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PAPER

Cysteine as a sustainable sulfur reagent for the protecting-group-free synthesis of sulfur-containing amino acids: biomimetic synthesis of L-ergothioneine in water†

Irene Erdelmeier,* Sylvain Daunay, Remi Lebel, Laurence Farescour and Jean-Claude Yadan

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Biomass-derived cysteine was used as a sustainable sulfur source for the synthesis of rare sulfur-containing amino acids, such as L-ergothioneine (**4**), which might be a new vitamin, and various L- or D-2-thiohistidine compounds. Key in this simple, one-pot two-step procedure in water is a bromine-induced regioselective introduction of cysteine followed by a novel thermal cleavage reaction in the presence of thiols, a safer alternative to hazardous red phosphorus. Besides avoiding hazardous sulfur reagents, the new protecting-group-free approach reduces drastically the total number of steps, compared to described procedures. The main drawback, *i.e.* handling of liquid bromine as an activating and oxidizing reagent in water, was addressed by evaluating four alternative methods using *in situ* generation of bromine or HOBr, and first encouraging results are described.

Introduction

Sulfur-containing essential amino acids such as cysteine (**1**) and methionine (**2**) are widely distributed in nature, and have important functions as building blocks in proteins. The sulfur is present in these amino acids as a redox-active thiol or thioether function, which is essential to their physiological role (Fig. 1).[1]

Another interesting, but less well known class of sulfur-containing amino acids are thiohistidine compounds, such as 2-thiohistidine (**3a**) or L-ergothioneine (**4**), in which the sulfur is part of a rare imidazole-2-thione structure.[2]

2-Thiohistidine (**3a**) is used as an antioxidant, skin whitening[3] and photo-protective agent.[4,5] L-Ergothioneine (**4**) is a natural, non-proteinogenic amino acid. It has been first isolated from *Claviceps purpurea* in 1909,[6] and is almost ubiquitous in living organisms.[7] Biosynthesized in fungi (such as *Neurospora crassa*)[8] and mycobacteria[9] it is incorporated in plants and ingested by animals and humans through the diet, from which it is absorbed and concentrated in most tissues through a recently discovered specific organic cation transporter (OCTN1 or ETT[10]). This discovery, together with the suggestion that L-ergothioneine might be a new vitamin,[11] spurred the search for sustainable supply and the interest in further development of thiohistidine compounds, such as **3a** and **4**.

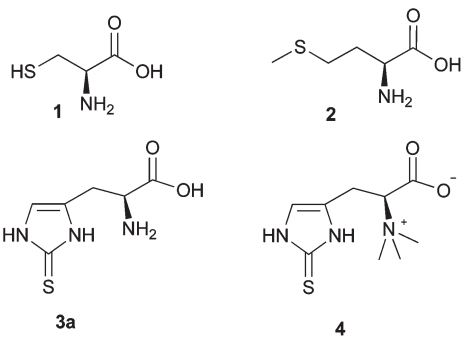


Fig. 1 Sulfur-containing amino acids.

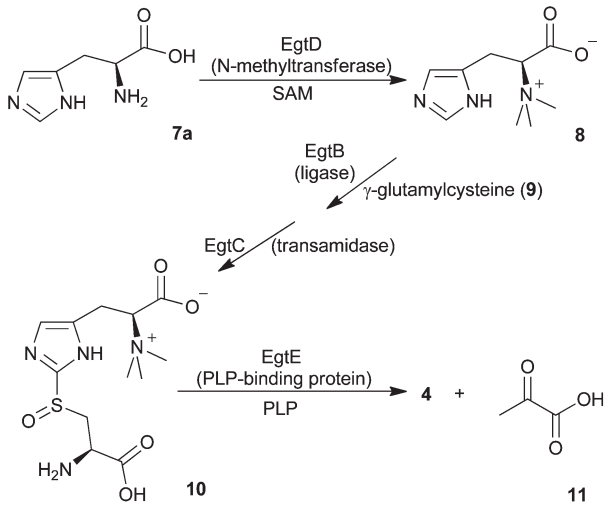
Both amino acids have been synthesized previously. For example, 2-thiohistidine is prepared in 5 steps from histidine, using phenyl chlorothionoformate[12] (**5**, PCTF, prepared from thiophosgene, see path A, Scheme S1, ESI†) or potassium thiocyanate (**6**) in an acidic medium (see Scheme S2, ESI†)[13] as a sulfur source (Fig. 2).

Fig. 2 Commonly used sulfur reagents.

Despite the apparently simple structure of **3a**, selective sulfur introduction required protection of competing reactive sites in histidine, such as the carboxyl and amino-function, increasing

TETRAHEDRON, Parc Biocitech, 102 Avenue Gaston Roussel, 93230 Romainville, France. E-mail: irene.erdelmeier@tetrahedron.fr; Fax: +331 41837508; Tel: +331 41837501

†Electronic supplementary information (ESI) available: Complete reaction schemes for the previously described synthesis of 2-thiohistidine and L-ergothioneine using phenylchlorothionoformate (**S1**) and KSCN (**S2**) as sulfur source, Figures of the ^{1}H NMR-analysis of the reaction mixture (**S3**) and copies of spectral data (^{1}H NMR and ^{13}C NMR) for compounds **3a–3c**, **4**, **12a–12c**, **13**, and **16**. See DOI: 10.1039/c2gc35367a



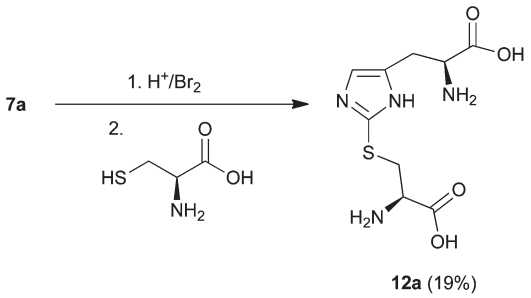
Scheme 1 Biosynthesis of ergothioneine.

the number of steps, leading to the use of several different organic solvents, gaseous HCl, and chromatographic separations. The synthesis of ergothioneine has been achieved using the same sulfur reagents in 7 and 9 steps, respectively, due to unavoidable protection of the carboxyl and the amino groups as well as ultimately of the nucleophilic sulfur function, to establish the trimethyl ammonium group (see path B, Schemes S1 and S2, ESI†).

Contrary to these chemical syntheses, the biosynthesis of ergothioneine in microorganisms such as *Neurospora crassa* is straightforward in 4 steps from histidine. In a first step,[14] histidine (**7a**, Scheme 1) is methylated threefold by a specific *N*-methyltransferase, using S-adenosyl methionine (SAM) as a methylating agent, to give L-hercynine (**8**). Interestingly, sulfur is introduced selectively in the 2-position of hercynine *via* an oxidative, enzyme-catalyzed and iron-dependent reaction with γ-glutamylcysteine (**9**)[15] (and not cysteine as initially suggested)[16] to yield the key intermediate **10** in two steps. Finally, **10** is cleaved to L-ergothioneine (**4**) and pyruvic acid (**11**) by a lyase-catalyzed reaction in the presence of pyridoxal phosphate (PLP).[17]

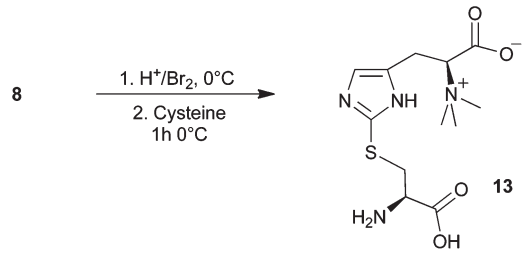
The biosynthetic pathway to **4** is appealing from the green-chemistry viewpoint, as it is a short, protecting-group-free synthesis, using a non-hazardous sulfur source in water as an innocuous reaction medium.[18] Protecting groups have been introduced to achieve chemo- and regioselective transformations,[19] as exemplified in the previous syntheses of **3a** and **4**, but substantial efforts have been developed in the last decade to meet the challenge of chemo- and regioselectivity without protecting steps. Protecting-group-free synthesis has a tremendous potential to improve "the economies of synthesis",[20] as demonstrated in the synthesis of indole-based marine natural products, alkaloids, terpenoids, antibiotics, and azasugars.[21] More recently, impressive examples of protecting-group-free synthesis in water have been described,[22] combining the discovery of unusual reactivities in water (as for example the rate-accelerating "on-water-effect"[23]) with simplified product purification or isolation.

In the present study, we describe a biomimetic approach to ergothioneine from easily available hercynine,[24] combining the



Scheme 2 First example of cysteinyl-histidine synthesis (ref. 26).

Table 1 Reaction parameters for oxidative cysteine introduction[a]



Entry	Concn (M)	HCl (equiv.)	Br_2 (equiv.)	Cys (equiv.)	Yield[b] (%)
1	0.1	1	1	1	9
2	0.1	1	1	3	42
3	0.5	1	1.3	3	50/(46)[c]
4	0.5	0.5	1.3	3	30
5	0.5	1.5	1.3	3	32
6	0.5	1	1.3	5	62/(58)[c]

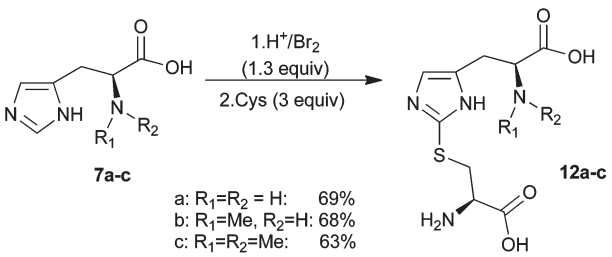
[a] Reaction conditions: hercynine (**8**, 5 mmol, 1.0 equiv.) and HCl (2.5–7.5 mmol, 0.5–1.5 equiv.) at 0–2 °C in water (10–50 mL), dropwise Br_2-addition in 1–2 min (5.0–6.5 mmol, 1–1.3 equiv.), cysteine (5–25 mmol, 1–5 equiv.) after 5–7 min. [b] Calculated yields by ^{1}H NMR of the crude reaction mixture. [c] Isolated yields after chromatography of the crude reaction mixture on a DOWEX 50WX2 200–400 (H+-form), **13** is obtained as tri-hydrochloride hydrate.

attractive features of the protecting-group-free biosynthesis in water with the advantageous use of cysteine as a renewable and readily accessible sulfur source.[25] The strategy could be generalized to the synthesis of various L- and D-thiohistidines, rendering this poorly explored class of sulfur-containing amino acids readily accessible in multi-gram quantities for further biological investigations.

Results and discussion

Our starting point was an intriguing reactivity of histidine (**7a**) observed by Ito.[26] After activation with bromine and addition of cysteine, a new histidine derivative **12a** was formed in low yield, characterized by an unusual thioether bond in the 2-position of the imidazole (Scheme 2).

Recognizing the close relationship to the *in vivo* formation of intermediate **10** in the biosynthesis of ergothioneine, we studied the feasibility to activate hercynine (**8**) and to induce a selective



Scheme 3 Optimized synthesis of cysteinyl-histidine thioethers.

reaction with cysteine, without protection of competing reactive sites.

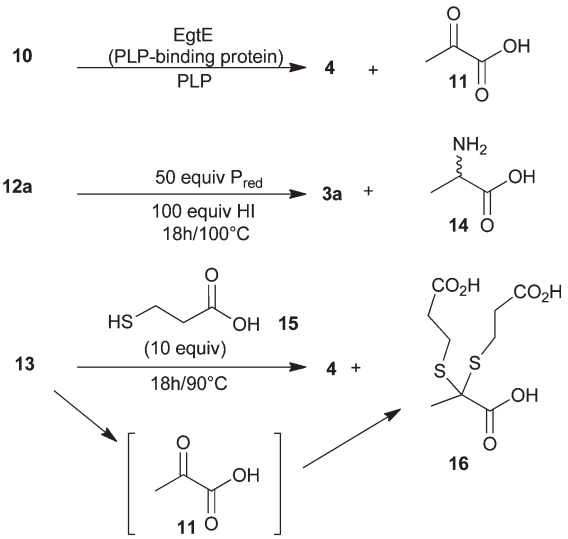
In a first experiment (Table 1, entry 1), one equivalent of bromine was added at 0 °C to an acidic aqueous solution of hercynine (0.1 M). Rapid consumption of bromine was indicated by discoloration of the reaction mixture, accompanied by the formation of an orange-red gummy solid. Analysis of the reaction mixture after addition of L-cysteine (1.0 equiv.) indicated the formation of the desired cysteinyl thioether **13**, but only in traces (9%)[27] besides a multitude of non-identified products. As further investigations showed that the major part (88%) of hercynine was converted, we speculated that the reactive intermediate formed by the reaction of bromine with hercynine was unstable, and degraded before reacting to a significant extent with cysteine. Therefore, to favor the reaction with cysteine *vs.* competitive degradation, several parameters were studied, such as cysteine, acid, and bromine equivalents, and hercynine concentration.

Interestingly, using three equivalents of cysteine instead of one increased the yield of thioether **13** in the reaction mixture to 42% (Table 1, entry 2). Slower dropwise addition of bromine in slight excess (1.3 equiv.) and a higher hercynine concentration (0.5 M instead of 0.1 M) were found to be beneficial, and provided **13** in 50% yield (Table 1, entry 3). The strong influence of the amount of acid was revealed by comparing the reaction in the presence of 1.0, 0.5, and 1.5 equivalents (Table 1, entries 3–5), one equivalent leading to the highest yield of **13**. Finally, the best result was obtained with 5 equivalents of cysteine, providing **13** in 62% yield (58% isolated yield, Table 1, entry 6) in the crude reaction mixture.

In order to study the general scope of the reaction, the optimized reaction conditions were applied to L-histidine (**7a**), as well as *N*-methyl- and *N*,*N*-dimethylhistidine[24] (**7b** and **7c**, respectively). To our delight, using an excess of 3 equivalents of cysteine, the known compound **12a** could be obtained from **7a** with an isolated yield of 69% (compared to 19% reported previously[26]). Similarly, the new *N*-methylated cysteinyl-histidine thioethers **12b** and **12c** were obtained with an isolated yield of 68 and 63% (Scheme 3), from **7b** and **7c**, respectively.

Striving towards a biomimetic synthesis of ergothioneine, we searched for a direct conversion of cysteinyl thioether **13** to ergothioneine itself, knowing that **13** is structurally related but not identical to the biosynthetic sulfoxide intermediate **10**.

While the biological transformation of thioether **10** is PLP-dependent and catalyzed by a β-lyase (Scheme 1), the sole related chemical transformation described in the literature is the hydrolysis of the cysteinyl thioether **12a**, derived from histidine.



Scheme 4 Reaction pathways for the cleavage of cysteinyl thioethers.

Using an excess of 50 equivalents of red phosphorus in 100 equivalents of hydroiodic acid for 18 h at 100 °C, **12a** is cleaved to 2-thiohistidine (**3a**) and D,L-alanine (**14**, Scheme 4).[26]

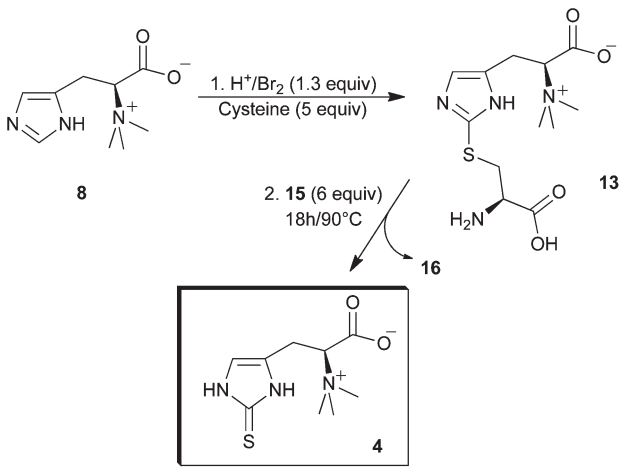
These conditions appeared unacceptable not only due to the multiple chromatographic purifications which are resource, energy and time-consuming, but also to the use of hazardous red phosphorus, in particular in such a high excess. Red phosphorus is classified as highly toxic and highly inflammable,[28] requiring a high level of risk management.

Searching for safer alternatives, we discovered that, under heating for 18–24 h at 90 °C in the presence of thiols (10 equiv.), **13** cleaved slowly to ergothioneine (80% isolated yield) and pyruvic acid (**11**), the latter being converted *in situ* to the corresponding dithioketal.[29] By choosing an appropriate and easily available thiol, such as 3-mercaptopropionic acid (**15**),[30] the dithioketal **16** is formed smoothly in this new thermal cleavage reaction (Scheme 4 and Fig. S4, ESI†).[31]

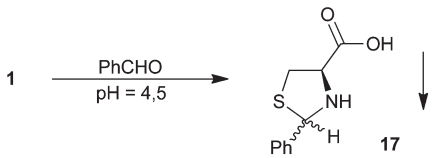
With regard to industrial production, another important advantage of this safer alternative to the red phosphorus pathway became evident upon treatment of the aqueous reaction mixture: despite being a tricarboxylic acid, **16**, together with the excess of **15**, can be easily removed by extraction with ethyl acetate after cleavage, therefore facilitating the purification of the very polar ergothioneine without chromatography. In comparison, D,L-alanine, obtained as a side product in the red phosphorus cleavage, cannot be extracted, and has to be separated by other means from the desired amino acid, as in Ito's example for the cleavage of thioether **12a** *via* several chromatographic separations and crystallizations.

Taking advantage of the clean and complete conversion of thioether **13**, the two steps – formation of thioether **13** and cleavage to ergothioneine – could be integrated in a streamlined one-pot procedure without isolation of the intermediate **13** (Scheme 5).

Carrying a zwitterionic betaine function, ergothioneine is highly water-soluble (>300 g L^{-1}), and could not be preferentially crystallized in satisfying yield from the crude mixture, which contained in particular cysteine (used in excess) and salts.



Scheme 5 One-pot synthesis of L-ergothioneine.



Scheme 6 Cysteine scavenging.

Interestingly, cysteine can be scavenged directly from an aqueous mixture at room temperature with benzaldehyde (Scheme 6). An optimisation study showed that a slight excess of benzaldehyde[32] (1.16 equiv. compared to cysteine) was sufficient to remove cysteine completely after pH adjustment to 4–5. Moreover, omitting ethanol as the usually employed organic co-solvent resulted in precipitation of the corresponding phenylthiazolidine-carboxylic acid **17**[33] (Scheme 6), which was isolated by simple filtration in a purity >95%. Besides improving the isolation of ergothioneine, this operation allows for a potential recovery and recycling of cysteine used in excess, as the transformation of **17** to cystine (the industrial cysteine precursor)[34] is known.[35]

For the final isolation of ergothioneine, the salt content was significantly reduced to allow for its direct crystallization from the aqueous mixture. For optimal process and material efficiency, electrodialysis (ED) as a powerful desalination technology was explored, instead of ion-exchange chromatography requiring two consecutive cation- and anion-exchange columns. ED is a high-throughput membrane-based technique which is easily adaptable on an industrial scale, and commonly used in water treatment for desalination of seawater,[36,37] removal of inorganic trace contaminants from bore water sources,[38] or tartrate stabilization in wine.[39]

In a typical set-up, as shown in Fig. 3, the aqueous reaction mixture is cycled repeatedly through the system (about 100–150 L h^{-1}), consisting of an electrical cell containing a membrane pack with multiple layers of alternating anion- and cation-selective membranes. Anions and cations are exchanged simultaneously against OH^- and H^+, respectively, driven by low electrical tension (10–20 V). Contrary to ion-exchange column chromatography, where the ions are absorbed on a resin, the ions

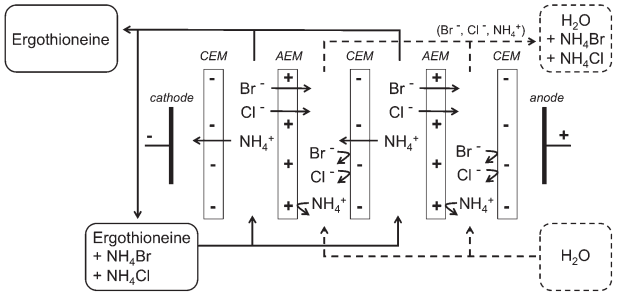


Fig. 3 Desalination *via* electrodialysis: simultaneous exchange of cations and anions (CEM = cation exchange membrane, AEM = anion exchange membrane).

migrate in ED through the membranes and collect in a "concentrate", which can be diluted or exchanged conveniently against water. The membranes can be used indefinitely in theory without saturation, but process impurities can induce scaling and occluding. In short, compared to classical ion exchange resin chromatography, the process density of exchange *via* electrodialysis is higher by orders of magnitude, combined with less material use (resin *vs.* membranes) and lower processing costs. This makes electrodialysis an interesting technology for green process design, but despite its powerful advantages, it has been only scarcely applied in organic chemistry until now.[40]

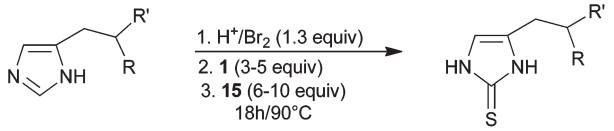
Implementing this efficient and environmentally friendly technique for the desalination of the reaction mixture with a 20 membrane-pair cell following cysteine scavenging finally allowed the crystallization of the desired highly polar ergothioneine, and its isolation in high purity (>99%) and a 40% global yield over the two steps (Table 2, entry 1).

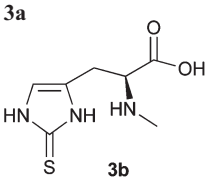
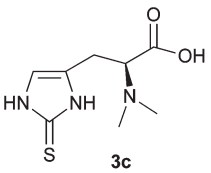
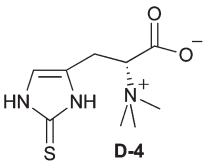
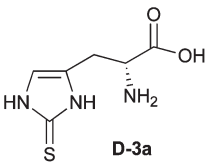
While there is room for improvement, in particular to increase the overall yield, this novel synthetic approach to ergothioneine *via* hercynine, readily available from histidine, constitutes a robust framework for further optimisations. It is not only 3–5 steps shorter than the previously described chemical synthesis,[41] but amazingly also one step shorter than the biosynthesis itself; since cysteine can be used as a sulfur source instead of the dipeptide γ-glutamyl cysteine.

The general feasibility of the two-step one-pot strategy was demonstrated by synthesizing a series of 2-thiohistidine-derived compounds **3a** to **3c**, which could be isolated in 46 to 49% global yield (Table 2, entries 2–4), respectively. Depending on the water solubility of the corresponding final compound,[42] the treatment could be significantly simplified compared to the isolation procedure for ergothioneine. Interestingly, also the non-natural enantiomers D-ergothioneine (**D-4**) and D-thiohistidine (**D-3a**) are easily accessible from D-hercynine and D-histidine, respectively (entries 5 and 6), using L-cysteine as a sulfur source and the same reaction sequence.

Noteworthy is in particular the rapidity and ease of preparation of 2-thiohistidine (**3a**). As the least water-soluble compound in this series, it can be isolated directly from the crude aqueous reaction mixture in 49% global yield, after extraction of the dithioketal **16**, despite the presence of salts and excess cysteine. Cysteine used in excess can then be recovered from the filtrate as cystine *via* room-temperature oxidation using H_2O_2 and filtration of the precipitate. To illustrate the potential for up-scaling,

Table 2 One-pot synthesis of ergothioneine and 2-thiohistidine compounds



Entry	Substrate	R/R′	Product	Yield[a] (%)
1	**8**	NMe_3^+/CO_2^-	**4**	40
2	**7a**	NH_2/CO_2H	**3a**	49
3	**7b**	$NHMe/CO_2H$	 **3b**	48
4	**7c**	NMe_2/CO_2H	 **3c**	46
5	**D-8**	NMe_3^+/CO_2^-	 **D-4**	37
6	**D-7a**	NH_2/CO_2H	 **D-3a**	51

[a] Isolated yield.

enantiomerically pure L-2-thiohistidine was prepared in two days on a hundred-gram scale, without any isolation of the intermediate, chromatography or recrystallization. Using a renewable and non-toxic sulfur source, this new manufacturing process for **3a** provides not only a significant gain in time, but also compares favorably with the previously described 5-step processes in terms of process mass intensity (PMI, eqn (1)). PMI is a green chemistry "yardstick" used in the pharmaceutical industry to drive more sustainable processes, and related to Sheldon's *E*-factor (E = PMI − 1) (Table 3).[43]

Table 3 PMI-comparison of three different processes for 2-thiohistidine

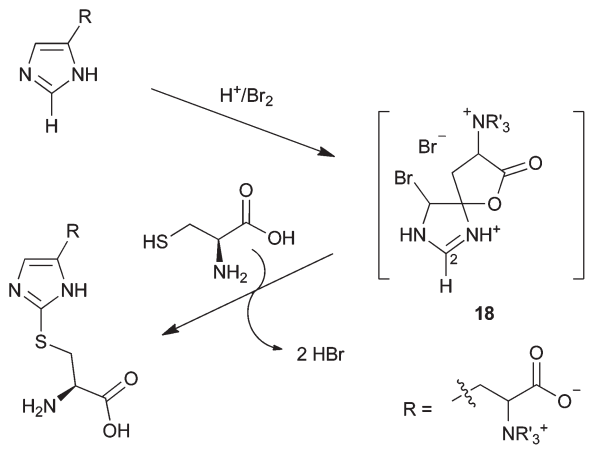
$$\text{Process Mass Intensity (PMI)} = \frac{\text{raw materials (kg)}}{\text{product (kg)}} \quad (1)$$

	Cysteine-process	KSCN-process	PCTF-process
PMI excluding water	104	257	>500
PMI including water	133	416	>500

Evaluation of alternatives to liquid bromine

The here presented biomimetic approach represents major improvements in the spirit of green chemistry by (i) using water as a reaction medium, (ii) reducing the total number of steps by avoiding protection, (iii) integrating two steps in one-pot, (iv) avoiding hazardous red phosphorus in the cleavage step and in particular (v) using cysteine as a renewable sulfur reagent. The main drawback of this method is the use of elemental bromine, which is toxic, corrosive and volatile.

The role of bromine in this reaction sequence is twofold. First it reacts with the aromatic imidazole ring as an electrophile, forming a reactive intermediate. We have not yet been able to identify unambiguously its structure, but by analogy to previous studies,[44] formation of a labile bromolactone **18** can be assumed (Scheme 7). **18** could react with cysteine as a nucleophile, yielding the desired cysteinyl thioether after aromatization. In an overall redox and mass balance, bromine serves furthermore as an oxidant, which is reduced to hydrogen bromide, the hydrogens originating formally from the thiol function of cysteine and the 2-position of the imidazole ring as a consequence of the overall oxidative cysteine introduction.



Scheme 7 A possible mechanism of oxidative cysteine introduction.

Numerous studies described in this journal[45] and others[46] investigated safer alternatives for the use of elemental bromine. Hutchinson and coworkers[47] proposed the use of HBr–H_2O_2 as a greener bromination procedure, generating bromine *in situ*. Other alternative procedures are based on the use of carrying agents supporting bromine. Eissen and Lenoir[48] evaluated 24 alternative methods under environmental, health and safety aspects, and in particular the mass index and *E*-factor, concluding that the avoidance of molecular bromine by most of the investigated methods is not a significant improvement due to higher waste production and solvent demand. Nonetheless, the most competitive alternatives considered are variations of the *in situ* generation of bromine, as for example by reaction of HBr with H_2O_2 (method A, eqn (2)), or also to a minor extent with Oxone (method B, eqn (3)).[49] Recently, the use of a bromide/bromate couple[50] in an acidic medium was introduced as another alternative to generate bromine (method C, eqn (4)) or HOBr (method D, eqn (5)), depending on the ratio used.[51]

Table 4 Oxidative cysteine introduction using alternative methods for bromine/HOBr generation

Entry	Substrate	Method[a]	Product	Yield[b] (%)
1	**7a**	A	**12a**	50
2	**7a**	B	**12a**	54
3	**7a**	C	**12a**	70
4	**7a**	D	**12a**	64
5	**8**	B	**13**	34
6	**8**	C	**13**	53
7	**8**	D	**13**	65
8	**7a**	C	**3a**	48

[a] Method A: H_2O_2 was stirred with HBr for 18 h before addition of histidine/hercynine followed by cysteine at 0 °C. Method B: HBr was added to an aqueous Oxone-solution and stirred for 15 min before addition of histidine/hercynine followed by cysteine at 0 °C. Method C: HCl was added dropwise at 0 °C to an acidic solution of histidine or hercynine, NaBr and $NaBrO_3$ (5 : 1) and stirred for 5 min before the addition of cysteine. Method D: HCl was added dropwise at 0 °C to an acidic solution of histidine or hercynine, NaBr and $NaBrO_3$ (2 : 1) and stirred for 5 min before the addition of cysteine. [b] Calculated yields by ^{1}H NMR of the crude reaction mixture.

$$\text{(A)}\ 2HBr + H_2O_2 \rightarrow Br_2 + 1H_2O \quad (2)$$

$$\text{(B)}\ 2\ HBr + 0{,}5\ Oxone \rightarrow Br_2 + 1\ H_2O + 1{,}5\ KHSO_4 + 0{,}5\ K_2SO_4 \quad (3)$$

$$\text{(C)}\ 5NaBr + 1NaBrO_3 + 6HCl \rightarrow 3Br_2 + 6NaCl + 3H_2O \quad (4)$$

$$\text{(D)}\ 2NaBr + 1NaBrO_3 + 3HCl \rightarrow 3HOBr + 3NaCl \quad (5)$$

To evaluate the alternatives to liquid bromine in the here described oxidative sulfur introduction, the four procedures A–D were compared (Table 4). The *in situ* formation of bromine by oxidation of HBr with H_2O_2 is slow. Hutchinson's procedure is performed by heating the reaction mixture for several hours, using an excess of H_2O_2 and HBr. Since we previously observed the thermal instability of the bromine-activated histidine intermediate, bromine was therefore generated *in situ* prior to the addition of histidine at 0 °C, to avoid decomposition. As shown in Table 4, the desired thioether **12a** could indeed be obtained following the *in situ* generation of bromine with H_2O_2, but only with a comparatively lower yield, due to incomplete conversion (see Table 4, entry 1). This can be explained by competitive decomposition of H_2O_2 catalyzed by bromine or HBr, in an acidic medium,[52] which renders complete oxidation of HBr difficult to achieve. While the *in situ* generation of bromine using Oxone is significantly quicker, the reaction yields of the desired thioether **12a** (Table 4, entry 2) or **13** (Table 4, entry 5) are still lower than upon oxidative cysteine introduction using liquid bromine.

Conversely, good reaction yields ranging from 64 to 70% of the desired thioether **12a** (Table 4, entries 3 and 4) and 53–65% of the thioether **13** (Table 4, entries 6 and 7) could be obtained with the alternative protocols C and D, comparable or even slightly better than the result obtained by direct addition of liquid bromine.

The use of *in situ* generated bromine is also compatible with the two-step one-pot procedure for the synthesis of 2-thiohistidine (**3a**), which could be isolated in 48% yield (using method C, Table 4, entry 8).

These encouraging examples illustrate first attempts to avoid the handling and use of liquid bromine for the oxidative sulfur introduction. In our view, a detailed case-to-case assessment is necessary to evaluate, in a holistic approach, which method is the best for each specific amino acid by considering environmental, safety and health data of the reagents together with reaction efficiency and reagent prices. Further issues to be considered are the manufacturing scale, the treatment and isolation procedure, and the fact that more waste (in the form of inorganic salts) is generated by the alternative methods.

Conclusion

Applying a biomimetic approach, various sulfur-containing amino acids such as L-ergothioneine (**4**)[53] and 2-thiohistidine (**3a**)[54] have been synthesized in a simple, one-pot two-step procedure in water. It encompasses oxidative regioselective introduction of cysteine as a renewable and non-toxic sulfur reagent followed by a novel thermal cleavage reaction in the presence of thiols as a safer alternative to a reaction implying hazardous red phosphorus. Inspired by the biosynthesis, this new chemical approach is one step shorter, as the histidine–sulfur bond can be formed directly with cysteine (instead of γ-glutamyl-cysteine in the biosynthesis).[55]

Addressing the main drawback of this approach, *i.e.*, the handling of liquid bromine as an activating and oxidizing reagent in water, first encouraging results were obtained in the evaluation of four alternative methods, using *in situ* generation of bromine or HOBr directly in the reaction vessel.

Besides the benefit of avoiding hazardous sulfur reagents, taking advantage of the unusual reactivity of the bromine activated histidine derivatives towards cysteine in water drastically reduces the total number of steps compared to classical approaches. As derivatization with protecting groups becomes unnecessary, generation of waste as well as the use of organic solvents are minimized.[56]

L-Ergothioneine is a highly polar and water-soluble betaine amino acid, and to improve its isolation from the aqueous reaction mixture in high purity, electrodialysis was used for desalination prior to crystallization. As a time-, resource- and material-efficient alternative to ion-exchange chromatography, electrodialysis was shown to be an interesting technology for organic synthesis and green process design, and may have broader potential for the treatment and purification of reactions designed in water.

Experimental section

General

Chemicals were purchased from commercial suppliers and used as received. ^{1}H NMR spectra were recorded at 400 MHz with D_2O (4.79 ppm) or D_2O–DCl as the solvent and internal standard ('NH' protons did not appear in ^{1}H NMR spectra because of D_2O exchange, for D_2O–DCl-solutions, 2-(trimethylsilyl)-1-propanesulfonic acid sodium salt[57] was used as the internal

standard). Chemical shifts are reported in parts per million (δ) downfield from TMS. Spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants are reported in hertz (Hz). ^{13}C NMR spectra were obtained at 75 MHz with D_2O or D_2O–DCl as the solvent. Mass spectra were recorded by electrospray ionization mass-spectrometry (ESI-MS) or atmospheric pressure chemical ionization (APCI-MS). Melting points were determined on a Stuart Scientific apparatus. Purifications of compounds **13** and **12a–c** were performed by chromatography on a DOWEX 50WX2 200–400 (H^+-form). Desalination was performed *via* electrodialysis (10 V) using a cell package with 20 membrane-pairs (cation-exchange PC-SK, anion-exchange PC-SA, ED200-020, PC Cell, Germany). *N*-Methyl-histidine hydrochloride (**7b**·HCl), *N*,*N*-dimethyl-histidine hydrochloride hydrate (**7c**·HCl·H_2O) and L-hercynine (**8**) were prepared according to ref. 24. Other reagents, such as L-histidine hydrochloride hydrate (**7a**·HCl·H_2O), cysteine (**1**), 3-mercaptopropionic acid (**15**), and benzaldehyde, were commercially available and used without further purification.

Optimization study: preparation of 3-{2-[(2-amino-2-carboxyethyl)thio]-1*H*-imidazol-4-yl}-2-(trimethylammonio)-propanoate (13)

For the optimization of reaction conditions, hercynine (**8**, 986 mg, 5 mmol) and concd hydrochloric acid (2.5–7.5 mmol, 0.5–1.5 equiv.) were dissolved in water (10–50 mL) and cooled in an ice-bath. Br_2 (800–1040 mg, 5.0–6.5 mmol, 1.0–1.3 equiv.) was added dropwise, and cysteine (0.61–3.03 g, 5–25 mmol, 1–5 equiv.) after 5–7 min. After stirring for 1 h at 0 °C, 200 µL of the reaction mixture were diluted in 500 µL D_2O, filtered, and the solution analyzed by ^{1}H NMR (400 MHz, see Fig. S3, ESI†).

Column chromatography (gradient 0.5–2 N HCl, DOWEX 50WX2-400) of the filtered reaction mixture provided compound **13** in the form of a slightly yellow hygroscopic solid as trihydrochloride hydrate (1.295 g, 58% using 5 equiv. cysteine; 1.065 g, 46% using 3 equiv. cysteine); mp (dec) 97 °C; δ_H (400 MHz, D_2O) 3.36 (9H, s), 3.50 (1H, dd, *J* 14.2 and 11.8 Hz), 3.63 (1H, dd, *J* 14.2 and 3.8 Hz), 3.73 (1H, dd, *J* 15.5 and 4.7 Hz), 3.84 (1H, dd, *J* 15.5 and 4.7 Hz), 4.31 (1H, dd, *J* 11.8 and 3.8 Hz), 4.49 (t, *J* 4.7 Hz, 1H) and 7.51 (1H, s); δ_C (75 MHz, D_2O) 23.0, 35.4, 52.7, 53.0, 74.5, 121.3, 129.1, 138.0, 168.6 and 169.4; *m*/*z* (ESI) 317 (MH^+). Found: C, 32.10; H, 5.59; N, 12.22. Calc. for $C_{12}H_{20}N_4O_4S \times 3HCl \times H_2O$: C, 32.48; H, 5.68; N, 12.63%.

General procedure A: synthesis of compounds 12a–12c

Following the procedure described for **13**, using 1.3 equiv. Br_2 at 0 °C and 3 equiv. cysteine, compounds **12a** (69%), **12b** (68%), and **12c** (63%) were obtained from L-histidine (**7a**), α-*N*-methyl-L-histidine (**7b**), and α-*N*,*N*-dimethyl-L-histidine (**7c**), respectively, after column chromatography (gradient 0.5–2.0 N HCl, DOWEX 50WX2-400) of the filtered reaction mixture.

2-Amino-3-{[4-(2-amino-2-carboxyethyl)-1*H*-imidazol-2-yl]thio}-propanoic acid (12a)

Compound **12a** was isolated as tri-hydrochloride hydrate following general procedure A as a pale yellow hygroscopic solid; mp (dec) 108 °C; δ_H (400 MHz, D_2O) 3.39 (1H, dd, *J* 15.9, 6.9 Hz), 3.46 (1H, dd, *J* 15.9, 6.9 Hz), 3.68 (1H, dd, *J* 15.4, 4.7 Hz), 3.83 (1H, dd, *J* 15.4, 4.7 Hz), 4.32 (1H, t, *J* 6.9 Hz) and 4.34 (1H, t, *J* 4.7 Hz), 7.51 (1H, s); δ_C (75 MHz, D_2O) 25.9, 35.6, 52.5, 53.1, 121.3, 129.9, 138.0, 169.9 and 170.8; *m*/*z* (ESI) 275 (MH^+). Found: C, 26.80; H, 4.71; N, 13.79. Calc. for $C_9H_{14}N_4O_4S \times 3HCl \times H_2O$: C, 26.91; H, 4.77; N, 13.95%.

2-Amino-3-({4-[2-carboxy-2-(methylamino)ethyl]-1*H*-imidazol-2-yl}thio)propanoic acid (12b)

Compound **12b** was isolated as tri-hydrochloride hydrate following general procedure A as a pale yellow hygroscopic solid; mp (dec) 110 °C; δ_H (400 MHz, D_2O) 2.83 (3H, s), 3.40 (1H, dd, *J* 15.8, 7.8 Hz), 3.50 (1H, dd, *J* 15.8, 5.4 Hz), 3.70 (1H, dd, *J* 15.4, 4.8 Hz), 3.83 (1H, dd, *J* 15.4, 4.8 Hz), 4.16 (1H, dd, *J* 7.8, 5.4 Hz), 4.36 (1H, t, *J* 4.8 Hz) and 7.52 (1H, s); δ_C (75 MHz, D_2O) 24.6, 32.1, 35.4, 52.8, 60.0, 121.3, 129.5, 137.7, 169.4 and 169.9; *m*/*z* (APCI) 290. Found: C, 29.30; H, 4.80; N, 13.57%. Calc. for $C_{10}H_{16}N_4O_4S \times 3HCl \times H_2O$: C, 28.89; H, 5.09; N, 13.48%.

2-Amino-3-({4-[2-carboxy-2-(dimethylamino)ethyl]-1*H*-imidazol-2-yl}thio)propanoic acid (12c)

Compound **12c** was isolated as tri-hydrochloride hemi-hydrate following general procedure A as a pale yellow hygroscopic solid; mp (dec) 102 °C; δ_H (400 MHz, D_2O) 3.01 (6H s), 3.42 (1H, dd, *J* 15.3, 9.8 Hz), 3.54 (1H, dd, *J* 15.3, 4.4 Hz), 3.70 (1H, dd, *J* 15.5, 4.8 Hz), 3.81 (1H, dd, *J* 15.5, 4.8 Hz), 4.30 (1H, dd, *J* 9.8, 4.4 Hz), 4.46 (1H, t, *J* 4.8 Hz) and 7.52 (1H, s); δ_C (75 MHz, D_2O) 22.4, 35.5, 41.3 (broad), 52.7, 66.6, 121.2, 130.1, 137.4 and 169.4;[58] *m*/*z* (ESI) 303 (MH^+). Found: C, 31.40; H, 5.12; N, 13.12%. Calc. for $C_{11}H_{18}N_4O_4S \times 3HCl \times 0.5H_2O$: C, 31.40; H, 5.27; N, 13.32%.

L-Ergothioneine (4)

(A) Formation of 4 and 2,2-bis[(2-carboxyethyl)thio]propanoic acid (16) by cleavage of compound 13 in the presence of 15. Compound **13**·3HCl·H_2O (2.22 g, 5 mmol) and 3-mercaptopropionic acid (**15**, 4.4 mL, 5.31 g, 50 mmol, 10 equiv.) were dissolved in water (20 mL) and heated under stirring for 18 h at 90–100 °C (see Fig. S4, ESI†). The solution was cooled to rt, extracted with ethyl acetate (20 mL × 4, to remove the excess of **15** and dithioketal **16**), and the pH adjusted to 6 by addition of a 20% ammonia solution. After lyophilization, the powder obtained was dissolved in an ethanol–water mixture and treated with charcoal. After filtration, crystallization provided L-ergothioneine (**4**) as a white powder (917 mg, 80%).

Compound **16** was synthesized independently to confirm the structure: a mixture of pyruvic acid (**11**, 4 mL, 5.0 g, 55.6 mmol), **15** (9.86 mL, 11.9 g, 111.2 mmol) and water

(2 mL) was heated under stirring at 90 °C for 18 h. After the addition of water (20 mL), filtration and washing with water, ethyl acetate and pentane, a white solid was obtained, which was recrystallized in acetonitrile (containing 1% dithiothreitol). Compound **16** was obtained as fine colorless crystals (6.13 g; 37%); mp 139 °C; δ_H (400 MHz, D_2O + NaOD (pH 7)) 1.74 (3H, s), 2.48 (4H, m) and 2.82 (4H, m); δ_C (75 MHz, D_2O + NaOD (pH 7)) 27.0, 27.5, 37.5, 64.8, 178.0 and 181.6; *m/z* (APCI) 305 ($M + Na^+$). Found: C, 38.40; H, 4.99. Calc. for $C_9H_{14}O_6S_2$: C, 38.28; H, 4.99%.

(B) One-pot-procedure for the synthesis of L-ergothioneine (4). Hercynine (183.0 g, 0.9 mol) and concd HCl (37%, 75.1 mL, 0.9 mol) were dissolved in water (1.8 L), and the solution cooled to 0 °C. Under vigorous stirring and continued cooling, Br_2 (60.1 mL, 186.0 g, 1.17 mol, 1.3 equiv.) was added rapidly, followed by cysteine (556.3 g, 4.5 mol, 5 equiv.) 5–7 min later, and the mixture stirred for 1 h at 0 °C. 3-Mercaptopropionic acid (579 g, 5.4 mol, 6 equiv.) was added, and the mixture heated for 18 h at 95 °C. After cooling to rt, the clear orange solution was extracted with ethyl acetate, and the pH of the aqueous phase adjusted to 4.5 by addition of a 20% ammonia solution. Under stirring and cooling with a water bath, benzaldehyde (482.3 g, 4.5 mol, 5 equiv.) was added rapidly. After 5–10 min, phenylthiazolidine-4-carboxylic acid (**17**, δ_H (400 MHz, d_6-DMSO)[33] 3.00–3.17 (1H, m), 3.25–3.42 (1H, m), 3.89 (0.45H, t, *J* 7.8 Hz), 4.22 (0.55H, dd, *J* 6.7, 4.7 Hz), 5.50 (0.45H, s), 5.67 (0.55H, s) and 7.20–7.54 (5H, m)) precipitated as a white solid, which was filtered, and rinsed several times with water. The combined filtrates were washed with ethyl acetate, and desalinated by electrodialysis. The obtained slightly yellow solution was concentrated under vacuum. Crystallization provided L-ergothioneine (**4**) in the form of white crystals (83.0 g, 40%); mp (dec) 251 °C; $[\alpha]_D$ +125.2 (*c* 1.0 in H_2O) (lit.,[12] mp 262–263 °C; $[\alpha]_D$ +126.6 (*c* 1.0 in H_2O)); δ_H (400 MHz, D_2O) 3.18 (2H, m), 3.28 (9H, s), 3.90 (1H, dd, *J* 11.7, 4.0 Hz), 6.80 (1H, s); δ_C (75 MHz, D_2O) 23.4, 52.7, 77.5, 115.7, 124.3, 156.6, and 170.5; *m/z* (ESI) 230 (MH^+). Found: C, 47.30; H, 6.59; N, 18.29%. Calc. for $C_9H_{15}N_3O_2S$: C, 47.14; H, 6.59; N, 18.32%.

D-(−)-Ergothioneine (D-4)

Following the same protocol, D-ergothioneine (**D-4**, 1.7 g, 37%) was obtained starting from D-hercynine (4.0 g, 20 mmol), Br_2 (1.34 mL, 4.17 g, 26 mmol, 1.3 equiv.) and L-cysteine (12.41 g, 100 mmol, 5 equiv.), *via* desalination with Amberlite IRA 410 resin (HCO_3^--form); mp (dec) 250 °C; $[\alpha]_D$ −124.6 (*c* 1.0 in H_2O). Found: C, 47.10; H, 6.55; N, 18.40%. Calc. for $C_9H_{15}N_3O_2S$: C, 47.14; H, 6.59; N, 18.32%. Spectral data were identical in all respects with those of the above enantiomer.

L-(−)-2-Thiohistidine (3a)

Compound **7a**·HCl·H_2O (317.6 g, 1.5 mol) was dissolved in water (3 L), and the solution cooled to −2–0 °C. Under vigorous stirring and continued cooling, Br_2 (100 mL, 311 g, 1.95 mol, 1.3 equiv.) was added rapidly, followed by cysteine (562 g, 4.5 mol, 3 equiv.) 5–7 min later, and the mixture stirred for 1 h at 0 °C. 3-Mercaptopropionic acid (960 g, 9 mol, 6 equiv.) was added, and the mixture heated for 18 h at 95 °C. After cooling to rt, the clear brown solution was extracted with ethyl acetate. The aqueous phase was placed in a water bath at 40 °C under nitrogen, and the pH of the solution adjusted to 6.5. L-2-Thiohistidine (**3a**) precipitated as a white solid (135.0 g, 49%), which was filtered, rinsed several times with water and finally ethanol, and dried; mp (dec) 314 °C; $[\alpha]_D$ −10.7 (*c* 1.0 in HCl 1 N) (lit.,[26] mp > 280 °C; $[\alpha]_D$ −10.6 (*c* 0.5 in HCl 1 N)); δ_H (400 MHz, D_2O + DCl) 3.22 (1H, dd, *J* 15.9, 7.3 Hz), 3.30 (1H, dd, *J* 15.9, 5.9 Hz), 4.36 (1H, dd, *J* 7.3, 5.9 Hz) and 6.92 (1H, s); δ_C (75 MHz, D_2O + DCl) 25.7, 52.2, 116.5, 123.6, 156.8, and 170.7; *m/z* (ESI) 188 (MH^+).

D-(+)-2-Thiohistidine (D-3a)

Following the same protocol as described for the synthesis of L-2-thiohistidine, D-2-thiohistidine (**D-3a**, 7.6 g, 51%) was obtained starting from D-histidine·HCl·H_2O (16.77 g, 80 mmol); mp (dec) 316–318 °C; $[\alpha]_D$ +11.3 (*c* 1.0 in HCl 1 N). Found: C, 38.10; H, 4.88; N, 22.45 Calc. for $C_6H_9N_3O_2S$: C, 38.49; H, 4.84; N, 22.44%. Spectral data were identical in all respects with those of the above enantiomer.

2-(Methylamino)-3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)-propanoic acid (3b)

Following the same protocol as described for the synthesis of L-ergothioneine (without desalination), compound **3b**·H_2O (5.28 g, 48%) was obtained in the form of a white powder starting from compound **7b**·HCl (10.28 g, 50 mmol), Br_2 (3.34 mL, 10.39 g, 65 mmol, 1.3 equiv.) and L-cysteine (18.17 g, 150 mmol, 3 equiv.), after cleavage of intermediate **12b** in the presence of 3-mercaptopropionic acid (31.8 g, 300 mmol, 6 equiv.), scavenging of the excess of cysteine with benzaldehyde (15.9 g, 150 mmol, 3 equiv.) and crystallization; mp (dec) 234 °C; $[\alpha]_D$ +36.7 (*c* 1.0 in HCl 1 N); δ_H (400 MHz, D_2O + DCl) 2.81 (3H, s), 3.31 (2H, m), 4.27 (1H, t, *J* 6.2 Hz) and 6.93 (1H, s); δ_C (75 MHz, D_2O + DCl) 24.8, 32.2, 60.0, 116.4, 123.2, 156.9 and 170.1; *m/z* (APCI) 202 (MH^+). Found: C, 38.30; H, 5.82; N, 18.87%. Calc. for $C_7H_{11}N_3O_2S \times H_2O$: C, 38.34; H, 5.98; N, 19.16%.

2-(Dimethylamino)-3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)-propanoic acid (3c)

Following the same protocol as described for the synthesis of L-ergothioneine (without desalination), compound **3c**·0.5H_2O (10.25 g, 46%) was obtained in the form of a white powder starting from compound **7c**·HCl·H_2O (23.76 g, 100 mmol), Br_2 (6.68 mL, 20.7 g, 130 mmol, 1.3 equiv.) and L-cysteine (37.0 g, 300 mmol, 3 equiv.), after cleavage of intermediate **12c** in the presence of 3-mercaptopropionic acid (64.3 g, 600 mmol, 6 equiv.), scavenging of the excess of cysteine with benzaldehyde (30.7 g, 300 mmol, 3 equiv.) and crystallization; mp (dec) 200 °C; $[\alpha]_D$ +5.9 (*c* 1.0 in HCl 1 N); δ_H (400 MHz, D_2O + DCl) 3.02 (6H, s), 3.30 (1H, dd, *J* 15.6, 8.5 Hz), 3.40 (1H, dd, *J* 15.6, 5.5 Hz), 4.32 (1H, dd, *J* 8.5, 5.5 Hz) and 6.94 (1H, s); δ_C

(75 MHz D_2O + DCl) 22.9, 40.9, 43.1, 66.7, 116.2, 123.8, 156.3 and 169.5; *m/z* (APCI) 216 (MH^+). Found: C, 42.80; H, 6.18; N, 18.65%. Calc. for $C_8H_{13}N_3O_2S \times 0.5H_2O$: C, 42.84; H, 6.29; N, 18.74%.

Oxidative cysteine introduction using alternative methods (Table 4)

Method A (Table 4, entry 1): Hydrogen peroxide (30%, 736 mg, 6.5 mmol, 1.3 equiv.) was added dropwise to a solution of hydrobromic acid (2.19 g, 1.47 mL, 13 mmol, 2.6 equiv.) in water (10 mL). After stirring for 18 h at room temperature in a closed vessel, the orange-red solution was cooled to 0 °C. Histidine hydrochloride hydrate (1.05 g, 5 mmol, 1 equiv.) was added and, after 5 min at 0 °C, cysteine (1.85 g, 15 mmol, 3 equiv.). After stirring for 1 h at 0 °C, 200 μL of the reaction mixture were diluted in 500 μL D_2O, filtered, and the solution analyzed by ^1H NMR (400 MHz).

Method B (Table 4, entries 2 and 5): Hydrobromic acid (2.19 g, 1.47 mL, 13 mmol, 2.6 equiv.) was added dropwise to a cooled solution of Oxone (2.0 g, 6.5 mmol, 1.3 equiv.) in water (10 mL) at 0 °C. After stirring for 15 min at room temperature in a closed vessel, histidine (5 mmol, 1 equiv. for entry 2) or hercynine (**8**, 986 mg, 5 mmol, 1 equiv. for entry 5) was added and, after 5 min at 0 °C, cysteine (1.85 g, 15 mmol, 3 equiv. for entry 2; 3.09 g, 25 mmol, 5 equiv. for entry 5). After stirring for 1 h at 0 °C, 200 μL of the reaction mixture were diluted in 500 μL D_2O, filtered, and the solution analyzed by ^1H NMR.

Method C (Table 4, entries 3 and 6): Hydrochloric acid (37%, 1.2 g, 1.1 mL, 13 mmol, 2.6 equiv.) was added dropwise to a cooled solution of histidine hydrochloride hydrate (5 mmol, 1 equiv. for entry 3) or hercynine hydrochloride (5 mmol, 1 equiv. for entry 6), sodium bromide (1.14 g, 11 mmol) and sodium bromate (0.33 g, 2.2 mmol) in water (10 mL) at 0 °C. Cysteine (1.85 g, 15 mmol, 3 equiv. for entry 3; 3.09 g, 25 mmol, 5 equiv. for entry 6) was added after 5 min at 0 °C. After stirring for 1 h at 0 °C, 200 μL of the reaction mixture were diluted in 500 μL D_2O, filtered, and the solution analyzed by ^1H NMR.

Method D (Table 4, entries 4 and 7): Hydrochloric acid (37%, 0.64 g, 0.55 mL, 6.6 mmol, 1.3 equiv.) was added dropwise to a cooled solution of histidine hydrochloride hydrate (5 mmol, 1 equiv. for entry 4) or hercynine hydrochloride (5 mmol, 1 equiv. for entry 7), sodium bromide (0.45 g, 4.33 mmol) and sodium bromate (0.33 g, 2.2 mmol) in water (10 mL) at 0 °C. Cysteine (1.85 g, 15 mmol, 3 equiv. for entry 4; 3.09 g, 25 mmol, 5 equiv. for entry 7) was added after 5 min at 0 °C. After stirring for 1 h at 0 °C, 200 μL of the reaction mixture were diluted in 500 μL D_2O, filtered, and the solution analyzed by ^1H NMR.

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24 L-Hercynine can be prepared in two steps and 73% global yield from L-histidine: V. N. Reinhold, Y. Ishikawa and D. B. Melville, *J. Med. Chem.*, 1968, **11**, 258–260.

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27 As **13** had been synthesized in small amounts in the study of the biosynthesis of ergothioneine (see ref. 16) from chloroalanine and

ergothioneine, its described ^{1}H NMR-spectrum could be compared to the ^{1}H NMR of the crude reaction mixture (Fig. S3, ESI†). Besides non-converted hercynine (about 12%) and other unidentified hercynine-derived products with aromatic protons, a signal at 7.43 ppm, attributed to H-5 in **13**, could indeed be detected, its integration accounting for *ca.* 9% of the total aromatic protons

28 (*a*) European Chemical Substances Informations System (ESIS). 2000. IUCLID Dataset for phosphorus. European Commission Joint Research Centre. http://esis.jrc.ec.europa.eu/doc/IUCLID/datasheets/7723140.pdf, last accessed May 2012; (*b*) Hazardous Substances Data Bank (HSDB). 2010. Entry for phosphorus. United States National Library of Medicine http://toxnet.nlm.nih.gov/, last accessed May 2012.

29 The transient formation of **11**, which is also a byproduct in the biosynthesis of ergothioneine, is probably due to immediate hydrolysis of dehydroalanine as the primary cleavage product, which could not be detected in the reaction mixture

30 3-Mercaptopropionic acid (CAS-Nr. 107-96-0) is registered in the context of the REACH-regulation, and the dossier available at: http://apps.echa.europa.eu/registered/data/dossiers/DISS-97d8e287-1c3b-58ba-e044-00144f67d031/DISS-97d8e287-1c3b-58ba-e044-00144f67d031_DISS-97d8e287-1c3b-58ba-e044-00144f67d031.html, last accessed May 2012.

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32 For a summary of data, see OECD SIDS-document for benzaldehyde, CAS-Nr. 100-52-7, http://www.inchem.org/documents/sids/sids/100527.pdf, last accessed May 2012.

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42 The water-solubility ranges from >300 g L^{-1} for **4** to ~2 g L^{-1} for **3a** as the least water-soluble compound in this series

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55 Theoretically, a shorter reaction sequence yielding ergothioneine in one step and with higher atom economy could be imagined by using Na_2S instead of cysteine, comparable to the one-step synthesis of 2-thiohistidine as reported by Ito in 12% yield (ref. 26). In a comparative evaluation of the Na_2S reaction with bromine-activated hercynine on a 5 mmol-scale, we detected small amounts of ergothioneine (2–4%). As in an industrial perspective the use of Na_2S in an acidic medium (generating H_2S *in situ*) or the direct use of gaseous H_2S is not recommendable and sustainable for safety and environmental reasons (see for example: hydrogen sulfide: human health aspects. (Concise International Chemical Assessment Document; 53). World Health Organization: Geneva, 2003), further optimization of this reaction pathway was not considered, despite its high atom economy

56 The use of organic solvents impacts strongly the environmental footprint of a process, see for a quantitative assessment: R. K. Henderson, C. Jimenez-Gonzalez, D. J. C. Constable, S. R. Alston, G. G. A. Inglis, G. Fisher, J. Sherwood, S. P. Binks and A. D. Curzons, *Green Chem.*, 2011, **13**, 854–862.

57 The use of this and other internal references related to ^{1}H NMR studies of histidine-derivatives is mentioned in this reference: T. Tynkkynen, M. Tiainen, P. Soininen and Laatikainen, *Anal. Chim. Acta*, 2009, **648**, 105–112.

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