal side of the proton pump,[10] i.e., the H^+/K^+-ATPase of the parietal cell in the oxyntic mucosa of the stomach. Omeprazole is rapidly converted to the sulfenamide at low pH (Scheme 1). In the (human) body this is exclusively achieved in the tubuvesicular and canalicular structures of the parietal cell, i.e., the structures carrying the proton pump. Omeprazole is also a weak base and accumulates in these acidic compartments. Once there, it is rapidly converted to the sulfenamide which is effectively prevented from reentering the systemic circulation by its charge, thereby accumulating in the vicinity of the target enzyme resulting in a selective and specific mode of action in the human body.[11]

The sulfide **3** is not subject to the same inherent reactivity and complex chemistry and thus could provide a simpler, more attractive class of antimicrobials than omeprazole itself. This is further strengthened by the finding that the antibacterial activity of the sulfide is specific for *Helicobacter* spp.,[12] suggesting the possibility of developing a very selective drug.

In this paper we report the in vitro structure−activity relationship (SAR) of some 2-[[(2-pyridyl)methyl]thio]-1*H*-benzimidazoles, i.e., sulfides with the generic structure **6**, and the in vivo evaluation of one selected derivative **18** (Chart 1).

Chemistry

Design Concepts. Twenty-one sulfides were randomly selected and tested for anti *H. pylori* activity. Disregarding structure **21**, the selected compounds,

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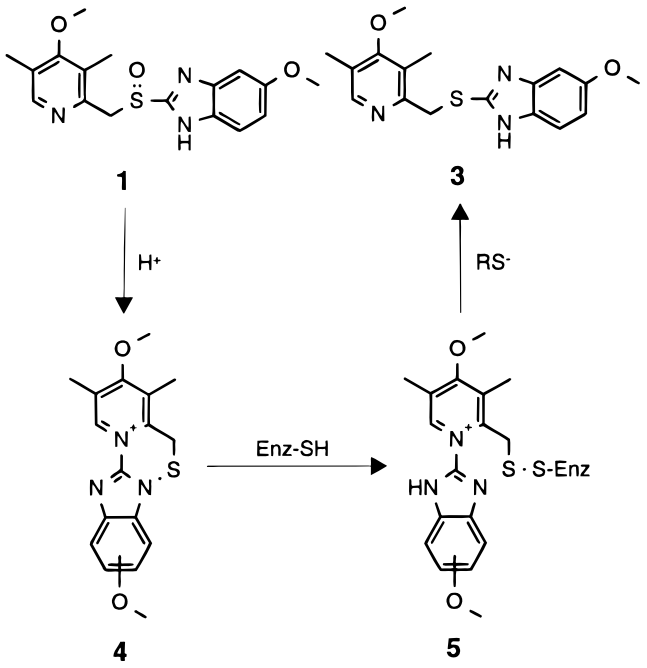
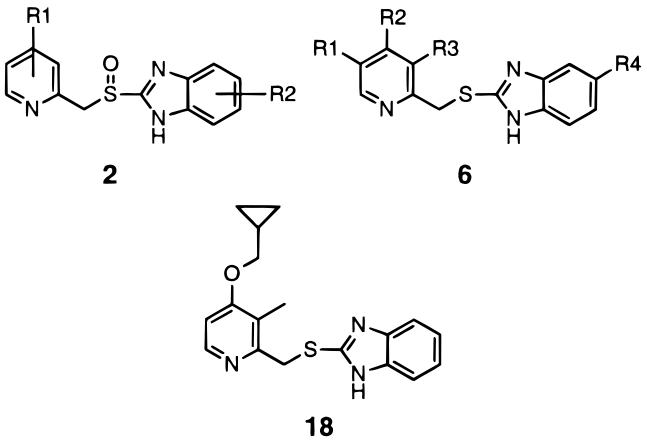
Scheme 1[a]



[a] Omeprazole (**1**) is a prodrug which at low pH readily furnishes the active species, the sulfenamide **4**. The sulfenamide in turn covalently modifies cysteine residues in the H^+/K^+-ATPase (the proton pump) to form an enzyme−inhibitor complex **5**. When Enz-SH equals R-SH, as is the case when the antibacterial activity of omeprazole is studied in Brucella broth or by Agar dilution techniques, the disulfide corresponding to **5** can react with available thiolates (RS^-) to generate the sulfide **3**.

Chart 1[a]



[a] The generic structure of a proton pump inhibitor **2**, the generic structure of the 2-[[(2-pyridyl)methyl]thio]-1*H*-benzimidazoles (**6**) investigated in this study, and the sulfide (**18**) selected for in vivo evaluation.

corresponding to structures **3** and **7**−**26** in Table 1, could be divided into three principal groups, those carrying no ($R_1 = R_3 = H$), one ($R_1 = H$, $R_3 = CH_3$), or two ($R_1 = R_3 = CH_3$) methyl groups in the pyridine ring, respectively. A second categorization was based on the absence or presence of substituents in the benzimidazole moiety, cf. R_4 in Table 1. Thus, the SAR analysis discussed below was carried out on three sets of data: one subset where $R_4 = H$ (12 compounds), a second subset where $R_4 \neq H$ (nine compounds), and a third (complete) set which comprised all 21 compounds.

The sulfides are amphoteric, and charged species will to different extents contribute to the overall lipophilicity, cf. Scheme 2. Initial attempts to account for the presence of these charged species by including pK_a values as variables in the SAR proved not to be straightforward. Protonation of the pyridine nitrogen was hard to distinguish from protonation on the benzimidazole moiety such that pK_a values were ambiguous. A superior means of estimating the contribution of these charged species to the overall lipophilicity was provided by chromatographic retention time k'.[13] Relating each retention time to a reference compound (**3**) had the advantage of compensating for variations between different experiments as well as yielding relative[14] (k'_0) rather than absolute lipophilicities.

In an attempt to identify other pertinent SAR variables, each structure in addition to each substituent (R_1 through R_4 in Table 1) was described by a number of geometrical and quantum chemistry parameters calculated by the TSAR-software (V2.31; Oxford Molecular Ltd. Oxford, UK). Initial approximations of molecular conformations were calculated by COBRA and optimized by VAMP.

The contribution of the measured lipophilicity index k'_0 and each of the calculated TSAR variables to the SAR model was then checked by the partial least square (PLS) method using the SIMCA program[15] to decide whether they indeed qualified as variables. A model based on 21 entries, compound **3** and compounds **7**−**26**, was then built and used to predict the activities of four additional compounds, structures **27**−**30**, which were synthesized and tested.

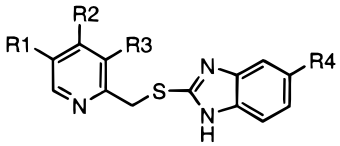
Syntheses. The syntheses of the target compounds are outlined in Scheme 3.

2,4-Disubstituted Pyridines. 2-Methyl-4-nitropyridine *N*-oxide[16] (**31**) was alkoxylated under basic conditions to furnish the alkoxy derivatives **32** or **33**. These were rearranged according to Katada[17] to furnish the (hydroxymethyl)pyridines **34** and **35**, respectively. The 2-methyl- and the 2,4-dimethylpyridines **36** and **37** were both reacted similarly to yield the 2-(hydroxymethyl)-pyridine (**38**)[18] and the 2-(hydroxymethyl)-4-methylpyridine (**39**), respectively. The 4-*tert*-butyl derivative **40** and the 4-chloro analogue **41** were both synthesized by the Minisci procedure[19,20] starting from compounds **42** and **43**, respectively. The thiomethyl compound **44** was obtained by treating the chloro derivative **41** with sodium thiomethoxide. The 4-substituted 2-(hydroxymethyl)pyridines so prepared could readily be activated with thionyl chloride and reacted with the appropriate 2-mercapto-1*H*-benzimidazoles to furnish target compounds **7**−**13**.

2,3,4-Trisubstituted Pyridines. 2,3-Dimethyl-4-nitropyridine *N*-oxide[16] (**45**) or 2,3-dimethyl-4-chloropyridine *N*-oxide (**46**) were alkoxylated under basic conditions to afford intermediates **47**−**51** or intermediates **52** and **53**, respectively. Again, the Katada conditions[21] were employed to prepare the (hydroxymethyl)-pyridines **54**−**60**. Activation with thionyl chloride and coupling with the appropriate 2-mercapto-1*H*-benzimidazoles afforded target compounds **17**−**19** and **27**−**30**. Target compounds **14**,[16] **15**,[16] **16**,[22] **20**,[16] and **21**[16] were prepared as described previously.

2,3,4,5-Tetrasubstituted Pyridines. 2,3,5-Trimethylpyridine (**61**) was oxidized to the corresponding *N*-oxide **62**. Nitration of **62** in the 4-position followed

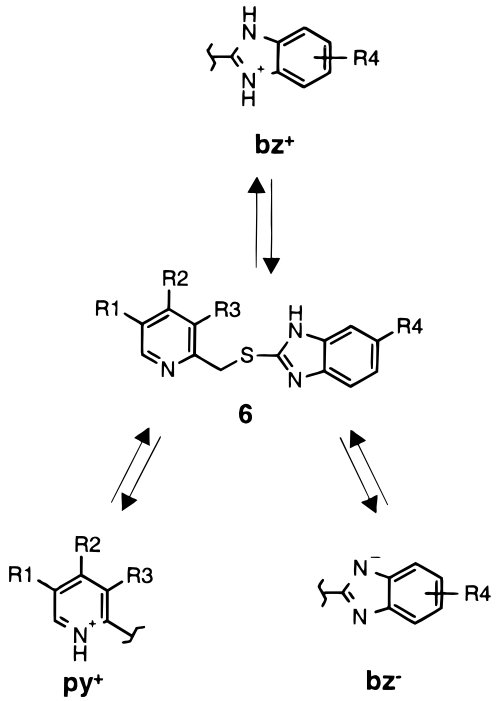
Table 1. Compounds Selected and Tested for Anti *H. pylori* Activity (Compounds **27**−**30** Were Synthesized To Test the PLS Model)



compd no.	R_1	R_2	R_3	R_4	MBC (μg/mL)	log MBC (nmol/mL)[a]	log k'_o[b]	MR[c] for R_2	mp (°C)	microanalysis
7	H	OCH_3	H	H	64	2.37	−0.30	6.15	126−127	NC
8	H	OCH_2-c-Pr	H	H	32	2.01	0.13	18.99	131−133	NC
9	H	H	H	H	128	2.72	−0.39	0.89	130−131	NC
10	H	Cl	H	H	64	2.36	−0.08	5.39	ND	NC
11	H	CH_3	H	H	128	2.70	−0.21	5.5	145−146	NC
12	H	*t*-Bu	H	$COCH_3$	64	2.27	0.18	18.28	ND	NC
13	H	SCH_3	H	OCH_3	256	2.91	−0.16	12.6	131−132	NC
14	H	OEt	CH_3	H	2	0.82	0.19	10.87	*d*	*d*
15	H	OCH_2CF_3	CH_3	H	4	1.05	0.28	11.6	*d*	*d*
16	H	$O(CH_2)_3OCH_3$	CH_3	H	2	0.77	0.07	22.06	*e*	*e*
17	H	OCH_3	CH_3	H	32	2.05	−0.08	6.15	>250 dec	NC
18	H	OCH_2-c-Pr	CH_3	H	1 (0.5−1)[f]	0.48	0.46	18.14	151−152	$C_{18}H_{19}N_3OS \cdot {}^1/_8EtOAc$[g]
19	H	$O(CH_2)_2OCH_3$	CH_3	H	2	0.78	−0.17	17.2	127−128	NC
20	H	OCH_3	CH_3	CH_2OH	128	2.61	−0.66	6.15	*d*	*d*
21	H	OCH_3	OCH_3	F	4	1.05	0.44	18.99	*d*	*d*
22	CH_3	OCH_3	CH_3	H	8	1.43	0.05	6.16	130	NC
3	CH_3	OCH_3	CH_3	OCH_3	32	1.99	0.00	6.15	*d*	*d*
23	CH_3	OCH_3	CH_3	*t*-Bu	8	1.35	0.86	6.15	84−86	NC
24	CH_3	H	CH_3	Cl	8	1.42	0.45	0.89	177−179	NC
25	CH_3	H	CH_3	$COCH_3$	128	2.61	−0.11	0.89	>90 dec	NC
26	CH_3	H	CH_3	CH_3	32	2.05	0.23	0.89	199−200	NC
27	H	O-*i*-Bu	CH_3	H	1 (<1)[h]	0.48 (0.42)[h]	0.75	19.95	153	$C_{18}H_{21}N_3OS$[g]
28	H	OCH_2CHCH_2	CH_3	H	1 (<4)[h]	0.51 (1.02)[h]	0.26	15.32	149	$C_{17}H_{17}N_3OS \cdot {}^1/_2H_2O$[g]
29	H	OCH_2CCH	CH_3	H	2 (<8)[h]	0.81 (1.24)[h]	−0.01	13.83	ND	$C_{17}H_{15}N_3OS \cdot {}^1/_2MeOH$[i]
30	H	OCH_2Ph	CH_3	H	8 (<1)[h]	1.34 (0.24)[h]	0.67	30.77	188−190	$C_{21}H_{19}N_3OS$[g]
Am[j]					0.05 (0.05)[f]					

[a] Molar units were used in the PLS model to account for differences in molecular weight. [b] Chromatographic retention values were used as a measure of lipofilicity as defined in refs 13 and 14. [c] Molecular refraction. [d] Reference 16. [e] Reference 22. [f] MBC values for *H. felis*. [g] C, H, N, S. [h] Activities predicted by the PLS model. [i] C, H, N. [j] Amoxicillin. ND, not determined. NC, not checked, purity was determined by two independent HPLC analyses.

Scheme 2[a]



[a] Charged Species, py^+, bz^-, and bz^+, will to different extents contribute to the overall lipophilicity of the compounds (**6**) that were studied.

by alkoxylation of the incipient nitro *N*-oxide **63** with sodium methoxide yielded the methoxylated *N*-oxide **64**. Both *N*-oxides **62** and **64** could be rearranged[21] to the (hydroxymethyl)pyridines **65** and **66**, respectively. Coupling of these with the proper 2-mercapto-1*H*-benzimidazoles provided target compounds **22**−**26**. Compound **3** was prepared as described previously.[16]

Except for derivative **67**, the required 2-mercapto-1*H*-benzimidazoles were commercially available or prepared as described earlier.[16] Briefly, 4-acetylacetanilide (**68**) was nitrated and reduced to furnish 4-acetylphenylenediamine (**69**) which was converted to **67** by treatment with potassium ethyl xanthogenate, cf. Scheme 4.

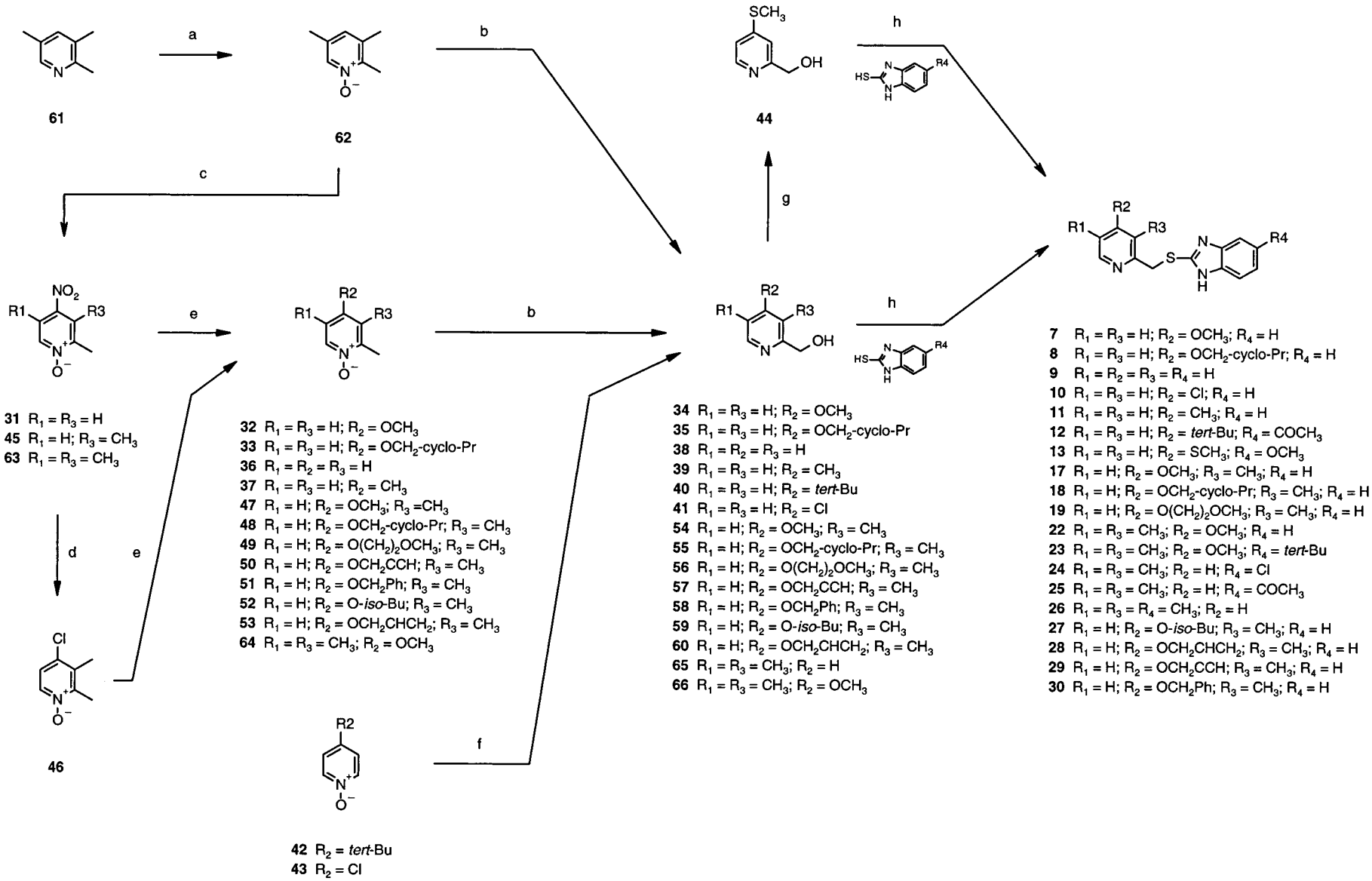
Microbiology and Pharmacology

Bacterial Strains. The *H. pylori* strain used in the antibacterial activity studies was ATCC 43504 from the American Type Culture Collection, Rockville, MD. The *Helicobacter felis* strain used in the animal studies was CS1 (ATCC 49179) and was obtained from Professor Adrian Lee (University of New South Wales, Sydney, Australia) who originally isolated this strain from a cat stomach.[23]

Stock cultures were stored at −70 °C in Brucella broth (Difco; pH 7.0) supplemented with 10% fetal calf serum (FCS) and 20% glycerol. The FCS was inactivated at 56 °C for 30 min prior to use.

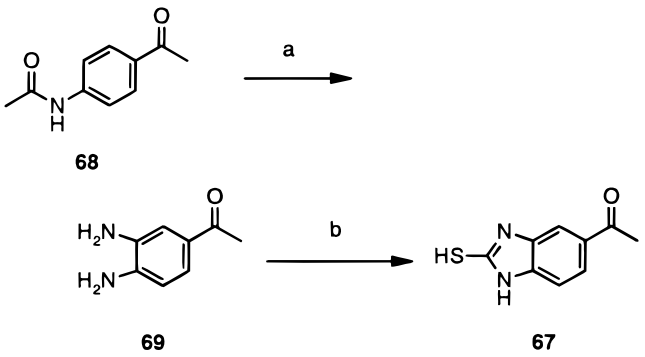
Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs). Brucella broth (Difco; pH 7.0) supplemented with 10% FCS was preferred for determinations of MICs. Solid medium for determining

Scheme 3. Synthetic Routes Toward Target Compounds **7**−**13**, **17**−**19**, and **22**−**30**[a]



[a] (a) H_2O_2/HOAc; (b) (1) $(CH_3CO)_2O$, Δ; (2) NaOH, Δ; (c) HNO_3, Δ; (d) HCl, Δ, autoclave; (e) base, ROH; (f) Minisci conditions, cf. refs 19 and 20; (g) $NaSCH_3$; (h) (1) $SOCl_2$; (2) NaOH and appropriately substituted mercaptobenzimidazole. Note that some of the synthetic building blocks have become commercially available but are still included in the scheme for reasons of clarity. Procedures for their preparation are not included in the experimental section.

Scheme 4. Synthesis of Compound **67**[a]



[a] (a) (1) HNO_3, 0 °C; (2) HCl, Δ; (3) H_2, Pd/C; (b) $K^{+-}SCSOEt$, Δ.

MBCs was Colombia blood agar containing 42.5 g/L Colombia Agar Base II and 15 g/L Bacto Agar (both from Oxoid), 7% horse blood, and 1% IsoVitaleX at pH 7.3 ± 0.2 (BBL Microbiology System). Bacteria were grown under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2) at 37 °C in an automatic CO_2-O_2 incubator for both MIC and MBC determinations.

Compounds were tested in twofold serial dilutions ranging from 0.5 to 256 μg/mL with an initial cell count of approximately 10^6 CFU/mL. In order to overcome solubility problems, compounds were dissolved *and diluted* in dimethyl sulfoxide before being added to the test medium. After incubation for 72 h, MICs were determined by reading the optical density at 560 nm. Then 10 μL from each well was transferred to a large Colombia blood agar plate (120 × 120 × 17 mm) which was incubated for additional 72 h to determine MBCs. MIC was defined as the lowest concentration of a given compound completely inhibiting growth whereas MBC was defined as the lowest concentration of a given compound furnishing less than 10 colonies/spot.

Animals. Female BALB/C mice (Bomholt Breeding Center, Denmark) were housed in Makrolon II cages four by four. They were fed with autoclaved commercial food pellets and sterile water ad libitum. Mean weight at arrival was 20 g. The animals were allowed to rest at least 1 week after arrival before being used in any experiment. Infected animals and controls were killed at appropriate time points by CO_2 and cervical dislocation. The study was approved by the local ethics committee.

Inoculation of Animals. *H. felis* was cultured on Skirrow's selective blood agar medium (37 g of Oxoid CM271 and 100 mL of defibrinated horse blood was used for 1000 mL) supplemented with 5 μg/mL amphotericin B (Squibb), 10 μg/mL vancomycin (Lilly), 3 μg/mL polymyxin B (Sigma), and 5 μg/mL trimethoprim (Sigma) under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2) at 37 °C in a humidified incubator for 3 days. Bacteria were harvested with phosphate-buffered saline (pH 7), and each animal was challenged with approximately 10^9 bacteria each day for three consecutive days by means of oro-gastric intubation. None of the animals exhibited any adverse symptoms throughout their life span that could be attributed to a persistent *H. felis* infection of the gastric mucosa.

In Vivo Studies. Animals were inoculated 6 weeks prior to the start of the experiment. Four animals each were randomly assigned to either control or treatment groups. Compound **18** (125 μmol/kg, 41 mg/kg) and amoxicillin (15 μmol/kg, 6.25 mg/kg) included as a positive control were both given bid for 4 days (mornings and afternoons) by means of oral dosing by gavage. Compound **18** was given as a suspension in carbonate-buffered 0.5% carboxymethylcellulose (pH 9), and amoxicillin was given as an aqueous unbuffered suspension of the trihydrate form (Astra Pharmaceuticals). The volume administered at each dosing occasion was 5 mL/kg.

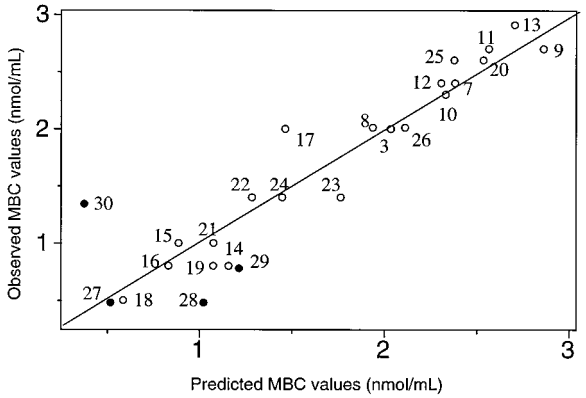


Figure 1. A PLS plot displaying predicted versus observed antibacterial activities. Entries used in the PLS analysis, ○; predicted entries, ●. Molar units were used to account for differences in molecular weight.

Urease Test, Vital Microscopy, and Histology. The animals were immediately sacrificed at the end of treatment. The abdomen was opened and the stomach removed. The stomach was opened along the greater curvature and briefly rinsed in saline. After division along the smaller curvature, half of the stomach was analyzed by direct microscopy. Small samples of mucosal scrapings from the antrum and the body, respectively, were microscopically checked for the presence of the typical spiral and rod-shaped often highly motile bacteria that are indicative of an *H. felis* infection. In addition, two small sections of tissue (approximately 3 × 3 mm) again from both the antrum and the body were checked in a rapid urease test.[24] The other half of the stomach was analyzed by histology. After fixation in phosphate-buffered saline (pH 7) with 4% formaldehyde, the tissue was trimmed and embedded in paraffin. Sections of 5 μm were cut and stained with Whartin-Starry silver stain or with Haematoxylin and Eosin stain. Sectioning, from the antrum all the way to the most proximal part of the corpus, was done to get representative specimen from all parts of the stomach. The degree of infection was then assessed microscopically by examining stained sections and estimating the bacterial density on a scale from 0 to 5. All sections were coded prior to the analyses.

Results

The MBC values of the test compounds against *H. pylori*, the measured lipophilicities, and one of the calculated variables (MR for the R_2 substituent) are given in Table 1. A PLS plot[15] displaying predicted *versus* observed antibacterial activities is shown in Figure 1.

The SAR analysis suggested the measured lipophilicity index k'_o to be the most important variable for

determining activity. The numerical values of this variable centered around 0 (the reference compound **3**) and ranged from polar −0.66 to nonpolar 0.86 and were quite evenly distributed throughout the entire range as shown in Table 1. This is a remarkable finding since the compounds were selected entirely at random. Five of the calculated variables also proved to be pertinent for explaining the biological variation. These were the molecular refraction (MR) and the molecular volume (MV) for the R_3 substituent, the MV and MR for the R_2 substituent, and the MV for the whole structure, in that order. Two PLS components were found to be statistically significant by cross validation ($R^2 = 0.92$), and the predictive power of the model was quite good with a Q^2 of 0.85. Examination of the PLS regression coefficients showed that in order to optimize potency both k'_o and the five calculated variables mentioned above should attain the highest numerical values possible since they all made favorable negative contributions to the numerical value of the MBCs. When the PLS analysis was repeated on either of the subsets defined in the Design Concepts section above, virtually identical results to those obtained with the entire data set were arrived at. That is, the biological variation was best accounted for by changes in k'_o and in the substituents on the pyridine ring. We interpreted this finding as justifying that compound synthesis for the first round of optimization studies could be limited to sulfides without an R_4 substituent since substitution on the benzimidazole moiety had a smaller effect than substitution on the pyridine ring.

Validation of the model by permuting the Y column in the PLS matrix (the experimentally determined MBC values) and reanalyzing these artificial data sets furnished models with far less predictive power than with the authentic data set. This finding suggests that the SAR was not due to an overfit or a chance correlation.

To test and benefit from the model, the four compounds **27**, **28**, **29** (probing k'_o while trying to keep MR for the R_2 substituent constant), and **30** (aiming at an increase in both k'_o and MR for the R_2 substituent) were synthesized and tested. As shown in both Table 1 and Figure 1, the analysis underestimated the activities of compounds **28** and **29** in comparison with that of compound **27** because of the former compounds' low k'_o and MR. The new results showed that k'_o could be both increased *and* decreased without losing potency while MR for the R_2 substituent remained (roughly) constant. This finding was quite contrary to that expected. Similarly, increasing both k'_o and MR for the R_2 substituent as in **30** did not make the compound more potent as predicted, but resulted in loss of potency.

The control compound amoxicillin, given orally at 15 μmol/kg (6.25 mg/kg) bid for 4 days, reduced bacterial cell counts as estimated by the scoring method in all areas of the stomach of animals infected with *H. felis*. Compound **18**, given orally at 125 μmol/kg (41 mg/kg) bid for 4 days, did not markedly affect bacterial cell counts in any region of the stomach of animals infected with *H. felis* when compared to the controls, cf. Figure 2. Compound **18** did, however, reduce stimulated gastric acid secretion in the chronic fistula rat model[25] when given as a *single* oral dose of 95 μmol/kg (31 mg/kg) as shown in Figure 3. Acid output was inhibited

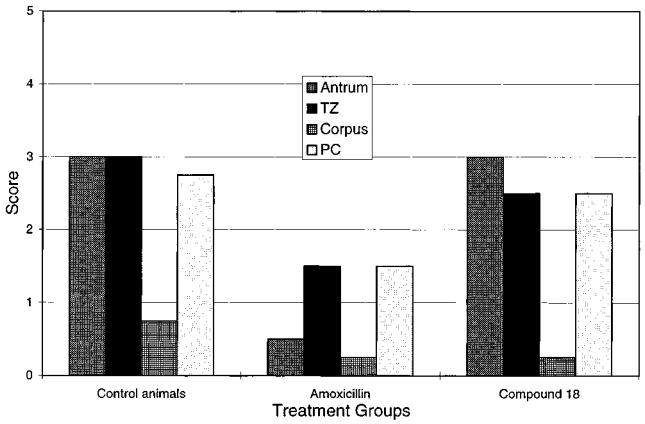


Figure 2. The *H. felis* infected mouse. Effects of amoxicillin (15 μmol/kg, 6.25 mg/kg) and test compound **18** (125 μmol/kg, 41 mg/kg) on stomach bacterial cell counts. The compounds were give orally, twice daily, for 4 days. The degree of infection was assessed by microscopically examining stained sections of the stomach and estimating the bacterial density on a scale from 0 to 5.

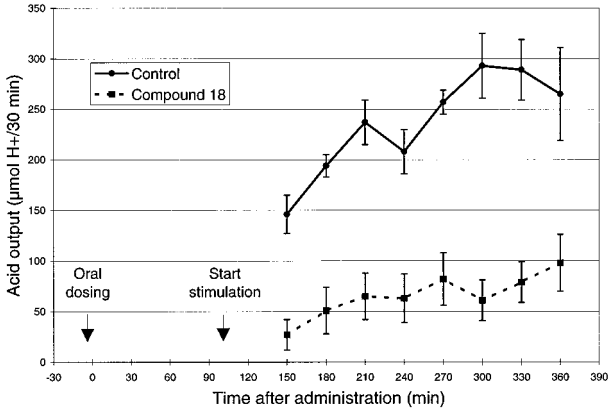


Figure 3. Inhibition of stimulated gastric acid secretion in the female chronic fistula rat model. Acid output after a single oral dose of compound **18** (95 μmol/kg, 31 mg/kg).

by some 80% 2 h after dosing and remained potently inhibited throughout the entire experiment. Similarly, both volume and acidity were also strongly inhibited throughout the whole experiment (data not shown).

As an *H. felis* rather than an *H. pylori* animal model was used to check for in vivo efficacy, *H. felis* MBC values were also determined. The susceptibility of *H. felis* was consistently higher, usually by a factor of 2, when compared to *H. pylori* (data not shown except for test compound **18** and the reference compound amoxicillin, cf. Table 1).

Discussion

The in vitro potencies of the compounds discussed in this paper are not fully optimized but could probably be improved by another 1 or 2 orders of magnitude as judged by other reports discussing similar structures.[26−29] Our contribution lies in that we established a SAR and that we carried one compound through a conceptual test and checked it for in vivo efficacy. This is significant in that close to 30 patents have appeared in recent years claiming compounds with the generic structure **6** as anti *Helicobacter* agents. Animal data, however, has never been provided, and the current study is the first one to appear discussing in vivo efficacy. A possible explana-

tion for this could be that only until recently have reliable and practical *H. pylori* animal models become available.[30] This is indeed the chief reason for why we in the present study chose to use an established *H. felis* animal model[31,32] rather than an experimental *H. pylori* model.

Unfortunately antibacterial activity could not be demonstrated in the mouse infected with *H. felis*. Instead we observed a very potent acid secretion inhibition despite the fact that intact sulfides **6** do not act on the proton pump in vitro or in vivo.[33] A possible explanation accounting for the observed acid secretion inhibition could be that compound **18** in vivo is oxidized to some extent to the corresponding sulfoxide, yielding a proton pump inhibitor in a similar manner as discussed for the prototype sulfide **3** by Lindberg et al. and Brändström et al.[10,34] On the basis of this analogy, it is our opinion that antibacterial activity of sulfides with the generic structure **6** can never be fully separated from activity on the proton pump.

The acid secretion inhibition we observe could increase the pH in the stomach, providing more favorable circumstances for bacterial cell growth rate in this otherwise hostile environment. For the proton pump inhibitor omeprazole, this is the case and a heavy bacterial colonization of the corpus region of the stomach has been observed in humans upon prolonged therapy. Interestingly, it has been postulated that it is such bacterial cell growth-rate promotion that makes *H. pylori* susceptible to antimicrobial therapies when combined with omeprazole.[5]

Whereas the antibacterial activity obviously has the potential of decreasing bacterial cell counts in vivo, the proton pump inhibitory effect (less acid secretion) promotes bacterial cell growth (in vivo).[35] The latter effect seemingly is the dominant one, i.e., cell growth rate promotion by potent acid inhibition overrides any antibacterial activity. One could argue that an increase of 1 or 2 orders of magnitude in antibacterial activity could reverse the situation to make the desired antibacterial effect the dominant one. This, however, is not the case as we have tested one such "potentiated" compound.[36] Hence, our results strongly suggest that sulfides with the generic structure **6** do not qualify for development as new anti *H. pylori* therapeutics unless structural changes are made that prevent metabolic activation yielding the proton pump inhibitor structure **2**.

The PLS model was not good enough to allow us to predict exact MBCs of new compounds. The reason for this could be that we did not account for an important factor such as molecular shape in our calculations. The model did, however, guide us in a qualitative ranking of the 21 randomly selected sulfides and, more importantly, suggested parameters for improving activity. Most of the biological variation could be attributed to properties of the R_2 substituent where the data set had the most structural variation. Virtually identical properties as for the R_2 substituent were found to be of importance when explaining the biological variation by whole-molecule properties. This finding is most likely due to a strong correlation between R_2 substituent properties and whole-molecule properties.

To conclude, we established a SAR between 21 2-[[(2-pyridyl)methyl]thio]-1*H*-benzimidazoles (**6**) and their in vitro anti *H. pylori* activity. Chiefly, larger and more lipophilic compounds, i.e., compounds with such R_2 substituents, generally had lower MBC values. We also conclude that the 2-[[(2-pyridyl)methyl]thio]-1*H*-benzimidazoles (**6**) as a compound class are compromised by their propensity to become potent proton pump inhibitors upon metabolic oxidation in vivo and hence lack the proper structural features to qualify for development as new anti *H. pylori* therapeutics.

Experimental Section

General. Chemicals, reagents, and solvents were purchased from any of the major vendors if nothing else is stated or referenced. NMR spectra were recorded on Bruker or Varian instruments. Mass spectra were recorded on Micromass spectrometers. Melting points were taken on a Büchi instrument.

Chromatography. The following analytical HPLC and GC methods were used unless otherwise stated. Method A: LiChrosphere 60 RP-select B (125 × 4.00 mm, 5 μm), MeCN/0.025 M Na_2HPO_4 buffer (pH 6.5), 0.8 mL/min, $\lambda = 214-287$ nm. Method B: the same as method A with the exception that MeCN/0.025 M Na_2HPO_4 buffer (pH 7.6) was used as the eluent. Method C: Phenomenex Kromasil 10 C8 (250 × 4.60 mm, 10 μm), MeCN/0.1 M NH_4OAc buffer (pH 7), 2 mL/min, $\lambda = 254$ nm. Method D: Waters symmetri C8 (50 × 2.1 mm, 3.5 μm), MeCN/0.1 M NH_4OAc buffer (pH 7), 0.4 mL/min, $\lambda = 254$ nm. Method E: Novapak C18 (150 × 3.9 mm, 4 μm), 40% MeCN/0.025 M Na_2HPO_4 buffer (pH 7.4), 0.8 mL/min, $\lambda = 280$ nm. This method was also used to determine the retention times needed to calculate k'.[13] The void volume was determined by injecting sodium dichromate. Method F: OV-1 fused silica (film thickness 2.65 μm, 10 m × 0.53 mm id), 70 °C to 280 °C at 20 °C/min.

Chemistry. The following standard preparative procedures were used. **Method I**. The appropriate pyridine *N*-oxide (*x* mmol) was dissolved in Ac_2O (10−40%, w/v, employing the more concentrated solution when working on a larger scale) and added dropwise to acetic anhydride (3*x* mL) heated to 110 °C. The resulting solution was stirred at 110 °C until all the starting material was consumed as judged by TLC. Excess reagent was evaporated leaving the corresponding 2-[(acetyloxy)methyl]pyridine which was used without further purification. **Method II**. The appropriately substituted 2-[(acetyloxy)methyl]pyridine (*x* mmol) was dissolved in MeOH (7*x* mL). Aqueous NaOH (1.1*x* mmol, 2 M) was added, and the solution was heated to reflux until all the starting material was consumed as judged by TLC. The solvents were evaporated, and the residue was partitioned between H_2O and CH_2Cl_2. The organic layer was collected, dried over $MgSO_4$, and evaporated, leaving the corresponding 2-(hydroxymethyl)pyridine in a reasonably pure state. **Method III.** The appropriately substituted 2-(hydroxymethyl)pyridine (*x* mmol) was dissolved in CH_2Cl_2 (2.5*x* mL) and cooled to 0 °C. $SOCl_2$ (1.3*x* mmol) dissolved in CH_2Cl_2 (2.5*x* mL) was added dropwise and the resulting mixture allowed to react at 0 °C until all the starting material was consumed as judged by TLC or direct inlet EI-MS. The reaction was quenched with isopropyl alcohol and taken to dryness. The residue was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$ (5%, v/v). The organic layer was collected, dried over $MgSO_4$, and concentrated, leaving a quantitative yield of the corresponding 2-(chloromethyl)pyridine. The appropriately substituted 2-mercapto-1*H*-benzimidazole (1.1*x* mmol) was suspended in MeOH (3*x* mL) and treated with aqueous NaOH (1.1*x* mmol, 2 M). The appropriate 2-(chloromethyl)pyridine (*x* mmol) dissolved in MeOH (2*x* mL) was added, and the mixture was heated to reflux until TLC showed complete conversion of starting materials. The solvents were evaporated, and the residue was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$ (5%, v/v). The organic layer was collected, dried over $MgSO_4$, and concentrated, furnishing the desired 2-[[(2-pyridyl)methyl]thio]-1*H*-benzimidazole **6**.

2-[[(4-Methoxy-2-pyridyl)methyl]thio]-1*H*-benzimidazole (7). The title compound was prepared on a 3.5 mmol scale according to method III and recrystallized from EtOAc saturated with ammonia, affording 549 mg (60%) of beige crystals: 500 MHz ^{1}H NMR (CH_2Cl_2-d_2) δ 3.92 (s, 3H), 4.35 (s, 2H), 6.88 (dd, 1H), 6.96 (d, 1H), 7.22 (m, 2H), 7.57 (m, 2H), 8.52 (d, 1H). Method A (28 to 70% MeCN in 20 min, λ = 285 nm), 99% at t_R = 8.8 min; method E, 98.9% at t_R = 3.2 min.

2-[[[4-[(Cyclopropylmethyl)oxy]-2-pyridyl]methyl]thio]-1*H*-benzimidazole (8). The title compound was prepared on a 1.65 mmol scale according to method III, affording 540 mg (95%) of the desired product as a solid: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 0.37 (m, 2H), 0.68 (m, 2H), 1.27 (m, 1H), 3.88 (d, 2H), 4.28 (s, 2H), 6.79 (dd, 1H), 6.87 (d, 1H), 7.19 (m, 2H), 7.46 (b, 1H), 7.65 (b, 1H), 8.44 (d, 1H). Method A (40 to 70% MeCN in 20 min, λ = 280 nm), 96% at t_R = 8.25 min; method E, 96.5% at t_R = 6.5 min.

2-[[(2-Pyridyl)methyl]thio]-1*H*-benzimidazole (9). 2-(Chloromethyl)pyridine (247 g, 1.5 mol) was added to a mixture of 2-mercapto-1*H*-benzimidazole (225 g, 1.5 mol) in EtOH (95%, 1500 mL) and NaOH (120 g, 30 mol) in H_2O (100 mL). The mixture was refluxed for 2 h, cooled, and diluted with H_2O (2000 mL). The precipitate was collected, dried, and recrystallized from toluene. The crystals were treated with charcoal in isopropyl alcohol (1500 mL) acidified with concentrated HCl (101 mL). The solid collected from the chilled filtrate was recrystallized from isopropyl alcohol, washed with diethyl ether, and dried, leaving 277 g (70%) of white crystalline material: 300 MHz ^{1}H NMR (DMSO-d_6) δ 4.99 (s, 2H), 7.36 (m, 2H), 7.50 (distorted t, 1H), 7.63 (m, 2H), 7.75 (d, 1H), 8.01 (dt, 1H), 8.63 (d, 1H) 8.82 (b, NH). Analytical LC; LiChrosorbe RP-8 (150 × 3.00 mm, 5 μm), 40% MeCN/0.025 M Na_2HPO_4 buffer (pH 7.6), 0.8 mL/min, λ = 200 nm, 99.7% at t_R = 4.3 min; method E, 99.0% at t_R = 2.8 min.

2-[[(4-Chloro-2-pyridyl)methyl]thio]-1*H*-benzimidazole (10). The title compound was prepared on a 5.3 mmol scale according to method III and purified on silica gel (CH_2Cl_2/MeOH, 97/3 to 90/10), furnishing 950 mg (64%) of white crystalline material: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 4.41 (s, 2H), 7.21 (m, 2H), 7.29 (dd, 1H), 7.44 (m, 2H), 7.67 (m, 1H), 8.53 (d, 1H). Direct inlet MS (EI) for $C_{13}H_{10}ClN_3S$ *m/z* (relative intensity) 275 (M^+, 100), 277 (M + 2, 40). Method D (30% MeCN), 98.5% at t_R = 3.6 min; method E, 99.7% at t_R = 4.2 min.

2-[[(4-Methyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (11). The title compound was prepared on a 24 mmol scale according to method III and recrystallized from EtOAc saturated with ammonia, affording 3.9 g (65%) of solid material: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.39 (s, 3H), 4.32 (s, 2H), 7.12 (distorted dd, 1H), 7.20 (coinciding signals, m, 3H), 7.56 (b, 2H), 8.51 (d, 1H). Direct inlet MS (EI) for $C_{14}H_{13}N_3S$ *m/z* (relative intensity) 255 (M^+, 100), negative EI 254 (M − H, 100). Method D (30% MeCN), 98.0% at t_R = 2.5 min; method E, 98.8% at t_R = 3.6 min.

5-Acetyl-2-[[(4-*tert*-butyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (12). The title compound was prepared on a 4.0 mmol scale according to method III and purified on silica gel (EtOAc saturated with ammonia), furnishing 947 mg (70%) of colorless oil: 500 MHz ^{1}H NMR (CH_2Cl_2-d_2) δ 1.37 (s, 9H), 2.67 (s, 3H), 4.44 (s, 2H), 7.41 (dd, 1H), 7.48 (d, 1H), 7.61 (d, 1H), 7.89 (dd, 1H), 7.22 (d, 1H), 8.61 (d, 1H). Method A (40 to 70% MeCN in 20 min, λ = 280 nm), 98.1% at t_R = 8.2 min; method E, 98.2% at t_R = 7.7 min.

5-Methoxy-2-[[(4-thiomethoxy-2-pyridyl)methyl]thio]-1*H*-benzimidazole (13). The title compound was prepared on a 7.1 mmol scale according to method III using EtOH rather than MeOH. Recrystallization from CH_3CN/isopropyl alcohol (1/1, v/v) afforded 1.2 g (67%) of white crystalline material: 90 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.40 (s, 3H), 3.79 (s, 3H), 4.58 (s, 2H), 6.89 (dd, 1H), 7.06 (dd, 1H), 7.16 (d, 1H), 7.55 (d, 1H), 7.36 (d, 1H), 8.38 (d, 1H). Method D (30% MeCN), 99.2% at t_R = 3.1 min; method E, 99.5% at t_R = 3.9 min.

2-[[(4-Methoxy-3-methyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (17). The title compound was prepared on a 48 mmol scale according to method III using EtOH rather than MeOH. Recrystallization from CH_3CN afforded 10.1 g (73%) of white crystalline material: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.26 (s, 3H), 3.89 (s, 3H), 4.38 (s, 2H), 6.76 (d, 1H), 7.18 (m, 2H), 7.46 (b, 1H), 7.62 (b, 1H), 8.37 (d, 1H). Analytical LC; LiChrosorbe RP-18 (125 × 4.00 mm, 5 μm), 40% MeCN/0.025 M Na_2HPO_4 buffer (pH 7.6), 0.8 mL/min, λ = 214−287 nm, 98.2% at t_R = 5.2 min; method E, 96.7% at t_R = 4.4 min.

2-[[[4-[(Cyclopropylmethyl)oxy]-3-methyl-2-pyridyl]methyl]thio]-1*H*-benzimidazole (18). The title compound was prepared on a 3.22 mmol scale according to method III and recrystallized from EtOAc, leaving 600 mg (57%) of white crystalline material: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 0.38 (m, 2H), 0.67 (m, 2H), 1.29 (m, 1H), 2.29 (s, 3H), 3.89 (d, 2H), 4.37 (s, 2H), 6.71 (d, 1H), 7.17 (m, 2H), 7.45 (b, 1H), 7.62 (b, 1H), 8.33 (d, 1H). Method B (40% MeCN, λ = 280 nm), 98.2% at t_R = 14.2 min; method E, 97.3% at t_R = 13.8 min.

2-[[[4-[(2-Methoxy-ethyl)oxy]-3-methyl-2-pyridyl]methyl]thio]-1*H*-benzimidazole (19). The title compound was prepared on a 0.42 mmol scale according to method III and recrystallized from EtOAc saturated with ammonia leaving 57 mg (35%) of white crystalline material: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.29 (s, 3H), 3.46 (s, 3H), 3.80 (m, 2H), 4.20 (m, 2H), 4.38 (s, 2H), 6.77 (d, 1H), 7.18 (m, 2H), 7.48 (b, 1H), 7.60 (b, 1H), 8.36 (d, 1H). Method A (40 to 70% MeCN in 20 min, λ = 280 nm), 99% at t_R = 6.5 min; method D (30% MeCN), 99.1% at t_R = 2.6 min.

2-[[(4-Methoxy-3,5-dimethyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (22). $SOCl_2$ (861 mL) in CH_2Cl_2 (3.7 L) was added dropwise to a refluxing solution of **66**[37] (1032 g, 6.04 mol) in CH_2Cl_2 (6 L) over a period of 1.5 h and reacted for 30 min. The mixture was taken to dryness and the residue recrystallized from EtOH−diethyl ether, leaving 1235 g (90%) of white crystalline 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride which was reacted with 2-mercapto-1*H*-benzimidazole on a 99.1 mmol scale according to method III. Recrystallization from CH_3CN afforded 22.5 g (84%) of white crystalline material: 90 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.25 (s, 3H), 2.32 (s, 3H), 3.79 (s, 3H), 4.43 (s, 2H), 7.26 (m coinciding with residual $CHCl_3$ in $CHCl_3$-*d*, estimated 2H), 7.62 (m, 2H), 8.32 (s, 1H), 9.41 (b, NH). Analytical LC; LiChrosorbe RP-8 (150 × 4.00 mm, 5 μm), 40% MeCN/0.025 M Na_2HPO_4 buffer (pH 7.6), 0.8 mL/min, λ = 280 nm, 98.8% at t_R = 8.4 min; method E, 98.3% at t_R = 5.5 min.

5-*tert*-Butyl-2-[[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (23). The title compound was prepared from 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (*cf.* the synthesis of **22**) and 5-*tert*-butyl-2-mercapto-1*H*-benzimidazole[16] on a 19.3 mmol scale according to method III. Recrystallization from diethyl ether furnished 6.7 g (97%) of white crystalline material: 500 MHz ^{1}H NMR (DMSO-d_6) δ 1.31 (s, 9H), 2.18 (s, 3H), 2.27 (s, 3H), 3.71 (s, 3H), 4.65 (s, 2H), 7.19 (dd, 1H), 7.36 (d, 1H), 7.40 (s, 1H), 8.17 (s, 1H). Method B (52% MeCN, λ = 300 nm), 94.1% at t_R = 8.8 min; method E, 89.8% at t_R = 32.6 min.

5-Chloro-2-[[(3,5-dimethyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (24). $SOCl_2$ (17.6 mL, 245 mmol) in CH_2Cl_2 (200 mL) was added dropwise to **65** (24.6 g, 163 mmol) in CH_2Cl_2 (300 mL) and allowed to react at 0 °C for 30 min. Isopropyl alcohol was added, and the mixture was taken to dryness. Recrystallization from CH_3CN furnished 25 g (79%) of white crystalline 2-(chloromethyl)-3,5-dimethylpyridine hydrochloride. The chloromethyl compound was reacted with 5-chloro-2-mercapto-1*H*-benzimidazole[16] on a 4.99 mmol scale according to method III. Recrystallization from EtOAc−diethyl ether afforded 826 mg (54%) of white crystalline material: 300 MHz ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H), 2.37 (s, 3H), 4.68 (s, 2H), 7.16 (dd, 1H), 7.45 (m, 2H), 7.52 (s, 1H), 8.19 (s, 1H). Method A (46 to 70% MeCN in 20 min, λ = 280 nm), 99% at t_R = 10.4 min; method E, 93.7% at t_R = 13.4 min.

5-Acetyl-2-[[(3,5-dimethyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (25). The title compound was prepared from 2-(chloromethyl)-3,5-dimethylpyridine hydrochloride (cf. the synthesis of **24**) and 5-acetyl-2-mercapto-1*H*-benzimidazole

(**67**) on a 4.99 mmol scale according to method III. Recrystallization from EtOAc−CH_2Cl_2−diethyl ether afforded 416 mg (27%) of white crystalline material: 300 MHz ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 2.38 (s, 3H), 2.58 (s, 3H), 4.72 (s, 2H), 7.45 (s, 1H), 7.50 (b, 1H), 7.76 (b, 1H), 8.07 (b, 1H), 8.19 (s, 1H). Method A (40 to 70% MeCN in 20 min, λ = 280 nm), 97.3% at t_R = 6.5 min; method E, 98.5% at t_R = 4.6 min.

5-Methyl-2-[[(3,5-dimethyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (26). The title compound was prepared from 2-(chloromethyl)-3,5-dimethylpyridine hydrochloride (cf. the synthesis of **24**) and 2-mercapto-5-methyl-1*H*-benzimidazole on a 15.6 mmol scale affording 3.2 g (72%) of white solid: 90 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.35 (s, 3H), 2.41 (s, 3H), 2.48 (s, 3H), 4.38 (s, 2H), 7.08 (d, 1H), 7.44 (m, 2H), 7.54 (s, 1H), 8.38 (s, 1H). Method A (46 to 70% MeCN in 20 min, λ = 280 nm), 98.1% at t_R = 6.6 min; method E, 95.1% at t_R = 8.6 min.

2-[[(4-Isobutyloxy)-3-methyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (27). The title compound was prepared on a 0.36 mmol scale according to method III and purified on silica gel (CH_2Cl_2/MeOH, 98/2), furnishing 146 mg (74%) of white solid: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 1.06 (d, 6H) 2.15 (m, 1H), 2.28 (s, 3H), 3.79 (d, 2H), 4.37 (s, 2H), 6.73 (d, 1H), 7.18 (m, 2H), 7.54 (b, 2H), 8.34 (d, 1H). FAB-MS (3-nitrobenzyl alcohol) for $C_{18}H_{21}N_3OS$ *m/z* (relative intensity) 328 (M + H, 100). Method B (70% MeCN, λ = 280 nm), 99.3% at t_R = 4.5 min; method C (60% MeCN), 98.8% at t_R = 6.1 min.

2-[[(4-[(3-Allyl)oxy]-3-methyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (28). The title compound was prepared on a 0.14 mmol scale according to method III and purified on silica gel (CH_2Cl_2/MeOH, 98/2), furnishing 28 mg (64%) of white solid: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.28 (s, 3H), 4.39 (s, 2H), 4.60 (m, 2H), 5.38 (m, 2H), 6.04 (m, 1H), 6.72 (d, 1H), 7.17 (m, 2H), 7.55 (b, 2H), 8.33 (d, 1H). FAB-MS (3-nitrobenzyl alcohol) for $C_{17}H_{17}N_3OS$ *m/z* (relative intensity) 312 (M + H, 100). Method A (40 to 70% MeCN in 20 min, λ = 280 nm), 97.2% at t_R = 12.5 min; method D (30% MeCN), 99.3% at t_R = 7.5 min.

2-[[(3-Methyl-4-[(3-propargyl)oxy]-2-pyridyl)methyl]-thio]-1H-benzimidazole (29). Methanesulfonyl chloride (19 μL, 0.25 mmol) was added to Et_3N (43 μL, 0.31 mmol) in dry CH_2Cl_2 (0.5 mL) at 0 °C and allowed to equilibrate for 5 min in an inert atmosphere. Compound **57** (9 mg, 0.081 mmol) dissolved in dry CH_2Cl_2 (0.5 mL) was added, and the mixture was reacted for 1 h at 0 °C. The mixture was treated with ice and diluted with CH_2Cl_2. The organic layer was collected, dried over $MgSO_4$, and evaporated, leaving 12 mg (62%) of 2-(methanesulfonylmethyl)-3-methyl-4-[(3-propargyl)oxy]pyridine. LiCl (10.5 mg, 0.25 mmol) was added to the (methanesulfonylmethyl)pyridine in dry CH_3CN (1 mL) and allowed to react at ambient temperature for 20 h in an inert atmosphere. The solvent was evaporated and the residue partitioned between CH_2Cl_2 and aqueous $NaHCO_3$ (5%, w/v). The organic layer was collected, dried over $MgSO_4$, and evaporated. Flash chromatography (CH_2Cl_2/MeOH, 98/2) afforded 8.1 mg (81%) of 2-(chloromethyl)-3-methyl-4-[(3-propargyl)oxy]pyridine as a white solid which was reacted with 2-mercapto-1*H*-benzimidazole as described in method III. Purification on silica gel (CH_2Cl_2/MeOH, 98/2) furnished 9.9 mg (78%) of white solid: 400 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.27 (s, 3H), 2.57 (t, 1H), 4.38 (s, 2H), 4.78 (d, 2H), 6.90 (d, 1H), 7.17 (m, 2H), 7.48 (b, 1H), 7.60 (b, 1H), 8.38 (d, 1H). FAB-MS (3-nitrobenzyl alcohol) for $C_{17}H_{15}N_3OS$ *m/z* (relative intensity) 310 (M + H, 100). Method A (40 to 70% MeCN in 20 min, λ = 280 nm), 95.9% at t_R = 9.5 min; method D (30% MeCN), 96.9% at t_R = 5.1 min.

2-[[(4-(Benzyloxy)-3-methyl-2-pyridyl)methyl]thio]-1*H*-benzimidazole (30). The title compound was prepared on a 1.81 mmol scale according to method III, furnishing 363 mg (49%) of white solid: 400 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.31 (s, 3H), 4.37 (s, 2H), 5.13 (s, 2H), 6.80 (d, 1H), 7.17 (dd, 2H), 7.32−7.42 (m:s, 5H), 7.44 (b, 1H), 7.61 (b, 1H). 8.33 (d, 1H). FAB-MS (3-nitrobenzyl alcohol) for $C_{21}H_{19}N_3OS$ *m/z* (relative intensity) 362 (M + H, 100). Method B (58% MeCN, λ = 280 nm), 97.8% at t_R =5.7 min; method C (60% MeCN), 99.1% at t_R = 4.2 min.

2-(Hydroxymethyl)-4-methoxypyridine (34). A suspension of **31** (11.1 g, 72 mmol) in methanol (70 mL) was carefully treated with potassium *tert*-butoxide (9.6 g, 85.7 mmol) and reacted at reflux until TLC showed complete conversion of starting material. The mixture was concentrated and the residue partitioned between H_2O (75 mL) and CH_2Cl_2. The organic layer was collected, dried over Na_2SO_4, and evaporated, leaving 7.0 g (70%) **32** as a yellow oil which was rearranged according to method I, furnishing a quantitative yield of dark oily 2-[(acetyloxy)methyl]-4-methoxypyridine. The dark oil (9.18 g, 50 mmol) was hydrolyzed according to method II and the resulting product purified on silica gel (MeOH/CH_2Cl_2, 1/9), affording 4.1 g (59%) of yellow solid: 500 MHz ^{1}H NMR (CHCI3-*d*) δ 3.84 (s, 3H), 4.70 (s, 2H), 6.71 (dd, 1H), 6.87 (d, 1H), 8.31 (d, 1H). Method F, 98.4% at t_R = 3.4 min.

4-[(Cyclopropylmethyl)oxy]-2-(hydroxymethyl)pyridine *N*-Oxide (35). NaH (55% in mineral oil, 18.7 g, 0.43 mol) was carefully added to cyclopropyl methyl alcohol (200 mL) at 0 °C and allowed to form a solution. The mixture was slowly added to **31** (60 g, 390 mmol) dissolved in cyclopropyl methyl alcohol (200 mL) warmed to 50 °C. The resulting mixture was heated to 75 °C and reacted for 2 h. Excess alcohol was evaporated and the residual oil treated with charcoal, filtered, and dried *in vacuo*, leaving 63 g (90%) of **33** as a brown oil which was rearranged according to method I, furnishing a quantitative yield of dark oily 2-[(acetyloxy)-methyl]-4-[(cyclopropylmethyl)oxy]pyridine. The dark oil (60 g, 270 mmol) was hydrolyzed according to method II and the product purified by Kugelrohr distillation (0.3 mbar, 170−190 °C), leaving 22 g (40%) of colorless oil: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 0.36 (m, 2H), 0.67 (m, 2H), 1.28 (m, 1H), 3.85 (m, 2H), 4.69 (s, 2H), 6.72 (dd, 1H), 6.77 (d, 1H), 8.33 (d, 1H). GC−MS (negative EI) for $C_{10}H_{13}NO_2$ *m/z* (relative intensity) 178 (M − H, 80); other prominent peaks were 150 (30), 124 (45). Method F, 95.3% at t_R = 6.8 min.

2-(Hydroxymethyl)-4-methylpyridine (39). Compound **37** (69.6 g, 566 mmol) was reacted according to method I, leaving 81.6 g (87%) of 2-[(acetyloxy)methyl]-4-methylpyridine which was hydrolyzed in aqueous HCl (10% v/v, 220 mL) at reflux for 30 min. The mixture was taken up in H_2O, neutralized with solid K_2CO_3, and extracted three times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and evaporated. Distillation (0.7 mbar, 80 °C) afforded 26.2 g (40%) of colorless oil: 90 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.38 (s, 3H), 4.78 (s, 2H), 7.1D (d, 1H), 7.22 (s, 1H), 8.52 (d, 1H).

4-*tert*-Butyl-2-(hydroxymethyl)pyridine (40). Dimethyl sulfate (14.1 mL, 150 mmol) was added dropwise to a solution of **42** (22.4 g, 148 mmol) in MeOH (170 mL), stirred for 15 min, and then heated to reflux for 2 h. The pH of the mixture was adjusted to 2.5 with aqueous NaOH (10 M), and a solution of ammonium persulfate (33.7 g, 150 mmol) in H_2O (60 mL), with an internal pH of 7.0, was added at a rate of 2.5 mL/min. The pH of the reaction mixture was continuously adjusted to 2.5 by adding aliquots of H_2SO_4 (2 M). After completion, the mixture was refluxed for 1.5 h and then stirred at room temperature for 20 h. MeOH (170 mL) was added, and precipitated materials were removed by filtration. The filtrate was taken to dryness and the residue partitioned between CH_2Cl_2 and aqueous NaOH (2 M). The organic layer was collected, dried over Na_2SO_4, and evaporated. Distillation (15 mbar, 136−150 °C) afforded 5.93 g (24%) of colorless oil: 500 MHz ^{1}H NMR (CH_2Cl_2-d_2) δ 1.32 (s, 9H), 4.75 (s, 2H), 7.20 (dd, 1H), 7.23 (d, 1H), 8.45 (d, 1H).

4-Chloro-2-(hydroxymethyl)pyridine (41). A solution of **43** (6.47 g, 50 mmol) and trimethyloxonium tetrafluoroborate (7.77 g, 52 mmol) in CH_2Cl_2 (150 mL) was stirred for 2 h at ambient temperature. The solvent was evaporated and the residue taken up in MeOH (150 mL) and heated to near boiling. Ammonium persulfate (2.28 g, 10 mmol) dissolved in H_2O (10 mL) was added, and the mixture was heated to reflux for 30 min. A second portion of ammonium persulfate (1.14 g, 5 mmol) in H_2O (5 mL) was added, and the mixture was refluxed for another 30 min. The solvents were evaporated, and the residue was partitioned between CH_2Cl_2 and aqueous

Na_2CO_3 (10% w/v). The organic layer was washed with H_2O, dried over $MgSO_4$, and evaporated, leaving 4.5 g (63%) of pale yellow oil: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 4.75 (s, 2H), 7.23 (dd, 1H), 7.33 (d, 1H), 8.47 (d, 1H).

2-(Hydroxymethyl)-4-thiomethoxypyridine (44). Compound **41** (2.1 g, 15 mmol) was dissolved in ethanolic sodium thiomethoxide[38] (0.6 M, 35 mL) and refluxed for 18 h. The solvent and excess methyl mercaptan were removed,[38] and the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was collected, dried over $MgSO_4$, and evaporated, leaving 1.4 g (62%) of colorless oil: 90 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.50 (s, 3H), 4.73 (s, 2H), 7.08 (d, 1H), 7.23 (s, 1H), 8.42 (d, 1H).

4-Chloro-2,3-dimethylpyridine *N*-Oxide (46). A solution of **45** (4.0 g, 23.6 mmol) in HCl (12 M, 8 mL) was heated to 170 °C in a Teflon-coated reaction vessel in an autoclave for 18 h. After cooling, the reaction mixture was poured onto ice and neutralized with solid K_2CO_3. The aqueous phase was extracted five times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. Flash chromatography (CH_2Cl_2/MeOH, 95/5) furnished 3.18 g (85%) of white crystalline material: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.39 (s, 3H), 2.55 (s, 3H), 7.13 (d, 1H), 8.08 (d, 1H). Method F, 88% at t_R = 8.8 min.

2-(Hydroxymethyl)-4-methoxy-3-methylpyridine (54). A solution of **45** (47.6 g, 280 mmol) in MeOH (600 mL) was treated with K_2CO_3 (44.4 g, 320 mmol) at reflux for 20 h. The mixture was filtered and the solvent evaporated, leaving 35.5 g (82%) of crystalline **47** which was rearranged according to method I, furnishing 2-[(acetyloxy)methyl]-4-methoxy-3-methylpyridine. The (acetyloxy)methylpyridine was hydrolyzed in HCl (10% v/v, 100 mL) at 90 °C for 1.5 h. The pH was adjusted to 12 with NaOH (2 M) and the aqueous layer extracted with CH_2Cl_2. The organic layer was collected, dried over Na_2SO_4, and evaporated, leaving 25.3 g (72%) of brown crystals: 300 MHz ^{1}H NMR (DMSO-d_6) δ 2.10 (s, 3H), 3.84 (s, 3H), 4.53 (d, 2H), 4.99 (t, OH) 6.96 (d, 1H), 8.27 (d, 1H).

4-[(Cyclopropylmethyl)oxy]-2-(hydroxymethyl)-3-methylpyridine (55). Benzyltri-*n*-butylammonium chloride (6.5 g, 20.8 mmol) and $KHCO_3$ (6.2 g, 62 mmol) in H_2O (10 mL) were added to a mixture of **45** (5.18 g, 31 mmol) and cyclopropyl methyl alcohol (130 mL) in CH_3CN (30 mL) and heated to reflux for 24 h. The mixture was taken to dryness and the product purified on preparative HPLC (C8, 500 × 100 mm, AcCN/0.1 M NH_4OAc, 1/4 to 6/4), leaving a quantitative yield of white solid **48**. Rearrangement of **48** (4.1 g, 21 mmol) according to method I furnished 3.5 g (48%) of 2-[(acetyloxy)-methyl]-4-[(cyclopropylmethyl)oxy]-3-methylpyridine, which was hydrolyzed according to method II at ambient temperature. Flash chromatography (CH_2Cl_2/MeOH saturated with ammonia, 97/3) afforded 2.0 g (69%) of white solid: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 0.36 (m, 2H), 0.64 (m, 2H) 1.27 (m, 1H), 2.05 (s, 3H), 3.76 (d, 2H), 4.63 (s, 2H), 6.65 (d, 1H), 8.25 (d, 1H).

2-(Hydroxymethyl)-4-[(2-methexyethyl)oxy]-3-methylpyridine (56). K_2CO_3 (6.6 g, 48 mmol) was added to **45** (6.7 g, 40 mmol) in 2-methoxyethyl alcohol (65 mL) and heated to reflux for 20 h. The mixture was taken to dryness, and the residue was taken up in CH_2Cl_2. Solids were filtered off, and the filtrate was dried over $MgSO_4$, treated with charcoal, refiltered, and evaporated, leaving 4.2 g (53%) of **49**. Rearrangement of **49** (900 mg, 4.6 mmol) according to method I fumished 1.0 g (94%) of 2-[(acetyloxy)methyl]-4-[(2-methoxyethyl)oxy]-3-methylpyridine which was hydrolyzed according to method II, leaving 530 mg (63%) of white solid: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.07 (s, 3H), 3.45 (s, 3H) 3.79 (m, 2H), 4.18 (m, 2H), 4.65 (s, 2H), 6.72 (d, 1H), 8.29 (d, 1H).

2-(Hydroxymethyl)-3-methyl-4-[(3-propargyl)oxy]pyridine (57). Benzyltri-*n*-butylammonium chloride (9.3 mg, 0.030 mmol) and K_2CO_3 (83 mg, 0.60 mmol) were added to **45** (50 mg, 0.30 mmol) in a CH_3CN/propargyl alchohol mixture (3/1, v/v, 1 mL), and the mixture was heated to 90 °C for 3 h. The mixture was taken to dryness and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was collected, dried over $MgSO_4$, and evaporated, leaving 46 mg (86%) of **50** as a yellow solid which was rearranged according to method I, furnishing 57 mg (99%) of 2-[(acetyloxy)methyl]-3-methyl-4-[(3-propargyl)oxy]pyridine. The crude (acetyloxy)methylpyridine was hydrolyzed according to method II and the product purified on silica gel (CH_2Cl_2/MeOH, 98/2), leaving 16.2 mg (35%) of white solid: 300 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.06 (s, 3H), 2.56 (m, 1H), 4.66 (s, 2H), 4.78 (m, 2H), 6.86 (d, 1H), 8.33 (d, 1H). Direct inlet MS (EI) for $C_{10}H_{11}NO_2$ *m/z* (relative intensity) 177 (M^+, 100).

4-(Benzyloxy)-2-(hydroxymethyl)-3-methylpyridine (58). Benzyltri-*n*-butylammonium chloride (93 mg, 0.30 mmol) and K_2CO_3 (830 mg, 6.0 mmol) were added to **45** (500 mg, 3.0 mmol) in a CH_3CN/benzyl alcohol mixture (3/1, v/v, 5 mL) and heated to 90 °C for 20 h. The mixture was taken to dryness and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was collected, dried over $MgSO_4$, and evaporated. Flash chromatography (CH_2Cl_2/MeOH, 95/5) afforded 393 mg (57%) **51** as a white solid. Rearrangement of **51** (980 mg, 4.27 mmol) according to method I furnished 1.11 g (96%) of 2-[(acetyloxy)methyl]-4-(benzyloxy)-3-methylpyridine as a tanned oil which was hydrolyzed according to method II. Flash chromatography (CH_2Cl_2/MeOH, 96/4) afforded 528 mg (56%) of white solid: 400 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.09 (s, 3H), 4.65 (s, 2H), 5.13 (s, 2H), 6.76 (d, 1H), 7.30−7.47 (m:s, 5H), 8.27 (d, 1H). FAB-MS (3-nitrobenzyl alcohol) for $C_{14}H_{15}NO_2$ *m/z* (relative intensity) 230 (M + H, 100).

2-(Hydroxymethyl)-4-(isobutyloxy)-3-methylpyridine (59). NaH (60% in mineral oil, 7.1 g, 17.8 mmol) was added in small portions to isobutyl alcohol (200 mL) at 0 °C and stirred until a solution was formed. Compound **46** (14.0 g, 89 mmol) was cautiously added, and the mixture was reacted at reflux for 20 h. The mixture was taken to dryness and the residue purified on silica gel (CH_2Cl_2/MeOH, 95/5), leaving 11.0 g (63%) of **52** as a white solid. Rearrangement of **52** (6.79 g, 35 mmol) according to method I furnished 8.16 g (98%) of 2-[(acetyloxy)methyl]-4-(isobutyloxy)-3-methylpyridine. The (acetyloxy)methylpyridine (9.1 g, 38 mmol) was hydrolyzed according to method II, and the product was purified on silica gel (CH_2Cl_2/MeOH, 19/1), leaving 6.0 g (81%) of white solid: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 1.05 (d, 6H), 2.05 (s, 3H), 2.14 (m, 1H), 3.78 (d, 2H), 4.65 (s, 2H), 4.91 (b, OH), 6.69 (d, 1H), 8.28 (d, 1H). Direct inlet MS (EI) for $C_{11}H_{17}NO_2$ *m/z* (relative intensity) 195 (M^+, 10); other prominent peaks were 180 (20), 152 (70), 138 (100). Method A (46 to 70% MeCN in 20 min, λ = 280 nm), 98.1% at t_R = 6.6 min.

4-[(3-Allyl)oxy]-2-(hydroxymethyl)-3-methylpyridine (60). NaH (60% in mineral oil, 260 mg, 6.4 mmol) was cautiously added to allyl alcohol (5 mL, dried over 4 Å molecular sieves) and stirred until a solution was obtained. Compound **46** (500 mg, 3.2 mmol) dissolved in allyl alcohol (5 mL) was added dropwise, and the mixture was allowed to react for 20 h. The mixture was taken to dryness and the residue partitioned between CH_2Cl_2 and H_2O. The organic layer was collected, dried over $MgSO_4$, and evaporated. Recrystallization from EtOAc−petroleum ether (40−60 °C) afforded 492 mg (86%) of white crystalline **53**. Rearrangement of **53** (200 mg, 0.90 mmol) according to method I furnished 199 mg (99%) of tanned solid 2-[(acetyloxy)methyl]-4-[(3-allyl)oxy]-3-methylpyridine which was hydrolyzed according to method II. Flash chromatography (CH_2Cl_2/MeOH, 95/5) afforded 97 mg (60%) of white solid: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.07 (s, 3H), 4.60 (d, 2H), 4.65 (s, 2H), 5.32 (d, 1H), 5.43 (d, 1H), 6.04 (m, 1H), 6.70 (d, 1H), 8.28 (d, 1H). Direct inlet MS (EI) for $C_{10}H_{13}NO_2$ *m/z* (relative intensity) 179 (M^+, 40); other prominent peaks were 164 (20), 148 (40), 138 (90); negative EI 178 (M − H, 60).

2,3,5-Trimethylpyridine *N*-Oxide (62). Aqueous H_2O_2 (30%, 60 mL) was added to **61** (121 g, 1.0 mol) in acetic acid (350 mL), and the mixture was stirred for 3 h at 90 °C. The mixture was cooled, and a second portion of aqueous H_2O_2 (30%, 25 mL) was added, after which the mixture was stirred for another 20 h at 90 °C. The solvent was evaporated[39] (toluene was used to remove remaining traces of acetic acid by means of azeotropic distillation). The pH was adjusted to

10 with NaOH (10 M, ~110 mL), CH_3CN (250 mL) was added, and precipitated materials were filtered off. The filtrate was evaporated, leaving 104 g (76%) of white solid: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.24 (s, 3H), 2.32 (s, 3H), 2.47 (s, 3H), 7.00 (s, 1H), 8.12 (s, 1H). Analytical GC; 10% OV-101 on Chromasorb W-HP glass column, (80−100 mesh, 2 m × 2 mm id), 140 to 200 °C at 4 °C/min, 94% at t_R = 6.86 min.

3,5-Dimethyl-2-(hydroxymethyl)pyridine (65). Rearrangement of **62** (13.7 g, 100 mmol) according to method I furnished in quantitative yield 2-[(acetyloxy)methyl]-3,5-dimethylpyridine, which was hydrolyzed according to method II. Flash chromatography (CH_2Cl_2/MeOH, 92/8) afforded 10.3 g (75%) of clear oil: 500 MHz ^{1}H NMR ($CHCl_3$-*d*) δ 2.18 (s, 3H), 2.31 (s, 3H), 4.66 (s, 2H), 7.28 (s, 1H), 8.21 (s, 1H). Analytical GC; OV-1 fused silica column (film thickness 0.33 μm, 11 m × 0.2 mm id), 100 to 230 °C at 4 °C/min, 94.7% at t_R = 4.8 min.

5-Acetyl-2-mercapto-1*H*-benzimidazole (67). Compound **68** (67.3 g, 380 mmol) was cautiously added to fuming HNO_3 (174 mL) at such a rate that the temperature did not exceed 5 °C. The mixture was poured onto ice−water (2.5 L) and the precipitate collected. Recrystallization from absolute EtOH afforded 50.2 g (59%) of white crystalline 4-acetyl-2-nitroacetanilide. The acetanilide (18 g, 81 mmol) was added to a solution of HCl (12 M, 71 mL) in H_2O (24 mL) and heated to reflux for 30 min. The mixture was cooled, and the precipitate was collected, leaving 14.1 g (97%) of white crystalline 4-acetyl-2-nitroaniline. The aniline (8.0 g, 44.4 mmol) was dissolved in EtOH (200 mL) and hydrogenated over Pd/C (5%) for 16 h. The mixture was filtered, and the filtrate was treated with diethyl ether saturated with HCl. The precipitate was collected and washed with diethyl ether, leaving 7.2 g (73%) of yellow crystalline 4-acetylphenylenediamine hydrochloride. A solution of the hydrochloride (11.11 g, 49.8 mmol) in EtOH/H_2O (6.6/1, 210 mL) was charged with potassium ethyl xanthogenate (13.0 g, 81 mmol) and stirred for 15 min. Potassium carbonate (3.44 g, 25 mmol) was added, and the mixture was heated to reflux for 16 h. Warm water (70 °C, 185 mL) was added, and dark red crystals were precipitated by adjusting the pH to 5 with a mixture of acetic acid (5.98 mL) in H_2O (4.4 mL). The solid was collected, dissolved in aqueous NaOH (2 M), and treated with charcoal. Solids were removed, and the product was reprecipitated from the filtrate by acidification with acetic acid to pH 5. The material was collected and dried *in vacuo*, leaving 7.2 g (70%) of yellow crystals: 300 MHz ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H), 7.24 (d, 1H), 7.69 (s, 1H), 7.76 (d, 1H). Method A (16 to 70% MeCN in 20 min, λ = 280 nm), 96.5% at t_R = 4.9 min.

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