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Biocatalytic and chemical routes to all the stereoisomers of methionine and ethionine sulfoxides

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Abstract

Biotransformations of the *N*-phthaloyl derivatives of D- and L-methionine and of D- and L-ethionine by *Beauveria bassiana* ATCC 7159 or *Beauveria caledonica* ATCC 64970 produce the corresponding (S_S) sulfoxides in good yield and diastereomeric excess. Pure (S_SS_C) diastereomers can be obtained from L-series substrates by crystallisation of the biotransformation extract, and the corresponding (S_SR_C) products obtained from D-series substrates by chromatography of the biotransformation extract. Hydrogen peroxide-catalysed oxidation of the *N*-phthaloyl derivatives of D- and L-methionine and of D- and L-ethionine gives diastereomeric mixtures from which the (S_SS_C) and (R_SR_C) diastereomers can be obtained by crystallisation, and the (S_SR_C) and (R_SS_C) diastereomers obtained by chromatography. *N*-Cbz- and *N*-*t*-Boc methionines are also converted to sulfoxides with predominant (S_S) configuration by both *B. bassiana* and *B. caledonica*, but the isolated yields and d.e. of products were generally lower than those obtained from the *N*-phthaloyl substrates.

Removal of the *N*-phthaloyl group from diastereomerically pure methionine and ethionine sulfoxides gave the corresponding amino acid sulfoxides in high yield; removal of *N*-Cbz and *N*-*t*-Boc groups from protected methionine sulfoxides was also achieved without loss of configuration at sulfur. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Sulfoxides of the thia-amino acids methionine (**1**), ethionine (**2**), and (*S*)-methylcysteine (**3**) possess a wide variety of biological properties. Oxidation of methionine residues in proteins has been the target of considerable interest in recent years, as it often results in significant structural and functional changes in the protein.[1] This oxidation is reversible, and is performed by a methionine sulfoxide reductase enzyme that functions as an in vivo antioxidant by transferring excess oxidising equivalents onto methionine during times of cellular oxidative stress.[2] The reversible oxidation of methionine is also implicated in

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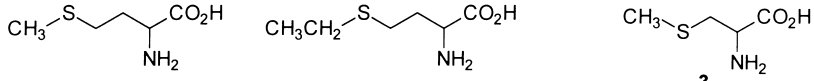
other biological functions such as the modulation of potassium channel activity,[3] and its role in the oxidation of high density lipoproteins, leading to a reduced ability of the latter to transport cholesterol to the liver for excretion, has recently been established.[4,5] It is also implicated in the ageing process by demonstration of the effect of methionine sulfoxidation on the activity of the human keratinocyte growth factor[6] and on the immuno-peptide thymosin β-4;[7] the in vivo oxidation of methionine to the sulfoxide is an age-dependent process in humans.[8]



1 **2** **3**

The carcinogenic properties of ethionine (**2**) have been linked to its in vivo oxidation to sulfoxide,[9] while (*S*)-methylcysteine sulfoxide (**3**-sulfoxide) is responsible for the anti-bacterial action of cabbage[10] and possesses the ability to lower serum cholesterol levels.[11] It is also the substrate for the enzyme cysteine sulfoxide lyase, whose activity in damaged vegetable tissue leads to the production of the volatile sulfur compounds responsible for the characteristic 'rotting vegetable' smells.[12]

With the exception of L-methionine sulfoxide isolated from the blowfly *Phormia regina*,[13] and (*S*)-methyl-L-cysteine sulfoxide from cabbage,[14] both of which have the (S_SS_C) configuration, no data are available on the configuration at sulfur in naturally occurring amino acid sulfoxides from other sources. Prior to our preliminary report, no diastereoselective method for the oxidation of a sulfur-containing amino acid to its sulfoxide appears in the literature.[15] The potential relevance of the configuration at sulfur to the biological activity of oxidised amino acids is clearly indicated by a report on the differential toxicity of the diastereomers of an (*S*)-pentachlorobutadienylcysteine sulfoxide metabolite of hexachlorobutadiene.[16]

The value of biocatalytic oxidation as a method for the stereoselective oxidation of sulfides to sulfoxides is well established,[17,18] and this method has been used for the oxidation of methionine by *Penicillium citreo-viride*, but no stereochemical details were reported.[19] In view of the ability of fungal biocatalysts to oxidise a wide variety of prochiral sulfides to chiral sulfoxides, we have investigated such catalysts for the diastereoselective oxidation of a range of *N*-protected thia-amino acids, and now report their use for the diastereoselective oxidation of methionine and ethionine derivatives which, together with complementary chemical oxidations, can lead to the preparation of all the stereoisomers of methionine and ethionine sulfoxides.

2. Results

Screening experiments for the ability of fungi to oxidise *N*-phthaloyl-D,L-methionine were carried out using established oxidative biocatalysts[18,20] such as *Aspergillus niger* ATCC 9142, *Aspergillus ochraceous* ATCC 18500, *Curvularia lunata* ATCC 12017, *Mortierella isabellina* ATCC 42613, *Helminthosporium* species NRRL 4671, *Rhizopus arrhizus* ATCC 11145, and *Beauveria bassiana* ATCC 7159. Of these, only the latter gave sulfoxide product in >10% conversion, producing in unoptimised biotransformation of *N*-phthaloyl-L-methionine an 85% yield of a product subsequently identified as predominantly the (S_SS_C) sulfoxide (d.e. 60%), and from *N*-phthaloyl-D-methionine 88% of the (S_SR_C) sulfoxide (d.e. 74%), as summarised in Table 1. This led to the investigation of other *Beauveria* species, and *B. caledonica* ATCC 64970 was identified as a superior catalyst for the oxidation of *N*-phthaloyl-L-methionine to the (S_SS_C) sulfoxide, giving a quantitative conversion to the (S_SS_C) sulfoxide (d.e. 90%), while *N*-phthaloyl-D-methionine was converted to the (S_SR_C) sulfoxide in lower yield but with high d.e. (92%) (Table 1).

Table 1
Sulfoxide products obtained from the biotransformation of *N*-protected methionine substrates

Methionine configuration	Sulfoxide											
	B. bassiana						*B. caledonica*					
	N-Protecting group						*N*-Protecting group					
	Phth		Cbz		t-boc		Phth		Cbz		t-boc	
	Y*	DE*	Y	DE	Y	DE	Y	DE	Y	DE	Y	DE
L (S_C)	85	60	46	40	45	58	98	90	45	60	40	64
D (R_C)	88	74	46	32	41	60	66	92	80	80	65	84

* Y = Yield, DE = Diastereomeric Excess

Table 2
Sulfoxide products obtained from the biotransformation of *N*-phthaloylethionine substrates

Ethionine configuration	Sulfoxide			
	B. bassiana		*B. caledonica*	
	Y*	DE*	Y	DE
L (S_C)	74	86	63	80
D (R_C)	48	76	50	73

* Y = Yield, DE = Diastereomeric Excess

The configuration of the predominant diastereomer obtained from biocatalytic oxidations of *N*-phthaloyl-L-methionine was determined following its crystallisation to homogeneity, comparison of analytical data with those reported for (S_SS_C)-*N*-phthaloylmethionine sulfoxide,[21] and conversion to (S_SS_C)-methionine sulfoxide[22] by hydrazinolysis. The product obtained from *N*-phthaloyl-D-methionine was diastereomeric with that obtained from the L-isomer, and thus identified as (S_SR_C)-*N*-phthaloylmethionine sulfoxide, subsequently converted to (S_SR_C)-methionine sulfoxide.[22] Other *N*-protected methionine substrates, also summarised in Table 1, gave consistently lower yields or diastereomeric purities of non-crystalline products which were not readily separable into individual diastereomers by chromatography, and were thus characterised following removal of the protecting group by comparison with authentic standards of methionine sulfoxides. Mixtures of the latter give baseline-resolved ^{13}C NMR signals for the individual diastereomers at 75 MHz.

In view of the superior results obtained from *N*-phthaloylmethionines, only this protecting group was used for investigation of the biotransformation of *N*-protected L- and D-ethionine substrates. The results, summarised in Table 2, parallel those obtained from the corresponding methionines in terms of stereoselectivity of oxidation, but in this instance no significant differences were observed between the two *Beauveria* strains employed.

The product from biotransformations of *N*-phthaloyl-L-ethionine could be crystallised to homogeneity, and was identified as the (S_SS_C) diastereomer by determination of its X-ray crystallographic structure (Fig. 1). The product from *N*-phthaloyl-D-ethionine could not be purified by crystallisation, but chroma-

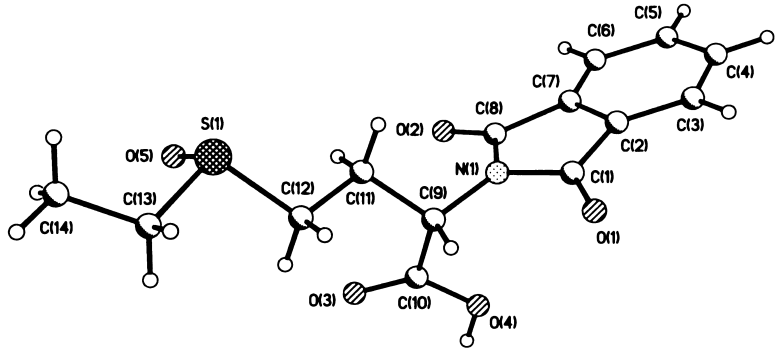


Figure 1. (S_SS_C)-*N*-Phthaloylethionine

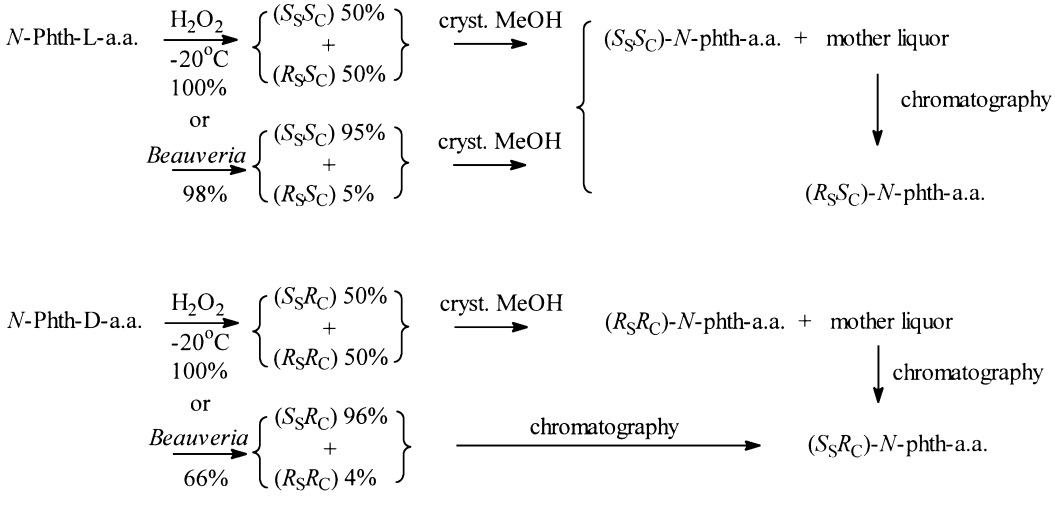
tography gave a 46% yield of sulfoxide, d.e. 95%, which was diastereomeric with that obtained from the L-isomer of substrate, and thus identified as (S_SR_C)-*N*-phthaloylethionine.

N-Phthaloyl-L- and D-methionine and *N*-phthaloyl-L- and D-ethionine were all oxidised by hydrogen peroxide non-stereoselectively but in quantitative yields to the corresponding sulfoxides. From these mixtures, the (S_SS_C) and (R_SR_C) diastereomers could be obtained in pure form by crystallisation, and the (S_SR_C) and (R_SS_C) diastereomers obtained from the corresponding mother liquors by chromatography.

Removal of the *N*-phthaloyl group from *N*-phthaloylmethionine and -ethionine sulfoxides was achieved by hydrazinolysis, avoiding the classical acidic work-up that leads to racemisation of the configuration at sulfur, and yielded the corresponding amino acid sulfoxides in good yield. The configurations of products in the methionine series were determined by comparison with literature data for standards of known configuration, and configurations in the ethionine series were based on correlations with the structure of Fig. 1.

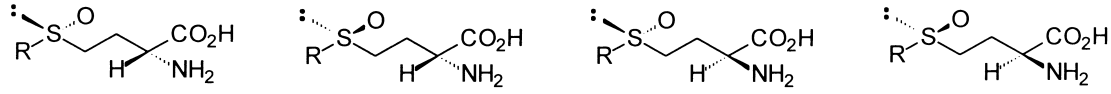
3. Discussion

A flow chart for the preparation of methionine and ethionine sulfoxides is presented in Scheme 1, below.



Scheme 1. Summary of routes for the preparation of methionine and ethionine sulfoxides

Specific biotransformation yields and percentage compositions given in Scheme 1 refer to conversions of *N*-phthaloyl-L- and D-methionines by *B. caledonica*, but the stereochemical relationships outlined in the Scheme are relevant to all the isomers of *N*-phthaloylmethionines and -ethionines. The stereostructures are shown below for methionine (R=CH_3) and ethionine (R=C_2H_5) sulfoxides.



(S_SR_C)-a.a. sulfoxides (R_SR_C)-a.a. sulfoxides (S_SS_C)-a.a. sulfoxides (R_SS_C)-a.a. sulfoxides

The routes outlined in Scheme 1 for the preparation of diastereomerically defined *N*-phthaloylmethionine and -ethionine sulfoxides, and thence for the corresponding amino acid sulfoxides, present a simple method for the preparation of the latter, and are superior to existing resolution-based methods for the preparation of the individual diastereomers of methionine sulfoxide.[22] No methods for the preparation of the corresponding ethionine sulfoxides have previously been reported.

The biocatalytic approach is currently being extended to the preparation of other naturally occurring amino acid sulfoxides, and these results will be presented in due course.

4. Experimental

4.1. Materials and methods

Melting points were determined on a Kofler hot stage and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance series 300 spectrometer in $CDCl_3$ using residual $CHCl_3$ as the internal standard unless otherwise stated; chemical shifts are reported in ppm (δ) and the signals quoted as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). The ^{13}C NMR spectra were recorded at 75 MHz on the same spectrometer in $CDCl_3$, CD_3OD, or D_2O solution as stated below. Mass spectra were obtained in EI mode (unless otherwise stated) using a Kratos 1S spectrometer. IR spectra were obtained using a Mattson Research Series FTIR spectrometer. Optical rotations were recorded at ambient temperature in the stated solvent using a Rudolph Autopol 3 polarimeter. Diastereomeric excess (d.e.) was determined by ^{13}C NMR analysis of signals α and β to sulfur. TLC was performed on Merck silica gel F_{254} plates, 0.2 mm, and column chromatography used Merck silica gel 9385, 230–400 mesh. *Beauveria bassiana* ATCC 7159 was maintained on Sabouraud dextrose agar slopes, grown at 26°C and stored at 4°C, and *Beauveria caledonica* ATCC 64970 on potato dextrose agar slopes, grown at 24°C and stored at 4°C.

4.2. Preparation of substrates

N-Cbz- and *N*-*t*-Boc-methionines were commercial samples (Sigma). *N*-Phthaloyl-D- and L-methionines and D- and L-ethionines were prepared from the amino acids in 95–98% yield by the method of Bose.[23]

N-Phthaloyl-D-methionine: m.p. 123–124°C; ^{1}H NMR δ 2.08 (3H, s), 2.42–2.62 (4H, m), 5.15 (1H, m), and 7.75/7.89 (each 2H, m) ppm; ^{13}C NMR δ ($CDCl_3$) 15.7, 28.2, 31.2, 51.0, 124.1, 132.1, 134.7, 167.9, and 175.1 ppm; MS m/z (%) 279 (12), 205 (100), 187 (86), 132 (29); $[\alpha]_D$ +47.46 (c 5.0, MeOH).

N-Phthaloyl-L-methionine: m.p. 122–124°C (lit.[24] m.p. 124°C); spectral data as above; $[\alpha]_D$ −47.50 (c 5.0, MeOH) (lit.[25] $[\alpha]_D$ −47.5 (c 5.2, MeOH)).

N-Phthaloyl-D-ethionine: m.p. 77–79°C; ^{1}H NMR δ 1.22 (3H, t), 2.38–2.66 (6H, m), 5.15 (1H, m), 7.70/7.90 (each 2H, m), and 8.85 (1H, br.s) ppm; ^{13}C NMR δ ($CDCl_3$) 15.0, 26.1, 28.7, 28.8, 51.1, 124.0, 132.1, 134.7, 167.9, and 174.8 ppm; MS m/z (%) 293 (13), 205 (100), 187 (97), 132 (24); $[\alpha]_D$ +45.7 (c 1.5, MeOH); calcd for $C_{14}H_{15}NO_4S$ C 57.32, H 5.15, N 4.77%, found C 57.55, H 5.14, N 4.77%.

N-Phthaloyl-L-ethionine: m.p. 78–80°C; spectral data as above; $[\alpha]_D$ −47.2 (c 2.5, MeOH); calcd for $C_{14}H_{15}NO_4S$ C 57.32, H 5.15, N 4.77%, found C 57.33, H 5.15, N 4.69%.

4.3. Biotransformation procedures

4.3.1. B. bassiana

A growth medium (3 L) composed of glucose (10 g) and corn steep liquor (20 g) per L of distilled water, adjusted to pH 4.85 with 1 M NaOH was distributed in 15 1 L Erlenmeyer flasks which were stoppered with foam plugs and sterilised by autoclaving at 121°C for 20 min. The flasks were allowed to cool, then inoculated under sterile conditions with *B. bassiana* taken from a 3-day-old agar slope. The flasks were allowed to stand overnight at 27°C, then placed on a rotary shaker (1 in. orbit) at 180 rpm, 27°C. After 3 days, a solution of the appropriate substrate (1.0 g) in 95% ethanol (30 mL) was added, and growth allowed to continue for a further 3 days. The fungal mass was removed by filtration, and the filtrate adjusted to pH 3 and continuously extracted with dichloromethane for 4 days. The extract was treated with decolourising carbon in chloroform solution, and then evaporated to give a residue that was treated as described below.

4.3.2. B. caledonica

This was grown in 1 L Erlenmeyer flasks as described above in a medium of potato dextrose broth at 24–26°C. The substrate was added 6 days after inoculation of the culture, and biotransformation allowed to proceed for a further 3 days. Subsequent procedures were as described above. *B. caledonica* could also be grown and used for biotransformation in the glucose/corn steep liquor medium described for *B. bassiana*. No qualitative or quantitative differences in product produced using the two media were observed.

4.4. Characterisation of biotransformation products

Spectral data are listed below under the appropriate substrate headings for products from biotransformations with *B. bassiana*. The yield and d.e. of products from *B. caledonica* are listed in Tables 1 and 2.

4.4.1. N-*Phthaloyl*-D-*methionine*

(S_SR_C)-*N*-Phthaloylmethionine sulfoxide. The crude biotransformation extract from *B. bassiana* (1.8 g from 2 g substrate) was analysed by ^{1}H and ^{13}C NMR and found to consist largely of *N*-phthaloyl-D-methionine sulfoxide, d.e. 74%. The material resisted crystallisation, and was purified by chromatography using an ethyl acetate/methanol gradient elution to yield 65% of material with d.e. 95% (^{13}C NMR), m.p. 180–183°C; ^{1}H NMR δ 2.45–2.8 (2H, m), 2.70 (3H, s), 2.9–3.05 (2H, m), 5.0 (1H, m), and 7.75/7.88 (each 2H, m) ppm; ^{13}C NMR δ (CD_3OD) 23.03, 36.98, 51.03, 52.75, 123.30, 132.38, 134.51, 168.64, and 174.41 ppm; MS m/z (%) 295 (2), 279 (11), 232 (18), 205 (100), 187 (95), 132 (33); $[\alpha]_D$ +63.0 (c 0.96, EtOH), determined following conversion to methionine sulfoxide (see below) to be the (S_SR_C) diastereomer.

4.4.2. N-*Phthaloyl*-L-*methionine*

(S_SS_C)-*N*-Phthaloylmethionine sulfoxide. The crude biotransformation extract from *B. bassiana* (3.5 g from 4 g substrate) was analysed by ^{1}H and ^{13}C NMR and found to consist largely of *N*-phthaloyl-L-methionine sulfoxide, d.e. 60%. The material was crystallised by dissolving in hot methanol (30 mL), the volume of the solution reduced to ca. 15 mL by boiling, and the solution then allowed to cool to room temperature to yield 2.56 g (64%) of material with d.e. ≥95% (^{13}C NMR), m.p. 216–218°C (lit.[21] m.p. 215–217°C); ^{1}H NMR δ 2.45–2.8 (2H, m), 2.70 (3H, s), 2.9–3.05 (2H, m), 4.90 (1H, m), and 7.75/7.88 (each 2H, m) ppm; ^{13}C NMR δ (CD_3OD) 23.25, 37.24, 51.37, 53.0, 123.29, 132.37, 134.51, 168.63, and 174.41 ppm; MS m/z (%) 295 (2), 279 (14), 232 (18), 205 (100), 187 (93), 132 (34); $[\alpha]_D$ +12.5 (c 0.65, EtOH) (lit.[21] $[\alpha]_D$ +13 (c 0.35, EtOH)), confirmed by conversion to methionine sulfoxide (see below) as the (S_SS_C) diastereomer.

4.4.3. N-*Phthaloyl*-D-*ethionine*

(S_SR_C)-*N*-Phthaloylethionine sulfoxide. The crude biotransformation extract from *B. bassiana* (1.52 g from 1.5 g substrate) was analysed by ^{1}H and ^{13}C NMR and found to consist largely of *N*-phthaloyl-D-ethionine sulfoxide, d.e. 76%. The material resisted crystallisation, and was purified by chromatography using an ethyl acetate/methanol gradient elution to yield 46% of material with d.e. 95% (^{13}C NMR), m.p. 163–165°C; ^{1}H NMR δ (CD_3OD) 1.30 (3H, t), 2.58–3.0 (6H, m), and 4.78 (1H, m), and 7.80/7.90 (each 2H, m) ppm; ^{13}C NMR δ (CD_3OD) 6.10, 22.77, 45.22, 47.90, 51.09, 123.47, 132.12, 134.73, 168.19, and 170.54 ppm; MS (FAB-NBA) m/z (%) 310 (M+H, 80), 288 (72), 232 (52), 186 (94), 176 (100); $[\alpha]_D$ +41.4 (c 1.14, MeOH); calcd for $C_{14}H_{15}NO_5S$ C 54.36, H 4.89, N 4.53%, found C 54.12, H 5.16, N 4.46%.

4.4.4. N-*Phthaloyl*-L-*ethionine*

(S_SS_C)-*N*-Phthaloylethionine sulfoxide. The crude biotransformation extract from *B. bassiana* (2.02 g from 2 g substrate) was analysed by ^{1}H and ^{13}C NMR and found to consist largely of *N*-phthaloyl-L-ethionine sulfoxide, d.e. 86%. The material was crystallised by dissolving in hot methanol (30 mL), the volume of the solution reduced to ca. 15 mL by boiling, and the solution then allowed to cool to room temperature to yield 1.1 g (50%) of material with d.e. ≥95% (^{13}C NMR), m.p. 218–220°C; ^{1}H NMR δ 1.32 (3H, t), 2.5–3.05 (6H, m), 4.94 (1H, m), and 7.72/7.88 (each 2H, m) ppm; ^{13}C NMR δ 5.98, 22.94, 45.35, 47.80, 51.52, 123.47, 132.12, 134.73, 168.19, and 170.54 ppm; MS (FAB-NBA) m/z (%) 310 (M+H, 100), 232 (46), 186 (44); $[\alpha]_D$ −21.1 (c 0.7, EtOH); calcd for $C_{14}H_{15}NO_5S$ C 54.36, H 4.89, N 4.53%, found C 54.34, H 5.06, N 4.54%, identified by crystallography as the (S_SS_C) diastereomer. Material for X-ray crystallography was obtained by slow evaporation of a methanol/ethyl acetate solution to give monoclinic crystals, space group $P2_1$, *a*=5.4708(15) Å, *b*=9.783(4) Å, *c*=13.341(4) Å, α=90°, β=91.489(19)°, γ=90°, *V*=713.8(4) Å^3. Details are deposited in the Cambridge Crystallographic Data Centre and are available as supplementary information.

4.4.5. N-*Cbz*-D-*methionine*

(S_SR_C)-*N*-Cbz-D-methionine sulfoxide. The crude biotransformation product (1.04 g from 1 g substrate), d.e. 32% (^{13}C NMR), was subjected to chromatography using an ethyl acetate/methanol gradient elution to yield 46% of material with d.e. 30% (^{13}C NMR); oil; ^{1}H NMR δ 2.22–2.38 (2H, m), 2.57/2.63 (total 3H, ratio 35:65, s), 2.81–2.88 (2H, m), 4.47 (1H, m), 5.09 (2H, s), 6.06/6.16 (total 1H, d, exchanges D_2O), 7.34 (5H, m), and 9.30 (1H, br.s, exchanges D_2O) ppm; ^{13}C NMR δ ($CDCl_3$), 25.84/26.26 (35:65), 37.54/37.70 (35:65), 49.45, 53.04/53.29 (65:35), 67.53, 128.58, 128.63, 128.96, 136.54, 156.54, and

173.51/175.04 ppm; IR ν_{max} 3200–3500, 2900–3100, 1700, 1600, and 1050 cm^{-1}, determined following conversion to methionine sulfoxide (see below) to be the (S_SR_C) diastereomer.

4.4.6. N-*Cbz*-L-*methionine*

(S_SS_C)-*N*-Cbz-L-methionine sulfoxide. The crude biotransformation product (0.544 g from 0.5 g substrate), d.e. 40% (^{13}C NMR), was subjected to chromatography using an ethyl acetate/methanol gradient elution to yield 46% of material with d.e. 40% (^{13}C NMR); oil; ^1H NMR as in the preceding paragraph except for δ 2.57/2.63 (total 3H, ratio 70:30); ^{13}C NMR δ ($CDCl_3$), 25.84/26.26 (70:30), 37.54/37.70 (70:30), 49.45, 53.04/53.29 (30:70), 67.53, 128.58, 128.63, 128.96, 136.54, 156.54, and 173.51/175.04 ppm; IR as above, determined following conversion to methionine sulfoxide (see below) to be the (S_SS_C) diastereomer.

4.4.7. N-t-*Boc*-D-*methionine*

(S_SR_C)-*N*-*t*-Boc-D-methionine sulfoxide. The crude biotransformation product (0.72 g from 1 g substrate), d.e. 60% (^{13}C NMR), was subjected to chromatography using an ethyl acetate/methanol gradient elution to yield 41% of material with d.e. 60% (^{13}C NMR); oil; ^1H NMR δ 1.43 (9H, s), 2.25 (2H, m), 2.68/2.71 (total 3H, ratio 22:78, s), 2.8 (2H, m), 4.16 (1H, m), and 6.32 (1H, br.s, exchanges D_2O) ppm; ^{13}C NMR δ ($CDCl_3$) 25.01/26.28 (21:79), 28.55, 37.23/38.0 (20:80), 49.22/49.82 (19:81), 53.92, 79.09, 155.93, and 177.57 ppm, determined by conversion to methionine sulfoxide (see below) to be the (S_SR_C) diastereomer.

4.4.8. N-t-*Boc*-L-*methionine*

(S_SS_C)-*N*-*t*-Boc-L-methionine sulfoxide. The crude biotransformation product (0.6 g from 1 g substrate), d.e. 58% (^{13}C NMR) was subjected to chromatography using an ethyl acetate/methanol gradient elution to yield 45% of material with d.e. 60% (^{13}C NMR); oil; ^1H NMR δ 1.44 (9H, s), 2.24–2.36 (2H, m), 2.66/2.71 (total 3H, ratio 80:20, s), 2.88–2.94 (2H, m), 4.41 (1H, m), and 5.66/5.73 (total 1H, br.s, exchanges D_2O); ^{13}C NMR δ ($CDCl_3$) 25.01/26.28 (78:22), 28.55, 37.23/38.0 (79:21), 49.22/49.82 (81:19), 54.06, 79.08, 155.93, and 177.48 ppm, determined by conversion to methionine sulfoxide (see below) to be the (S_SS_C) diastereomer.

4.5. Hydrogen peroxide oxidations

4.5.1. N-*Phthaloyl*-D-*methionine*

Separate solutions of *N*-phthaloyl-D-methionine (2.79 g, 10 mmol) in methanol (50 mL) and hydrogen peroxide (1.246 g of 30 wt%, 11 mmol) in methanol (50 mL) were cooled to −20°C and then mixed. The mixture was allowed to stand at −20°C overnight, then evaporated to dryness to give 2.95 g (100%) of crude product, d.e. ca. 0%. This was dissolved in 10 mL of boiling methanol, and the solution allowed to cool to room temperature, yielding 1.3 g of product, d.e. 80–85%. One further recrystallisation from methanol (10 mL) then afforded 0.85–0.9 g of diastereomerically pure product, m.p. 217–218°C, $[\alpha]_D$ −12.5 (c 0.585, EtOH), spectral data identical with those reported above for the (S_SS_C) diastereomer, determined following conversion to methionine sulfoxide (see below) to be the (R_SR_C) diastereomer. Evaporation of the mother liquors gave a sample enriched in the (S_SR_C) diastereomer (d.e. 76%), whose purity remained unchanged on crystallisation from a wide variety of solvents, but which, on chromatography as described above, gave the (S_SR_C) diastereomer, m.p. 179–181°C, spectral data as described above, $[\alpha]_D$ +62.5 (c 1.1, EtOH), d.e. 95%.

4.5.2. N-*Phthaloyl*-L-*methionine*

Oxidation of *N*-phthaloyl-L-methionine by the procedure in the preceding paragraph, followed by purification of the product as described, gave 0.85–0.9 g of diastereomerically pure sulfoxide, m.p. and spectral data identical with those reported above and previously reported,[21] determined following conversion to methionine sulfoxide (see below) to be the (S_SS_C) diastereomer. Evaporation of the mother liquors gave a sample enriched in the (R_SS_C) diastereomer (d.e. 72%), whose purity remained unchanged on crystallisation from a wide variety of solvents, but which, on chromatography as described above, gave the (R_SS_C) diastereomer, m.p. 180–183°C, spectral data identical with those described above, $[\alpha]_D$ −61.8 (c 1.25, EtOH), d.e. 95%.

4.5.3. N-*Phthaloyl*-D-*ethionine*

Separate solutions of *N*-phthaloyl-D-ethionine (1.354 g) in methanol (25 mL) and hydrogen peroxide (0.74 g of 30 wt%) in methanol (25 mL) were cooled to −20°C and then mixed. The mixture was allowed to stand at −20°C overnight, then evaporated to dryness to give 1.45 g (100%) of crude product, d.e. ca. 0%. This was dissolved in 8 mL of boiling methanol, and the solution allowed to cool to room temperature, yielding 0.6 g of product, d.e. 80–85%. One further recrystallisation from methanol (3 mL) then afforded 0.4 g of a diastereomerically pure product, m.p. 217–219°C, spectral data as reported above for the (S_SS_C) isomer, $[\alpha]_D$ +21.0 (c 0.71, EtOH), which was determined to be the (R_SR_C) isomer by comparison with the authentic (S_SS_C) isomer. Evaporation of the combined mother liquors gave material enriched in the (S_SR_C) diastereomer, d.e. 70%, whose purity remained unchanged on crystallisation from a wide variety of solvents, but which, on chromatography as described above, gave the (S_SR_C) diastereomer, m.p. 165–168°C, spectral data identical with those described above, $[\alpha]_D$ +37.8 (c 1.33, EtOH), d.e. 95%.

4.5.4. N-*Phthaloyl*-L-*ethionine*

Oxidation as described in the preceding paragraph gave a comparable yield of the (S_SS_C) isomer, m.p. 218–220°C, $[\alpha]_D$ −21.4 (c 0.95, EtOH), identified by comparison with an authentic sample (above). Evaporation of the combined mother liquors gave material enriched in the (R_SS_C) diastereomer, d.e. 74%, whose purity remained unchanged on crystallisation from a wide variety of solvents, but which, on chromatography as described above, gave the (R_SS_C) diastereomer, m.p. 164–166°C, spectral data identical with those described above, $[\alpha]_D$ −39.8 (c 1.35, EtOH), d.e. 95%.

4.6. Removal of protecting groups

4.6.1. N-*Phthaloylmethionine sulfoxides*

A solution of (S_SS_C)-*N*-phthaloylmethionine sulfoxide (0.45 g) in 95% ethanol (10 mL) was treated with hydrazine hydrate (0.10 g, 95%), and the resulting mixture stirred and heated to reflux on a water bath for 2 h. The mixture was then evaporated to dryness, dissolved in distilled water (10 mL), and filtered. The filtrate was evaporated to give 0.27 g of material, which was redissolved in distilled water (4 mL) and the resulting solution clarified by filtration through a 0.45 μ Millipore™ filter. Acetone (25 mL) was added to the filtrate and the mixture was allowed to stand overnight at room temperature. The product was then removed by filtration, washed with acetone and vacuum dried to give 0.18 g (72%) of (S_SS_C)-methionine sulfoxide, m.p. >260°C (dec.) (lit.[22,26] m.p. 250–255°C (dec.)); ^{1}H NMR δ (D_2O) 2.13–2.32 (2H, m), 2.59 (3H, s), 2.78–3.08 (2H, m), and 4.10 (1H, m) ppm; ^{13}C NMR δ (D_2O) 23.49, 37.01, 48.26, 51.97, and 171.09 ppm; $[\alpha]_D$ +98.2 (c 0.5, H_2O) (lit.[22] +99.7 (c 0.5, H_2O)). Also obtained from the corresponding *N*-phthaloyl material by an identical procedure were (R_SR_C)-methionine sulfoxide, m.p.

>260°C (dec.) (lit.[22,26] m.p. 250–255°C (dec.) for the (S_SS_C) enantiomer); spectral data identical with those reported above; $[\alpha]_D$ −99.3 (c 0.7, H_2O), (R_SS_C)-methionine sulfoxide, m.p. 235°C (dec.) (lit.[22,26] m.p. 239°C (dec.); ^{1}H NMR δ (D_2O) 2.13–2.32 (2H, m), 2.59 (3H, s), 2.78–3.08 (2H, m), and 4.10 (1H, m) ppm; ^{13}C NMR δ (D_2O) 23.71, 37.01, 48.38, 52.09, and 171.09 ppm; $[\alpha]_D$ −78 (c 0.5, H_2O) (lit.[22] −77 (H_2O)), and (S_SR_C)-methionine sulfoxide, m.p. 235–240°C (dec.) (lit.[22,26] m.p. 239°C (dec.) for the (R_SS_C) enantiomer); spectral data identical with those reported above; $[\alpha]_D$ +78 (c 0.5, H_2O).

4.6.2. N-*Phthaloylethionine sulfoxides*

A solution of (S_SS_C)-*N*-phthaloylethionine sulfoxide (0.373 g) in 95% ethanol (10 mL) was treated with hydrazine hydrate (0.075 g, 95%), and subsequent procedures carried out as described above for the preparation of (S_SS_C)-methionine sulfoxide. After purification the product (0.152 g, 77%) had m.p. 247–250°C (dec.); ^{1}H NMR δ (D_2O) 1.14 (3H, t), 2.12 (2H, m), 2.65–2.9 (4H, m), and 3.70 (1H, m) ppm; ^{13}C NMR δ (D_2O) 6.31, 24.26, 44.76, 45.90, 53.66, and 173.52 ppm; $[\alpha]_D$ +50.0 (c 1.04, H_2O); calcd for $C_6H_{13}NO_3S$ C 40.21, H 7.31, N 7.81%, found C 40.28, H 7.68, N 7.91%. Also obtained from the corresponding *N*-phthaloyl material by an identical procedure were (R_SR_C)-ethionine sulfoxide, m.p. 250°C (dec.); spectral data identical with those reported above; $[\alpha]_D$ −49.3 (c 0.5, H_2O), (R_SS_C)-ethionine sulfoxide, m.p. 219–221°C (dec.); ^{1}H NMR δ (D_2O) 1.18 (3H, s), 2.08–2.25 (2H, m), 2.68–3.0 (4H, m), and 3.70 (1H, m) ppm; ^{13}C NMR δ (D_2O) 6.34, 24.48, 44.64, 45.96, 53.97, and 173.54 ppm; $[\alpha]_D$ −13.2 (c 0.7, H_2O); calcd for $C_6H_{13}NO_3S$ C 40.21, H 7.31, N 7.81%, found C 40.04, H 7.30, N 7.82%, and (S_SR_C)-methionine sulfoxide, m.p. 219–221°C (dec.); spectral data identical with those reported above; $[\alpha]_D$ +12.7 (c 0.8, H_2O).

4.6.3. N-*Cbz-methionine sulfoxides*

A solution of *N*-Cbz-L-methionine sulfoxide obtained by biotransformation of *N*-Cbz-L-methionine using *B. bassiana* (53 mg, d.e. 40%) in dry chloroform (3 mL) under argon at room temperature was treated with 65 μL of TMS iodide (1.2 equiv.), and the reaction warmed and monitored for the disappearance of starting material by TLC (typically 3–5 h at 35–45°C). On completion, the reaction was quenched by the addition of methanol (260 μL), stirred for a further 5 min, then evaporated to dryness. The residue was dissolved in a mixture of ether (3 mL) and 1 M H_2SO_4 (3 mL), and the aqueous layer washed with ether (3×3 mL). The aqueous layer was then adjusted to pH 5.75 with 1 M $Ba(OH)_2$, clarified by filtration (Celite and Millipore™ filtration) and evaporated to dryness to give a sample of methionine sulfoxide (22.5 mg, 80%), determined by ^{13}C NMR analysis (D_2O) by comparison and in admixture with authentic diastereomers to consist of a mixture of (S_SS_C) and (R_SS_C) diastereomers in the ratio 70:30. Deprotection of *N*-Cbz-D-methionine sulfoxide (from biotransformation of *N*-Cbz-D-methionine by *B. bassiana*) was performed in an analogous way.

4.6.4. N-t-*Boc-methionine sulfoxides*

A solution of *N-t*-Boc-L-methionine sulfoxide obtained by biotransformation using *B. bassiana* (116 mg, d.e. 58%) in water (5 mL) was treated with 2.6 mL (6 equiv.) of 1 M H_2SO_4. The resulting solution was stirred overnight at room temperature, then adjusted to pH 5.75 with 1 M $Ba(OH)_2$, clarified by filtration (Celite and Millipore™ filtration) and evaporated to dryness to give a sample of methionine sulfoxide (62 mg, 85%), determined by ^{13}C NMR analysis (D_2O) by comparison and in admixture with authentic diastereomers to consist of a mixture of (S_SS_C) and (R_SS_C) diastereomers in the ratio 80:20. Deprotection of *N-t*-Boc-D-methionine sulfoxide obtained by biotransformation of *N-t*-Boc-D-methionine using *B. bassiana* was performed in an exactly analogous experiment.

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