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Multigram-scale HPLC enantioseparation as a rescue pathway for circumventing racemization problem during enantioselective synthesis of ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate

Hong-Ngoc Pham^{1,2} | Axelle Arrault¹  | Nicolas Vanthuyne³  | Samir Acherar¹ 

Abstract

Racemic ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate is a key synthon for the design of promising therapeutic drugs. It is mainly synthesized from racemic ethyl 2,3-dibromopropionate and 2-aminophenol in presence of K_2CO_3 in refluxed acetone. Unfortunately, synthesis of (*R*)- and (*S*)-enantiomers using the enantioselective version of this reaction, which should normally be performed with a double S_N2 mechanism, is unsuitable due to a racemization process involving the dehydrobromination of enantiopure ethyl 2,3-dibromopropionate into ethyl 2-bromoacrylate. For the first time, the enantioselective version is studied (ee \approx 55–66%), and the percentage of racemization process has estimated to around 34–46% after determination of the optimal experimental conditions for analytical HPLC enantioseparation of racemate. The influence of the experimental and purification conditions on the racemization rate is also studied. The results indicate that racemization occurs faster at the beginning of the reaction but the initiation of the double S_N2 process takes place more faster to limit the racemization rate. The study of the influence of experimental conditions (reaction times, temperature, solvent or type of base, etc.) on the degree of racemization of the (*R*)- enantiomer is performed and shows that despite changes in the experimental conditions, the synthesis of the (*R*)- enantiomer is always accompanied by a racemization rate which is difficult in reducing. In parallel, (*R*)- and (*S*)-enantiomers are obtained in high enantiopurity (ee > 99.5%) by preparative HPLC enantioseparation of racemate on multigram scale and characterized by optical rotation measurements, ECD and UV spectra.

1 | INTRODUCTION

Since essential biomolecules needed for human body appeared as enantiomerically pure, chiral drugs have been and being a vital topic in the past decades as approximately 56% of marketed medicines are used in one of their enantiomeric form.^{1,2} Chiral molecules have the same chemical behavior in an achiral environment but may contain different biological activities such in the special case of as ibuprofen³ or thalidomide.^{4,5} Those compounds have been reported that only one optically active isomer can exhibit beneficial pharmacological activities than the other.

Due to the extraordinary diversity and multi-functions of heterocycles, heterocycle-based molecules are becoming key structural in development of new drugs with two third of marketed drugs containing heterocycles.⁶ 1,4-Benzoxazine derivatives are a fascinating class of heterocyclic compounds which can exhibit biological properties such as antibacterial,^{7,8} treatment against cardiovascular diseases,^{9,10} anti-hypertensive,¹¹ angiogenesis inhibitor,¹²⁻¹⁴ anti-thrombotic,^{15,16} or neuroprotective factor.^{17,18} Among them, various promising therapeutic drugs (e.g., anti-tumour,¹⁹ procognitive,²⁰ and EGFR kinase inhibitor²¹) were described in the literature

starting from our targeted ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate scaffold in a racemic form **rac-1** (Figure 1). In 2005, an overview of different strategies to provide racemic 1,4-benzoxazine analogs has been published,²² but only few reports brought up procedures to obtain enantiomerically pure analogs. These procedures use metal²³⁻²⁷ or phase transfer²⁸ catalyzed asymmetric reactions, kinetic resolution with enzyme^{29,30} or chiral resolving agents,³¹⁻³³ stereoselective aryl addition,³⁴ asymmetric baker's yeast reduction,³⁵ and chiral 1,2-cyclic sulfamidates nucleophilic cleavage.³⁶

It is interesting to note that, while our targeted scaffold **1** is a key synthon for the design of promising therapeutic drugs (Figure 1), its synthesis and characterizations (optical rotation and electronic circular dichroism [ECD]) as enantiomers were never described in the literature. **Rac-1** is mainly synthesized by reacting 2-aminophenol and racemic ethyl 2,3-dibromopropionate **rac-2** with anhydrous K₂CO₃ in refluxing dry acetone,^{9,11,37-40} but to date, its enantioselective version has never been reported for two reasons. First, both enantiomers of **2** have been just recently isolated by our team through a preparative HPLC enantioseparation of racemate on multigram scale.⁴¹ Second, this reaction should be proceeded via a

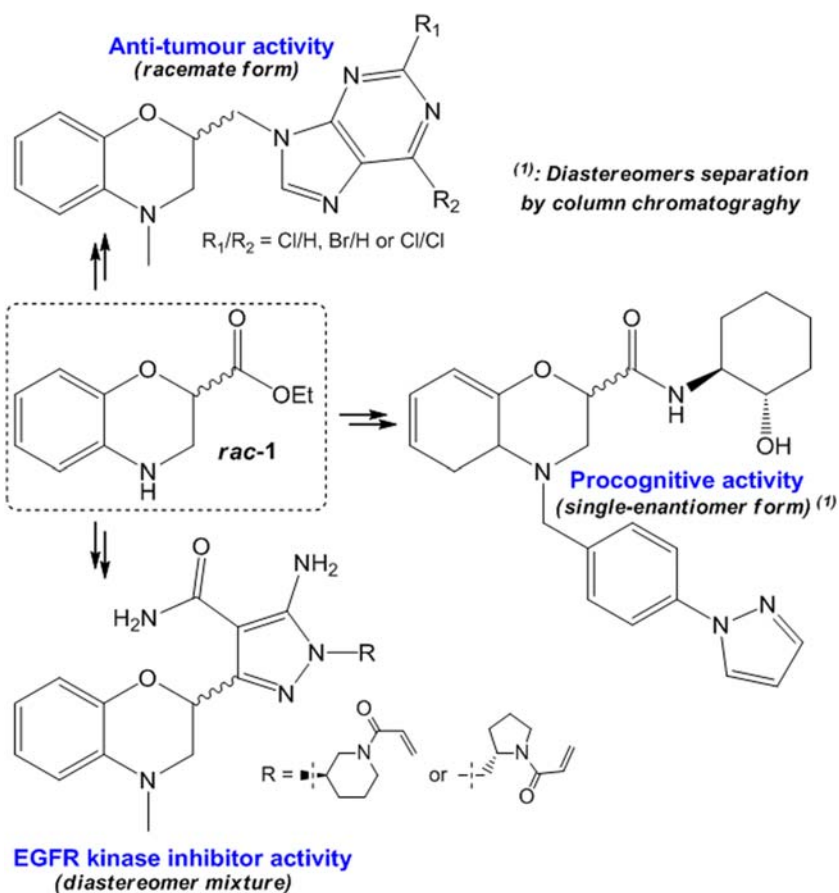


FIGURE 1 Examples of designed therapeutic drugs starting from **rac-1**

double S_N2 reaction, but a racemization is proposed to occur through a relay mechanism involving (i) the dehydrobromination of **2**, achieving the ethyl 2-bromoacrylate **3**; (ii) the Michael addition of the amine group of 2-aminophenol on the primary carbon; and (iii) the S_N2 reaction of the phenolate anion on the α-bromoester group to afford **rac-1** (Figure 2).

The goal of this work is to use analytical and preparative HPLC enantioseparation of **rac-1** for the first time (i) to investigate the enantioselective version of this reaction with (*R*)-**2** and to evaluate the percentage of racemization that occurred during the synthesis of (*R*)-**1** and (ii) to offer a cost-efficient alternative solution for the obtainment of both pure enantiomers of **1** in multigram scale. This work shows that (i) a minor amount of racemization (≈ 34–46%) occurred during the synthesis of (*R*)-**1** (ee ≈ 55–66%) and (ii) preparative HPLC enantioseparation of **rac-1** on multigram scale afforded both enantiomers with high enantiomeric excess (ee > 99.5%).

2 | MATERIALS AND METHODS

2.1 | Chemicals

Unless otherwise stated, all chemicals were purchased as the highest purity commercially available and were used without further purification. Dry acetone was obtained by distillation over P₂O₅ under an argon atmosphere, and

other reagent-grade solvents were used as received. 2-Aminophenol (99%, 109.13 g/mol) and ethyl 2,3-dibromopropionate racemate **rac-2** (≥ 98%, 259.92 g/mol) were purchased from Sigma-Aldrich. (*R*)-Ethyl 2,3-dibromopropionate (*R*)-**2** (ee > 99.5%, [α]_D²⁵ = + 12 (CH₂Cl₂, c = 3.29)) was obtained previously by our team⁴¹ through preparative HPLC enantioseparation of commercially available **rac-2** on multigram scale. Ethanol, heptane, and acetonitrile were HPLC grade and were purchased from VWR.

2.2 | Characterization

Reactions were monitored by thin-layer chromatography (TLC) using aluminum-backed silica gel plates (Macherey-Nagel ALUGRAM[®] SIL G/UV254). TLC spots were viewed under ultraviolet light or/and by heating the plate after treatment with a staining solution of phosphomolybdic acid or ninhydrin. Product purifications were performed using Geduran 60 H Silica Gel (63–200 mesh). NMR spectra were recorded on a Bruker Advance 300 spectrophotometer in CDCl₃ solvent at 298 K. CDCl₃ (δ = 7.26 and 77.0 ppm) residual peaks were used as internal references for ¹H and ¹³C NMR, respectively. Chemical shifts (δ) were reported in parts per million (ppm), and coupling constants (*J*) were given in hertz (Hz). Multiplicity was defined as Ar for aromatic, s for singlet, d for doublet, t for triplet, q for quadruplet, m for multiplet, and br for broad or combinations

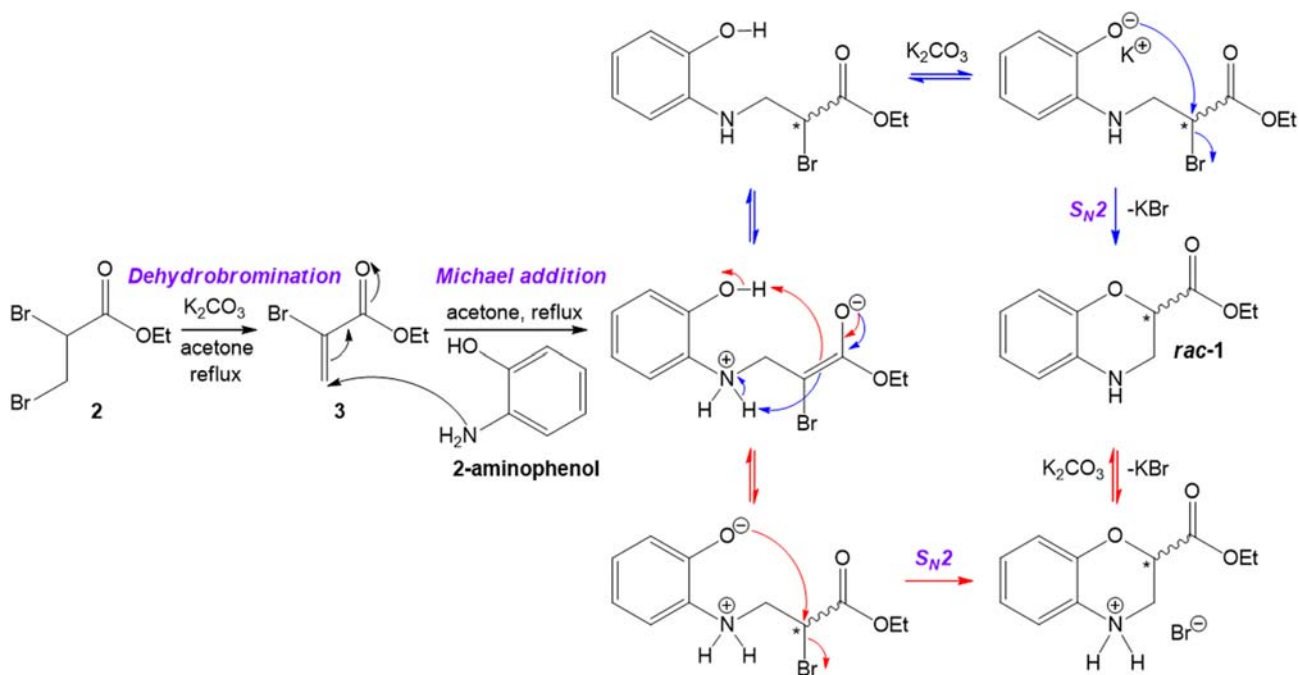


FIGURE 2 Proposed mechanism for the synthesis of **rac-1** from 2-aminophenol and **2**

thereof. Electrospray mass spectroscopy (ESI-MS) was performed on a ToF-Q HR spectrometer. HRMS spectra were obtained by the ESI method.

2.3 | Synthesis of racemic and (*R*)-ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate, *rac*-1 and (*R*)-1

To a stirring solution of 2-aminophenol (2.00 g, 18.3 mmol, 1.0 equiv.) and anhydrous potassium carbonate (5.57 g, 40.3 mmol, 2.2 equiv.) in dry acetone (30 mL), was added *rac*-2 or (*R*)-2 (5.24 g, 20.2 mmol, 1.1 equiv.). The reaction was refluxed at 60°C and allowed to stir for 16 h. After filtration on celite to remove carbonate salts, the resulting filtrate was concentrated in vacuo, diluted in CH₂Cl₂ (30 mL) and washed with water (10 mL). The organic layer was dried over MgSO₄ and concentrated in vacuo. The resulting crude material was purified by flash chromatography (Petroleum ether/EtOAc, 30/70, v/v) to afford *rac*-1 or (*R*)-1 in good yields (83–85%) as yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 1.27 (t, 3H, *J* = 6.9 Hz, CH₃), 3.50–3.65 (m, 2H, N-CH₂), 3.77 (br s, 1H, NH), 4.25 (q, 2H, *J* = 6.9 Hz, O-CH₂), 4.80 (dd, 1H, *J* = 6.9 and 3.3 Hz, CH [CO]), 6.63 (dd, 1H, *J* = 7.8 and 1.8 Hz, CH_{Ar}), 6.72 (td, 1H, *J* = 7.5 and 1.8 Hz, CH_{Ar}), 6.78 (td, 1H, *J* = 7.5 and 1.8 Hz, CH_{Ar}), 6.92 (dd, 1H, *J* = 7.8 and 1.8 Hz, CH_{Ar}); ¹³C NMR (75 MHz, CDCl₃) δ 14.80 (CH₃), 43.27 (N-CH₂), 62.31 (O-CH₂), 73.39 (CH [CO]), 116.74 (CH_{Ar}), 117.70 (CH_{Ar}), 120.64 (CH_{Ar}), 122.35 (CH_{Ar}), 132.77 (N-C_{Ar}), 143.83 (O-C_{Ar}), 170.00 (C=O); HRMS (ESI) calculated for C₁₁H₁₃NO₃ [M + Na]⁺ *m/z* 230.0793, found 230.0799 (see Appendix S1 for a copy of 1D and 2D NMR spectra).

2.4 | Synthesis of ethyl 2-bromoacrylate, 3

A solution of (*R*)-2 (500 mg, 1.92 mmol, 1.0 equiv.) and anhydrous potassium carbonate (532 mg, 3.85 mmol, 2.0 equiv.) in dry acetone (5 mL) was refluxed at 60°C and allowed to stir for 16 h. The reaction progress and the conversion yield (%) were estimated using analytical HPLC analysis (see Section 2.5) by comparing the ratio of peak areas of 3 and (*R*)-2 (retention times of 7.8 and 9.9 min, respectively). At the end of the reaction, the crude reaction product comprising 3 and residual (*R*)-2 was concentrated in vacuo. ¹H NMR of 3 (300 MHz, CDCl₃) δ 1.29 (t, 3H, *J* = 6.9 Hz, CH₃), 4.24 (q, 2H, *J* = 6.9 Hz, O-CH₂), 6.21 (s, 1H, =CH), 6.90 (s, 1H, =CH) (see Appendix S1 for a copy of ¹H NMR spectrum).

2.5 | Analytical achiral HPLC monitoring of the dehydrobromination of (*R*)-2

Analytical achiral HPLC monitoring of reaction progress of synthesis of 3 through dehydrobromination of (*R*)-2 at intervals of 0, 10, 60, 120, 180, 240, 300, 360, 420, 480, 540, and 960 min was performed using a Shimadzu LC-20A HPLC system, consisting of a degasser (DGU-20A3), two pumps (LC-20AT), an UV photo-diode array detector (SPD-M20A), and an oven (CTO-20A). Separations of peaks for (*R*)-2 and 3 were achieved using a Macherey-Nagel Nucleosil 300-7 C4 column (250 × 4 mm). The mobile phase was consisted of CH₃CN/H₂O (v/v, containing 0.1% of TFA) from 5/95 to 100/0 in 20 min, then 100/0 to 5/95 in 5 min. The flow rate and the UV detection were 1.0 mL/min and 254 nm, respectively.

2.6 | Enantioselective HPLC

Analytical and preparative HPLC experiments were performed on an Agilent 1260 Infinity unit (pump G1311B, autosampler G1329B, DAD G1315D), with Jasco OR-1590 online polarimeter and UV (254 nm) detectors. The analytical (250 × 4.6 nm) and preparative columns were purchased from Chiral Technologies Europe (Illkirch, France), Phenomenex (Le Pecq, France) or Regis Technologies (Morton Grove, USA) (see Appendix S2 for more details). HPLC grade heptane, hexane, and ethanol were degassed and filtered on a 45 μm Millipore membrane before use. Retention times *R_t* in minutes, retention factors $k_i = (R_t - R_{t_0})/R_{t_0}$ and enantioselectivity factor $\alpha = k_2/k_1$ and resolution $R_s = 1.18 (R_{t_2} - R_{t_1})/(w_1 + w_2)$ are given. *R_{t0}* was determined by injection of 1,3,5-tri-*tert*-butylbenzene, and *w_i* was the peak width at half-height.

2.6.1 | Analytical HPLC

Twenty microliter of a 3 mg/mL solution in ethanol was injected on the chiral column and detected with an UV (254 nm) and polarimeter detectors. A mixture of heptane/EtOH (70/30, v/v) as mobile phase with a flow-rate of 1 mL/min was used.

2.6.2 | Preparative HPLC

The preparative HPLC on multigram scale was performed starting from a solution of ~12 g of synthesized *rac*-1 in 180 mL of hexane/EtOH (50/50, v/v). Every

8 min, 200 μ L of this solution (900 injections) were injected (stacked method) in the preparative Lux-Cellulose-2[®] (250 \times 10 mm, 5 μ m) column using a mobile phase of hexane/EtOH (70/30, v/v), a flow-rate of 5 mL/min, and UV (254 nm) detection. Both enantiomers of **1** were separated and collected in two fractions of around 5.7 g with high purity (ee > 99.5%).

2.7 | Chiroptical characterization

2.7.1 | Optical rotation measurements

Optical rotations of both enantiomers of **1** (CH_2Cl_2 , $c = 0.2$) were measured on a JASCO P-2000 polarimeter with a sodium lamp ($\lambda = 589$ nm) or a halogen lamp ($\lambda = 578, 546, 436, \text{ and } 405$ nm) in a 10 cm cell thermostated at 25°C with a Peltier controlled cell holder.

2.7.2 | ECD and UV spectra

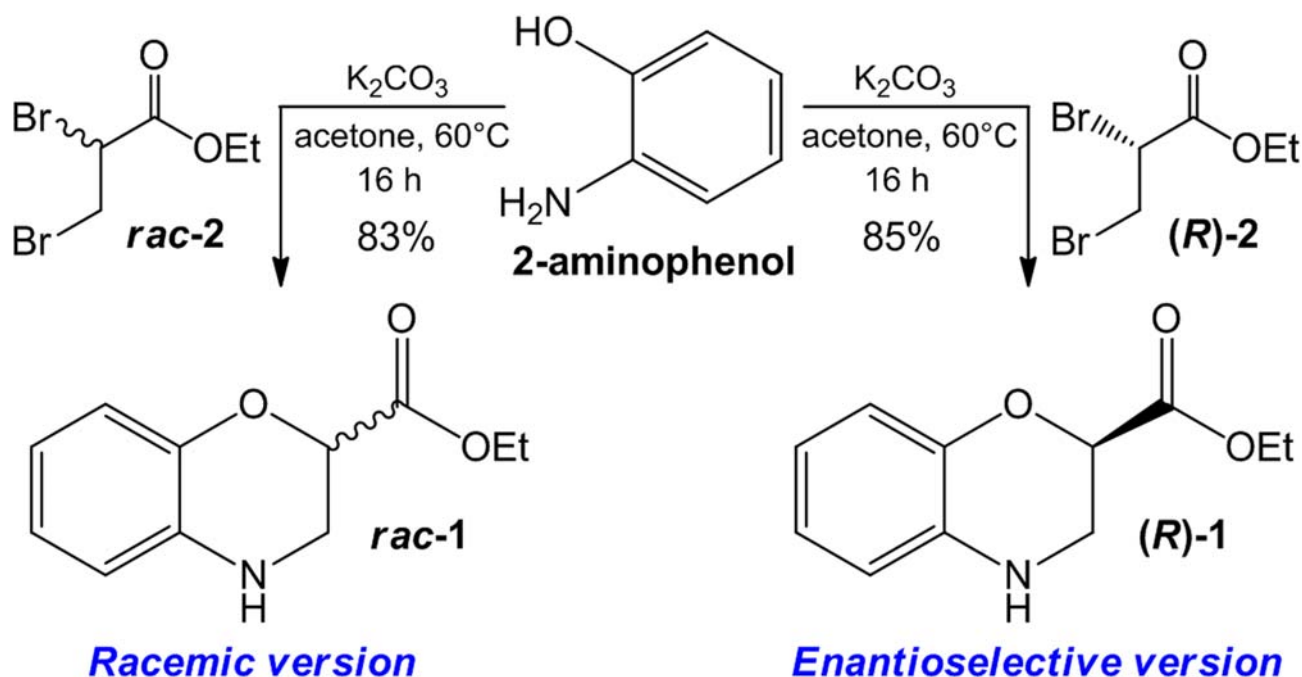
ECD and UV spectra of both enantiomers of **1** in acetonitrile ($c = 0.61$ mM) were measured on a JASCO J-815 spectrometer equipped with a JASCO Peltier cell holder PTC-423 to maintain the temperature at $25.0 \pm 0.2^\circ\text{C}$. A CD quartz cell of 1 mm of optical pathlength was used. The CD spectrometer was purged with nitrogen before recording each spectrum, which was baseline subtracted.

The baseline was always measured for the same solvent and in the same cell as the samples. The spectra are presented without smoothing and further data processing; 0.1 nm as intervals, scanning speed 50 nm/min, band width 2 nm, and 3 accumulations per sample were used as acquisition parameters.

3 | RESULTS AND DISCUSSION

3.1 | Racemic and enantioselective synthesis of (*R,S*)- and (*R*)-ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate, *rac*-**1** and (*R*)-**1**

The racemic synthesis of *rac*-**1** on multigram scale (83% yield) is carried out by a mainly used procedure using 2-aminophenol and *rac*-**2** with anhydrous K_2CO_3 in refluxed dry acetone during 16 h (Scheme 1). The first enantioselective version of this reaction (85% yield, synthesis performed twice) is also achieved using (*R*)-**2** ($[\alpha]_{\text{D}}^{25} = +12$ (CH_2Cl_2 , $c = 3.29$), which was recently isolated for the first time by our team⁴¹ (Scheme 1). This reaction should normally be performed through a double $\text{S}_{\text{N}}2$ process and afforded (*R*)-**1** from (*R*)-**2**. For this type of reaction, it is generally postulated, however, that a racemization process occurs during the synthesis, as already described above, in Figure 2. The racemic synthesis of *rac*-**1** allowed us to investigate the analytical and preparative HPLC enantioseparation of *rac*-**1** in



SCHEME 1 Synthesis of *rac*-**1** and (*R*)-**1**

multigram scale. The enantioselective synthesis of (*R*)-**1**, for its part, allowed us to determine, for the first time, the percentage of racemization in this reaction by measurement of the enantiomeric excess (ee).

3.2 | Analytical and preparative HPLC enantioseparation of (*R,S*)-ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate, *rac*-**1**

To determine the ee of the synthesized (*R*)-**1** and, at the same time, to estimate the percentage of racemization occurring during the synthesis, analytical chiral HPLC separation of *rac*-**1** is critical. Therefore, a screening of 16 different chiral stationary phases (CSPs, 250 × 4.6 mm) using a mixture of heptane/EtOH (70/30, v/v) as mobile phase with a flow rate of 1 mL/min under UV polarimetric detection is conducted to find the best suitable phase for the separation of both enantiomers of **1**. The screening results are reported in Table 1 (see Appendix S2 for HPLC chromatograms). These results show that a poorly baseline separation is obtained only for Chiralpak AS-H, Chiralpak ID and (*S,S*)-Whelk-O1

(*R*_s < 1.5, entries 1, 12, and 16, Table 1). Good baseline separation is observed for all other CSPs (*R*_s > 2.3) with the best values for Lux-Cellulose-2[®] and Lux-Cellulose-3[®] (*R*_s > 10, entries 3 and 4, Table 1). The polarimeter detector reveals an elution order of (–) and (+). Among these two CSPs, Lux-Cellulose-2[®] is chosen because of its better separation factor ($\alpha = 2.16$) and lower retention times for both eluted enantiomers (*R*_t = 5.70 and 8.87 minutes for (–)-**1** and (+)-**1**, respectively (Figure 3A and entry 3, Table 1).

Based on the analytical HPLC results, preparative HPLC enantioseparation of *rac*-**1** on multigram scale is successfully applied to access both enantiomers of our targeted scaffold **1** with high enantiopurity and their characterizations (optical rotation and electronic circular dichroism [ECD] are also investigated). This cost-efficient strategy uses a preparative Lux-Cellulose-2[®] CSP (250 × 10 mm, 5 μm), hexane/EtOH (70/30, v/v) as mobile phase with a flow rate of 5 mL/min under UV detection at 254 nm. About 12 g of *rac*-**1** is diluted in hexane/EtOH (50/50, v/v, 180 mL), and enantiomers are separated using a stacked injection technique (i.e., 900 injections of 200 μL every 8 min). Two highly enantiopure eluted fractions (5.7 g, ee > 99.5%) are

TABLE 1 Screening results for analytical HPLC enantioseparation of *rac*-**1**

Entry	Column	Heptane/EtOH (v/v)	First eluted enantiomer		Second eluted enantiomer		α^c	<i>R</i> _s ^d
			<i>R</i> _{t1} ^a	<i>k</i> ₁ ^b	<i>R</i> _{t2} ^a	<i>k</i> ₂ ^b		
1	Chiralpak AS-H	70/30	4.43(–)	0.50	4.66(+)	0.58	1.15	0.82
2	Chiralcel OD-3	70/30	6.32(–)	1.14	7.99(+)	1.71	1.50	6.08
3	Lux-Cellulose-2	70/30	5.70(–)	0.93	8.87(+)	2.01	2.16	10.09
4	Lux-Cellulose-3	70/30	14.56(–)	3.94	22.01(+)	6.46	1.64	10.06
5	Lux-Cellulose-4	70/30	5.23(–)	0.77	7.17(+)	1.43	1.85	8.43
6	Lux-Amylose-1	70/30	6.79(+)	1.30	8.23(–)	1.79	1.37	5.23
7	Lux-Amylose-2	70/30	5.94(–)	1.01	6.79(+)	1.30	1.29	3.89
8	Chiralpak AZ-H	70/30	5.31(–)	0.80	7.02(+)	1.38	1.72	6.38
9	Chiralpak IA	70/30	6.13(+)	1.08	7.25(–)	1.46	1.35	2.85
10	Chiralpak IB	70/30	5.39(–)	0.83	6.00(+)	1.03	1.25	2.60
11	Chiralpak IC	70/30	5.09(–)	0.72	5.77(+)	0.95	1.32	2.90
12	Chiralpak ID	70/30	5.13(–)	0.74	5.44(+)	0.84	1.14	0.98
13	Chiralpak IE	70/30	5.49(–)	0.86	6.00(+)	1.04	1.20	2.30
14	Chiralpak IF	70/30	5.45(–)	0.85	7.21(+)	1.45	1.70	5.60
15	Chiralpak IG	70/30	6.66(–)	1.26	7.36(+)	1.49	1.19	2.46
16	(<i>S,S</i>)-Whelk-O1	70/30	6.90(–)	1.34	7.33(+)	1.49	1.11	1.20

^aRetention time (min).

^bRetention factor.

^cEnantioselectivity factor.

^dResolution.

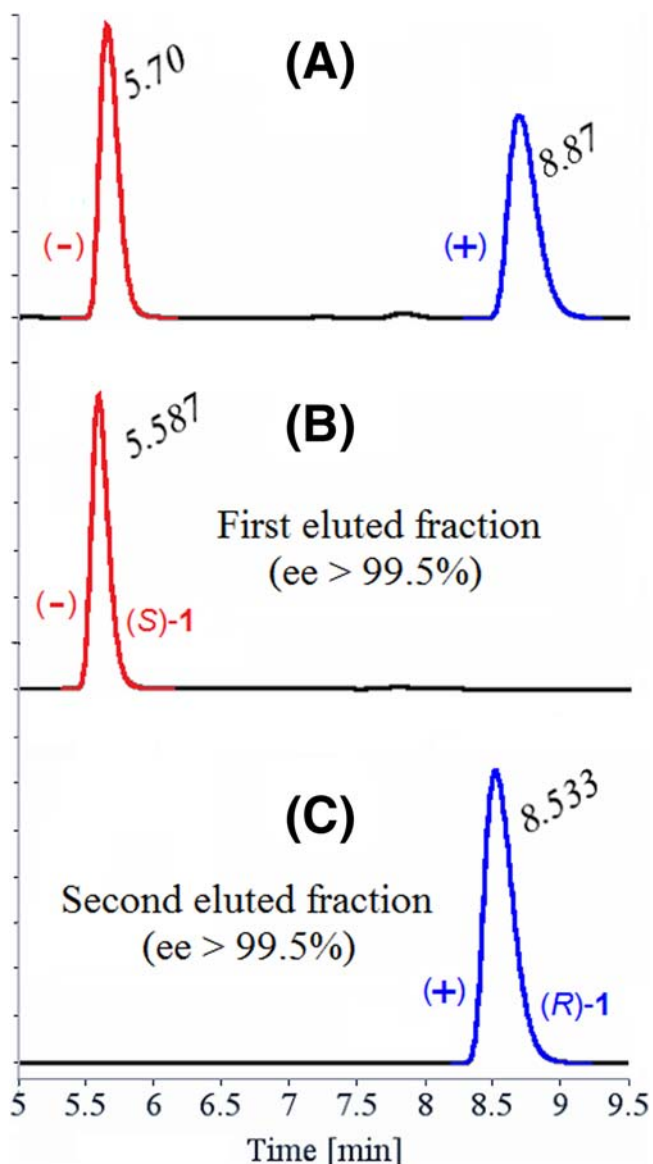


FIGURE 3 (A) Analytical HPLC enantioseparation of *rac*-**1** and (B,C) preparative HPLC chromatograms of the separated enantiomers from *rac*-**1** under UV (254 nm) detection

obtained at a retention time of 5.59 and 8.53 min, respectively (Figure 3B,C). The two separated enantiomers of **1** are characterized by optical rotation measurements and ECD and UV spectra (see Appendix S1 for characterization data). Two highly enantiopure eluted fractions show opposite values in optical rotation and ECD spectra, and same UV spectra. The assignment of the absolute configuration of both eluted fraction (i.e., the (*S*)-enantiomer is the first eluted) is based on the analytical HPLC chromatogram of the synthesized (*R*)-**1** (see Section 3.3) and the comparison of calculated UV and ECD spectra for (*S*)-enantiomer (DFT and TD-DFT calculations) with experimental ones for the first eluted

fraction (see Appendix S1 for more details about the absolute configuration determination by comparison of calculated and experimental ECD and UV spectra). Thanks to this assignment and ECD spectra, we can conclude that (*R*)-**1** has a positive and a negative maximum of same intensity at 205 and 219 nm, respectively, with an additional negative maximum of lower intensity at 254 nm, and a specific rotation $[\alpha]_D^{25} = +37$ (CH_2Cl_2 , $c = 0.19$) (see Appendix S1 for characterization data).

3.3 | Determination of the percentage of racemization accompanying the synthesis of (*R*)-ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate, (*R*)-**1**

The ee value of the synthesized (*R*)-**1** is determined by analytical chiral HPLC with the best chromatographic conditions obtained in Section 3.2 (entry 3, Table 1). The analytical HPLC chromatograms of (*R*)-**1** obtained from the two repeated enantioselective synthesis is given in Figure 4A,B. Ee values of $\approx 55\%$ and $\approx 66\%$ are obtained for the synthesized (*R*)-**1** highlighting the formation of the undesired (*S*)-enantiomer in $\approx 23\%$ and $\approx 17\%$, respectively. These data are used to estimate the percentage of the racemization process compared to that of double $\text{S}_{\text{N}}2$ process. It is interesting to note that the percentage of racemization is evaluated at around 34–46% (Figure 4A,B) indicating that the racemization is not the major mechanism involved in this reaction.

To confirm that the racemization is only due to the formation of the ethyl 2-bromoacrylate **3** intermediate as mentioned in Figure 2, the influence of the experimental (i.e., anhydrous K_2CO_3 , refluxed dry acetone, 16 h), and purification (i.e. flash chromatography (Petroleum ether/EtOAc, 30/70, v/v)) conditions on (*R*)-**1** and/or (*R*)-**2** is investigated (Table 2 and Figure 4C). First, to be sure that experimental conditions and purification step do not induce a racemization of (*R*)-**1**, pure enantiomer of (*R*)-**1** isolated by preparative chiral HPLC (ee > 99.5%) is placed in dry acetone under reflux for 16 h in presence of anhydrous K_2CO_3 (entry 1, Table 2) or passed through a silica gel column chromatography using Petroleum ether/EtOAc (30/70, v/v) as eluent (entry 2, Table 2). In both cases, analytical chiral HPLC chromatogram of the recovered (*R*)-**1** shows no racemization (ee > 99.5%), indicating that the racemization only occurs before the formation of (*R*)-**1**. Second, to confirm that racemization of **1** is proceeding by the postulated mechanism involving ethyl 2-bromoacrylate **3** as intermediate, enantiopure (*R*)-**2** (ee > 99.5%) is brought into solution in dry acetone with anhydrous K_2CO_3 and refluxed for 16 h (entry

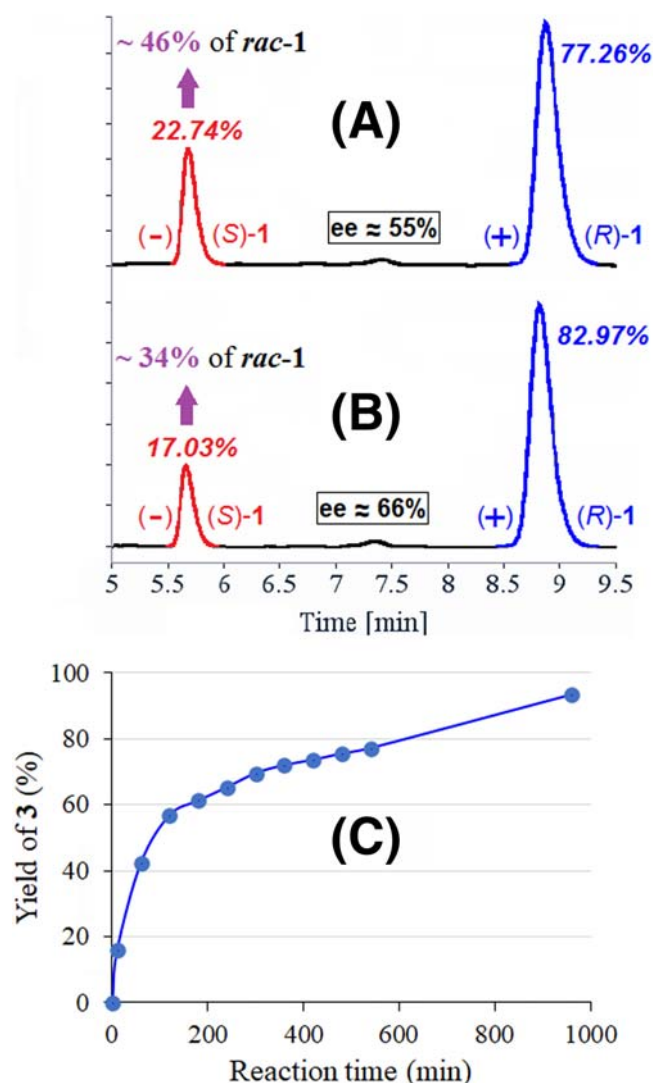


FIGURE 4 (A,B) Analytical HPLC chromatograms of the synthesized (*R*)-1 (synthesis performed twice) and (C) progress of the synthesis of **3** from (*R*)-2 monitored by achiral HPLC under UV detection (254 nm)

3, Table 2). The reaction progress and the conversion yield (%) of (*R*)-2 into **3** are estimated using analytical achiral HPLC analysis (see Section 2.5) by comparing the ratio of peak areas of **3** and (*R*)-2 (retention times of 7.8 and 9.9 min, respectively) (Figure 4C). The monitoring of the reaction indicates a fast dehydrobromination of (*R*)-2 into **3** during the first 2 h (i.e., conversion of about 57% of (*R*)-2 to **3**), followed by a slower increase of dehydrobromination during the rest of the reaction, reaching a maximum of around 93% within 16 h (Figure 4C). Given that percentage of racemization in the enantioselective synthesis of (*R*)-2 does not exceed 46% (Figure 4A,B), this means that the first step in the double S_N2 process (i.e., substitution of primary alkyl bromide by the amine group) proceeds very quickly in less than 1–2 h to limit the dehydrobromination reaction (Scheme 2).

Finally, we decided to study the influence of experimental conditions on the degree of racemization of (*R*)-1. To achieve this, we resynthesized (*R*)-1 at a 50-mg scale of 2-aminophenol by changing the reaction conditions (reaction times, temperature, solvent or type of base, etc.) under the same experimental procedure described in Section 2.3 (Table 3). All the results are compared to the initial conditions used to synthesize (*R*)-1 (entry 1, Table 3). The use of lower reaction times (4 and 10 h, entries 2 and 3, Table 3) reduced the isolated yield of (*R*)-1 while maintaining a similar degree of racemization (\approx 30–40%), and it is also noteworthy that the TLC spots of the starting materials disappear after approximately 2 h. These findings confirm that the racemization and the first S_N2 reaction in the double S_N2 process occur quickly (less than 2 h) as postulated in Scheme 2 and that the second S_N2 reaction in the double S_N2 process yielding (*R*)-1 takes more time to proceed. Decreasing the temperature from 60°C (entry 1, Table 3) to 40°C and 20°C (entries

TABLE 2 Study of the racemization process of (*R*)-1

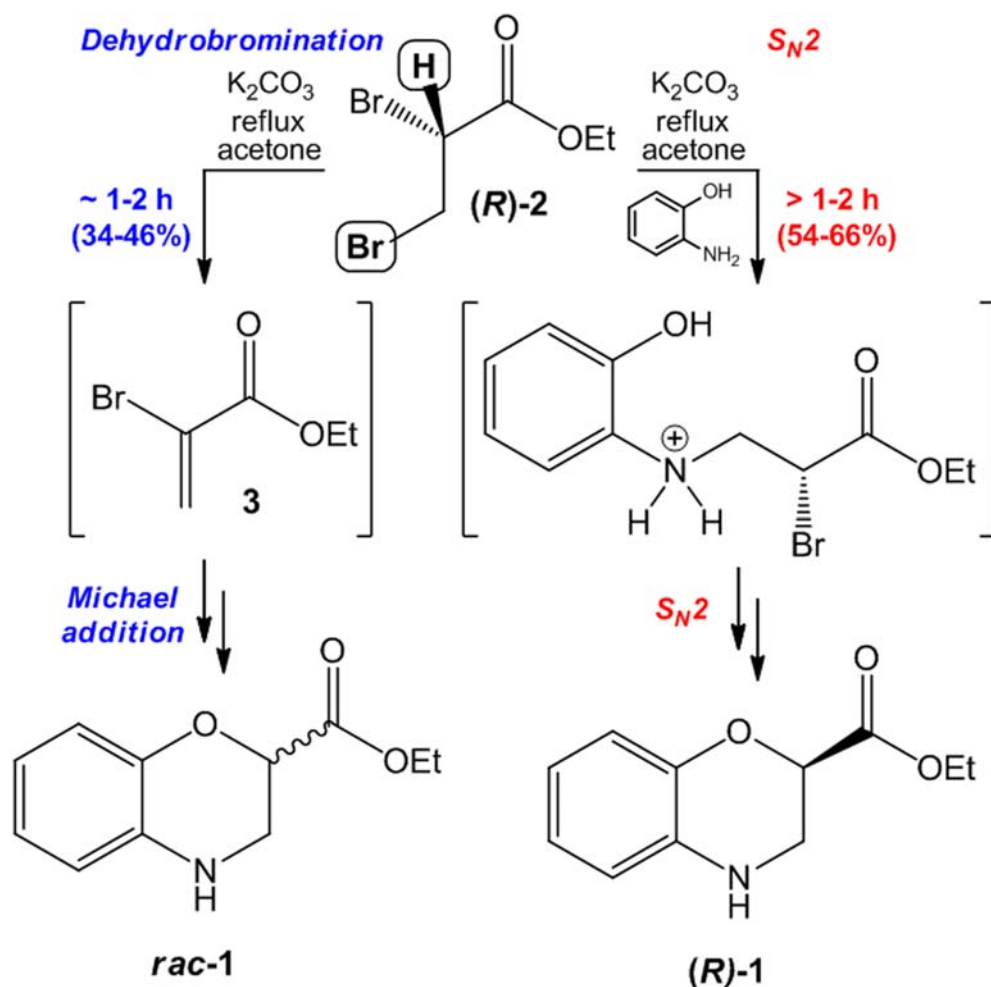
Entry	Compound ^a	Conditions	Results
1	(<i>R</i>)-1	Experimental ^b	The resulted mixture was extracted with CH ₂ Cl ₂ and concentrated in vacuo. No racemization was observed by analytical chiral HPLC analysis using Lux-Cellulose-2 [®] CSP.
2	(<i>R</i>)-1	Purification ^c	The collected fraction was concentrated in vacuo. No racemization was observed by analytical chiral HPLC analysis using Lux-Cellulose-2 [®] CSP.
3	(<i>R</i>)-2	Experimental ^b	The reaction progress (i.e., formation of 3 from (<i>R</i>)-2) was monitored by achiral HPLC using Nucleosil 300–7 C4 column (see Figure 4C).

^aee > 99.5%.

^banhydrous K₂CO₃ (2.2 equiv.), refluxed dry acetone, 16 h.

^cflash chromatography (Petroleum ether/EtOAc, 30/70, v/v).

SCHEME 2 Racemization and double S_N2 processes involving in the synthesis of (*R*)-1



4 and 5, Table 3) does not drastically reduce the degree of racemization ($\approx 22-28\%$) but does negatively affect the isolated yield of (*R*)-1 by lowering it to 20–45%. Aware that the use of K_2CO_3 with (*R*)-2 is responsible for the formation of **3** and so the racemization of (*R*)-1, we investigated the influence of K_2CO_3 addition and equivalent (entries 6–8, Table 3) and also the influence of (*R*)-2 addition (entries 9 and 10, Table 3). No reaction is observed by using one equivalent of K_2CO_3 or with addition of K_2CO_3 or (*R*)-2 after 1 h, emphasizing the importance of the presence of K_2CO_3 and (*R*)-2 from the beginning of the reaction. However, only a moderate yield of 20–34% is achieved with addition of K_2CO_3 or (*R*)-2 after 30 min without significant improvement in degree of racemization. The solvent's change from refluxed acetone (entry 1, Table 3) to refluxed acetonitrile (MeCN) or dichloromethane (DCM) (entries 11 and 12, Table 3) has a negative impact on the isolated yield of (*R*)-1 without any improvement in the degree of racemization. In the initial conditions (entry 1, Table 3), K_2CO_3 ($pK_a \approx 10.3$) is used to deprotonate 2-aminophenol ($pK_a \approx 9.7$), but these basic conditions are also sufficient for the

dehydrobromination of (*R*)-2 (i.e., deprotonation of the H-atom alpha to the ester group). The replacement of K_2CO_3 with weaker bases such as 2,6-lutidine ($pK_a \approx 6.7$) or $NaHCO_3$ ($pK_a \approx 6.4$) (entries 13 and 14, Table 3) is unsuccessful, while the use of NEt_3 (entry 15, Table 3) having a similar basicity ($pK_a \approx 10.8$) led to a high degree of racemization (90%). This high racemization can be explained by the fact that the synthesis of **3** through dehydrobromination of (*R*)-2 in the presence of NEt_3 (1.1 eq.) in acetone at room temperature is achieved with 97% yield in 30 min.⁴² Finally, a stronger base (KOH, $pK_a \approx 13.5$) is also tried (entry 15, Table 3) after necessary modifications in the experimental conditions (i.e., refluxed acetone is replaced by refluxed toluene to avoid the formation of enolate from acetone and a Dean-Stark is also used to remove water from the reaction medium). This last attempt gives (*R*)-1 in 9% isolated yield with high degree of racemization (70%). In summary, this study revealed that notwithstanding the changes in the experimental conditions, the synthesis of (*R*)-1 is always accompanied by a racemization rate which is difficult in reducing.

TABLE 3 Study of experimental conditions influence on the degree of racemization of (*R*)-1

Nc1ccc(O)cc1 + CCOC(=O)C[C@H](Br)C[C@H](Br)C $\xrightarrow[\text{Time}]{\text{Base, Dry solvent, T (}^\circ\text{C)}}$ CCOC(=O)C[C@H]1CNc2ccccc2O1

2-aminophenol (1.0 eq, 50 mg) + (*R*)-2 (ee > 99.5%, 1.1 eq) \rightarrow (*R*)-1

Entry	Base	Dry solvent	T (°C)	Time (h)	Yield of (<i>R</i>)-1 (%) ^a	Ee of (<i>R</i>)-1 (%)	Racemization (%)
Initial conditions ^b							
1	K ₂ CO ₃ (2.2 eq.)	Acetone refluxed	60	16	85	66–55	34–46
Influence of reaction time ^b							
2	K ₂ CO ₃ (2.2 eq.)	Acetone refluxed	60	10	46	60	40
3	K ₂ CO ₃ (2.2 eq.)	Acetone refluxed	60	4	32	70	30
Influence of temperature							
4	K ₂ CO ₃ (2.2 eq.)	Acetone	40	16	40	78	22
5	K ₂ CO ₃ (2.2 eq.)	Acetone	20	16	25	72	28
Influence of K ₂ CO ₃ addition and equivalent							
6 ^c	K ₂ CO ₃ (2.2 eq.)	Acetone refluxed	60	16	34	80	20
7 ^d	K ₂ CO ₃ (2.2 eq.)	Acetone refluxed	60	16	0 ^e	—	—
8	K ₂ CO ₃ (1.0 eq.)	Acetone refluxed	60	16	0 ^e	—	—
Influence of (<i>R</i>)-2 addition							
9 ^f	K ₂ CO ₃ (2.2 eq.)	Acetone refluxed	60	16	20	73	27
10 ^g	K ₂ CO ₃ (2.2 eq.)	Acetone refluxed	60	16	0 ^e	—	—
Influence of solvent							
11	K ₂ CO ₃ (2.2 eq.)	DCM refluxed	40	16	13	62	38
12	K ₂ CO ₃ (2.2 eq.)	MeCN refluxed	80	16	46	68	32
Influence of type of base							
13	NaHCO ₃ (2.2 eq.)	Acetone refluxed	60	16	0 ^e	—	—
14	2,6-lutidine (2.2 eq.)	Acetone refluxed	60	16	0 ^e	—	—
15	Et ₃ N (2.2 eq.)	Acetone refluxed	60	16	78	10	90
Influence of type of base and solvent							
16 ^h	KOH (2.2 eq.)	Toluene refluxed	112	16	9	30	70

^aIsolated yield after silica gel chromatography purification.

^bThe TLC spots of the starting materials disappear after a reaction time of around 2 h.

^cAddition of K₂CO₃ after 30 min.

^dAddition of K₂CO₃ after 1 h.

^eNo conversion was observed on TLC.

^fAddition of (*R*)-2 after 30 min.

^gAddition of (*R*)-2 after 1 h.

^hWith KOH, refluxed dry acetone (60°C) was replaced by refluxed dry toluene (112°C) to avoid the formation of enolate from acetone and a Dean-Stark was also used to remove water from the reaction medium.

4 | CONCLUSIONS

In this work, the enantioselective version of the known ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate synthesis from (*R*)-ethyl 2,3-dibromopropionate and 2-aminophenol in the presence of K_2CO_3 in refluxing acetone was investigated for the first time. This reaction normally should be proceeded via a double S_N2 reaction, but a racemization is postulated to occur through a dehydrobromination of (*R*)-ethyl 2,3-dibromopropionate, yielding the ethyl 2-bromoacrylate. The objectives of this study were to quantify the racemization rate on carrying out this enantioselective synthesis and to offer a cost-efficient alternative for the obtainment of both pure enantiomers. To achieve these goals, analytical HPLC enantioseparation of the synthesized racemic form was investigated and successfully run. Based on the best analytical enantioseparation results (Lux-Cellulose-2[®] CSP, $\alpha = 1.70$ and $R_s = 10.09$), the synthesized (*R*)-enantiomer indicated an ee around 55–66%, highlighting a racemization rate of ≈ 34 –46%. The influence study of the experimental and purification conditions on the racemization was performed and revealed that (i) racemization only occurred before the formation of the (*R*)-enantiomer and (ii) despite a very fast dehydrobromination of (*R*)-ethyl 2,3-dibromopropionate with K_2CO_3 in refluxing acetone, the initiation of the double S_N2 process took place more faster to limit the racemization rate. Accordingly, because racemization occurred during the preparation of the molecule and not at a later stage, a study of the influence of experimental conditions (reaction times, temperature, solvent or type of base, etc.) on the degree of racemization of (*R*)-**1** was performed. The results revealed that despite various changes in the experimental conditions, a racemization rate that was difficult to reduce, always accompanied the synthesis of the (*R*)-enantiomer. In parallel, the preparative HPLC enantioseparation of the synthesized racemic form in multigram scale (12 g) led to the obtention of both pure enantiomers in high enantiopurity (ee > 99.5%), offering a cost-efficient solution to overcome the problem of racemization. Both enantiomers were characterized by optical rotation measurements and ECD and UV spectra. The absolute configuration assignment of both eluted enantiomers (i.e., the (*S*)-enantiomer is the first eluted) was supported by the analytical HPLC chromatogram of the synthesized (*R*)-enantiomer and the comparison of calculated UV and ECD spectra for (*S*)-enantiomer (DFT and TD-DFT calculations) with experimental ones for the first eluted fraction. The obtained (*R*)- and (*S*)-enantiomers of ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate will serve as key starting building blocks for synthesizing

broadly varied new enantiopure heterocyclic therapeutic drugs or as β -amino acid analogs for designing new pseudopeptides and foldamers.

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