Development of Biotransamination Reactions towards the 3,4-Dihydro-2*H*-1,5-benzoxathiepin-3-amine Enantiomers

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Abstract: The stereoselective synthesis of chiral amines is an appealing task nowadays. In this context, biocatalysis plays a crucial role due to the straightforward conversion of prochiral and racemic ketones into enantiopure amines by means of a series of enzyme classes such as amine dehydrogenases, imine reductases, reductive aminases and amine transaminases. In particular, the stereoselective synthesis of 1,5-benzoxathiepin-3-amines have attracted particular attention since they possess remarkable biological profiles; however, their access through biocatalytic methods is unexplored. Amine transaminases are applied herein in the biotransamination of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one, finding suitable enzymes for accessing both target amine enantiomers in high conversion and enantiomeric excess values. Biotransamination experiments have been analysed, trying to optimise the reaction conditions in terms of enzyme loading, temperature and reaction times.

1. Introduction

We have reported several (*RS*)-benzo-fused seven-membered rings with oxygen and sulfur atoms in 1,5 relative positions with interesting anti-proliferative activities against the MCF-7 cancer cell line. The most active compounds are **1** and **2** [1] (Figure 1). Other compounds, such as **3** [2] and **4** [3], exhibited more potent anti-ischemic effects than reference compounds, whilst **5** can be the prototype for the design of more potent anti-proliferative agents [4] (Figure 1). The (3*R*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-amine core appears in red in compounds **3–5** (Figure 1). Such a (3*R*)-amino-1,5-benzoxathiepin scaffold has been obtained from L-cystine ((2*R*)-2-amino-3-[[(2*R*)-2-amino-2-carboxyethyl]disulfanyl]propanoic acid) [4,5]. The incorporation of α-amino acids into heterocyclic structures is an effective strategy for generating numerous peptidomimetics and combinatorial library scaffolds.
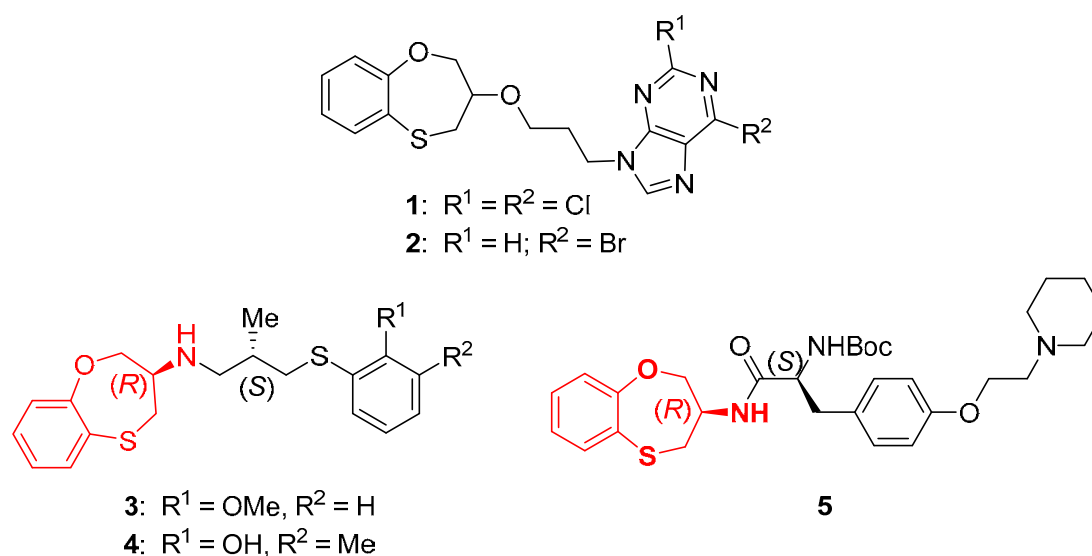
Figure 1. Benzo-fused seven-membered rings with oxygen and sulphur atoms in 1,5 relative positions (**1–5**) with interesting biological properties [1–5]. The (3*R*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-amine core appears in red in compounds **3–5**.

Due to the fact that the primary amine is a key functional group in all areas of chemistry, methods to generate molecules containing primary amine groups are of intense interest and impact on many research fields. The use of enzymes in organic synthesis has gained maturity in the last few decades, since the advances in enzyme immobilisation, modification and rational design allow for the application of improved biocatalysts for the development of a wide variety of stereoselective transformations [6–10]. In this context, the synthesis of chiral amines is particularly challenging, with the conversion of prochiral ketones into optically active amines receiving great attention in recent years [11–14] by using mainly imine reductases [15–17] and amine transaminases (ATAs) [18–23]. Taking into account the potential of ATAs in the single biotransamination of cyclic ketones [24–32], even as part of multienzymatic sequences [33–38], but especially since they have served as valuable biocatalysts in the production of pharmacologically active products [39–43], we have focused herein our efforts in the pursuit of an efficient biotransamination protocol for 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one (**6**).

2. Results and Discussion

The synthesis of the benzo-fused seven-membered ketone **6** is depicted in Scheme 1. 2-Mercaptophenol was alkylated with two equivalents of ethyl bromoacetate in refluxing acetone in the presence of dry potassium carbonate to give diester **7** (83%). Examination of the Dieckmann reaction of **3** showed that the reaction occurred smoothly when sodium ethoxide/ethanol was used as a base in dry tetrahydrofuran (THF) to give ethyl 3-oxo-3,4-dihydro-2*H*-1,5-benzothiepin-4-carboxylate **8** as the sole cyclised product in 90% yield. Decarboxylation of the β-ketoester **4** in boiling acetic acid containing aqueous sulfuric acid gave the 3,4-dihydro-2*H*-1,5-benzothiepin-3-one (**6**, 60%). Regioselectivity of the Dieckmann cyclisation was deduced based on the ^{1}H-NMR (CDCl$_3$) spectral data of the resulting product **8**, which exhibited two doublets (integrating each one for 1H) at δ 4.88 and 4.59 ppm (*J* = 17.5 Hz) assignable to the geminal methylene protons adjacent to the oxygen atom in the seven-membered ring. Compounds **7** and **8** have not been described previously, whilst ketone **6** was reported formerly by Sugihara et al. [44].
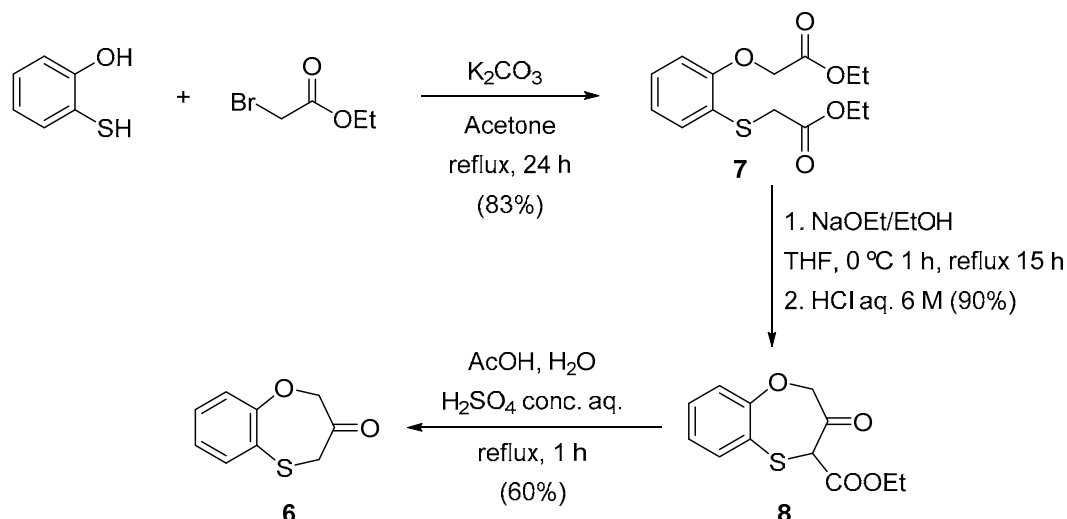
Scheme 1. Chemical synthesis of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one **6**.

Due to the amine transaminases catalytic mechanism, which involves two pairs of ketones and amines in equilibrium, the reductive amination of the substrate must be thermodynamically favoured in order to obtain high yields of the desired product [45,46]. In order to displace the equilibrium towards amine formation, the removal of the generated co-products by coupling different multienzyme networks is often required [20], but also worth noting is the use of sacrificial substrates, which normally range from the use of a large excess of a commercially available amine donor, typically isopropylamine [47], to "smart cosubstrates", mainly diamines, in a stoichiometric amount that are able to drive equilibrium by spontaneous cyclisation or aromatisation reactions [31,48–50]. Promisingly, we have found a favourable ΔG of -31.0 kJ/mol (calculated at M06-2X/6-311++G(3df,2p) level; see Section 3.8) for the transamination of **6** to **9** when using isopropylamine and acetone is formed as a by-product, probably due to ring strain instability. Figure 2 shows the charge density of the optimised geometry of the ketone **6**, where steric and electronic differences between the two substituents of the carbonyl group can be observed. This prompted us to study the biocatalytic process in depth.
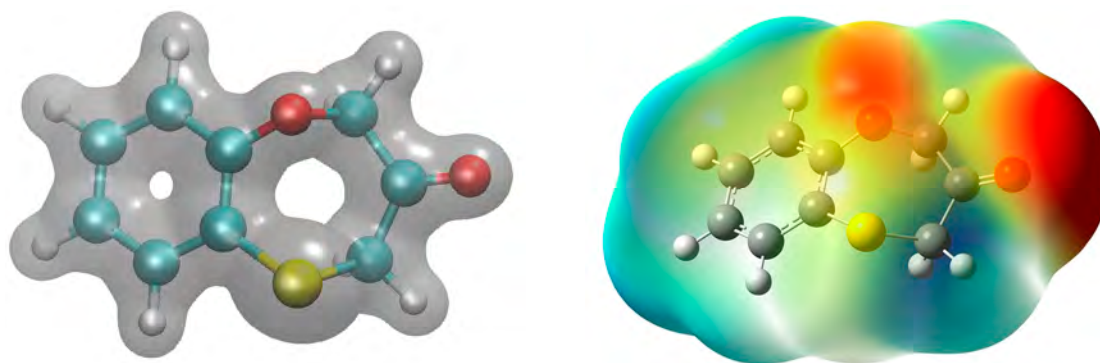
Figure 2. Optimised geometry of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one (**6**): electronic isodensity contour (**left**); colour-mapped with the electrostatic potential (**right**), where red and blue zones are related to the electrophilic and nucleophilic zones of the molecule, respectively.

The biotransamination of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one (**6**, 20 mM) was then studied in standard conditions previously employed in our research group [46,51]. These settings include the use of a large excess of isopropylamine as amine donor (1 M), pyridoxal 5′-phosphate (PLP, 1 mM) as cofactor, a 100 mM phosphate buffer pH 7.5 with acetonitrile (5% *v/v*) as cosolvent to favour the ketone solubility, at 30 °C and 250 rpm for 20 h (Scheme 2). Three different types of enzymes were employed:

(a) lyophilised *Escherichia coli* cells containing overexpressed ATAs; (b) commercially available ATAs from Codexis Inc.; (c) commercially available ATAs from Enzymicals AG.
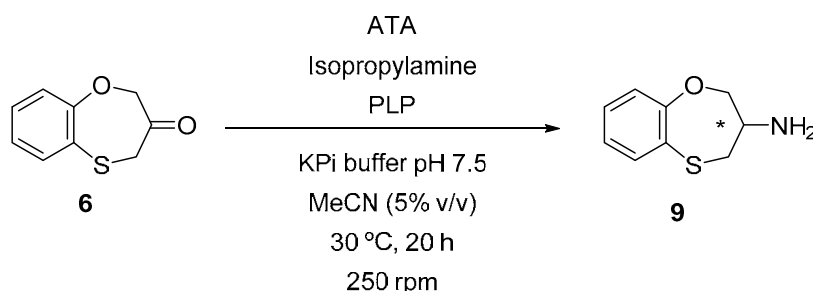
Scheme 2. Biotransamination of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one (**6**) into amine **9**, using ATAs.

Initially, for the biotransamination experiments made in house ATAs were used, all of them overexpressed in *Escherichia coli*. Some of them, such as the ones from *Chromobacterium violaceum* [52] or *Arthrobacter* species [53], displayed very low activity (<5%), while others such as *Arthrobacter citreus* [54] or the *Arthrobacter* species evolved variant named ArRmut11 [55] provided almost quantitative conversion but moderate (74% *ee*) or negligible stereoselectivity, respectively. Trying to improve both activity and selectivity values, commercially available ATAs were employed from two different commercial sources (Codexis Inc. and Enzymicals AG).

To start with, 30 Codexis enzymes were employed (Table 1), and we found that 19 of them led to the complete disappearance of the starting ketone. Remarkably, four enzymes from this kit provided the desired amine **9** in optical purities over 80% *ee*, the ATA-200 conducting to the (*S*)-**9** (entry 8), while the TA-P1-B04, TA-P1-F03 and TA-P1-G05 gave access to its amine antipode (entries 23, 24 and 26).

Table 1. Biotransamination of ketone **6** using Codexis ATAs [a].

Entry	Enzyme	Conversion (%) [b]	*ee* (%) [c]
1	ATA-7	<1	n.d.
2	ATA-13	30	n.d.
3	ATA-24	93	<1
4	ATA-25	96	<1
5	ATA-33	>99	<1
6	ATA-113	13	n.d.
7	ATA-117	2	n.d.
8	ATA-200	>99	85 (*S*)
9	ATA-217	6	n.d.
10	ATA-234	4	n.d.
11	ATA-237	>99	41 (*S*)
12	ATA-238	4	n.d.
13	ATA-251	>99	72 (*S*)
14	ATA-254	>99	56 (*S*)
15	ATA-256	>99	63 (*S*)
16	ATA-260	>99	79 (*S*)
17	ATA-301	>99	7 (*S*)
18	ATA-303	>99	<1
19	ATA-412	>99	55 (*S*)
20	ATA-415	>99	<1
21	TA-P1-A01	>99	62 (*R*)
22	TA-P1-A06	>99	50 (*R*)
23	TA-P1-B04	>99	82 (*R*)
24	TA-P1-F03	>99	90 (*R*)
25	TA-P1-F12	>99	28 (*R*)

Table 1. *Cont.*

Entry	Enzyme	Conversion (%) [b]	*ee* (%) [c]
26	TA-P1-G05	>99	93 (*R*)
27	TA-P1-G06	>99	67 (*R*)
28	TA-P2-A01	4	n.d.
29	TA-P2-A07	60	16 (*S*)
30	TA-P2-B01	99	19 (*R*)

[a] For reaction details, see Section 3.6. [b] Conversion values measured by GC analyses of the reaction crudes. [c] Enantiomeric excess (*ee*) of amine **9** determined by HPLC analyses after derivatisation of the reaction crude. These *ee* values were calculated for those reactions with conversions over 30% (n.d.: not determined).

Using the best found enzyme, TA-P1-G05 (entry 26, >99% conversion and 93% *ee*), the transamination of **6** was followed over time using two enzyme loadings (90% and 45% *w/w* enzyme vs. ketone); we observed a very fast conversion in the first 2 h and then a slower rate until complete depletion of the substrate occurred, after 6 h or 24 h, respectively (Figure 3).
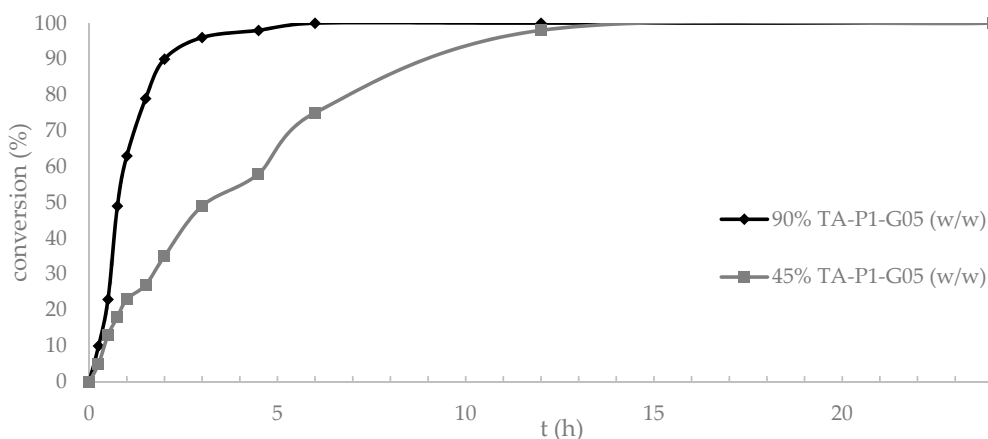
Figure 3. Study of the enzymatic transamination of ketone **6** with TA-P1-G05 over time employing: (◆) 90% of enzyme loading (*w/w*) or (■) 45% of enzyme loading (*w/w* vs. **6**).

Eight enzymes from Enzymicals AG were employed (Table 2), finding in three cases an amine with over 90% *ee* (entries 3, 7 and 8). Interestingly, the ATA08 from *Silicibacter pomeroyi* allowed the quantitative conversion of the ketone into the amine (*R*)-**9** (entry 7).

Table 2. Biotransamination of ketone **6** using Enzymicals AG ATAs [a].

Entry	Enzyme	Conversion (%) [b]	*ee* (%) [c]
1	ATA01 *Aspergillus fumigatus*	9	n.d.
2	ATA02 *Gibberella zeae*	<1	n.d.
3	ATA03 *Neosartorya fischeri*	29	90 (*S*)
4	ATA04 *Aspergillius oryza*	2	n.d.
5	ATA05 *Aspergillius terreus*	8	n.d.
6	ATA06 *Penicillium chrysogenum*	<1	n.d.
7	ATA07 *Mycobacterium vanbaalenii*	20	95 (*S*)
8	ATA08 *Silicibacter pomeroyi*	>99	91 (*R*)

[a] For reaction details, see Section 3.6. [b] Conversion values measured by GC analyses of the reaction crudes. [c] Enantiomeric excess of amine **9** determined by HPLC analyses after derivatisation of the reaction crude. These values were calculated for those reactions with conversions over 20% (n.d.: not determined).

In order to improve the conversion values towards the amine (*S*)-**9** the ATA03 *Neosartorya fischeri* (entry 3) and ATA07 *Mycobacterium vanbaalenii* (entry 7) were selected for optimization studies. So, new experiments were developed that includes the decrease of the substrate concentration, the use of longer reaction times, higher temperatures and enzyme loadings, and the performance of the biotransaminations without an organic cosolvent (Table 3). Interestingly, the best results were found when no cosolvent was employed, suggesting a deactivation of both enzymes in the presence of even low amounts of the organic solvent (MeCN, 5% *v/v*). In particular, the reduction of the substrate concentration from 20 to 10 mM of ketone **6** allowed higher conversions, although this limited its practical application. In addition, prolonged reaction times led to better conversions, while the use of higher temperatures led to a significant deactivation of the enzyme.

Table 3. Optimisation of the biotransamination of ketone **6** using selected enzymes [a].

Entry	Enzyme	[6] (mM)	Cosolvent [b]	T (°C)	t (h)	c (%) [c]
1	ATA03 *Neosartorya fischeri*	20	MeCN (5%)	30	20	29
2	ATA03 *Neosartorya fischeri*	10	none	30	48	86
3	ATA07 *Mycobacterium vanbaalenii*	20	MeCN (5%)	30	20	20
4	ATA07 *Mycobacterium vanbaalenii*	10	none	30	20	73
5	ATA07 *Mycobacterium vanbaalenii*	20	none	45	48	43
6 [d]	ATA07 *Mycobacterium vanbaalenii* [d]	20	none	30	65	91

[a] For reaction details, see Section 3.6. [b] Concentration values in *v/v* % indicated in brackets. [c] Conversion values measured by GC analyses of the reaction crudes. [d] Double the amount of enzyme was used (4 mg, 180% *w/w*).

Focusing on the scaling up of the biotransformations, we decided to move to higher substrate concentrations (50 mM of ketone) in order to produce a significant amount of the optically active amine (*R*)-**9**, which is a precursor of organic molecules with interesting biological profiles [2–5]. In this case, 225 mg of **6** were used, selecting TA-P1-G05 (entry 26, Table 1) as the ideal candidate since in standard conditions the amine (*R*)-**9** was formed in complete conversion and good selectivity (93% *ee*). The enzyme loading was reduced from an initial 90% *w/w* enzyme vs. substrate ratio to 33% to improve the economy of the process, and after 22 h quantitative conversion was also achieved, maintaining the selectivity and isolating the desired amine in 98% yield after a simple liquid-liquid extraction protocol (Scheme 3). Measurement of the optical rotation for the pure amine and its corresponding hydrochloride salt allowed us to unequivocally assign the absolute configuration by comparison with previously reported data [4,5].
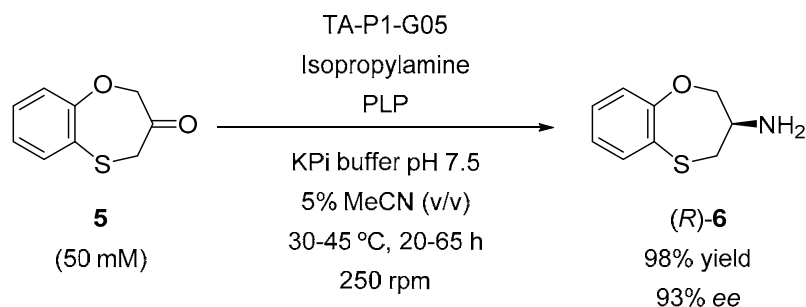
Scheme 3. Scale-up of the biotransamination towards the (3*R*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-amine (*R*-**9**).

3. Materials and Methods

3.1. General Materials and Methods

2-Mercaptophenol, ethyl bromoacetate and sodium ethoxide were purchased from Sigma-Aldrich, now Merck (Madrid, Spain). PLP as enzyme cofactor, other chemical reagents and solvents were obtained with the highest quality available from Sigma-Aldrich-Fluka (Steinheim, Germany). Amine transaminases were obtained from Codexis Inc. (Redwood City, CA, USA) and Enzymicals AG (Greifswald, Germany). Transaminases from *Chromobacterium violaceum* (2.1 U/mg), *Arthrobacter citreus* (0.9 U/mg), *Arthrobacter* species (0.6 U/mg) and the evolved ArRmut11 were overexpressed in *E. coli* and used as lyophilised cell lysates, as previously reported [26,56].

Melting point of compound **6** was measured in an open capillary in an Electrothermal digital melting point IA9200 apparatus (Cole-Parmer, Stone, UK) and is uncorrected. Elemental analyses were performed on a Thermo Scientific Flash 2000 analyzer (Thermo Flash & Carlo Erba Analyzers, Pennsauken, NJ, USA) and the measured values were indicated with the symbols of the elements or functions within ±0.4% of the theoretical values. NMR spectra were recorded on a Bruker AV300 MHz spectrometer (Bruker Co., Faellanden, Switzerland). All chemical shifts (δ) are given in parts per million (ppm) and referenced to the residual solvent signal as internal standard. High-resolution mass spectroscopy (HRMS) was performed on a VG AutoSpec Q high-resolution mass spectrometer (Fision Instrument, Milford, MA, USA). Measurement of the optical rotation values was carried out at 590 nm on an Autopol IV Automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA).

Gas chromatography (GC) analyses were performed for the determination of conversion values using a Hewlett-Packard HP-6890 chromatograph (Hewlett Packard, Palo Alto, CA, USA). A non-chiral HP-1 column (Agilent Technologies, Inc., Wilmington, DE, USA) was used with the following temperature programme: 90 °C (2 min) then 10 °C/minutes and finally 180 °C (0 min). The reaction crudes were analysed, obtaining the following retention times: 9.3 min for ketone **6** and 10.5 min for amine **9**.

High-performance liquid chromatography (HPLC) analyses were performed for enantiomeric excess value measurements using an Agilent 1260 Infinity chromatograph with UV detector at 210 nm (Agilent Technologies, Inc., Wilmington, DE, USA). A Chiralpak IA (25 cm × 4.6 mm) was used as chiral column at 30 °C (Chiral Technologies, Mainz, Germany), employing a mixture of n-hexane/2-propanol (90:10) as eluent with a 0.8 mL/min flow. The reaction crudes were derivatised as acetamides, obtaining the following retention times: 11.2 min for the (*R*)-**10** and 12.6 min for the (*S*)-**10** enantiomer (Figure 4).
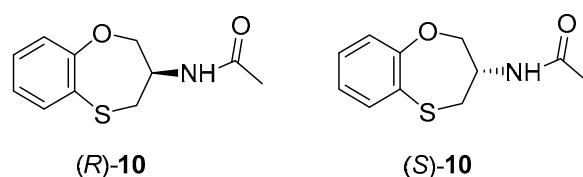
Figure 4. Structures of (*R*)- and (*S*)-**10**.

Thin-layer chromatography (TLC) analyses were conducted with Merck Silica Gel 60 F254 precoated plates (Merck KGaA, Darmstadt, Germany). They were visualised with UV and potassium permanganate stain. Column chromatography purifications were performed using Merck Silica Gel 60 (230–400 mesh, Merck KGaA, Darmstadt, Germany).

*3.2. General Procedure for the Synthesis of Ethyl 2-Ethoxycarbonylmethylthiophenoxyacetate (**7**)*

A mixture of 2-mercaptophenol (1 g, 7.925 mmol), ethyl bromoacetate (1.93 mL, 17,4 mmol) and dry K_2CO_3 (3.3 g, 23.8 mmol) in anhydrous acetone (20 mL) was added under argon atmosphere, and then stirred under reflux. After 24 h the solvent was evaporated under reduced pressure and the residue purified by column chromatography (EtOAc/n-hexane, 1:8), obtaining **7** as a colourless syrup. Yield 83%. ^{1}H NMR (300.13 MHz, CDCl$_3$) δ 7.43 (dd, J$_{HH}$ = 7.7, 1.7 Hz, 1H), 7.21 (td, J$_{HH}$ = 7.9, 1.7 Hz, 1H), 6.95 (td, J$_{HH}$ = 7.5, 1.2 Hz, 1H), 6.76 (dd, J$_{HH}$ = 8.2, 1.2 Hz, 1H), 4.70 (s, 2H), 4.26 (q, J$_{HH}$ = 7.1 Hz, 2H), 4.11 (q, J$_{HH}$ = 7.1 Hz, 2H), 3.72 (s, 2H), 1.28 (t, J$_{HH}$ = 7.1 Hz, 3H), 1.18 (t, J$_{HH}$ = 7.1 Hz, 3H) ppm. HRMS (ESI-TOF) (*m/z*) calcd. for $C_{14}H_{19}O_5S$ (M + H)$^+$ 299.0953, found 299.0955. Anal. Calcd for $C_{14}H_{18}O_5S$: C, 56.36; H, 6.08; S, 10.75. Found: C, 56.45; H, 5.89; S, 10.55.

*3.3. General Procedure for the Synthesis of Ethyl 3-Oxo-3,4-dihydro-2H-1,5-benzoxathiepin-4-carboxylate (**8**)*

To a mixture of diester **7** (1.37 g, 4.59 mmol) in THF (40 mL) at 0 °C, a solution of NaOEt (21% wt, 1.78 g, 5.51 mmol) in EtOH (2.06 mL) was added. The mixture was stirred at 0 °C for 1 h and then refluxed for 15 h. Solvent was evaporated under reduced pressure and the residue cooled to 0 °C, quenched first with water, and later with an aqueous HCl 6 M solution up to pH 6. The mixture was extracted with EtOAc (2 × 40 mL) and the organic fractions combined, dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. Compound **8** was purified by column chromatography (EtOAc/n-hexane, 1:7) as a colourless oil. Yield, 90%. ^{1}H NMR (300.13 MHz, CDCl$_3$) δ 7.22–7.12 (m, 1H), 7.08–6.85 (m, 3H), 4.88 (d, J$_{HH}$ = 17.5, 1H), 4.75 (s, 1H), 4.59 (d, J$_{HH}$ = 17.5, 1H), 4.29–4.15 (m, 2H), 1.21 (td, J$_{HH}$ = 7.1, 1.4 Hz, 3H) ppm. HRMS (ESI-TOF) (m/z) calcd. for $C_{12}H_{11}O_4S$ (M - H)$^+$ 251.0378, found 251.0376. Anal. Calcd for $C_{12}H_{12}O_4S$: C, 57.13; H, 4.79; S, 12.71. Found: C, 56.99; H, 4.98; S, 12.72.

*3.4. General Procedure for the Synthesis 3,4-Dihydro-2H-1,5-benzoxathiepin-3-one (**6**)*

A mixture of keto ester **8** (2.5 g, 11.9 mmol), acetic acid (4.16 mL), H_2SO_4 concentrated (4.16 mL) and H_2O (23.8 mL) was refluxed for 1 h. The reaction was cooled to 0 °C, and water was added and extracted with CH_2Cl_2 (2 × 40 mL). The organic fractions were combined, dried (anhydrous Na_2SO_4), filtered and the solvent was removed under reduced pressure. Compound **6** was purified by column chromatography (n-hexane and then, EtOAc/n-hexane 0.5:10) as a white solid, mp 29–31 °C, literature 28–31 °C [43]. Yield 60%. ^{1}H NMR (300.13 MHz, CDCl$_3$) δ 7.18 (dd, J$_{HH}$ = 8.0, 1.8 Hz, 1H), 7.14–7.07 (m, 1H), 7.01 (m, J$_{HH}$ = 8.1, 4.8, 1.5 Hz, 2H), 4.75 (s, OCH$_2$, 2H), 3.93 (s, SCH$_2$, 2H) ppm. HRMS (ESI-TOF) (m/z) calcd. for $C_9H_9O_2S$ (M + H)$^+$ 181.0323, found 181.0321.

*3.5. General Procedure for the Biotransamination of **6** Using ATAs Overexpressed in* Escherichia coli

The lyophilised cells of E. coli containing overexpressed transaminases (5 mg) were suspended in a 100 mM phosphate buffer pH 7.5 (475 μL) containing PLP (1 mM) and 2-propylamine (1 M). Then, a stock solution of ketone **6** in MeCN was added (25 μL of stock 0.4 M; final concentration 20 mM) and the mixture was shaken at 30 °C and 250 rpm for 20 h. After this time, the reaction was quenched by adding an aqueous 10 M NaOH solution (200 μL) and extracted with EtOAc (2 × 500 μL). The organic phases were combined and dried over anhydrous Na_2SO_4. The reaction crudes were analysed by GC to determine conversion values. Derivatisation were carried out in situ using acetic anhydride and K_2CO_3 for the measurement of the enantiomeric excesses through HPLC.

3.6. General Procedure for the Biotransamination of 6 Using Commercial ATAs

Transaminases from Codexis or Enzymicals AG (2 mg, 90% w/w) were suspended in a 100 mM phosphate buffer pH 7.5 (475 µL) containing PLP (1 mM) and 2-propylamine (1 M). Then, a stock solution of ketone **6** in MeCN was added (25 µL of stock 0.4 M; final concentration 20 mM) and the mixture was shaken at 30 °C and 250 rpm for 20 h. After this time, the reaction was quenched by adding an aqueous 10 M NaOH solution (200 µL) and extracted with EtOAc (2 × 500 µL). The organic phases were combined and dried over anhydrous Na_2SO_4. Reaction crude was analysed by GC to determine conversion values and in situ derivatisation was carried out using acetic anhydride and K_2CO_3 for the measurement of the enantiomeric excesses by HPLC.

3.7. Preparative Biotransamination of 6 under Optimised Conditions

Ketone **6** (225 mg, 1.25 mmol) was dissolved in MeCN (1.25 mL) and 100 mM phosphate buffer pH 7.5 (25 mL), containing PLP (0.5 mM) and 2-propylamine (1 M), and the TA-P1-G05 (75 mg, 33% w/w) were successively added. The mixture was shaken at 30 °C and 250 rpm for 22 h. The reaction was quenched by adding an aqueous NaOH 4 M solution (5 mL) and extracted with EtOAc (3 × 15 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, combined and the solvent removed under reduced pressure, affording the (*R*)-**9** amine (220 mg).

(3*R*)-3,4-Dihydro-2*H*-1,5-benzoxathiepin-3-amine (*R*)-**9**. Yield: 220 mg (98%). ¹H NMR (300.13 MHz, CDCl₃): δ 7.37 (dd, J_{HH} = 7.7, 1.7 Hz, 1H), 7.15 (ddd, J_{HH} = 8.1, 7.3, 1.7 Hz, 1H), 7.02–6.92 (m, 2H), 4.12–4.08 (m, 2H), 3.50–3.42 (m, 1H), 3.18 (dd, J_{HH} = 14.2, 3.2 Hz, 1H), 2.80 (dd, J_{HH} = 14.2, 5.7 Hz, 1H), 1.89 (br s, 2H) ppm. For the free amine (*R*)-**9** in 93% *ee* $[\alpha]_D^{20}$ = +32.6 (c = 0.1, MeOH), and for the hydrochloride salt (*R*)-**9**·HCl in 93% *ee* $[\alpha]_D^{20}$ = +41.2 (c = 0.1, MeOH); literature $[\alpha]_D^{20}$ = +48.9 (c = 0.35, MeOH) for the (*R*)-**9**·HCl in >99% *ee* [4].

3.8. Computational Methods

Calculations were performed using the Gaussian 09 package [57] at the M06-2X/6-311++G(3df,2p) level [58]. Molecular geometries of the studied compounds were optimised with tight convergence criteria and the frequencies were computed in order to obtain the thermal correction to the energy (298.15 K).

The molecular electrostatic potential of 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one (**6**) was computed at M06-2X/6-311++G(3df,2p) level with tight SCF procedure and generating the density and potential cubes to plot the isodensity surface, colour-coded with the electrostatic potential.

4. Conclusions

The synthesis of the 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-amine enantiomers has been possible by means of the stereoselective biotransamination of the 3,4-dihydro-2*H*-1,5-benzoxathiepin-3-one. A broad panel of commercially available amine transaminases were employed, finding after optimisation of parameters that affect the enzyme catalysis suitable reaction conditions for the access to both amine antipodes in high conversions and good selectivities. A scale-up experiment considering 50 mM substrate concentration was successfully achieved for the formation of the (*R*)-3,4-dihydro-2*H*-1,5-benzoxathiepin-3-amine (*R*-**9**), a valuable precursor of anti-proliferative agents.

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