Received: 2 February 2018 | Revised: 13 March 2018 | Accepted: 28 March 2018

DOI: 10.1002/chir.22968

REGULAR ARTICLE

WILEY

How do reaction conditions affect the enantiopure synthesis of 2-substituted-1,4-benzodioxane derivatives?

Valentina Straniero | Andrea Casiraghi | Laura Fumagalli | Ermanno Valoti

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Milan, Italy

Correspondence
Valentina Straniero, Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Luigi Mangiagalli 25, 20133 Milan, Italy.
Email: valentina.straniero@unimi.it

Abstract

Several biologically active compounds structurally include the enantiopure 2-substituted-1,4-benzodioxane scaffold. The straightforward racemization that affects reactions involving most of the common chemical reactives is thus a crucial issue. The developing of a completely stereo-controlled synthetic route that does not affect the enantiomeric excess is consequently mandatory. It is also important to set up a reliable chiral HPLC method, able to follow the reaction, and to improve the synthetic performances. Here, we report the chiral investigation of two different synthons, we specifically evaluated the synthetic pathways that could be run in order to afford them, avoiding the racemization processes, which could normally occur in basic conditions. In addition, we developed peculiar chiral HPLC methods in order to resolve the enantiomers, define the enantiomeric excess, and fully characterize these compounds.

KEYWORDS

1,4-benzodioxane, enantioselective reaction, enantiopure Weinreb amide, enantioseparation, racemate DSC analysis

1 | INTRODUCTION

The 1,4-benzodioxane scaffold is a common building block of several biologically active molecules, acting towards a wide variety of pharmacological targets. The adrenergic,[1-3] the catecholaminergic,[4-6] and the dopaminergic[7] systems are the most significant areas of interest in which they are involved in.

Recently, we deeply studied the 1,4-benzodioxane moiety and particularly the 2-substituted derivatives, in their interaction with the human enzyme farnesyl transferase (FTase)[8-10] and the prokaryotic protein filamentous temperature-sensitive Z (FtsZ),[11] developing potent antiproliferative and antimicrobial agents, respectively.

Abbreviations: CDI, (1,1'-Carbonyldiimidazole); DCM, (Dichloromethane); FTase, (Farnesyl transferase); FtsZ, (Filamentous temperature-sensitive Z); IPA, (Isopropyl alcohol); IPE, (Isopropyl ether); THF, (Tetrahydrofuran).

Working on FtsZ inhibitors, we have thoroughly demonstrated[12,13] that the two oxygen atoms of the 1,4-benzodioxane play an essential role in binding the target, and, moreover, that the stereochemistry of carbon 2 has a crucial outcome in interacting with FtsZ.

A similar behavior was already seen for WB4101 analogues[14,15] and other 1,4-benzodioxane lignan natural products.[16]

Preserving the correct and untouched chiral center is therefore mandatory, as well as monitoring each single synthetic step, in order to verify the goodness of the synthesis and to accomplish enantiopure derivatives. In the latest months, designing novel FtsZ inhibitors, we followed the idea used by Haydon and coworkers,[17] consisting in the introduction of a methyl or a hydroxymethyl pendant on the methylene linker between the benzodioxane moiety and the benzamide scaffold. As a result, we needed to obtain the enantiopure methyl

ketone 2 (Scheme 1), as a starting point for the subsequent synthetic steps.

We firstly considered the straightforward synthetic route shown in Scheme 1, applied by Gershengorn and coworkers few years ago.[18]

They started from the racemic 1,4-benzodioxane-2-carboxylic acid (I) and followed our procedure,[19] consisting in the resolution by a diastereomeric salts approach, using the *p*-methyl or *p*-nitro substituted 1-phenylethylamine enantiomers. The (*S*)-acid I was converted into the Weinreb amide 1 and then further transformed into methyl ketone 2.

However, the high costs of the substituted 1-phenylethylamines prompted us to consider other established resolving procedures.

Among them, optimal results were achieved via enzymatic resolutions of racemic 1,4-benzodioxan-2-carboxylic acid or esters[20-24] or by diastereomeric crystallization with the (+)-dehydroabietylamine.[25]

Pondering either yields and commercial availability, we followed the previously defined satisfactory method, in which the 1,4-benzodioxan-2-carboxylic acid was firstly resolved with the chiral dehydroabietylamine acetate and then converted into the methyl ester, affording crystallized (*S*)-II with high yield and high enantiomeric excess.[25]

We therefore aimed the first hydrolysis of the ester II and then the following of the above-mentioned Gershengorn procedure.

Nevertheless, we guessed if the basicity coming from a classical hydrolysis medium, as well as from the imidazole or from the Grignard reagent, could somehow compromise the enantiomeric purity of 1, 2 and I.

Indeed, profuse studies on carboxyl-containing derivatives, having a chiral carbon atom in α-position of the carboxylic function, such as esters of arylpropionic acids and amino acids, exhaustively demonstrated how they are highly susceptible to racemization, under basic treatment.

As a result, here, we report an extensive work, comprehensive of synthetic and analytic efforts, aimed to (1) achieve the (*S*)-carboxylic acid I, the (*S*)-Weinreb amide 1, and the (*S*)-methyl ketone 2, with high-yield and enantiopure conversions, starting from (*S*)-methyl ester II (Figure 1); (2) fully characterize the products, since no information on optical rotations and melting points of 1 and 2 are reported in literature so far; (3) follow in real-time the enantiomeric changes, by a suitable and reliable chiral HPLC analytic method, directly on the reaction crude.

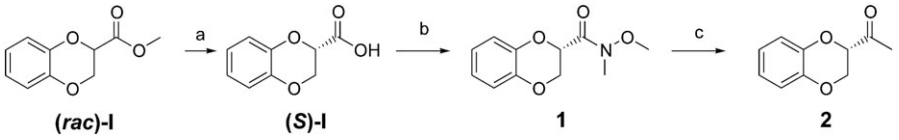
2 | MATERIALS AND METHODS

2.1 | General

All reactives (aqueous HCl 37%, thionyl chloride ($SOCl_2$), N,O-dimethyl hydroxylamine*HCl, MeMgBr 3.0 M in diethylether) were purchased from Sigma Aldrich and were used without further purification. The solvents, such as acetone, dichloromethane (DCM), tetrahydrofuran (THF), analytical grade n-hexane, analytical grade ethanol, and analytical grade isopropyl alcohol (IPA), were purchased from Sigma Aldrich.

Silica gel matrix, with fluorescent indicator 254 nm, was used in analytical thin-layer chromatography (TLC on aluminum foils), and silica gel (particle size 40-63 μm, Merck) was used in flash chromatography on Biotage IsoleraTM One; visualizations were accomplished with UV light (λ 254 nm).

^{1}H NMR spectra were measured by Varian Mercury 300 NMR spectrometer/Oxford Narrow Bore superconducting magnet operating at 300 MHz. Chemical shifts (δ) are reported in ppm relative to residual solvent as internal standard. Signal multiplicity is used according



SCHEME 1 Literature synthetic scheme for the obtainment of Weinreb amide (1) and methyl ketone (2); [Reactives and solvents: (a) [see Bolchi et al[19]; (b) 1,1'-Carbonyldiimidazole (CDI) (1.1 eq), CH_2Cl_2, 1 h; N,O-dimethyl hydroxylamine hydrochloride; (c) MeMgCl (1.5 eq), THF, 0°C, 30 min]

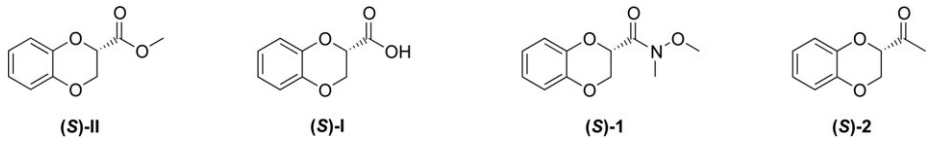


FIGURE 1 Compounds 1, 2, I, and II, which synthesis, HPLC analysis and thermal characterization are the objects of the present work

to the following abbreviations: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quadruplet, m = multiplet, and bs = broad singlet. Elemental analyses of the new substances are within 0.40% of theoretical values. IR spectra were recorded with a FT-IR Spectrum TwoTM Perkin Elmer Spectrometer. Melting point were determined by DSC analysis over a TA Instruments DSC 1020 apparatus.

Optical rotations were measured in a 1 dm cell of 1 or 5 mL capacity using a Jasco P − 1010 polarimeter. The enantioseparation analyses were performed on Lux 3μTM Cellulose-1 (150 × 4.6 mm), purchased from Phenomenex. The HPLC measurements were made on a Hewlett Packard instrument, equipped with a HP 1050 pump, a HP 1050 UV detector, and a Merck-Hitachi D-7000 Chromatographic Data Station Software HPLC System Manager.

2.2 | Chiral HPLC evaluation

The chiral HPLC method was set up, using a Phenomenex Lux 3 μ Cellulose-1 column, which was flushed with freshly prepared *n*-hexane/IPA (90:10, *v*/v) for 1 and 2 or *n*-hexane/IPA (85:15, v/v) + 1.5% formic acid for I, until column pressure was stable.

All the employed mobile phases were degassed with 10 minutes sonication before use; the investigated samples were prepared through dissolution either of the crude reaction mixtures or of the purified products in the selected mobile phase, at the approximate concentrations of 0.5-1 mg/m, filtered through a 0.45 μm filter and analyzed. The injection volume was 20 μL. Owing to the presence of an aromatic portion in each compound, chromatograms were recorded at 254 nm.

2.3 | Synthesis

2.3.1 | Synthesis of (*S*)-methyl 1,4-benzodioxan-2-carboxylate (II)

The methyl ester **II** was achieved following method D reported in reference 25. Further crystallization from isopropyl ether afforded 1.3 g of **II** as a white solid that was analyzed by chiral HPLC.[25] Optical purity, mp, and $[\alpha]_D^{25}$ were in line with literature data, as well as IR, ^{1}H-NMR, and ^{13}C-NMR spectra.[25]

2.3.2 | Synthesis of (*S*)-1,4-Benzodioxan-2-carboxylic acid (I)

II (1.23 g, 6.33 mmol) was dissolved in acetone (6 mL) and slowly amounted of a solution of aqueous HCl 10% (12 mL, 31.67 mmol) at 0°C. The mixture was firstly warmed to room temperature, then refluxed and stirred for 18 hours; after that time, the reaction mixture was concentrated under reduced pressure and extracted with DCM (2 × x10 mL). The collected organic layers were washed with brine (3 × 10 mL), dried over Na_2SO_4, filtered and concentrated, yielding 1.14 g (quantitative yield) of **I** as a white solid. Crystallization from 8 mL of toluene further enriched its enantiopurity; the e.e. resulted to be in the range of 98.0%-99.64%, as seen by chiral HPLC (see Section 2.2). $[\alpha]_D^{25}$ and mp were in line with literature data, as well as IR, ^{1}H-NMR, and ^{13}C-NMR spectra.[25]

2.3.3 | Synthesis of (*S*)-N-Methoxy-N-methyl-1,4-benzodioxane-2-carboxamide (1)

CDI procedure

1,1'-Carbonyldiimidazole (0.51 g, 3.06 mmol) was added portion-wise to a solution of I (0.5 g, 2.78 mmol) in DCM, at a specific temperature, depending on the trial (see Table 1). The reaction mixture was stirred at that temperature for a specific time, in a range of 0-6 hours, and then N,O-dimethyl hydroxylamine hydrochloride (0.27 g, 2.78 mmol) was added. The mixture was stirred at room temperature for 18 hours, then diluted with additional DCM (15 mL), washed with 10% aqueous HCl (3 × 10 mL), 10% aqueous $NaHCO_3$ (3×10 mL), and brine (3 × 10 mL), dried over Na_2SO_4, filtered and concentrated under vacuum to yield a residue that was purified by flash chromatography on silica gel. Elution with 7/3 cyclohexane/ethyl acetate gave 1 as a white solid. The enantiomeric purity was determined by chiral HPLC (see Section 2.2 for the method and Section 3 for the results).

mp, e.e., and $[\alpha]_D^{25}$ differed in a range depending on the trial (see Table 1), moving from 66.0°C, 0.0%, and 0.0°C (trial g) to 86.6°C, 98.4% and −13.4 (trial a), respectively.

Thionyl chloride method

Thionyl chloride (0.84 mL, 11.43 mmol) was added dropwise to a solution of I (1.03 g, 5.72 mmol) in DCM (10 mL) at 0°C. After stirring at 0°C for 15 minutes, the reaction mixture was warmed to room temperature, then refluxed and stirred for 1 hour. At completion, the solution was concentrated to dryness, and the crude resumed with DCM (10 mL) and slowly amounted of N,O-dimethyl hydroxylamine hydrochloride (1.11 g, 11.43 mmol). The mixture was stirred at room temperature for 18 hours, then diluted with additional DCM (15 mL), washed with 10% aqueous HCl (3 × 10 mL), 10% aqueous $NaHCO_3$ (3 × 10 mL) and brine (3 × 10 mL), dried over Na_2SO_4, filtered and concentrated under vacuum to yield a residue that was purified by

TABLE 1 Evaluation of the decrease of the e.e. (expressed as Δ % e.e.), during Weinreb amide (1) formation, considering the changes in reactives, temperatures, and activation times

Trial	Reactive	Temperature	Activation Time	Δ % e.e.
a)	CDI	−10°C	1 h	1.0%
b)	CDI	0°C	0′	1.2%
c)	CDI	0°C	15’	1.9%
d)	CDI	0°C	30’	3.5%
e)	CDI	0°C	1 h	10.3%
f)	CDI	RT	1 h	27.2%
g)	CDI	RT	6 h	100%
h)	$SOCl_2$	Reflux	/	0%

flash chromatography on silica gel. Elution with 7/3 cyclohexane/ethyl acetate gave 930 mg (73.0%) of 1 as a white solid. $[\alpha]_D^{25}$, mp, and e.e. resulted to be of 87.7°C for mp; 99.4% for the e.e., and −14.8 for $[\alpha]_D^{25}$ (c 1, $CHCl_3$) (see Table 1, trial h), respectively. The enantiomeric purity was determined by chiral HPLC (see Section 2.2 for the method and Section 3 for the results). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.00-6.97 (m, 1H), 6.89-6.85 (m, 3H), 5.05 (dd, *J* = 7.0, 2.6 Hz, 1H), 4.43 (dd, *J* = 11.4, 2.6 Hz, 1H), 4.26 (dd, *J* = 11.4, 7.0 Hz, 1H), 3.80 (s, 3H), 3.26 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 167.4, 142.99, 142.96, 121.8, 121.7, 117.3, 117.1, 70.4, 64.7, 61.8, 32.3. Anal. Calcd for $C_{11}H_{13}NO_4$ (223.08): C, 59.19; H, 5.87; N, 6.27; O, 28.67.

2.3.4 | Synthesis of (*S*)-2-Acetyl-1,4-benzodioxane (2):

Methylmagnesium bromide 3.0 M in diethylether (equivalents depending on the trial) was added dropwise to a solution of 1 (0.81 g, 3.63 mmol) in dry THF (15 mL) at 0°C or at room temperature under nitrogen atmosphere. The mixture was stirred at that temperature for 30 minutes and slowly poured into 10% aqueous HCl (20 mL) and ethyl acetate (20 mL) at 0°C. The organic layer was washed with brine (3 × 10 mL), dried over Na_2SO_4, filtered and concentrated under vacuum to yield a residue that was purified by flash chromatography on silica gel. Elution with 85/15 cyclohexane/ethyl acetate gave 2 as a white solid. Yields depending on the trial (see Table 2). The enantiomeric purity was determined by chiral HPLC (see Section 2.2 for the method and Section 3 for the results). mp, e.e. and $[\alpha]_D^{25}$ differed in a range depending on the trials (see Table 2); specifically moving from 36.6 °C, 58.8% and − 15.5 (trial k) to 67.9 °C, 99.4% and −95.9 (trial j). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.06–6.94 (m, 1H), 6.95–6.82 (m, 3H), 4.62 (dd, *J* = 5.3, 3.3 Hz, 1H), 4.43 (dd, *J* = 11.4, 3.3 Hz, 1H), 4.28 (dd, *J* = 11.4, 5.3 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 206.1, 143.2, 142.2, 122.1, 122.0, 117.5, 117.3, 78.2, 64.4, 26.8. Anal. Calcd for $C_{10}H_{10}O_3$ (178.06): C, 67.41; H, 5.66; O, 26.94.

3 | RESULTS AND DISCUSSION

Our synthetic and analytic investigations could be divided into the three different reactions: (1) the ester hydrolysis, avoiding basic conditions; (2) the Weinreb amide formation; (3) the methyl ketone obtainment. Here, we report the main results; the synthetic protocols were previously reported in Section 2.3, while further experimental data (NMR spectra, DSC curves, IR spectra, and HPLC chromatograms) are included in the Supporting information.

Firstly, after having assessed that basic hydrolysis of methyl ester (*S*)-**II** (e.e. >99%) unequivocally brought to racemization, we thought to accomplish the estereal hydrolysis in aqueous acidic conditions. However, the introduction of a co-solvent was mandatory for the complete dissolution of the ester itself.

TABLE 2 Evaluation of the decrease of the e.e. (expressed as Δ % e.e.), during methyl ketone (2) formation, considering changes in temperatures and equivalents

Trial	Reactive	Temperature	Time	Equivalents	Δ % e.e.
i)	MeMgBr	RT	30 min	1	25%
j)	MeMgBr	0°C	30 min	1	0%
k)	MeMgBr	0°C	30 min	1.5	40%

Therefore, we set-up that using five equivalents of aqueous HCl 10% in five volumes of acetone and stirring for 18 hours let the recovery of (*S*)-**I** carboxylic acid with a simple work-up and an high enantiomeric excess (>99%).

The second step required a more deep evaluation (see Table 1); we firstly applied the same conditions used by Gershengorn and coworkers (Trial **f**): 1 hour of activation time with CDI at room temperature and then 8 hours of stirring at the same temperature, after the addition of the N,O-dimethyl hydroxylamine hydrochloride. Regrettably, chiral HPLC assay showed a partial racemization, quantified to be of a 27% decrease vs the initial enantiomeric excess of the corresponding carboxylic acid. This result forced us to promptly investigate the activation step that is the treatment of **I** with CDI, causing the formation of the acylimidazole.

We considered as variables both the activation time and the reaction temperature, demonstrating that the higher is the temperature, the more significant is the racemization process.

Specifically, keeping 1 hour of activation time (trials **a**, **e**, and **f** in Table 1), the enantiomeric excess decreased of only 1% if the reaction was performed at −10°C (**a**), conversely it reached almost 10% if done at 0°C (**e**) and it rose at 27% if carried out at room temperature (**f**). Despite the racemization, in trials **e** and **f** 1-hour activation time let the complete conversion of the acid into the acylimidazole and an almost quantitative recover of the Weinreb amide. On the contrary, the low temperature of trial **a** limited the conspicuous racemization but strongly affected the conversion lowering the yield to less than 50%.

Moreover, we maintained the reaction temperature at 0°C, and we changed the activation time; as a result, we observed the longer is this time, the more conspicuous is the racemization. The Δ % e.e. moved from 1.2% if no activation time was used (**b**), to almost 2% for 15 minutes of activation (**c**), to 3.5% for 30 minutes (**d**), till more than 10% if the reaction took 1 hour of activation time, as explained before (**e**).

A complete racemization was measured in trial **g**, extending acylimidazole formation to 6 hours at room temperature. Unfortunately, the absence or the strong reduction in activation time significantly affected the conversion of the acid into the acylimidazole. Consequently, even if the racemization was almost untouched (**b** and **c**) the yield loss was quite significant.

This serious drawback prompted us not to evaluate lower temperatures or other reaction parameters but moved us to the use of a different amide synthetic procedure. The novel synthesis should avoid the basicity coming from the imidazole, which is an unavoidable byproduct of these reaction conditions. We then moved to the use of thionyl chloride, which should rapidly bring to the acyl chloride, responsible for the Schotten-Baumann reaction with the N,O-dimethyl hydroxylamine. Successfully, trial **h** brought to a high yield conversion, with unaffected enantiomeric excess (see Figure 2) and a simple recovery of the (*S*)-Weinreb amide **1** (mp: 86.1, $[\alpha]_D^{25} = -14.9$ (c 1, $CHCl_3$), as reported in Table 3).

Eventually, the third step was intensely investigated, due to the evident basicity generated from the Grignard reagent; the most interesting results are summarized in Table 2.

The starting point was the usage of the conditions applied by Gershengorn and coworkers (Trial **k**): 1.5 equivalents of MeMgBr, at 0°C for 30 minutes. Sadly, even if the conversion of the Weinreb amide into the methyl ketone was complete, we evaluated a 40% decrease of the enantiomeric excess.

We thus calculated how temperature and Grignard equivalents affects the racemization process. Firstly, in trial **j** we reduced the equivalents to a single one, observing no change in the enantiomeric excess (see Figure 3) and accomplishing pure (*S*)-methyl ketone (mp: 58.9, $[\alpha]_D^{25} = -95.9$ (c 1, $CHCl_3$), as reported in Table 3).

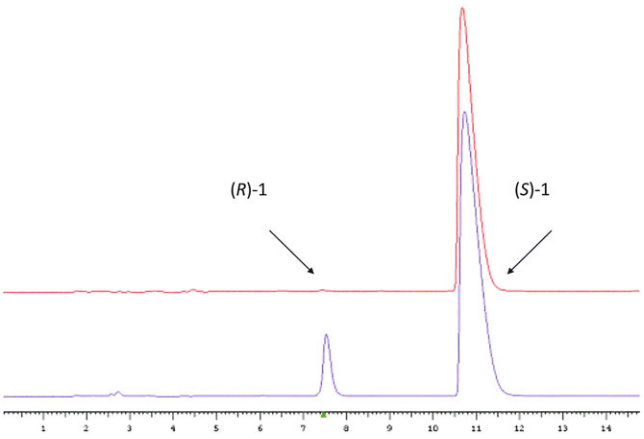


FIGURE 2 HPLC chromatograms comparison between (*S*)-1 (trial **h**, red line) and (*S*)-1 (trial **e**, blue line), using the method reported in Section 2.2

TABLE 3 Experimental data of 1 and 2

Compound	(*rac*)	(*S*)		
	mp	mp	$[\alpha]_D^{25}$ (c 1, $CHCl_3$)	e.e.
1	68.6°C	86.1°C	−14.9	99.4%
2	32.4°C	58.9°C	−95.9	99.4%

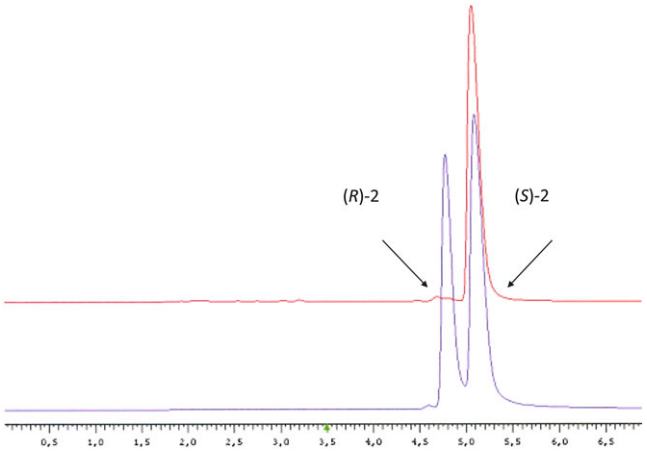


FIGURE 3 HPLC chromatograms comparison between (*S*)-2 (trial **j**, red line) and (*S*)-2 (trial **k**, blue line), using the method reported in Section 2.2

The conversion was complete, the global yield reasonable (assessed around 90%) and completely reliable, even scaling the reaction from tens of milligrams to a few grams.

Furthermore, in order to verify our hypothesis and stress the conditions, we increased the reaction temperature, operating at room temperature (**i**); as expected, the reaction outcome was negative with a loss of enantiopurity, quantified to be of 25%.

The purified compounds, as well as the reaction crudes, coming from the three synthetic steps, were analyzed on chiral HPLC, using a Phenomenex Lux 3 μ-Cellulose 1 column, which proved to be effective in resolving all the samples, as reported in Table 4.

Retention factors k_1 **(a)** e k_2 **(b)** refer respectively to (*R*) and (*S*) enantiomers and are calculated as $k_1 = (t_1 - t_0)/t_0$ and $k_2 = (t_2 - t_0)/t_0$, considering that t_1 and t_2 are the retention times of the former and the latter enantiomers and that t_0 is the solvent retention time (dead time). The separation factor α **(c)** is calculated as the ratio between k_2 and k_1, while resolution Rs (**d**) as 2 times the ratio $(t_2 - t_1)/(w_1 + w_2)$, in which w_1 and w_2 are the peak widths of the two enantiomers.

Furthermore, over the course of our studies on the resolution of 2-substituted 1,4-benzodioxanes we demonstrated by DSC and IR analyses that 1,4-benzodioxane-2-carboxylic acid I is a racemic compounds, whereas the respective methyl ester II is a conglomerate.[25]

We therefore moved to investigate the nature of the racemates of 1 and 2 because of the relevant information and suggestions that could come up. We firstly considered IR spectra, which revealed to be identical and superimposable for both the couples of derivatives, suggesting a presumed nature of conglomerate for 1 and 2 (see Supporting information for all the spectra).

Secondly, the DSC analyses of (*rac*)-1, (*S*)-1 and a number of their mixtures (Figure 4) allowed the construction of the binary phase diagram for mole fractions of (*S*)-1 ranging from 0.5 to 1 (Figure 5). The DSC curves of the differently proportioned mixtures in Figure 4 showed the presence of two peaks, the former at 68°C that is the fusion of the racemate, and the latter representing the fusion of the excess of (*S*)-1, whose temperature value increases with the increment of (*S*)-1 amount. In Figure 5 the

TABLE 4 Enantioseparation parameters

Chiral HPLC parameters				
Compound	k_1[a]	k_2[b]	α[c]	Rs[c]
I	7.13	8.26	1.16	1.60
1	3.17	5.02	1.58	3.01
2	1.50	1.65	1.10	0.41

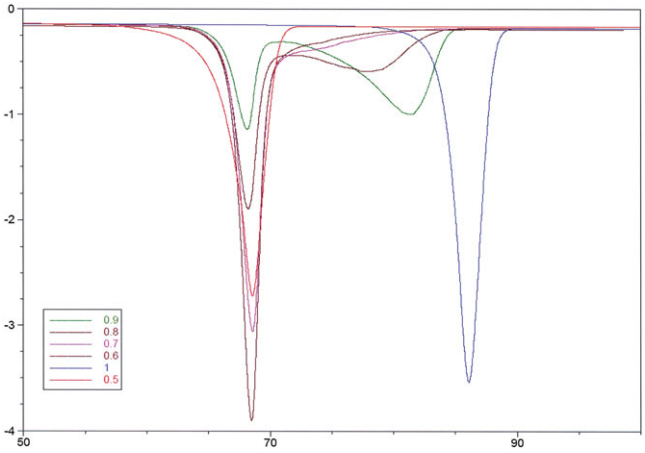


FIGURE 4 Superimposed DSC traces of pure (*S*)-1 (blue), (*rac*)-1 (orange) and mixtures with compositions ranging from 0.5 to 1 (other colors)

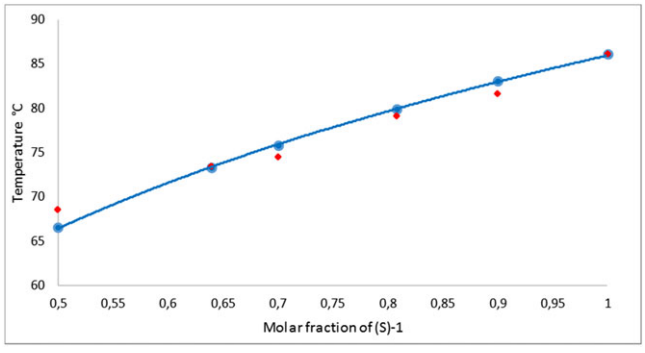


FIGURE 5 Binary melting-point phase diagram of **1**; the blue solid curve comes from the melting point values calculated based on the Schröder–van Laar equation

experimental melting points (red dots) are compared with the theoretical ones (blue dots), calculated using the Schröder–van Laar equation; they fit rather well, confirming our initial hypothesis of 1 as a conglomerate.

DSC analyses were performed also on **2** (Figure 6); they showed that the melting point of the racemate (32.4°C, as reported in Table 3) is more than 25 degree lower than that of the enantiopure (*S*)-**2** (58.9°C), supporting the identification of a conglomerate also for the methyl ketone.

Even if the very low melting point of the racemate limit the handling of the enantiomeric mixtures, the solid binary phase diagram for a number of mixtures of (*S*)-**2** and (*rac*)-**2** was constructed (Figure 7). The good fitting between the theoretical (blue dots) and the experimental (red dots) values of the melting points confirmed methyl ketone- **2** as a conglomerate, too.

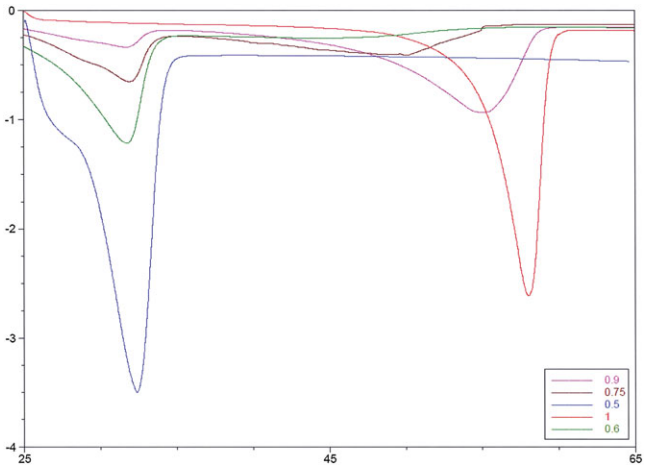


FIGURE 6 Superimposed DSC traces of pure (*S*)-**2** (red), (*rac*)-**2** (blue) and mixtures with compositions ranging from 0.5 to 1 (other colors)

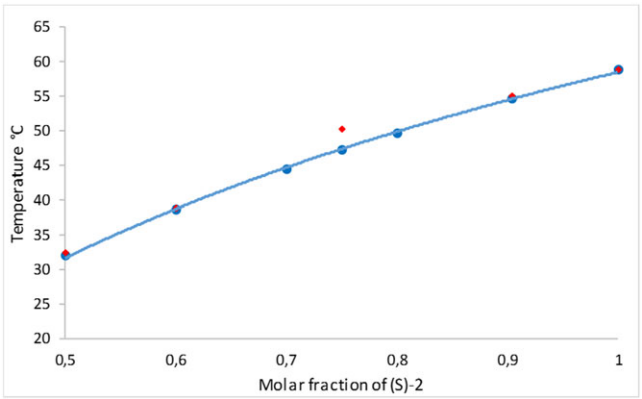


FIGURE 7 Binary melting-point phase diagram of **2**; the blue solid curve comes from the melting point values calculated based on the Schröder–van Laar equation

4 | CONCLUSION

We have developed a completely enantio-controlled synthetic pathway for the obtainment of interesting pharmacologic synthons, evaluating different reaction conditions, and setting a chiral HPLC method, able to fully quantify the enantiopurity of these derivatives.

Additionally, the characterization of the enantiomeric system of the two 1,4-benzodioxane derivatives as a conglomerate was unexploited so far and further enriched the knowledge on this class of compounds.

Finally, this broad study highlights the requirement of having a reliable and real-time enantioseparation method to follow and therefore to define a solid synthetic protocol.

ORCID

Valentina Straniero http://orcid.org/0000-0002-5089-0879

REFERENCES

1. Villa L, Valoti E, Villa AM, et al. Molecular properties of the WB4101 enantiomers and of its chiral methyl derivatives for alpha 1-adrenoceptor recognition. *Farmaco*. 1994;49:587-606.
2. Fumagalli L, Bolchi C, Colleoni S, et al. QSAR study for a novel series of ortho monosubstituted phenoxy analogues of alpha$_1$-adrenoceptor antagonist WB4101. *Bioorg Med Chem*. 2005; 13(7):2547-2559.
3. Pallavicini M, Budriesi R, Fumagalli L, et al. WB4101-related compounds: new, subtype-selective α_1-Adrenoreceptor antagonists (or inverse agonists?). *J Med Chem*. 2006;49(24): 7140-7149.
4. Pallavicini M, Moroni B, Bolchi C, et al. Synthesis and α4β2 nicotinic affinity of unichiral 5-(2-pyrrolidinyl)oxazolidinones and 2-(2-pyrrolidinyl)benzodioxanes. *Bioorg Med Chem Lett*. 2006; 16(21):5610-5615.
5. Bolchi C, Gotti C, Binda M, et al. Unichiral 2-(2′-pyrrolidinyl)-1,4-benzodioxanes: the 2 R,2′ S diastereomer of the N -methyl-7-hydroxy analogue is a potent α4β2- and α6β2-nicotinic acetylcholine receptor partial agonist. *J Med Chem*. 2011;54(21): 7588-7601.
6. Bolchi C, Valoti E, Gotti C, et al. Chemistry and pharmacology of a series of Unichiral analogues of 2-(2-Pyrrolidinyl)-1,4-benzodioxane, Prolinol phenyl ether, and Prolinol 3-Pyridyl ether designed as α4β2-nicotinic acetylcholine receptor agonists. *J Med Chem*. 2015;58(16):6665-6677.
7. Birch AM, Bradley PA, Gill JC, Kerrigan F, Needham PL. *N*-substituted (2,3-Dihydro-1,4-benzodioxin-2-yl)methylamine derivatives as D_2 antagonists/5-HT_{1A} partial agonists with potential as atypical antipsychotic agents. *J Med Chem*. 1999;42:3342-3355.
8. Bolchi C, Pallavicini M, Fumagalli L, et al. New RAS CAAX mimetics: design, synthesis, antiproliferative activity, and RAS

prenylation inhibition. *Bioorg Med Chem Lett*. 2009; 19(18):5500-5504.

9. Bolchi C, Pallavicini M, Bernini SK, et al. Thiazole- and imidazole-containing peptidomimetic inhibitors of protein farnesyltransferase. *Bioorg Med Chem Lett*. 2011;21(18): 5408-5412.
10. Straniero V, Pallavicini M, Chiodini G, et al. Farnesyltransferase inhibitors: CAAX mimetics based on different biaryl scaffolds. *Bioorg Med Chem Lett*. 2014;24(13):2924-2927.
11. Chiodini G, Pallavicini M, Zanotto C, et al. Benzodioxane-benzamides as new bacterial cell division inhibitors. *Eur J Med Chem*. 2015;89:252-265.
12. Straniero V, Pallavicini M, Chiodini G, et al. 3-(Benzodioxan-2-ylmethoxy)-2,6-difluorobenzamides bearing hydrophobic substituents at the 7-position of the benzodioxane nucleus potently inhibit methicillin-resistant Sa and Mtb cell division. *Eur J Med Chem*. 2016;120:227-243.
13. Straniero V, Zanotto C, Straniero L, et al. 2,6-Difluorobenzamide inhibitors of the bacterial cell division protein FtsZ: design, synthesis and structure activity relationship study. *ChemMedChem*. 2017;12(16):1303-1318.
14. Ferri V, Pallavicini M, Piccini D, Valoti E, Villa L. Synthesis, binding affinities for α-adrenoceptor and eudismic analysis of chiral benzodioxane derivatives and their chiral opened analogues. *Farmaco*. 1988;43:1153-1163.
15. Pallavicini M, Fumagalli L, Gobbi M, et al. QSAR study for a novel series of ortho disubstituted phenoxy analogues of α1-adrenoceptor antagonist WB4101. *Eur J Med Chem*. 2006; 41(9):1025-1040.
16. Pilkington LI, Barker D. Synthesis and biology of 1,4-benzodioxane lignan natural products. *Nat Prod Rep*. 2015;32(10):1369-1388.
17. Stokes NR, Baker N, Bennett JM, et al. Design, synthesis and structure–activity relationships of substituted oxazole–benzamide antibacterial inhibitors of FtsZ. *Bioorg Med Chem Lett*. 2014;24(1):353-359.
18. Engel S, Skoumbourdis AP, Childress J, et al. A virtual screen for diverse ligands: discovery of selective G protein-coupled receptor antagonists. *J Am Chem Soc*. 2008;130(15):5115-5123.
19. Bolchi C, Pallavicini M, Fumagalli L, et al. Highly efficient resolutions of 1,4-benzodioxane-2-carboxylic acid with *para* substituted 1-phenylethylamines. *Tet Asym*. 2005;16(9):1639-1643.
20. Kasture SM, Varma R, Kalkote UR, Nene S, Kulkarni BD. Novel enzymatic route for kinetic resolution of (±)1,4-benzodioxan-2-carboxylic acid. *Biochem Eng J*. 2005;27:66-71.
21. Khouili M, Lazar S, Guillaumet G, Coudert G. Synthesis of enantiomerically pure 7-hydroxy-2-substituted-2,3-dihydro-1,4-benzodioxin derivatives. *Tet Asym*. 1994;5:535-536.
22. Varma R, Kasture SM, Nene S, Kalkote UR. Lipases catalyzed enantioselective hydrolysis of (R,S)-methyl 1,4-benzodioxan-2-carboxylate intermediate for (S)-doxazosin mesylate. *World J Microbiol Biotechnol*. 2008;24(4):577-579.
23. Rouf A, Gupta AA, Mushtaq AA, et al. Chemoenzimatic synthesis of piperoxan, prosympal, dibozane, and doxazosin. *Tet:Asym*. 2012;23:1615-1623.
24. Rouf A, Mushtaq AA, Kumar B, Taneja SC. A facile approach to chiral 1,4-benzodioxane toward the syntheses of doxazosin, prosympal, piperoxan, and dibozane. *Tetrahedron Lett*. 2013;54(48):6420-6422.
25. Bolchi C, Fumagalli L, Moroni B, Pallavicini M, Valoti E. A short entry to enantiopure 2-substituted 1,4-benzodioxanes by efficient resolution methods. *Tet Asym*. 2003;14(23):3779-3785.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Straniero V, Casiraghi A, Fumagalli L, Valoti E. How do reaction conditions affect the enantiopure synthesis of 2-substituted-1,4-benzodioxane derivatives? *Chirality*. 2018;1–8. https://doi.org/10.1002/chir.22968