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Asymmetric reduction of α-thiocyanatoketones by *Saccharomyces cerevisiae* and *Mortierella isabellina*—a new route to optically active thiiranes

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Abstract—A simple method for the preparation of optically active 1-aryloxy- and 1-arylsulphanyl-3-thiocyanatopropan-2-oles from racemic 1-chloro-3-aryloxy- and 1-chloro-3-arylsulphanyl-2-propanols via 1-aryloxy- and 1-arylsulphanyl-3-thiocyanatopropan-2-ones has been developed. The enantiomerically enriched β-hydroxythiocyanates were obtained by a microbiological reduction of the thiocyanatoketones with *Saccharomyces cerevisiae* or *Mortierella isabellina* and subsequently converted into optically active thiiranes on treatment with a lithium hydroxide solution.

1. Introduction

The asymmetric reduction of the carbonyl group in β-substituted ketones is often catalyzed by baker's yeast. The broad scope of this biotransformation, its preparative simplicity, and usually good stereochemical results arising from precise facial recognition and high substrate tolerance of the yeast dehydrogenases, are the reasons for its common usage. Depending on the β-substituent, the ketone molecule can be reduced to a diol[1,2] or to an appropriately substituted secondary alcohol.[3] Some of these compounds found application as useful building blocks in the synthesis of various biologically active compounds. For example, the enantiomerically pure 1-substituted 3-aryloxypropan-2-ols are used as convenient intermediates in the preparation of drugs, such as β-receptor blockers,[4] or of chiral fragments in the optically active crown ethers.[1] 1-Azido-3-aryloxy-2-propanols are direct precursors of aziridines[4] and 1-amino-3-aryloxy-2-propanols,[4] the popular β-adrenolytic drugs commonly used in the treatment of hypertension and other circulatory disorders.[5] Baker's yeast dehydrogenases are also known to reduce some phthalimide[6]- or oxyphthalimide[7]- substituted ketones with the formation of the corresponding amino- or oxyaminoalcohol derivatives, which can be used as anesthetics, analgesics or chiral auxiliaries in asymmetric synthesis.[8,9]

From the point of view of synthetic chemistry β-hydroxythiocyanates, known as intermediates in thiirane synthesis, constitute as another interesting subclass of secondary alcohols. There are two known synthetic methods for preparing these alcohols: in the first, cyclic sulfates can be reacted with ammonium thiocyanate,[10] while the second method consists of the opening of oxiranes with thiocyanate anion in the presence of different catalysts, such as titanium isopropoxide,[11] triphenylphosphine thiocyanogen (TPPT),[12] titanium and zinc chlorides,[13] triphenylphosphine palladium,[14] crown ethers,[15] Selectfluor[16] or tetraarylporphyrins.[17] However, all of these methods to date have only been used for the preparation of racemic β-hydroxythiocyanates.

Recently,[18,19] we described a chemo-enzymatic procedure for the preparation of some enantiomerically enriched 1-substituted-3-thiocyanatopropan-2-ols and their acetates. These compounds appeared to be good substrates in the synthesis of optically active 2-(arylsulphanylmethyl)- and 2-(aryloxymethyl)thiiranes.[19]

Herein we report another method for the preparation of optically active β-hydroxythiocyanates, which consists of the synthesis of appropriate α-thiocyanatoketones and their stereoselective reduction by yeast (*Saccharomyces*

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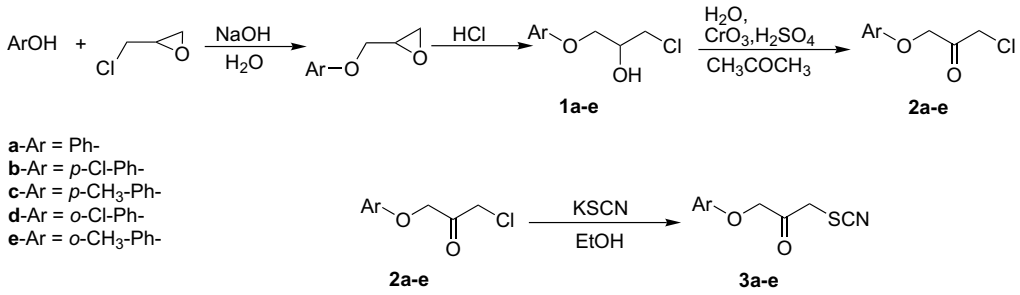


Scheme 1.

cerevisiae) and fungi (*Mortierella isabellina*) dehydrogenases.

2. Results and discussion

The starting glycidyl ethers were prepared in the Williamson reaction from the appropriate phenols and epichlorohydrin in an aqueous 10% sodium hydroxide solution. In our experiments the oxirane intermediates were not isolated; upon addition of an excess amount of hydrochloric acid the reaction mixture was stirred at room temperature for some time with TLC monitoring (Scheme 1) to give 1-chloro-3-aryloxypropan-2-ols **1a**–**e**. The yields of the reaction depended on the phenyl-ring substituent and usually ranged between 69% and 86%. Alcohols **1a**–**e** were oxidized to the corresponding 1-chloro-3-aryloxypropan-2-ones **2a**–**e** in the reaction with the Jones' reagent in an acetone solution at 5 °C. The yields of ketones **2a**–**e** are summarized in Table 1. Conversion of **2a**–**e** into the corresponding 1-aryloxy-3-thiocyanatopropan-2-ones **3a**–**e** was accomplished in good yields (Table 1) by refluxing with potassium thiocyanate in an ethanol solution.

Table 1. The yields of ketones **2a**–**e** and **3a**–**e**

Entry	Ar	Yield of **2a**–**e** (%)	Yield of **3a**–**e** (%)
a	Ph–	44.5	70
b	*p*-Cl–Ph–	86	72
c	*p*-CH_3–Ph–	43	81.5
d	*o*-Cl–Ph–	53	69
e	*o*-CH_3–Ph–	41	75

Our first experiments with the baker's yeast-catalyzed reduction of prochiral **3a**–**e** were carried out with **3a** added in a DMF solution to the growing yeast cultures (30 °C) suspended in an aqueous, glucose supplemented medium. Upon removal of the biomass and routine extraction with ethyl acetate only some 2-(phenoxymethyl)thiirane **5a** could be isolated by column chromatography from the complicated mixture of the reaction products; **4a** was not stable enough to stand the reaction conditions. In an attempt to increase the yield of **5a**, the crude mixture of products was treated with lithium hydroxide in a two-phase aqueous LiOH/THF system, which is known in converting β-hydroxythiocyanates in high efficiency into thiiranes[19] (Scheme 2). However, as can be seen in Table 2, the yields of thiiranes obtained in this way were similar to those noted in the non-catalyzed decomposition of β-thiocyanato alcohols.

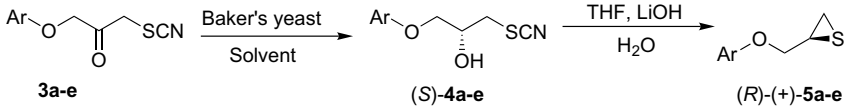
Table 2. The yields and ee (%) of (*R*)-thiirane **5a** obtained in reaction with[a] or without LiOH/THF system

Entry	Yeast g/1 mmol of substrate	Incubation time (h)	ee (%)	Yield of **5a**
1	3	21	82	13
2	3	48	88	38
3[a]	3	21	77	15
4[a]	2.5	24	88	30

[a] Reactions with LiOH/THF.

In order to avoid the laborious and time consuming extraction procedure and hoping to increase the yield of thiirane, we next investigated the microbiological reduction of **3a** in an organic solvent. Hexane, the solvent of choice in reactions with living cells, failed to dissolve **3a**. For the same reason, other highly non-polar solvents also proved unsuitable. Thus, *tert*-butyl methyl ether was chosen for the preliminary experiments. In a saturated solution (24 mL of the ether/1 mmol of **3a**), no reduction of the carbonyl group took place and the addition of a trace amount of water was necessary.

As shown in Table 3, the amount of yeast also affects the thiirane yield. A quantitative conversion of 1 mmol of **3a** into **5a** required at least 2 g of the yeast, but with 2.5 g, the reaction was twice as fast. Further increase in the amount of yeast did not accelerate the reaction, but decreased the product yield. Alcohol **4a** was probably more



Scheme 2.

Table 3. The yields of thiirane (*R*)-(+)-**5a** obtained in reaction catalyzed by different amounts of baker's yeast in *t*-BuOMe

Yeast g/1 mmol of substrate	Time (h)	ee (%)	*c* (%)	Thiirane **5a** yield (%)
1	168	93	Small conversion	Not isolated
2	45	93	100	79
2.5	24	93	100	77
3	20	93	100	72

strongly adsorbed by yeast than the starting ketone **3a**. Due to its instability, **4a** was not isolated but in situ converted into **5a** in the LiOH/THF system. Therefore, the progress of the **3a**→**4a** was monitored by observation of the disappearence of **3a** spot on TLC plates.

Three other organic solvents, diethyl ether, toluene and tetrahydrofuran, were investigated in the reaction besides *tert*-butyl methyl ether. The data in Table 4 reveal that only THF was completely unsuitable; it caused a total inhibition of the reaction. The other solvents gave fairly good results of a similar order.

Bioreductions of **3b–e** followed by transformations into thiiranes **5b–e** were performed under the optimal conditions developed with **3a** (1 mmol of the ketone, 24 mL of *tert*-butyl-methyl ether, 2.5 g of yeast, Scheme 2). The results of these experiments are summarized in Table 5. High enantiomeric excesses of thiiranes **5a–e** obtained in satisfactory yields makes the method of preparative interest.

Table 4. Solvent effect in reduction of **3a** catalyzed by baker's yeast

Solvent	Baker's yeast (g)	Time (h)	ee (%)	Thiirane **5a** yield (%)
H_2O	2.5	24	88	30
TBME	2.5	24	93	77
Et_2O	2.5	24	88.6	66
Et_2O	3	21		60
Toluene	2.5	24	92	67.5
Toluene	3	21		66
THF	2.5	—		

Table 5. The yields and enantiomeric excesses of thiiranes **5a–e**

Compound	Time (h)	ee (%)	Yield (%)
5a	24	93	77
5b	24	99	86.5
5c	24	92	79
5d	24	95	76
5e	24	96	91

1-Arylsulphanyl-3-thiocyanatopropan-2-ones **7a–c** are another group of α-thiocyanatoketones used by us in the synthesis of optically active thiiranes. They were prepared from **6a–c** by the chloride→thiocyanate exchange reaction (Scheme 3). Ketones **6a–c** were obtained by a known procedure[20] from the corresponding sodium benzenethiolates (prepared from benzenethiol and sodium hydroxide in an aqueous medium) and dichloroacetone in a methanol solution. The yields of 3-chloro- and 3-thiocyanatopropan-2-ones are summarized in Table 6.

The yeast-catalyzed reductions of 1-arylsulphanyl-3-thiocyanatopropan-2-ones **7a–c** (Scheme 4) were performed under the conditions which secured the best yields and selectivities with **3a–e.** The reactions were arrested after 24 h and the crude (*S*)-alcohols were converted into the corresponding (*R*)-thiiranes as described earlier. However, **7a–c** were reduced with substantially lower stereoselectivities (ee = 55–80%) than those noted with **3a–e**. Detailed results are shown in Table 7.

The bioreductions of **3a–c** were also carried out with living cells of *M. isabellina* DSM 1414 (**Mi**) in a 2% glucose solution at 30 °C (Scheme 5). Incubation of **3a–c** with a suspended culture of the fungi, and subsequent treatment with THF/LiOH (as previously described) gave the corresponding (*S*)-thiiranes **5a–c** as the major products in 20–57% isolated yields. Attempted separations of (*R*)-alcohols **4a–c** were unsuccessful because of their low stability. Detailed information with regards to the reactions is presented in Table 8. As can be seen, the enantiomeric excesses (ee%) and yields of **5a–c** prepared with *M. isabellina* were much lower than those obtained with baker's yeast.

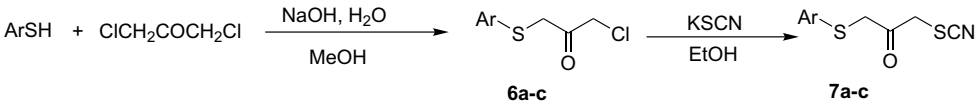
The absolute configurations of thiiranes **5a–e** and **9a–c** were determined by comparing their specific rotations with those described earlier.[19] They indicated an opposite

Table 6. The yields of ketones **6a–c** and **7a–c**

Compound	Ar	Yield of **6a–c** (%)	Yield of **7a–c** (%)
a	Ph–	89	38
b	*p*-Cl–Ph–	61	39
c	*p*-CH_3–Ph–	46	37

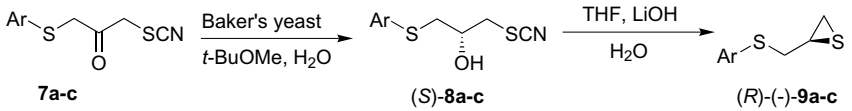
Table 7. Reduction of ketones **7a–c** catalyzed by baker's yeast in *t*-BuOMe

Substrate	Time (h)	ee (%)	Yield (%)
7a	24	55	73
7b	24	80	62.5
7c	24	56	80

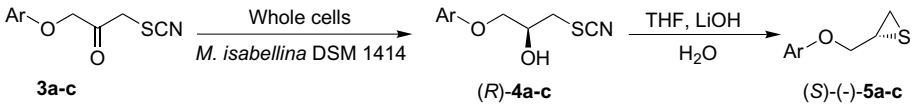


6a-c **7a-c**

Scheme 3.



Scheme 4.



Scheme 5.

Table 8. Yields and enantiomeric purities of (*S*)-thiiranes **5a**–**c** obtained by bioreduction of **3a**–**c** by *Mortierella isabellina* DSM 1414 (**Mi**)

Substrate	Ar	Incubation time (h)	*c* (%)	ee (%)	Yield (%)
3a	Ph–	24	100	27	57
3b	*p*-Cl–Ph–	168	100	13	20
3c	*p*-CH_3–Ph–	48	Low conversion	30	21
3c	*p*-CH_3–Ph–	168	100		38

stereopreference of baker's yeast and *M. isabellina* in the reduction of 1-aryloxy-3-thiocyanatopropan-2-ones. On the basis of the reaction mechanism proposed by Price and Kirk[21] for the transformation of β-thiocyanatoalcohols into thiiranes, it seems reasonable to assume that the configuration of the optically active alcohols **4a**–**e** and **8a**–**c** is opposite to that of the final thiiranes. In all reductions catalyzed by baker's yeast, an (*S*)-stereopreference of the resulting alcohol was observed. This is in agreement with Prelog's rule of stereocontrol observed in most baker's yeast reductions with *Re*-face[22] addition of a hydride anion. On the contrary, alcohols **4a**–**c** prepared by reductions with *M. isabellina* DSM 1414 (**Mi**) possess the opposite specific rotation, which suggests an *anti*-Prelog stereopreference.

3. Conclusions

The baker's yeast-induced asymmetric reduction of α-thiocyanatoketones was found to be a useful method for the preparation of the corresponding optically active β-hydroxythiocyanatopropane derivatives. In organic solvents, this transformation occurred with high stereoselectivity. The enantiomeric purities of the alcohols obtained were higher in the reductions of 1-aryloxy-3-thiocyanatopropan-2-ones than of 1-arylsulphanyl-3-thiocyanatopropan-2-ones. The transformations of the crude optically active β-hydroxythiocyanatopropane derivatives into the corresponding enantiomerically enriched thiiranes were carried out in a two-phase aqueous LiOH/THF system with Walden inversion and preservation of stereoselectivity. An opposite stereopreference of the baker's yeast and *M. isabellina* was observed in the reduction of 1-aryloxy-3-thiocyanatopropan-2-ones.

4. Experimental

4.1. General

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Mercury 400 MHz spectrometer in a $CDCl_3$ solution; IR spectra were taken on a Carl Zeiss Specord M80 instrument. Ees of the thiiranes **5a**–**e** were determined on a Thermo–Separation Products P-100 HPLC apparatus with Chiralcel OD-H column (in hexane:*iso*-propanol 95:5; 98:2, 99:1 0,8 mL/min) using the corresponding racemic compounds as references. Ees of the thiiranes **9a**–**c** were determined by chiral gas chromatography (Agilent Technologies 6890N, 30 m-long Cyclodex B (Agilent Technologies) capillary column). Optical rotations were measured in a $CDCl_3$ solution with a PolAAr 32 polarimeter. Elemental analyses were performed on a CHNSCl/O Perkin–Elmer type 2400 instrument. The reactions were monitored by TLC on silica gel 60 (230–400 mesh) and by GC with Hewlett-Packard Model 5890 II chromatograph with Helium as the carrier gas.

4.2. Microorganisms

The instant dry *S. cerevisiae* yeast was generously supplied by Mauri Foods Ltd. The DSM 1414 strain of *M. isabellina* was purchased from German Collection of Microorganisms and Cell Cultures (DSMZ).

4.3. Growth medium for *M. isabellina*

Malt extract (30 g) and soya peptone (3 g), were dissolved in 1 L of distilled water and the pH adjusted to 5.6. The liquid medium was sterilized at 120 °C for 15 min. The solid medium was prepared by adding 7.5 g of agar–agar to 500 ml of the liquid medium and sterilization was carried out under the same conditions. The microorganism was grown on a plate with agar medium for 3 days at 27 °C until it was well sporulated. Spores from the surface culture were used to inoculate flasks containing sterile liquid medium, next incubated at 30 °C for 3 days on a rotary shaker at 140 rpm. Then the fungal mycelium was collected by filtration from the growing culture.

4.4. General procedure for the synthesis of 1-chloro-3-aryloxypropan-2-one 2a–e

To a stirred solution of 1-chloro-3-aryloxypropan-2-ol **1a–e** (0.053 mol) in CH_3COCH_3 (116 mL) at 5 °C, a solution of H_2CrO_3 (prepared from H_2SO_4 (6.16 mL), CrO_3 (7.71 g) and H_2O (14 mL) was added dropwise over 2 h. Next the mixture was stirred for 1hr at room temperature and stopped by the dropwise addition of 2-propanol (16 mL). The inorganic precipitate was then filtered off and washed with CH_3COCH_3 (2×60 mL). The solvent was evaporated and to the resulting crude product H_2O (70 mL) was added. The mixture was extracted with $CHCl_3$ (3×60 mL) and the organic layer washed with water (3×50 mL), dried over anhydrous Na_2SO_4 and evaporated. Product was purified by chromatography on silica-gel column with *n*-hexane–dichloromethane (1:4 v/v) as the eluent. ^1H, ^{13}C spectra and IR data of the prepared ketones are reported below:

4.4.1. 1-Chloro-3-phenoxypropan-2-one 2a. Oil. ^1H NMR ($CDCl_3$): δ ppm: 4.43 (s, 2H (CH_2Cl)); 4.76 (s, 2H (CH_2OPh)); 6.89–7.34 (m, 5H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 46.81; 71.53; 114.41; 122.18; 129.81; 157.23; 199.06. IR (film, cm^{-1}) 1735 (ν_{CO}). ^1H NMR spectrum is identical with that given in the lit.[23]

4.4.2. 1-Chloro-3-(4-chlorophenoxy)propan-2-one 2b. Colourless crystals: mp 70–72 °C (lit.[24] 63–66 °C). ^1H NMR ($CDCl_3$): δ ppm: 4.38 (s, 2H (CH_2Cl)); 4.76 (s, 2H (CH_2OPh)); 6.82–7.29 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 46.45; 71.66; 115.83; 127.22; 129.70; 155.90; 198.39. IR (Nujol, cm^{-1}) 1740 (ν_{CO}).

4.4.3. 1-Chloro-3-(*p*-tolyloxy)propan-2-one 2c. Colourless crystals; mp 50–52 °C (lit.[25] 59 °C). ^1H NMR ($CDCl_3$): δ ppm: 2.30 (s, 3H (CH_3)); 4.42 (s, 2H (CH_2Cl)); 4.72 (s, 2H (CH_2OPh)); 6.78–7.12 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 20.42; 46.85; 71.75; 114.23; 130.20; 131.52; 155.16; 199.29. IR (Nujol, cm^{-1}) 1745 (ν_{CO}).

4.4.4. 1-Chloro-3-(2-chlorophenoxy)propan-2-one 2d. Colourless crystals; mp 51.5–53 °C ^1H NMR ($CDCl_3$): δ ppm: 4.57 (s, 2H (CH_2Cl)); 4.77 (s, 2H (CH_2OPh)); 6.82–7.42 (m, 4H (Ph)). ^{13}C NMR (CDCl3); δ ppm; 47.16; 72.44; 113.50; 123.02; 123.15; 127.93; 130.69; 152.84; 198.37. IR (Nujol, cm^{-1}) 1741 (ν_{CO}).

4.4.5. 1-Chloro-3-(*o*-tolyloxy)propan-2-one 2e. Colourless crystals; mp 46.5–49 °C (lit.[26] 50 °C). ^1H NMR ($CDCl_3$): δ ppm: 2.27 (s, 3H (CH_3)); 4.42 (s, 2H (CH_2Cl)); 4.77 (s, 2H (CH_2OPh)); 6.60–7.05 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 15.42; 47.18; 75.31; 113.51; 123.04; 123.17; 127.95; 130.72; 152.86; 198.40. IR (Nujol, cm^{-1}) 1740 (ν_{CO}).

4.5. General procedure of the synthesis of 1-chloro-3-arylsulphnylpropan-2-ones 6a–c

A suspension of sodium benzenethiolate prepared from benzenethiol (5.5 g, 0.05 mol) and sodium hydroxide (2 g, 0.05 mol) in water (50 mL) was added to a stirred solution of 1,3-dichloropropan-2-one (6.35 g, 0.05 mol) in methanol–water (1:3) mixture (50 mL) at 0 °C. The mixture was stirred at 0 °C for 7 h and then at room temperature for the next 10 h. The precipitated product was extracted with chloroform and the extract was washed with water, dried over Na_2SO_4 and evaporated to dryness. The solid residue of **6a** was recrystallized from Et_2O. The remaining crude products **6b–c** were purified by chromatography on a silica-gel column with *n*-hexane–ethyl acetate (10:1 v/v) as the eluent. ^1H, ^{13}C NMR spectra and IR data of the prepared ketones are reported below:

4.5.1. 1-Chloro-3-phenylsulphanylpropan-2-one 6a. Colourless crystals; mp 43–44 °C (lit.[27] 46–47 °C). ^1H NMR ($CDCl_3$): δ ppm: 3.83 (s, 2H (CH_2SPh)); 4.27 (s, 2H (CH_2Cl)); 7.22–7.37 (m, 5H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 41.22; 46.46; 127.48; 129.27; 129.28; 130.13; 197.13. IR (Nujol, cm^{-1}) 1728 (ν_{CO}).

4.5.2. 1-Chloro-3-(4-chlorophenylsulphanyl)propan-2-one 6b. Colourless crystals; mp 39–41 °C. ^1H NMR ($CDCl_3$): δ ppm: 3.82 (s, 2H (CH_2SPh)); 4.25 (s, 2H (CH_2Cl)); 7.26–7.28 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 41.27; 46.30; 129.47; 131.67; 132.01; 133.77; 196.84. IR (Nujol, cm^{-1}) 1720 (ν_{CO}). Anal. Calcd for $C_9H_8Cl_2OS$: C, 45.97; H, 3.43; Cl, 30.16; S, 13.64. Found: C, 45.89; H, 3.38; Cl; 30.01; S, 13.79.

4.5.3. 1-Chloro-3-(tolylsulphanyl)propan-2-one 6c. Oil. ^1H NMR ($CDCl_3$): δ ppm: 2.31 (s, 3H, (CH_3)); 3.77 (s, 2H (CH_2SPh)); 4.27 (s, 2H (CH_2Cl)); 7.10–7.28 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm; 21.01; 41.85; 46.49; 129.64; 130.07; 131.03; 137.94; 197.12. IR (Nujol, cm^{-1}) 1730 (ν_{CO}).

4.6. General procedure of thiocyanatoketones 3a–e and 7a–c preparation

A mixture of the appropriate 1-chloro-3-aryloxypropan-2-one **2a–e** (or 1-chloro-3-arylsulphanylpropan-2-one **6a–c**) (10 mmol), potassium thiocyanate (10 mmol), and ethanol (41 mL) was stirred under reflux conditions. The progress of the reaction was monitored by TLC, using *n*-hexane–ethyl acetate (3:1 v/v) as the eluent. After completion of the reaction, an equal volume of water was added and the resulting precipitate was filtered off, or exacted with CH_2Cl_2. Products **3a** and **3b** were purified by crystallization from EtOH, **3c–e** from Et_2O whilst ketones **7a–c** were purified by chromatography on silica-gel column with *n*-hexane–ethyl acetate (10:1 v/v) as the eluent. ^1H, ^{13}C NMR spectra, IR data, and elemental analyses of the ketones prepared are reported below:

4.6.1. 1-Phenoxy-3-thiocyanatopropan-2-one 3a. Colourless crystals; mp 65–66 °C. ^1H NMR ($CDCl_3$): δ ppm: 4.28 (s, 2H (CH_2SCN)); 4.69 (s, 2H (CH_2OPh)); 6.89–7.36 (m, 5H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 41.59; 71.93; 110.98; 114.34; 122.52; 129.97; 156.87; 199.27. IR (Nujol, cm^{-1}) 2150 (ν_{CN}); 1740 (ν_{CO}). Anal. Calcd for $C_{10}H_9NO_2S$: C, 57.95; H, 4.38; N, 6.76; S, 15.47. Found: C, 57.84; H, 4.40; N, 6.71; S, 15.44.

4.6.2. 1-(4-Chlorophenoxy)-3-thiocyanatopropan-2-one 3b. Colourless crystals; mp 89–90 °C. ^{1}H NMR ($CDCl_3$): δ ppm: 4.25 (s, 2H (CH_2SCN)); 4.66 (s, 2H (CH_2OPh)); 6.82–7.30 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 41.32; 72.09; 110.90; 115.70; 127.48; 129.81; 155.44; 198.48. IR (Nujol, cm^{-1}) 2155 (ν_{CN}); 1745 (ν_{CO}). Anal. Calcd for $C_{10}H_8NClO_2S$: C, 49.69; H, 3.34; N, 5.80; Cl, 14.67; S, 13.27. Found: C, 49.80; H, 3.70; N, 5.83; Cl, 14.37; S, 12.99.

4.6.3. 1-Thiocyanato-3-(*p*-tolyloxy)propan-2-one 3c. Colourless crystals; mp 70–71 °C. ^{1}H NMR ($CDCl_3$): δ ppm: 2.30 (s, 3H (CH_3)); 4.26 (s, 2H (CH_2SCN)); 4.65 (s, 2H (CH_2OPh)); 6.78–7.14 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 20.43; 41.58; 72.13; 111.01; 114.17; 130.35; 131.91; 154.83; 199.53. IR (Nujol, cm^{-1}) 2160 (ν_{CN}); 1745 (ν_{CO}). Anal. Calcd for $C_{11}H_{11}NO_2S$: C, 59.71; H, 5.01; N, 6.33; S, 14.49. Found: C, 59.53; H, 5.02; N, 6.30; S, 14.70.

4.6.4. 1-(2-Chlorophenoxy)-3-thiocyanatopropan-2-one 3d. Colourless crystals; mp 84.5–85.5 °C. ^{1}H NMR ($CDCl_3$): δ ppm: 4.36 (s, 2H (CH_2SCN)); 4.71 (s, 2H (CH_2OPh)); 6.84–7.43 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 41.34; 72.62; 110.89; 113.56; 123.09; 123.32; 128.06; 130.75; 152.52; 198.38. IR (Nujol, cm^{-1}) 2158 (ν_{CN}); 1740 (ν_{CO}). Anal. Calcd for $C_{10}H_8NClO_2S$: C, 49.69; H, 3.34; N, 5.80; Cl, 14.67; S, 13.27. Found: C, 49.53; H, 3.59; N, 5.73; Cl, 14.43; S, 13.24.

4.6.5. 1-Thiocyanato-3-(*o*-tolyloxy)propan-2-one 3e. Colourless crystals; mp 71.5–72.5 °C. ^{1}H NMR ($CDCl_3$): δ ppm: 2.30 (s, 3H (CH_3)); 4.29 (s, 2H (CH_2SCN)); 4.68 (s, 2H (CH_2OPh)); 6.68–7.21 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 16.29; 41.58; 71.96; 110.56; 110.97; 122.15; 126.64; 127.13; 131.36; 155.02; 199.28. IR (Nujol, cm^{-1}) 2162 (ν_{CN}); 1745 (ν_{CO}). Anal. Calcd for $C_{11}H_{11}NO_2S$: C, 59.71; H, 5.01; N, 6.33; S, 14.49. Found: C, 59.80; H, 5.03; N, 6.31; S, 14.52.

4.6.6. 1-(Phenylsulphanyl)-3-thiocyanatopropan-2-one 7a. Colourless crystals; mp 66.5–67.5 °C. ^{1}H NMR ($CDCl_3$): δ ppm: 3.78 (s, 2H (CH_2SPh)); 4.17 (s, 2H (CH_2SCN)); 7.26–7.36 (m, 5H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 41.62; 42.62; 111.01; 127.88; 129.52; 130.25; 132.85; 196.31. IR (Nujol, cm^{-1}) 2150 (ν_{CN}); 1725 (ν_{CO}). Anal. Calcd for $C_{10}H_9NOS_2$: C, 53.79; H, 4.06; N, 6.27; S, 28.72. Found: C, 54.01; H, 4.36; N, 6.14; S, 28.67.

4.6.7. 1-(4-Chlorophenylsulphanyl)-3-thiocyanatopropan-2-one 7b. Colourless crystals; mp 59–61 °C. ^{1}H NMR ($CDCl_3$): δ ppm: 3.76 (s, 2H (CH_2SPh)); 4.17 (s, 2H (CH_2SCN)); 7.27–7.32 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 41.45; 42.73; 110.85; 129.72; 131.21; 131.83; 134.28; 195.76. IR (Nujol, cm^{-1}) 2130 (ν_{CN}); 1715 (ν_{CO}). Anal. Calcd for $C_{10}H_8NClOS_2$: C, 46.60; H, 3.13; N, 5.43; Cl, 13.75; S, 24.88. Found: C, 46.65; H, 3.35; N, 5.40; S, 24.83.

4.6.8. 1-Thiocyanato-3-(*p*-tolylsulphanyl)propan-2-one 7c. Colourless crystals; mp 40.5–41.5 °C. ^{1}H NMR ($CDCl_3$): δ ppm: 2.33 (s, 3H (CH_3)); 3.72 (s, 2H (CH_2SPh)); 4.17 (s, 2H (CH_2SCN)); 7.13–7.27 (m, 4H (Ph)). ^{13}C NMR ($CDCl_3$); δ ppm; 21.07; 41.73; 43.28; 111.07; 129.00; 130.33; 131.15; 138.45; 196.31. IR (Nujol, cm^{-1}) 2140 (ν_{CN}); 1705 (ν_{CO}). Anal. Calcd for $C_{11}H_{11}NOS_2$: C, 55.67; H, 4.67; N, 5.90; S, 27.02. Found: C, 55.71; H, 4.71; N, 5.93; S, 27.11.

4.7. General procedure for the synthesis of optically active thiiranes

In a typical experiment, the appropriate ketone **3a**–**e** or **7a**–**c** (1 mmol) was dissolved in 24 mL of *tert*-butyl methyl ether and 2.5 g of Mauripan baker's yeast and 1.5 mL of H_2O was added. The mixture was shaken at room temperature and the conversion monitored by TLC with *n*-hexane:ethyl acetate (3:1 v/v) as the eluent. After 24 h, the reaction was stopped by filtering off the baker's yeast and the solvent was evaporated. The resulting crude optically active β-hydroxythiocyanate was used as the substrate in the thiirane synthesis. Each of the optically active β-hydroxythiocyanates **4a**–**e** was dissolved in 14 mL of THF and a solution of $LiOH{\cdot}H_2O$ (2 mmol) in 2.5 mL of H_2O was added. For compounds **7a**–**c** β-hydroxythiocyanates **8a**–**c** were dissolved in 28 mL of THF and a solution of $LiOH{\cdot}H_2O$ (4 mmol) in 5 mL H_2O was added. The mixture was stirred at room temperature and the progress of the reaction monitored by TLC using *n*-hexane:ethyl acetate (3:1 v/v) as the eluent. After completion of the reaction (about 2 h), the organic layer was separated and the solvent evaporated. The crude product was purified by chromatography on a short silica-gel column with *n*-hexane:ethyl acetate (7:1 v/v for elution of thiiranes **5a**–**e** and 15:1 for thiiranes **9a**–**c**). ^{1}H, ^{13}C NMR spectra, optical rotations and elemental analyses of the prepared thiiranes **5a**–**e** and **9a**–**c** are reported below:

4.7.1. (*R*)-(+)-2-(Phenoxymethyl)thiirane 5a. Oil, yield 72%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.32–2.62 (m; 2H ($CHCH_2S$)); 3.28 (m; 1H (CH)); 3.90 (dd; 1H (OCH_aH_bCH); $J_{HaCH} = 7.2$ Hz; $J_{HaHb} = 10$ Hz); 4.22 (dd; 1H (OCH_aH_bCH); $J_{HbCH} = 5.2$ Hz); 6.90–7.31 (m; 5H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 23.98; 31.37; 72.51; 114.63; 121.21; 129.53; 158.35. $[\alpha]_D^{20.5} = +16.5$ (*c* 2, $CHCl_3$) ee = 93%. ^{1}H NMR and ^{13}C NMR spectra are identical with those given in the lit.[18]

4.7.2. (*R*)-(+)-2-((4-Chlorophenoxy)methyl)thiirane 5b. Oil, yield 76%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.31–2.61 (m; 2H ($CHCH_2S$)); 3.25 (m; 1H (OCH_2CHCH_2)); 3.87–4.16 (m; 2H (OCH_2CH)); 6.82–7.24 (m; 4H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 24.01; 31.40; 72.62; 114.92; 126.59; 129.50; 156.45. $[\alpha]_D^{24} = +11.4$ (*c* 2.89 $CHCl_3$) ee = 95%. ^{1}H NMR and ^{13}C NMR spectra are identical with those given in the lit.[18]

4.7.3. (*R*)-(+)-2-(*p*-Tolyloxymethyl)thiirane 5c. Oil, yield 91%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.29 (s; 3H (CH_3)); 2.32–2.61 (dd; 2H ($CHCH_2S$)); 3.26 (m; 1H ($CH_aH_bCHCH_2$)); 3.86 (dd; 1H (OCH_aH_bCH); $J_{HaHb} = 10$ Hz); 4.19 (dd; 1H (OCH_aH_bCH)); 6.80–7.10 (m; 4H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 20.46; 24.02; 31.45; 72.76; 114.57; 129.95; 130.51; 156.27. $[\alpha]_D^{25} = +11.4$ (*c* 3.16, $CHCl_3$)

ee = 96%. ^{1}H NMR and ^{13}C NMR spectra are identical with those given in the lit.[18]

4.7.4. (*R*)-(+)-2-((2-Chlorophenoxy)methyl)thiirane 5d. Oil, yield 86.5%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.35–2.63 (m; 2H ($CH\mathit{CH_2}S$)); 3.32 (m; 1H ($OCH_2\mathit{CH}CH_2$)); 3.93 (dd; 1H ($OC\mathit{H_a}H_bCH$); $J_{HaCH} = 7.2$ Hz; $J_{HaHb} = 10.4$ Hz); 4.31 (dd; 1H ($OCH_a\mathit{H_b}CH$); $J_{HbCH} = 5.2$ Hz); 6.91–7.39 (m; 4H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 24.02; 31.02; 73.83; 114.43; 122.14; 123.34; 127.70; 130.41; 153.94. $[\alpha]_D^{25} = +3$ (*c* 2, $CHCl_3$) ee = 99%. Anal. Calcd for C_9H_9SOCl: C, 53.86; H, 4.52; S, 15.98; Cl, 17.67. Found: C, 53.76; H, 4.40; S, 16.09; Cl, 17.72.

4.7.5. (*R*)-(+)-2-(*o*-Tolyloxymethyl)thiirane 5e. Oil, yield 79%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.28 (s; 3H (CH_3)); 2.34–2.63 (m; 2H ($CH\mathit{CH_2}S$)); 3.31 (m; 1H ($OCH_2\mathit{CH}CH_2$)); 3.95 (dd; 1H ($OC\mathit{H_a}H_bCH$); $J_{HaCH} = 6.8$ Hz; $J_{HaHb} = 10.4$ Hz); 4.22 (dd; 1H ($OCH_a\mathit{H_b}CH$); $J_{HbCH} = 5.6$ Hz); 6.81–7.18 (m; 4H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 16.18; 23.82; 31.59; 72.63; 111.52; 120.90; 126.74; 127.04; 130.78; 156.52. $[\alpha]_D^{31.5} = +13.6$ (*c* 2.5, $CHCl_3$) ee = 92%. Anal. Calcd for $C_{10}H_{12}OS$: C, 66.63; H, 6.71; S, 17.79. Found: C, 66.54; H, 6.65; S, 17.34.

4.7.6. (*R*)-(−)-2-(Phenylsulphanylmethyl)thiirane 9a. Oil, yield 73%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.10 (dd; 1H ($CHC\mathit{H_a}H_bS$); $J_{HaCH} = 1.6$ Hz; $J_{HaHb} = 5.2$ Hz); 2.48 (dd; 1H ($CHCH_a\mathit{H_b}S$); $J_{HbCH} = 2.4$ Hz); 2.79 (dd; 1H ($PhSC\mathit{H_c}H_dCH$); $J_{HcCH} = 8.8$ Hz; $J_{HcHd} = 13.6$ Hz); 3.11 (m; 1H (CH)); 3.45 (dd; 1H ($PhSCH_c\mathit{H_d}CH$); $J_{HdCH} = 5.2$ Hz); 7.23–7.46 (m; 5H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 26.01; 33.55; 41.16; 127.06; 129.07; 131.06; 135.04. $[\alpha]_D^{29} = -32.8$ (*c* 5, $CHCl_3$) ee = 55%. ^{1}H NMR and ^{13}C NMR spectra are identical with those given in the lit.[18]

4.7.7. (*R*)-(−)-2-((4-Chlorophenylsulphanyl)methyl)thiirane 9b. Oil, yield 62.5%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.10 (dd; 1H ($CHC\mathit{H_a}H_bS$); $J_{HaCH} = 1.6$ Hz; $J_{HaHb} = 5.6$ Hz); 2.48 (dd; 1H ($CHCH_a\mathit{H_b}S$); $J_{HbCH} = 2.4$ Hz); 2.81 (dd; 1H ($PhSC\mathit{H_c}H_dCH$); $J_{HcCH} = 8.4$ Hz; $J_{HcHd} = 13.6$ Hz); 3.08 (m; 1H (CH)); 3.39 (dd; 1H ($PhSCH_c\mathit{H_d}CH$); $J_{HdCH} = 4.8$ Hz); 7.26–7.38 (m; 4H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 25.90; 33.33; 41.34; 129.27; 132.38; 133.21; 133.57. $[\alpha]_D^{25} = -44.95$ (*c* 5, $CHCl_3$) ee = 80%. ^{1}H NMR and ^{13}C NMR spectra are identical with those given in the lit.[19]

4.7.8. (*R*)-(−)-2-(*p*-Tolylsulphanylmethyl)thiirane 9c. Oil, yield 80%. ^{1}H NMR ($CDCl_3$) δ ppm: 2.06 (dd; 1H ($CHC\mathit{H_a}H_bS$); $J_{HaCH} = 1.6$ Hz; $J_{HaHb} = 5{,}6$ Hz); 2.34 (s; 3H (CH_3)); 2.46 (dd; 1H ($CHCH_a\mathit{H_b}S$); $J_{HbCH} = 2.4$ Hz); 2.73 (dd; 1H ($PhSC\mathit{H_c}H_dCH$); $J_{HcCH} = 8.8$ Hz; $J_{HcHd} = 13.6$ Hz); 3.09 (m; 1H (CH)); 3.40 (dd; 1H ($PhSCH_c\mathit{H_d}CH$); $J_{HdCH} = 4.4$ Hz); 7.11–7.37 (m; 4H (Ph)). ^{13}C NMR ($CDCl_3$) δ ppm: 21.07; 26.07; 33.74; 41.82; 129.85; 131.18; 131.88; 137.39. $[\alpha]_D^{25} = -37.9$ (*c* 5.38, $CHCl_3$) ee = 56%. ^{1}H NMR and ^{13}C NMR spectra are identical with those given in the lit.[19]

4.8. General procedure of reduction of ketones 3a–c by *M. isabellina*. Preparation of (*S*)-(2-aryloxymethyl)thiiranes

Wet mycelium (94 g) was suspended in 240 mL of 2% glucose solution and 1.93 mmol of the appropriate substrate **3a**–**c** dissolved in minimum volume of DMF was added. The mixture was shaken at 30 °C and 140 rpm and the conversion was monitored by gas chromatography. After the appropriate time, ethyl acetate (100 mL) was added to the flask, and stirring was continued for 30 min. The biomass was filtered off and the supernatant was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure. To the crude mixture, 28 mL of THF and a solution of LiOH (4 mmol) in 5 mL of H_2O were added. The mixture was stirred at room temperature and the progress of the reaction was monitored by TLC using *n*-hexane:ethyl acetate (3:1 v/v) as the eluent. After completion of the reaction (about 2 h) the organic layer was separated and the solvent was evaporated. The crude mixture was purified by chromatography on a short silica-gel column with *n*-hexane:ethyl acetate (7:1 v/v) as the eluent. The yields and enantiomeric excesses of the prepared (*S*)-(2-aryloxymethyl)thiiranes are summarized in Table 8.

4.8.1. (*S*)-(−)-2-(Phenoxymethyl)thiirane 5a. $[\alpha]_D^{20.5} = -4.86$ (*c* 1.64) ee = 27%.

4.8.2. (*S*)-2-((4-Chlorophenoxy)methyl)thiirane 5b. $[\alpha]_D^{24} = 0$ (*c* 0.92) ee = 13%.

4.8.3. (*S*)-(−)-2-(*p*-Tolyloxymethyl)thiirane 5c. $[\alpha]_D^{25} = -3.4$ (*c* 2.36) ee = 30%.

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