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Conflict of Interest: The authors declare that they have no conflict of interest.

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Enantiodivergent Synthesis of Halofuginone by *Candida Antarctica* Lipase B (CAL-B)-catalyzed Kinetic Resolution in Cyclopentyl Methyl Ether (CPME)

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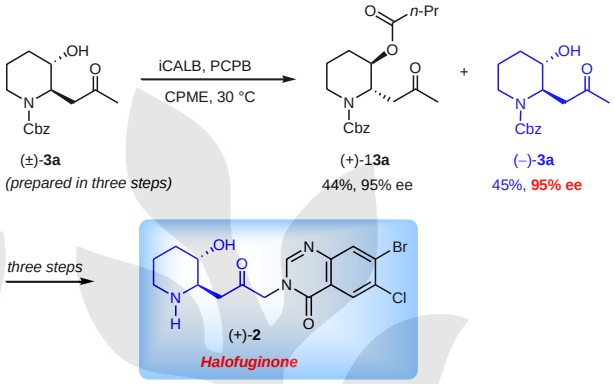

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Abstract The synthesis of both enantiomers of a key intermediate in the synthesis of halofuginone was accomplished by a *Candida antarctica* lipase B-catalyzed kinetic resolution of the corresponding racemate. When the resolution was carried out in the versatile solvent cyclopentyl methyl ether (CPME) using PCPB (p-chlorophenylbutyrate) as the acylating reagent, the highest enantiomeric ratio (*E*) values were measured and highly enantioenriched (95% ee) compounds could be obtained in a single iteration. As an example, one of the two enantiomers was used as a starting material to prepare (+)-halofuginone in a three-step procedure.

Key words Biocatalysis, Lipases, Kinetic resolution, Chiral alcohols, Halofuginone, Green solvent

Febrifugine (**1**, Figure 1) is a naturally occurring compound first isolated from the leaves of the Asian medicinal plant *Dichroa febrifuga*, traditionally used to reduce fever from malaria infection.[1] However, translation of febrifugine into a medicine failed because of serious side-effects such as nausea, vomiting and liver damage.[2] For this reason analogues were synthesized to improve the activity/toxicity profile. Amongst these, halofuginone (**2,** Figure 1), in which the metabolic vulnerable quinazoline protons in **1** were replaced by chlorine and bromine atoms, besides demonstrating to be an effective anti-protozoal drug against several species of *Eimeria* in poultry,[3] proved, in its racemic form, to possess a broad spectrum of pharmacological activities such as anti-fibrosis,[4] anti-tumor,[5] and growth-promoting functions.[6] As reports on the biological activity of the isolated enantiomers of **2** were rather limited,[7] in the recent decades many efforts were devoted to the enantioselective synthesis of halofuginone, and febrifugine as well, with the N-protected 3-hydroxypiperidine derivatives **3** (Figure 1), possessing (2*R*,3*S*) absolute configuration, being the key target intermediates.[8-10]

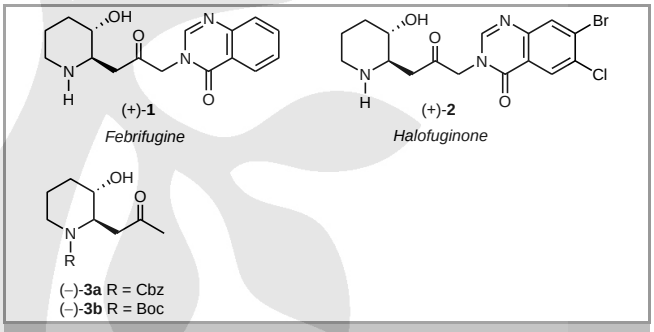
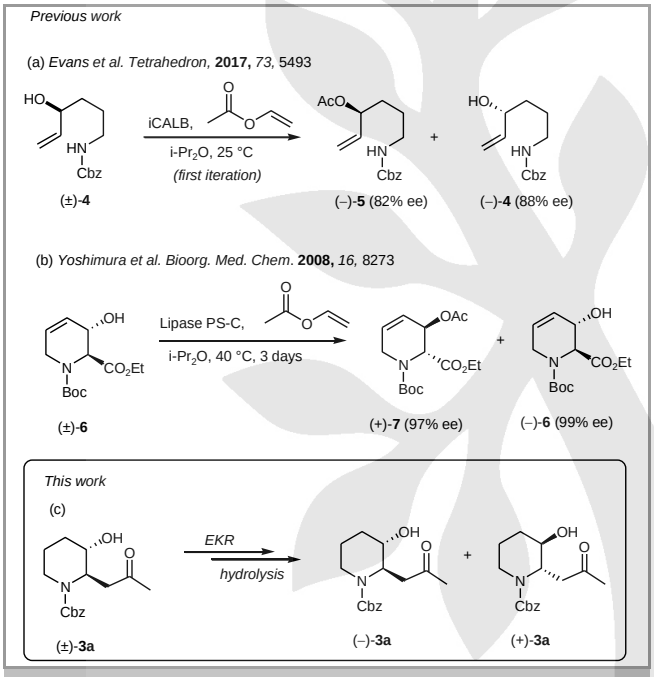


Figure 1 Structure of febrifugine (1), halofuginone (2) and 3-hydroxypiperidine derivative 3

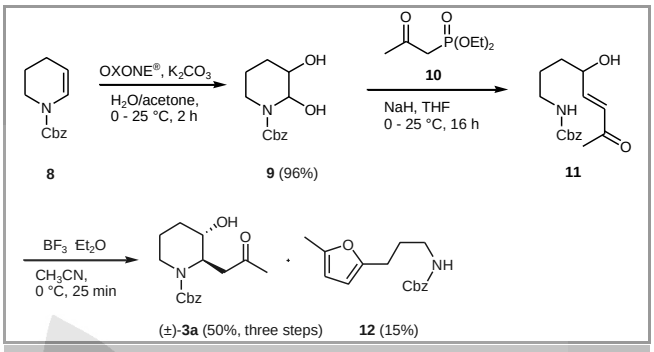
More recently explored strategies for the enantioselective synthesis of **3a** (R = Cbz) were based on the use of starting material from the chiral pool such as D-arabinose,[11] and the installation of new stereocenters by organocatalysis.[12,13] Despite the tremendous efficacy of enzymes in providing enantiopure compounds, to our knowledge only Evans did report on the enantiodivergent asymmetric synthesis of febrifugine, halofuginone and their hemiketals based on the kinetic resolution (KR) of a linear alcohol (**4**, Scheme 1, a) catalyzed by *Candida antarctica* lipase B,[14] which, however, required two iterations to provide one of the enantiomers [alcohol (+)-**4**] with high optical purity (98% ee) but in low (27%) overall yield. Encouraged by our recent results in the lipase-catalyzed KR of 3-hydroxypiperidine derivatives,[15a] the report by Yoshimura et al. on the efficient lipase-catalyzed KR of 3-hydroxypipecolic acid derivative **6** (Scheme 1, b), structurally similar to compound **3**,[16] and in continuation of our studies on the enantioselective synthesis of (poly)hydroxypiperidine alkaloids by enzyme[15b,15c] and transition metal catalysis,[15d-j] we decided to establish a practical, synthetic protocol for the synthesis of both

enantiomers of **3a** with high optical purity by lipase-catalyzed KR of the corresponding racemate (Scheme 1, c). Moreover, looking at a possible industrial application of our approach to **3a**, we decided to study this enzymatic process in both green and non-conventional media, in particular focusing on CPME (cyclopentyl methyl ether). This is a versatile solvent suitable for applications in biotechnology and biorefineries due to its valuable properties such as low peroxide formation rate, stability under basic and acidic conditions, relatively high boiling point and low water solubility and water content.[17] To the same purpose, for the synthesis of racemic **3a**, we also slightly modified the shortest known route from a commercial achiral precursor in order to reduce the number of chromatographic purifications. In this way we could establish an efficient protocol of possible industrial interest, and also useful to quickly prepare both enantiomers of halofuginone for comparative bioassays.



Scheme 1 Previous literature reports on EKR and the present work

Thus, commercially available, *N*-Cbz protected 1,2,3,4-tetrahydropyridine **8** (Scheme 2) was treated with OXONE® (2 equiv) in acetone/water and in the presence of K_2CO_3 (2 equiv) to obtain hemiaminal **9** (96% yield)[18] which, directly as a crude reaction mixture, was subjected to Horner-Wadsworth-Emmons olefination by reaction with phosphonate **10**[19] to give alcohol **11** with E geometry.[9] Treatment of crude **11** with $BF_3{\cdot}Et_2O$ in anhydrous acetonitrile eventually provided racemic **3a** in mixture with furan derivative **12** (15% by ^1H NMR).[20] The advantage of the HWE over the Wittig olefination of the same compound,[20a] is that only one chromatography of crude **3a** on silica gel is sufficient to separate this product from both **12** and residual unreacted phosphonate **10**.[20b] In this way, pure (±)-**3a** could be obtained in 50% yield over the three steps and the synthesis could be quickly repeated, usually on a 4 mmol scale, whenever **3a** was needed.



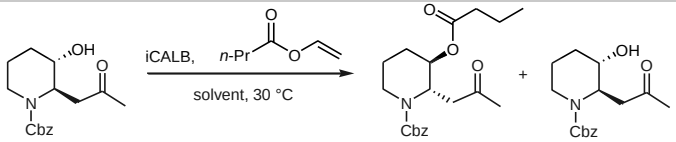
Scheme 2 Synthesis or racemic key intermediate **3**.

With racemic **3a** in hand, we studied its enzymatic kinetic resolution (EKR) via lipase-catalyzed esterification in solvents with low water content, focusing on the use of an immobilized enzyme, that is commercial iCALB (*Candida antarctica* lipase B immobilized on acrylic resin, Novozym® 435).[21] This choice stemmed from the fact that we successfully used iCALB for the kinetic resolution of hydroxylated piperidine derivatives.[15] This enzyme was used as provided by the supplier without any treatment prior use but we measured its esterification activity of 1-octanol in CPME using vinyl acetate as acylating reagent at 30 °C.[22] At a substrate/enzyme ratio of 1:10 [0.19 mmol of substrate (S) and 1.9 mg of immobilized enzyme (Enz)], substrate concentration 0.75 M, and 3.5 equiv of vinyl acetate, the conversion into product after 5 min was 49%, corresponding to an activity value of 9.8×10^{-6} mmol(S)/[min × mg(Enz)].

The results of this study are reported in Tables 1-2.

We started with vinyl butyrate as the acylating agent by screening different solvents (Table 1), i.e., THF (logP 0.46), for a comparison with a standard solvent we used in our previous studies,[15a-c] 2-MeTHF (logP 1.36), and CPME (logP 1.59).[23] On the bases of very recent literature on the use of Deep Eutectic Solvents (DES) as medium for biocatalyzed transformations, we employed also two DES[24] obtained by mixing choline chloride with urea and glycerol, respectively.[25] The reactions were monitored by GLC and stopped when the conversion was in the 40-50% range. The first experiments (entries 1-3) were carried out with solvents dried by distillation over Na/benzophenone. In all cases, the iCALB lipase preferentially catalyzed the esterification of the (2*S*,3*R*) enantiomer as determined by the sign of the optical rotation values (for **13a** after hydrolysis),[9] with comparable E (enantiomeric ratio) values,[26] although in CPME (and to a lesser extent in 2-MeTHF) the reaction was much faster (7 h). As interfacial activation is not known for this lipase,[27] the higher activity in the more lipophilic CPME could be related to the increased hydrophobicity of the hydration layer which facilitates the access of the substrate to the enzyme surface,[28] and is in line with the increased activity (and selectivity) observed in this solvent in other CALB-catalyzed resolution processes.[17] As the reaction was faster in CPME with an high E value, we continued our study with this solvent because of its special properties as mentioned above.[17]

Table 1 iCALB-catalyzed kinetic resolution of (±)-**3a** in different solvents[a]



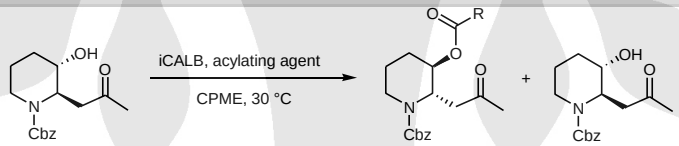
(±)-**3a** (+)-**13a** (–)-**3a**

Entry	Solvent	t (h)	*c* (%)[b]	(+)-**13a** ee (%)[c]	(–)-**3a** ee (%)[c]	*E*[d]
1	THF[e]	25	45	94	88	94
2	2-MeTHF[e]	7.5	39	97	64	127
3	CPME[e,f]	7	46	94	84	86
4	CPME[g]	8	48	92	87	68
5	CPME[h]	8.5	37	95	68	80
6	ChCl/Urea[i] (1:2)	118	16	93	24	35
7	ChCl/Urea (1:2) + CPME (10%)[i,j]	120	21	-	-	-
8	ChCl/Glycerol (1:2)[j]	22	2	-	-	-

[a] Reaction carried out on 0.2 mmol of substrate at 30 °C; substrate concentration: 0.76 M; enzyme (mg)/substrate (mmol) ratio: 100 mg/mmol; 3.5 equiv. of acylating agent.
[b] Reaction monitored by GLC; conversion determined by ^{1}H NMR spectroscopy.
[c] Determined by HPLC analysis on a HPLC Lux® 5 μm Amylose-1 (250 x 4.6 mm) column; for (+)-**13a** after hydrolysis to alcohol (+)-**3a**.
[d] *E* was calculated by using the formula $E = \ln[(1 - ee_S)/(1 + ee_S/ee_P)]/\ln[(1 + ee_S)/(1 + ee_S/ee_P)]$ as reported in ref. [26].
[e] Distilled over Na/benzophenone.
[f] Water content: 255 ppm.
[g] Commercial; water content: 1330 ppm.
[h] Dried over 4Å MS and reaction carried out in the presence of 4Å MS (130 mg/mmol$_s$).
[i] Carried out at 50 °C.
[j] Carried out at a c = 0.25 M.

We evaluated CPME as provided by the supplier, too, (entry 4) observing just a slight decrease of the *E* value (68) but not of the reaction rate. We measured the content of water in CPME before (1330 ppm) and after distillation (255 ppm) over Na/benzophenone by Karl Fischer titration. Our results show that the water content in commercial CPME is not sufficiently high to affect the reaction rate, for example by competing with the substrate, at least under our conditions, but has instead a small influence on the enantioselectivity.[29] The reaction rate was instead slight lower when carried out in the presence of 4Å molecular sieves (MS) and in CPME dried over 4Å MS for two days (entry 5).

Table 2 iCALB-catalyzed kinetic resolution of (±)-**3a** in CPME with different acylating agents[a]



(±)-**3a** (+)-**13a** R = *n*-Pr (+)-**13a'** R = Me (–)-**3a**

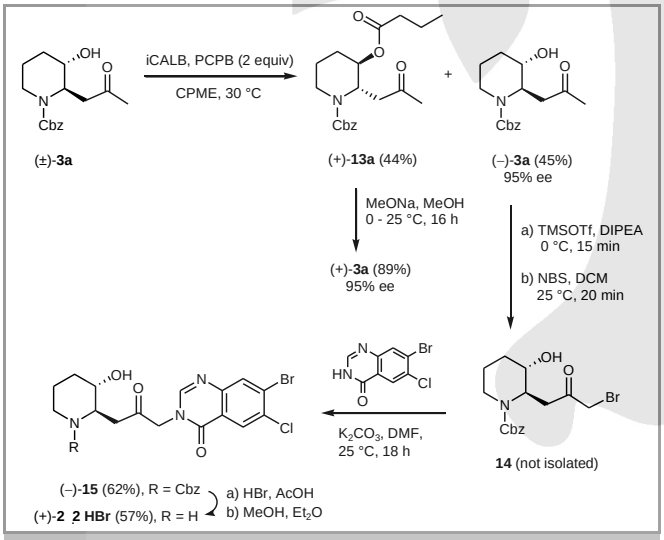
Entry	Acylating agent	t (h)	*c* (%)[b]	(+)-**13a/13a'** ee (%)[c]	(–)-**3a** ee (%)[c]	*E*[d]
1	VB	7	46	94	84	86
2	TFEB	96	39	98	66	197
3	PCPB	24	46	98	88	290
4[e]	PCPB	28	43	97	75	149
5	TCEA	49	42	95	74	85
6	VA	18	44	91	81	53
7	IPA	25	42	95	78	93
8[f]	PCPB	144 (6 days)	44	98	79	240
9[f]	VB	76	47	93	88	80
10[g]	PCPB	62	50	95	95	146
11[f,g]	VB	76	49	94	90	100

[a] Reaction carried out on 0.2 mmol of substrate at 30 °C; substrate concentration: 0.76 M; enzyme (mg)/substrate (mmol) ratio: 100 mg/mmol; CPME distilled over Na/benzophenone; 3.5 equiv. of acylating agent (VB = vinyl butyrate; VA = vinyl acetate; TFEB = trifluoroethyl butyrate; PCPB= p-Chlorophenyl butyrate; TCEA = trichloroethyl acetate; IPA = isopropenyl acetate).
[b] Reaction monitored by GLC; conversion determined by ^{1}H NMR spectroscopy.
[c] Determined by HPLC analysis on a HPLC Lux® 5 μm Amylose-1 (250 x 4.6 mm) column; for (+)-**13a**/**13a'** after hydrolysis to alcohol (+)-**3a**.
[d] E was calculated by using the formula $E = \ln[(1 - eeS)/(1 + eeS/eeP)]/\ln[(1 + eeS)/(1 + eeS/eeP)]$ as reported in ref. [26].
[e] CPME dried over 4Å MS.
[f] Enzyme (mg)/substrate (mmol) ratio: 25 mg/mmol.
[g] Carried out with 2 equiv. of acylating agent.

Disappointing results were instead obtained in the two DES we experimented (entries 6-8), in which reaction rates were unacceptably low despite several attempts of changing concentration, stirring rate, temperature, and acylating agent amount, and using crushed lipase or adding a co-solvent (data not shown). On the other hand, successful iCALB-catalyzed transesterification processes in the two DES we experimented used a large excess of alcohol to convert vinyl laurate.[25]

Our negative results are instead consistent with those obtained by Petrenz et al. who attempted a lipase-catalyzed KR of benzoin in DES with vinyl butyrate and found a much lower activity (10% conversion after 48 h) than in CPME and 2-MeTHF, likely because of mass transfer limitations due to the high DES viscosity.[30]

The next experiments were carried out in CPME distilled over Na/benzophenone by changing the acylating agent, in the attempt to further improve the E value (Table 2, entries 1-7). In all cases, the reaction was slower than with vinyl butyrate (entry 1) but we were very glad to find that with PCPB as the acylating agent the E value was very high (290) and the reaction was still reasonably fast (24 h to reach a 46% conversion). In general, the reaction was much faster when carried out with vinyl esters (entries 1,6-7) than with esters such as TFEB and TCEA (entries 2 and 5). Reducing the amount of enzyme to one fourth (25 mg per mmol of substrate) the reaction rate was, as expected, much lower (entries 8 and 9), without any significant effect on the enantiomeric ratio E. When reducing the amount of acylanting agent, while we did not observe any variation in the reaction rate with vinyl butyrate (cf. entries 9 and 11), the reaction did slow down significantly when we used PCPB (cf. entries 3 and 10). Despite this, we opted for the latter conditions for a larger scale KR of (±)-**3a** (and the next synthesis of enantiopure halofuginone) to avoid using a relatively strong excess of this acylating agent, the residue of which however, at the end of the process, can be separated by chromatography and recycled.



Scheme 3 Synthesis of (+)-halofuginone hydrobromide.

For the synthesis of (+)-halofuginone (Scheme 3) we first resolved 2 mmoles of (±)-**3a** by using iCALB under the conditions of Table 2, entry 10, and given the high enantioselectivity of the enzyme, only one iteration was necessary to obtain in good yields highly enantioenriched compounds (–)-**3a** (45%, 95% ee) and (+)-**13a** (44%). The hydrolysis of (+)-**13a**, carried out in the presence of MeONa in anhydrous methanol, then furnished alcohol (+)-**3a** (95% ee) in 89% yield. The next steps of the synthesis were carried out as reported,[14] that is by α-bromination of (–)-**3a** with NBS (*N*-bromosuccinimide) after generation of the corresponding silyl enol ether, followed by the reaction of **14** (as crude reaction mixture) with commercially available cebrazolone (7-bromo-6-chloro-4(1*H*)-quinazolinone) to give *N*-CBz-protected halofuginone (–)-**15** in 62% yield after chromatography. Finally, treatment with HBr in acetic acid provided (+)-halofuginone hydrobromide **2** in 57% yield. Of course, by the same approach, (–)-halofuginone with the same optical purity (95% ee) could be obtained from (+)-**3a**.

Concluding, we have demonstrated that the enantiodivergent synthesis of a key intermediate in the preparation of halofuginone can be accomplished by its enzymatic kinetic resolution using immobilized *Candida antarctica* lipase B. When the resolution was carried out in the CPME solvent, using PCPB (p-chlorophenylbutyrate) as the acylating reagent, the highest enantiomeric ratio (*E*) values were measured, providing almost enantiopure (95% ee) compounds in a single iteration of the kinetic resolution. Since the racemic material can be quickly obtained in three steps from a commercially available piperidine derivative, with only one chromatographic purification needed, our approach is an efficient and sustainable synthesis of halofuginone for further investigating the biological properties of both enantiomers of this biologically active compound. As an example, one of the two enantiomers was converted into enantiopure (+)-halofuginone according to a literature procedure.

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Anhydrous solvents were prepared according to the standard techniques. Commercially available reagents were used without further purification. Chromatographic separations were performed under pressure on silica gel 60 (Merck, 70–230 mesh) by using flash column techniques; R_f values refer to TLC carried out on 0.25 mm silica gel plates (F_{254}) with the same eluent indicated for column chromatography. ^{1}H (400 and 200 MHz) and ^{13}C NMR (100.4 MHz) spectra were recorded on Varian Inova (400 MHz) and Mercury (400 and 200 MHz) spectrometers in the specified deuterated solvent at 25 °C. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a Jeol ECZR600 spectrometer. Solvent reference lines were set at 7.26 and 77.0 ppm ($CDCl_3$), 2.50 and 39.5 ppm (DMSO-d_6) in ^{1}H and ^{13}C NMR spectra, respectively. Mass spectra were carried out by the direct inlet of a 10 ppm solution in CH_3OH on an LCQ Fleet Ion Trap LC/MS system (Thermo Fisher Scientific) with an electrospray ionization (ESI) interface in the positive ion mode. HPLC analyses were carried out with a Dionex Ultimate 3000 instrument, using a Lux 5 µm Amylose-1 column (250 x 4.6 mm) and eluting at 0.5 mL/min flow rate in isocratic 30% IPA, 70% hexane. Signals were monitored at 223 nm with a UV-detector. Compound **8** is commercially available or can be prepared as reported.[18] Compounds (+)-**2·2HBr**,[14] (±)-**3a**,[20] (–)-**3a**,[9] (+)-**3a**,[9] **9**,[18] **10**,[19] **11**,[20] **14**[14] and (–)-**15**[14] are known. *Candida antarctica* lipase B immobilized on acrylic resin (Novozym® 435) was purchased from Strem Chemicals, inc.

(2-Oxo-propyl)-phosphonic acid diethyl ester (10)[19]

Chloroacetone (2 mL, 25 mmol) was added to a suspension of KI (4.6 g, 27.5 mmol) in anhydrous CH_3CN (10 mL) and anhydrous acetone (10 mL) and the reaction was stirred at room temperature for 1 h. Then triethylphosphite (4.34 mL, 25 mmol) was added dropwise in 10 min. After 16 h, the reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with water (2 x 60 mL) and brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The yield of product **10** (80%) was calculated by

^{1}H-NMR, using CH_3NO_2 as internal standard. Crude **10** was then used without further purification.
^{1}H NMR (200 MHz, $CDCl_3$): δ 4.22–4.07 (m, 4 H), 3.08 (d, J = 22.8 Hz, 2 H), 2.32 (s, 3 H), 1.34 (t, J = 7.0 Hz, 6 H) ppm.

3-Hydroxy-2-(2-oxo-propyl)-piperidine-1-carboxylic acid benzyl ester [(±)-3a]

To a solution of **8** (696 μL, 3.6 mmol) and K_2CO_3 (1 g, 7.2 mmol) in acetone (12 mL) and water (12 mL) at 0 °C was added dropwise a solution of OXONE® (4.42 g, 7.2 mmol) in water (30 mL) in 5 min and the reaction mixture was stirred at room temperature for 2 h. Then the mixture was poured into ice and water (40 mL) and, after separation of the layers, the aqueous one was extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude intermediate **9** in 96% yield as a colorless oil, which was used in the next step without further purification.[18] ^{1}H NMR (200 MHz, $CDCl_3$) (mixture of diastereoisomers): δ 7.39–7.30 (m, 5 H), 5.77–5.70 (m, 1 H, major), 5.60–5.54 (m, 1 H, minor), 5.14 (s, 2 H), 3.96–3.77 (m, 1 H, major), 3.68–3.51 (m, 1 H, minor), 3.19 (td, J = 12.4, 3.6 Hz, 1 H, minor), 3.04 (td, J = 12.8, 3.2 Hz, 1 H, major), 2.34–2.21 (m, 1 H, major), 2.08–1.98 (m, 1 H, minor), 1.97–1.42 (m, 4 H) ppm. Crude **10** (1.67 g, 8.6 mmol) was dissolved in anhydrous THF (38 mL) and treated with NaH 60% (w/w) in mineral oil (344 mg, 8.6 mmol) for 30 min at 0 °C. A solution of crude hemiaminal **9** (867 mg, 3.45 mmol) in anhydrous THF (17 mL) was then added and the mixture allowed to warm to room temperature and stirred overnight. Then Et_2O (40 mL) was added and the organic layer was washed with a saturated aqueous solution of NH_4Cl (2 x 40 mL), brine (40 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent, the oily residue containing olefin **11** was used as such in the next step.[20] ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.37–7.32 (m, 5 H), 6.74 (dd, J = 16.0, 5.0 Hz, 1 H), 6.27 (dd, J = 16.0, 1.4 Hz, 1 H), 5.09 (s, 2 H), 4.94–4.85 (m, 1 H), 4.29–4.23 (m, 1 H), 3.32–3.17 (m, 2 H), 2.48–2.38 (m, 1 H), 2.26 (s, 3 H), 1.74–1.57 (m, 4 H) ppm. Crude olefin **11** (3.45 mmol) in acetonitrile (34.5 mL) was treated with $BF_3{\cdot}Et_2O$ (213 μL, 1.73 mmol) at 0 °C. After 25 min a saturated aqueous solution of $NaHCO_3$ (55 mL) was added and the product extracted with EtOAc (2 x 55 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane/EtOAc, 1 : 2, R_f = 0.29) to afford pure **(±)-3a** (582 mg, 2 mmol) in 50% yield as a yellow oil. Spectroscopic and analytical data are identical to those reported in the literature.[20]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.27 (m, 5 H), 5.11 (s, 2 H), 4.73 (m, 1 H), 4.13–4.00 (m, 1 H), 3.84–3.75 (m, 1 H), 2.86 (t, J = 12.0 Hz, 1 H), 2.63 (d, J = 7.6 Hz, 2 H) 2.14 (br s, 3 H), 1.89 (qt, J = 13.2, 4.8 Hz, 1 H), 1.79-1.71 (m, 1 H), 1.70–1.60 (m, 1 H), 1.45–1.36 (m, 1 H) ppm.

Lipase-Catalyzed Kinetic Resolution of (±)-3a

iCAL-B (200 mg) was added to a solution of **(±)-3a** (582 mg, 2 mmol) in anhydrous CPME (2.6 mL) at 30 °C and, after 10 min, *p*-chlorophenyl butyrate (795 mg, 4 mmol) was added. The reaction was left under stirring and the progress of EKR process was monitored by GLC. After 72 h, the conversion reached 50% and the reaction was stopped by filtration through a thin layer of Celite. The filter cake was washed with EtOAc (3 x 5 mL). After evaporation of the solvent, the crude product was purified by flash chromatography (*n*-hexane/EtOAc, 1 : 1) to give (+)-**13a** (318 mg, 44%; R_f = 0.40) as a colorless oil (95% ee), and (–)-**3a** (262 mg, 45%; R_f = 0.18) as a clear yellow oil.

3-Hydroxy-2-(2-oxopropyl)-piperidine-1-carboxylic acid benzyl ester [(-)-3a][9]

$[\alpha]_D^{20}$ – 19.9 (*c* 0.68, $CHCl_3$). Spectroscopic data identical to those reported for **(±)-3a**.

3-Butyryloxy-2-(2-oxopropyl)-piperidine-1-carboxylic acid benzyl ester [(+)-13a]

$[\alpha]_D^{20}$ + 50.8 (*c* 0.7, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.29 (m, 5 H), 5.13 (br s, 2 H), 4.83 (br d, J = 38.6 Hz, 2 H), 4.24–3.99 (br m, 1 H), 2.99–2.81 (m, 1 H), 2.72-2.60 (m, 2 H), 2.28–2.06 (br m, 5 H), 1.84–1.73 (br m, 3 H), 1.60–1.44 (br m, 3 H), 0.90 (t, J = 7.2 Hz, 3 H).

^{13}C NMR (100.4 MHz, $CDCl_3$) (mixture of rotamers): δ 205.9 and 205.0, 172.8, 155.7, 136.6, 128.4 (2 C), 127.9, 127.8 (2 C), 68.9, 67.2, 51.1, 43.4, 38.9, 36.2, 29.7, 23.7, 19.6, 18.3, 13.6 ppm.

MS (ESI): m/z (%) 745 ([2M + Na]$^+$, 100), 384 ([M + Na]$^+$, 55).

Elem. Analysis, calcd for $C_{20}H_{27}NO_5$: C 66.46, H 7.53, N 3.88; found: C 66.27, H 7.31, N 3.76.

3-Hydroxy-2-(2-oxo-propyl)-piperidine-1-carboxylic acid benzyl ester [(+)-3a][9]

To a solution of (+)-**13a** (318 mg, 0.88 mmol) in anhydrous MeOH (6 mL) cooled at 0 °C was added MeONa (95 mg, 1.76 mmol) and the reaction was stirred at room temperature overnight. A satd aqueous solution of NH_4Cl (880 μL) was then added and the reaction mixture concentrated under reduced pressure. Water (88 mL) and EtOAc (88 mL) were added to the residue and, after separation of the phases, the product was further extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the so obtained crude (+)-**3a** was purified by flash chromatography (*n*-hexane/EtOAc, 1 : 1; R_f = 0.18), affording pure (+)-**3a** (227 mg, 89%) as a clear yellow oil (95% *ee*). $[\alpha]_D^{20}$ + 23.2 (*c* 0.7, $CHCl_3$). Spectroscopic data identical to those reported for **(±)-3a**.

2-[3-(7-Bromo-6-chloro-4-oxo-4H-quinazolin-3-yl)-2-oxopropyl]-3-hydroxypiperidine-1-carboxylic acid benzyl ester [(–)-N-Cbz-protected halofuginone] [(–)-15][14]

DIPEA (502 μL, 2.88 mmol) and TMSOTf (521 μL, 2.88 mmol) were added to a solution of (–)-**3a** (262 mg, 0.90 mmol) in anhydrous DCM (16 mL), cooled at 0 °C (ice bath). After 20 minutes, NBS (224 mg, 1.26 mmol) was added, the ice bath removed and, after 20 minutes, the reaction was stopped by dilution with DCM (16 mL). The organic solvent was washed with water (2 x 16 mL), brine (16 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent, crude α-brominated intermediate **14** was obtained and used as such in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers): δ 7.38–7.28 (m, 5 H), 5.11 (s, 2 H), 4.72 (t, J = 6.8 Hz, 1 H), 4.05 (br s, 1 H), 3.83 (br s, 2 H), 3.01–2.87 (m, 2 H), 2.84 (d, J = 8.0 Hz, 2 H, major), 2.80 (d, J = 8.0 Hz, 2 H, minor), 2.85–2.79 (m, 1 H), 1.89 (qt, J = 12.8, 4.4 Hz, 1 H), 1.82–1.73 (m, 1 H), 1.72–1.63 (m, 1 H), 1.48–1.39 (m, 1 H) ppm. Crude **14** was dissolved in anhydrous DMF (10 mL) and reacted with 7-bromo-6-chloro-4(1*H*)-quinazolinone (cebrazolone, 234 mg, 0.90 mmol) in the presence of K_2CO_3 (124 mg, 0.90 mmol) at room temperature. After 18 h, the reaction mixture was diluted with EtOAc (20 mL), washed with water (2 x 20 mL) and, after separation of the phases, the product was further extracted with AcOEt (3 x 20 mL). The combined organic extracts were washed with brine (40 mL) and dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (EtOAc; R_f = 0.17) to afford (–)-*N*-Cbz-protected halofuginone (–)-**15** (307 mg, 62%) as a white solid. Spectroscopic and analytical data are in accordance with those reported.[14]

$[\alpha]_D^{21}$ – 21.4 (*c* 0.59, $CHCl_3$).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1 H), 8.00 (s, 1 H), 7.95 (br s, 1 H), 7.35–7.28 (m, 5 H), 5.11 (s, 2 H), 5.01–4.78 (m, 1 H), 4.71 (t, J = 6.4 Hz, 1 H), 4.07–3.95 (m, 1 H), 3.88 (s, 1 H), 3.08–2.96 (m, 1 H), 2.85–2.77 (m, 2 H), 2.60 (br s, 1 H), 1.90 (qt, J = 12.8, 4.4 Hz, 1 H), 1.83–1.72 (m, 2 H), 1.51–1.43 (m, 1 H), 0.90–0.80 (m, 1 H) ppm.

2-[3-(7-Bromo-6-chloro-4-oxo-4H-quinazolin-3-yl)-2-oxo-propyl]-3-hydroxy-piperidinium; bromide [(+)-2 · 2HBr][14]

(–)-*N*-Cbz-protected halofuginone (–)-**15** (307 mg, 0.56 mmol) in 33% HBr in AcOH (8.4 mL) was stirred at room temperature for 3 h. Then the reaction mixture was diluted with MeOH (8.4 mL), triturated with Et_2O (3 x 8.4 mL) and dried under reduced pressure to afford (+)-**2·2HBr** (184 mg, 57%) as a white solid with analytical and spectroscopic data in accordance with those reported in the literature.[14]

^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.79–8.67 (m, 2 H), 8.30 (s, 1 H), 8.22 (s, 1 H), 8.17 (s, 1 H), 5.14–5.08 (m, 2 H), 3.53 (td, J = 9.6, 4.2 Hz, 1 H), 3.35–3.29 (m, 1 H), 3.23 (dd, J = 17.4, 5.4 Hz, 1 H), 3.18–3.13 (m, 1 H), 2.93 (dd,

J = 17.4, 6.0 Hz, 1 H), 2.92–2.85 (m, 1 H), 1.94–1.89 (m, 1 H), 1.83–1.77 (m, 1 H), 1.68–1.59 (m, 1 H), 1.48–1.42 (m, 1 H) ppm.

^{13}C NMR (DMSO-d_6, 150 MHz) δ 200.7, 158.7, 149.6, 147.3, 132.5, 131.9, 128.5, 126.9, 121.8, 66.8, 56.2, 54.5, 43.0, 30.6, 20.2 ppm.

Acknowledgment

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Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO (this text will be deleted prior to publication)

References

(1) (a) Koepfli, J. B.; Mead, J. F.; Brockman Jr., J. A. *J. Am. Chem. Soc.* **1947**, *69*, 1837. (b) Koepfli, J. B.; Mead, J. F.; Brockman Jr., J. A. *J. Am. Chem. Soc.* **1949**, *71*, 1048.

(2) (a) Hirai, S.; Kikuchi, H.; Kim, H. -S.; Begum, K.; Wataya, Y.; Tasaka, H.; Miyazawa, Y.; Yamamoto, K.; Oshima, Y. *J. Med. Chem.* **2003**, *46*, 4351. (b) Zhu, S.; Meng, L.; Zhang, Q.; Wei, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1854; (c) Kikuchi, H.; Yamamoto, K.; Horoiwa, S.; Hirai, S.; Kasahara, R.; Hariguchi, N.; Matsumoto, M.; Oshima, Y. *J. Med. Chem.* **2006**, *49*, 4698. (d) Zhu, S.; Zhang, Q.; Gudise, C.; Wei, L.; Smith, E.; Zeng, Y.; *Bioorg. Med. Chem.* **2009**, *17*, 4496.

(3) (a) Anderson, A.; Goodall, E.; Bliss, G. W.; Woodhouse, R. N. *J. Chromatogr.* **1981**, *212*, 347. (b) Zhang, D. F.; Sun, B. B.; Yue, Y. Y.; Yu, H. J.; Zhang, H. L.; Zhou, Q. J.; Du, A. F. *Parasitol. Res.* **2012**, *111*, 695. (c) Ramadan, A.; Abo el-Sooud, K.; el-Bahy, M. M. *Egypt. Res. Vet. Sci.* **1997**, *62*, 175.

(4) (a) Granot, I.; Halevy, O.; Hurwitz, S.; Pines, M. *Biochim. Biophys.* **1993**, *1156*, 107. (b) Pines, M. *World J. Gastroenterol.* **2014**, *20*, 14778. (c) Yavas, G.; Calik, M.; Calik, G.; Yavas, C.; Ata, O.; Esme, H. *Med. Hypotheses* **2013**, *80*, 357.

(5) (a) Lamora, A.; Mullard, M.; Amiaud, J.; Brion, R.; Heymann, D.; Redini, F.; Verrecchia, F. *Oncotarget* **2015**, *6*, 14413. (b) Cook, J. A.; Choudhuri, R.; Degraff, W.; Gamson, J.; Mitchell, J. B. *Cancer Lett.* **2010**, *289*, 119. (c) Yee, K. O.; Connolly, C. M.; Pines, M.; Lawler, J. *Cancer Biol. Ther.* **2006**, *5*, 218.

(6) Casey, N. H.; Crosley, R. I.; Smith, G. A. *J. S. Afr. Vet. Assoc.* **1992**, *63*, 16.

(7) Linder, M. R.; Heckeroth, A. R.; Najdrowski, M.; Daugschies, A.; Schollmeyer, D.; Miculka, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4140.

(8) The absolute configuration of febrifugine was determined in 1999 by its total synthesis. Kobayashi, S.; Ueno, M.; Suzuki, R.; Ishitani, H.; Kim, H. -S.; Wataya, Y. *J. Org. Chem.* **1999**, *64*, 6833.

(9) McLaughlin, N. P.; Evans, P. *J. Org. Chem.* **2010**, *75*, 518, and references therein for a list of papers published prior to 2010.

(10) (a) For a review, see: Smullen, S.; McLaughlin, N. P.; Evans, P. *Bioorg. Med. Chem.* **2018**, *26*, 2199. (b) See, also: Zhang, J.; Yao, Q.; Liu, Z. *Molecules* **2017**, *22*, 1086, and references therein.

(11) Perali, R. S.; Bandi, A. *Tetrahedron Lett.* **2020**, *61*, 152151.

(12) Smullen, S.; Evans, P. *Tetrahedron Lett.* **2018**, *59*, 1627.

(13) Pansare, S. V.; Paul, E. K. *Synthesis* **2013**, *45*, 1863.

(14) Smullen, S.; Evans, P. *Tetrahedron* **2017**, *73*, 5493.

(15) (a) Begliomini, S.; Sernissi, L.; Scarpi, D.; Occhiato, E. G. *Eur. J. Org. Chem.* **2014**, 5448. (b) Occhiato, E. G.; Casini, A.; Guarna, A.; Scarpi, D. *Eur. J. Org. Chem.* **2011**, 6544. (c) Bartali, L.; Casini, A.; Guarna, A.; Occhiato, E. G.; Scarpi, D. *Eur. J. Org. Chem.* **2010**, 5831. (d) Occhiato, E. G.; Scarpi, D.; Guarna, A.; Tabasso, S.; Deagostino, A.; Prandi, C. *Synthesis* **2009**, 3611. (e) Bartali, L.; Scarpi, D.; Guarna, A.; Prandi, C.; Occhiato, E. G. *Synlett* **2009**, 913. (f) Scarpi, D.; Avataneo, O.; Prandi, C.; Venturello, P.; Occhiato, E. G. *Synthesis* **2012**, *44*, 3688. (g) Scarpi, D.; Bartali, L.; Casini, A.; Occhiato, E. G. *Eur. J. Org. Chem.* **2012**, 2597. (h) Scarpi, D.; Bartali, L.; Casini, A.; Occhiato, E. G. *Eur. J. Org. Chem.* **2013**, 1306. (i) Sernissi, L.; Petrovic´, M.; Scarpi, D.; Guarna, A.; Trabocchi, A.; Bianchini, F.; Occhiato, E. G. *Chem. Eur. J.* **2014**, *20*, 11187. (j) Scarpi, D.; Begliomini, S.; Prandi, C.; Oppedisano, A.; Deagostino, A.; Gómez-Bengoa, E.; Fiser, B.; Occhiato, E. G. *Eur. J. Org. Chem.* **2015**, 3251.

(16) Yoshimura, Y.; Ohara, C.; Imahori, T.; Saito, Y.; Kato, A.; Miyauchi, S.; Adachi, I.; Takahata, H. *Bioorg. Med. Chem.* **2008**, *16*, 8273.

(17) de Gonzalo, G.; Alcántara, A. R.; de María, P. D. *ChemSusChem* **2019**, *12*, 2083.

(18) Takeuchi, Y.; Azuma, K.; Oshige, M.; Abe, H.; Nishioka, H.; Sasaki, K.; Harayama, T. *Tetrahedron* **2003**, *59*, 1639.

(19) Schnaars, C.; Hansen, T. *Org. Lett.* **2012**, *14*, 2794.

(20) (a) Sukemoto, S.; Oshige, M.; Sato, M.; Mimura, K. -i.; Nishioka, H.; Abe, H.; Harayama, T.; Takeuchi, Y. *Synthesis* **2008**, 3081. (b) When we tried a Wittig olefination, we instead found that two consecutive chromatographic separations were necessary to obtain pure **3a**, one on silica gel to remove **12** and the excess of ylide, and a second one on alumina to remove triphenylphosphine oxide.

(21) (a) Akai, S.; Tanimoto, K.; Kita, Y. *Angew. Chem. Int. Ed.* **2004**, *43*, 1407. (b) Rotticci, D.; Norin, T.; Hult, K. *Org. Lett.* **2000**, *2*, 1373. (c) Orrenius, C.; Norin, T.; Hult, K.; Carrea, G. *Tetrahedron: Asymmetry* **1995**, *6*, 3023.

(22) Secundo, F.; Carrea, G.; Soregaroli, C.; Varinelli, D.; Morrone, R. *Biotechnol. Bioeng.* **2001**, *73*, 157.

(23) LogP is well known to influence enzymatic activity. See, for example: (a) Zieniuk, B.; Fabiszewska, A.; Białecka-Florjańczyk, E. *Bioprocess Biosyst Eng*. **2020**, *43*, 605. (b) Inagaki, T.; Tasada, K.; Kayahara, H. *Biosci. Biotech. Biochem*., 1994, 58, 1439.

(24) (a) Pätzold, M.; Siebenhaller, S.; Kara, S.; Liese, A.; Syldatk, C.; Holtmann, D. *Trends Biotechnol.* **2019**, *37*, 943. (b) Aranda, C.; de Gonzalo, G. *Molecules* **2020**, *25*, 3016. (c) Guajardo, N.; Domínguez de María, P. *ChemCatChem* **2019**, *11*, 3128. (d) Panić, M.; Radović, M.; Maros, I.; Tušek, A. J.; Bubalo, M. C.; Redovniković, I. R. *Process Biochem.* **2021**, *102*, 1.

(25) Durand, E.; Lecomte, J.; Baréa, B.; Piombo, G.; Dubreucq, E.; Villeneuve, P. *Process Biochem.* **2012**, *47*, 2081.

(26) The enantioselectivity in an enzymatic kinetic resolution process is expressed by the enantiomeric ratio E, which is the ratio between the specificity constants (kcat/KM) of the enzyme for the competing R and S enantiomers: E = $(kcat/KM)_R/(kcat/KM)_S$. E was calculated by using the formula E = $\ln[(1 - ee_s)/(1 + ee_s/ee_p)]/\ln[(1 + ee_s)/(1 + ee_s/ee_p)]$. Rakels, J. L. L.; Straathof, A. J. J.; Heijnen, J. *Enzyme Microb. Technol.* **1993**, *15*, 1051.

(27) Stauch, B.; Fisher, S. J.; Cianci, M. *J. Lipid Res.* **2015**, *56*, 2348.

(28) Zieniuk, B.; Fabiszewska, A.; Białecka-Florjańczyk, E. *Bioprocess Biosyst. Eng.* **2020**, *43*, 605.

(29) Léonard-Nevers, V.; Marton, Z.; Lamare, S.; Hult, K.; Graber, M. *J. Mol. Catal. B: Enzym.* **2009**, *59*, 90.

(30) Petrenz, A.; de María, P. D.; Ramanathan, A.; Hanefeld, U.; Ansorge-Schumacher, M. B.; Kara, S. *J. Mol. Catal. B: Enzym.* **2015**, *114*, 42.

Supporting information

Enantiodivergent Synthesis of Halofuginone by *Candida Antarctica* Lipase B (CAL-B)-catalyzed Kinetic Resolution in Cyclopentyl Methyl Ether (CPME)

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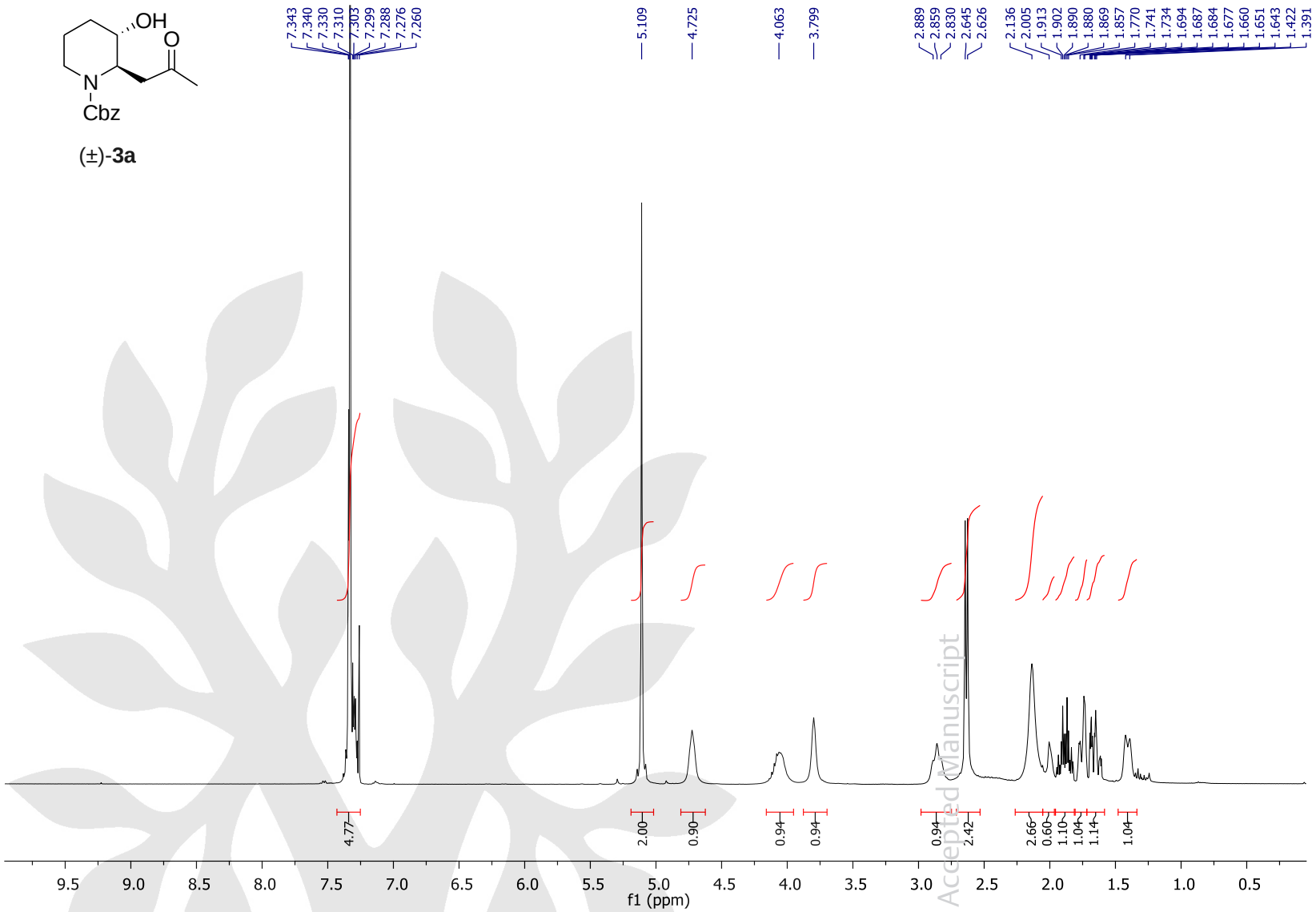
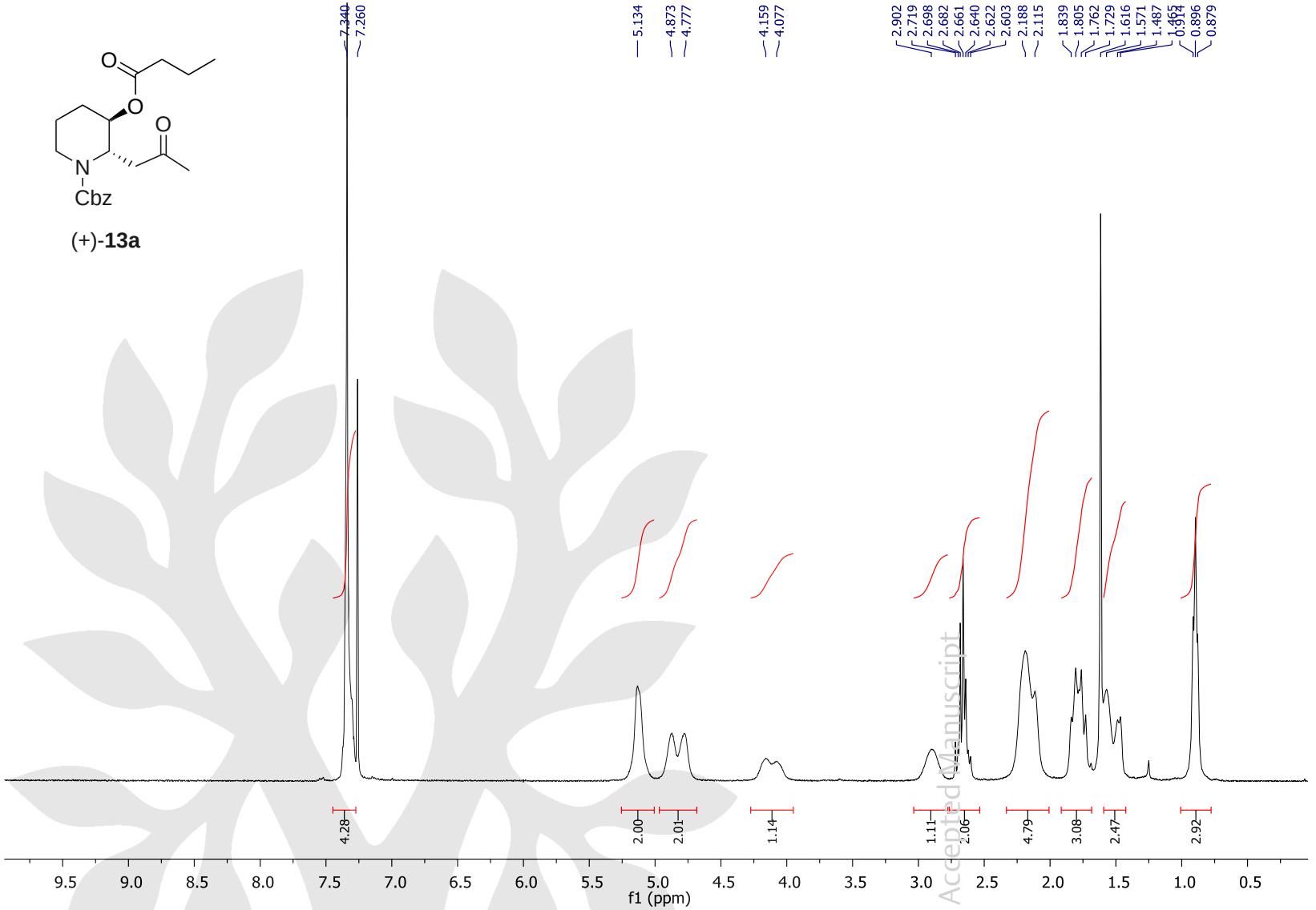


^{1}H NMR ($CDCl_3$, 400 MHz) of compound (±)-**3a**



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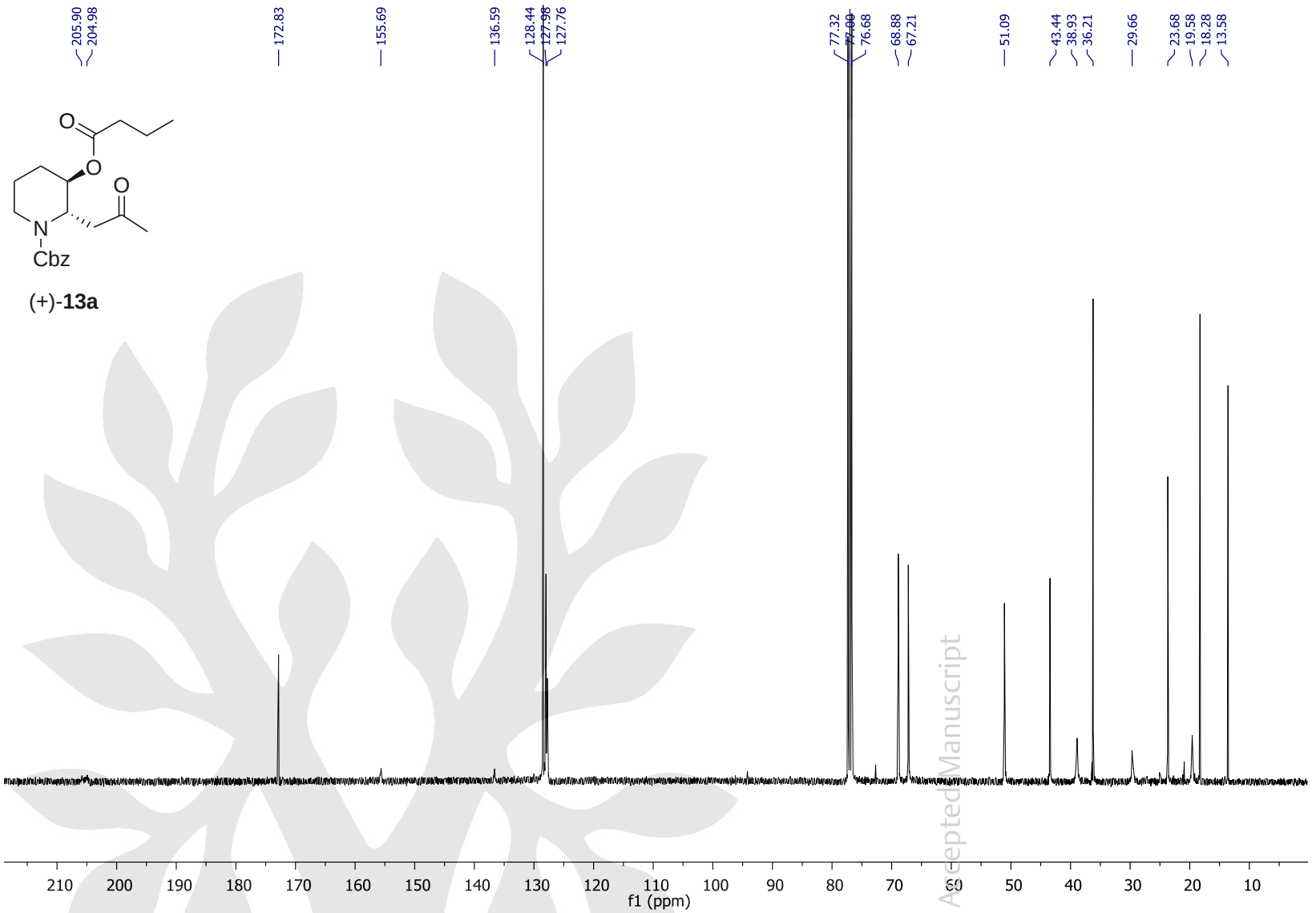

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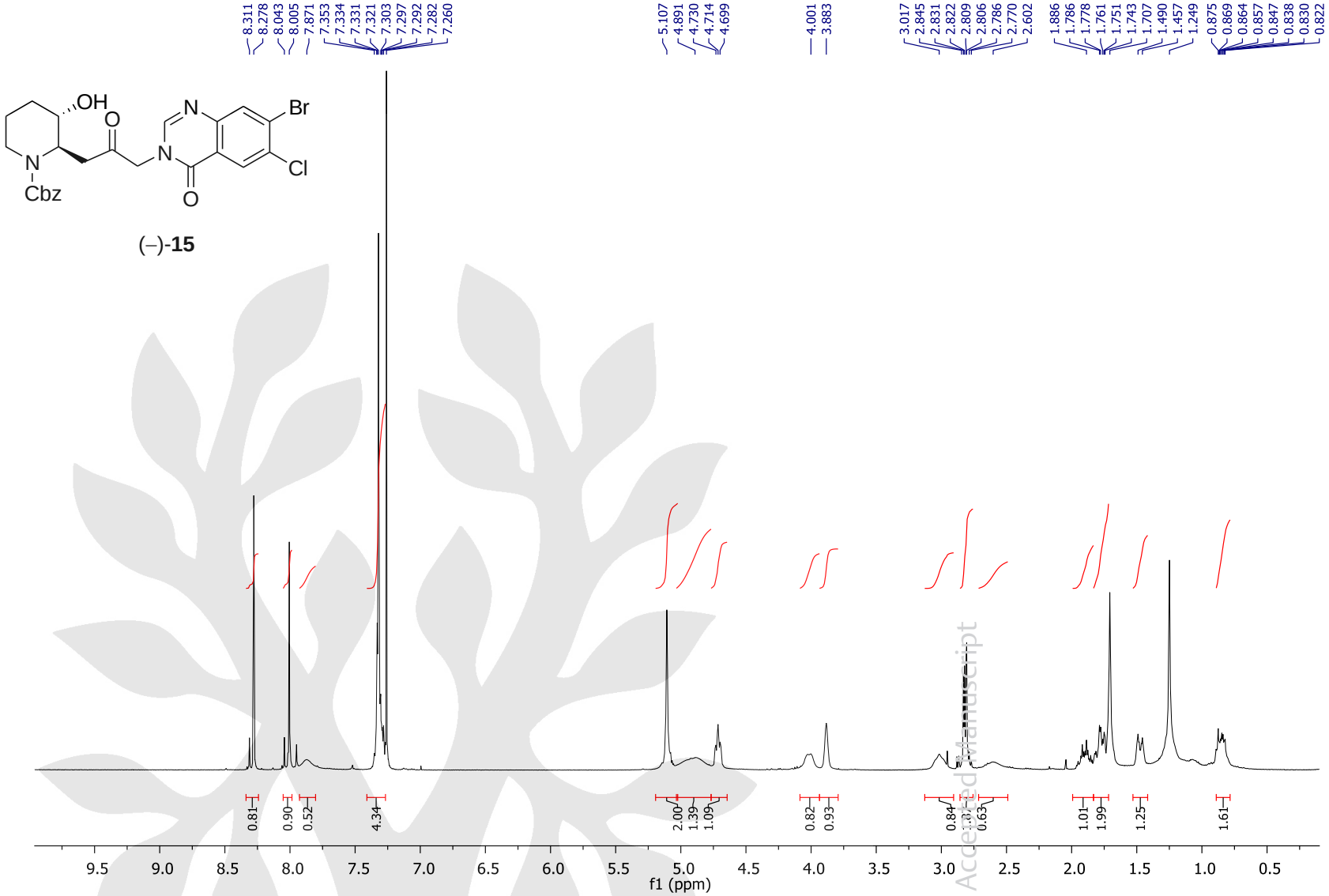

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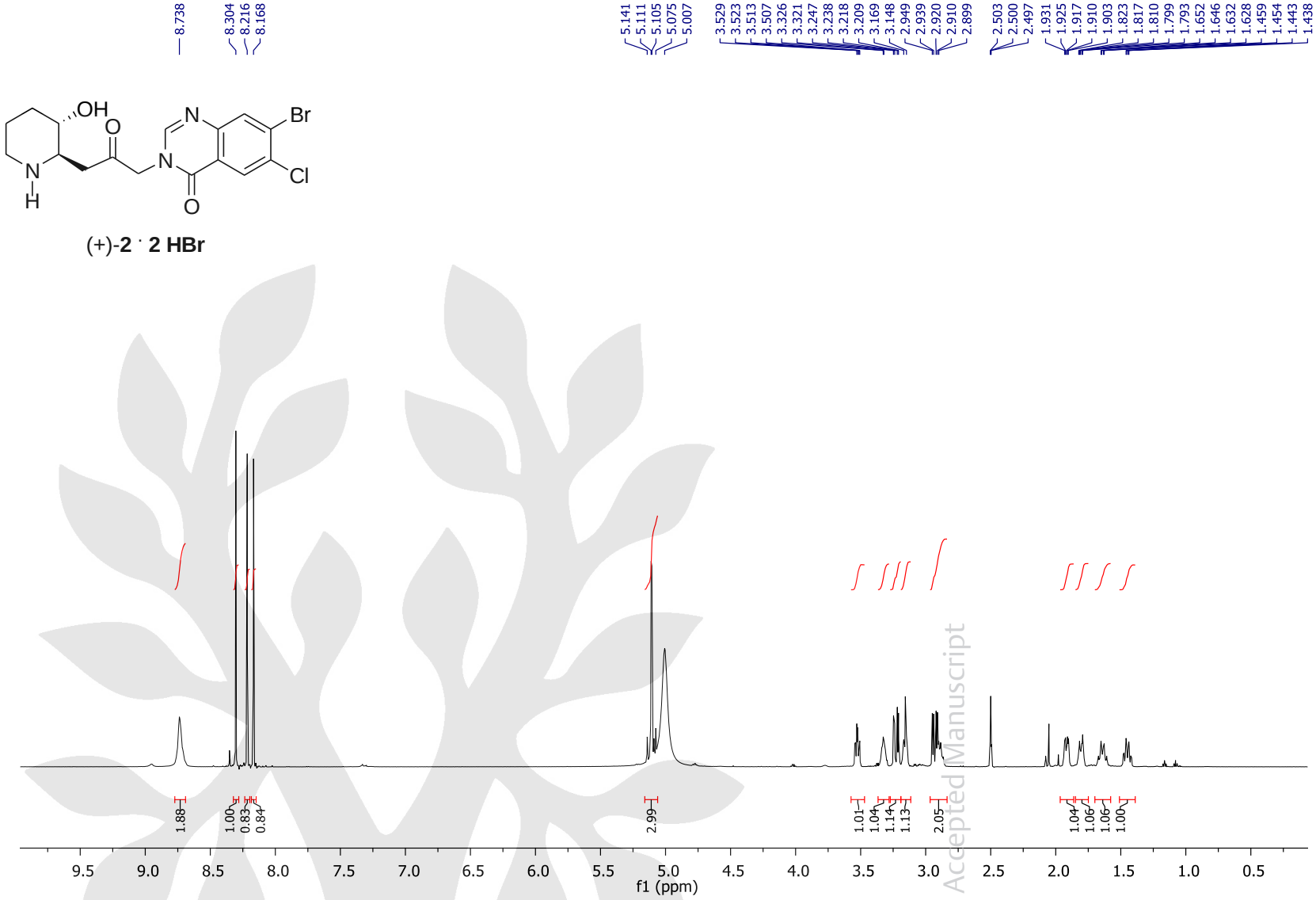
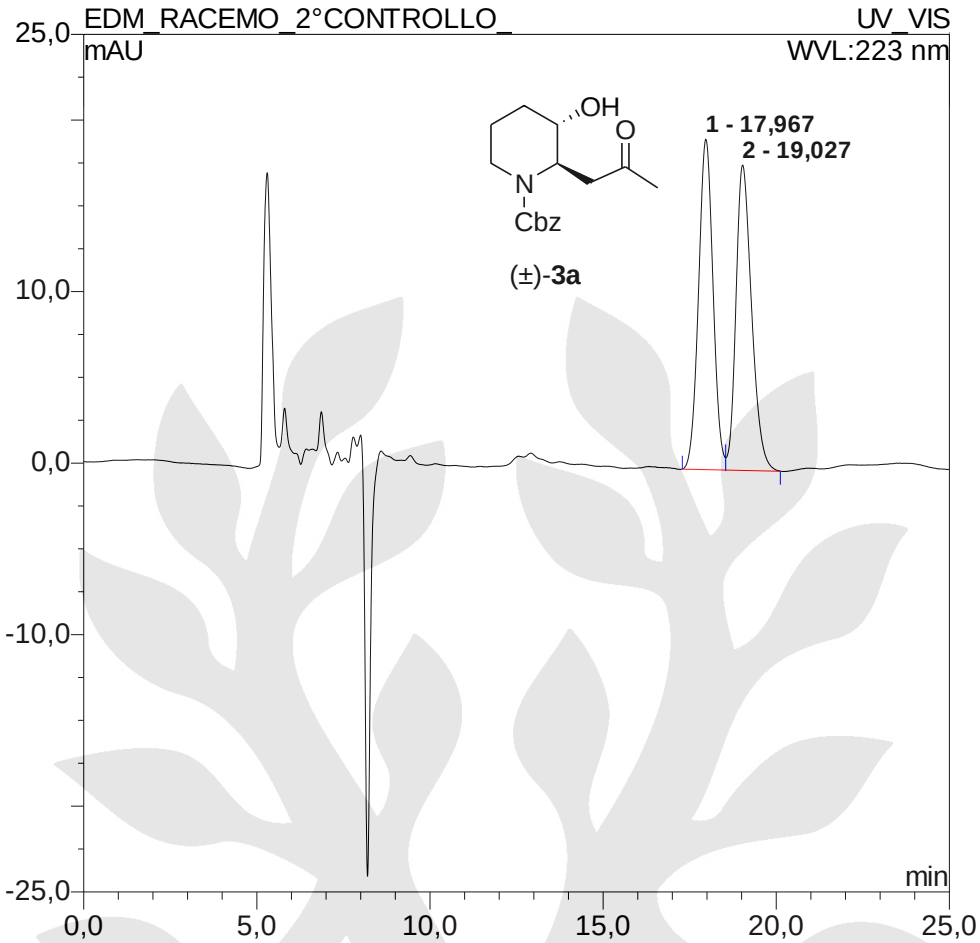


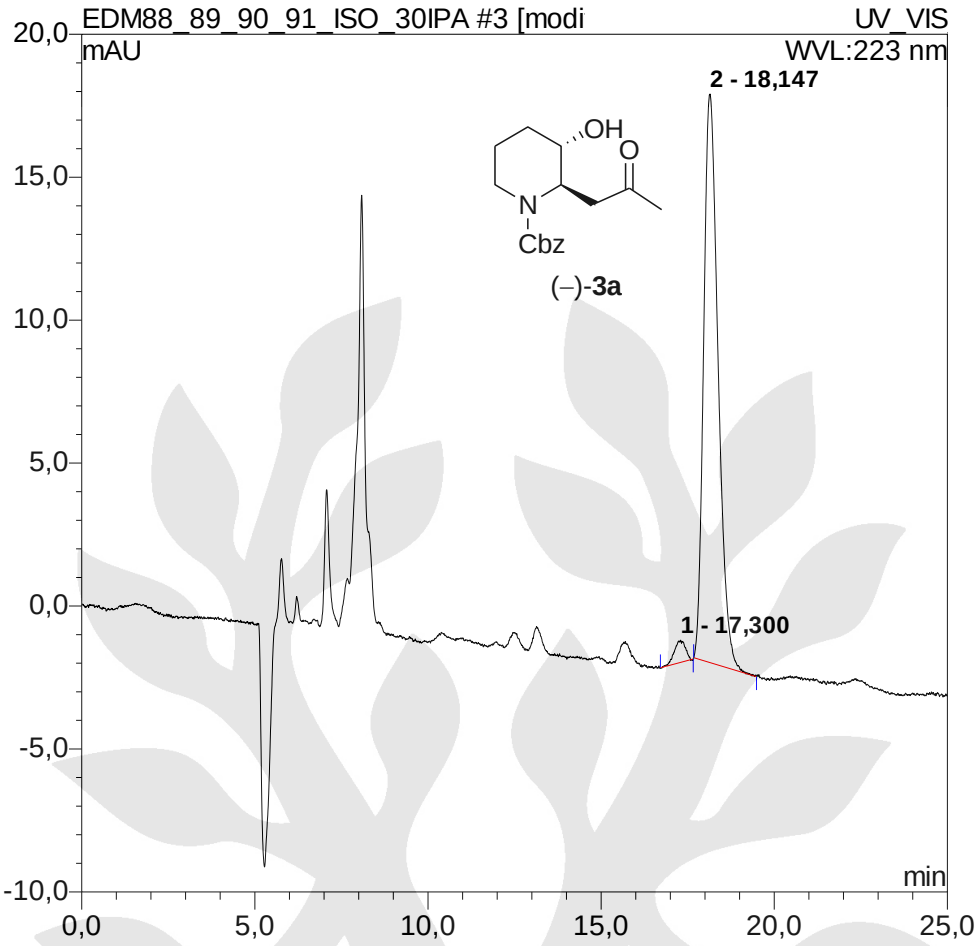
(+)-**2** · **2 HBr**

^{1}H NMR (DMSO-d6, 600 MHz) of compound (+)-**2·2HBr**



No.	Ret. Time min	Peak Name	Height mAU	Area mAU*min	Rel. Area %	Type
1	17,97	n.a.	19,269	9,357	50,01	BM
2	19,03	n.a.	17,801	9,353	49,99	MB
Total:			37,070	18,710	100,00	0,000

HPLC column: Lux 5 µm Amylose-1 column, 250 x 4.6 mm. Conditions: isocratic 30% IPA, 70% hexane.



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2	18,15	n.a.	19,892	9,613	97,11	BMB*
Total:			20,669	9,899	100,00	

HPLC column: Lux 5 µm Amylose-1 column, 250 x 4.6 mm. Conditions: isocratic 30% IPA, 70% hexane.

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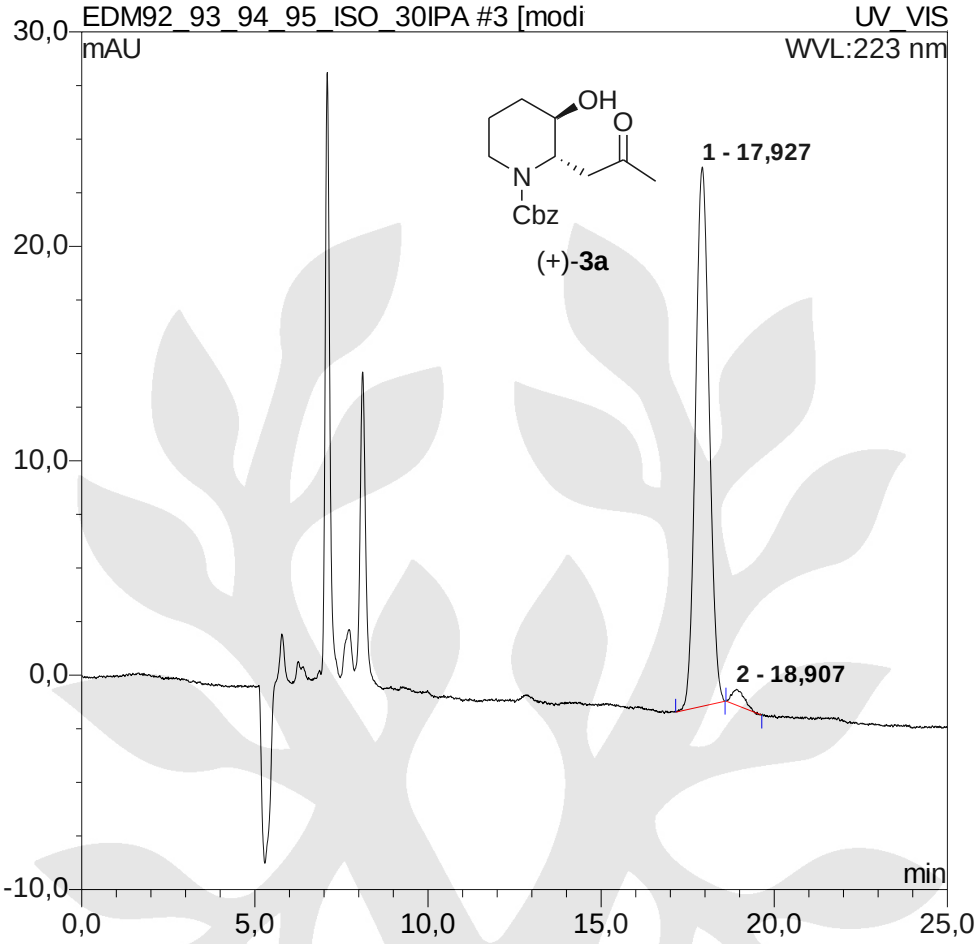

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Type
1	17,93	n.a.	25,152	11,549	97,37	BMB*
2	18,91	n.a.	0,744	0,312	2,63	BMB*
Total:			25,896	11,861	100,00	

HPLC column: Lux 5 µm Amylose-1 column, 250 x 4.6 mm. Conditions: isocratic 30% IPA, 70% hexane.