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Total synthesis of crispine A enantiomers through a *Burkholderia cepacia* lipase-catalysed kinetic resolution

Enikő Forró, László Schönstein, Ferenc Fülöp *

Institute of Pharmaceutical Chemistry, University of Szeged, H-6720 Szeged, Eötvös u 6, Hungary

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ABSTRACT

Both enantiomers of the antitumour-active alkaloid crispine A (ee = 95%) were synthesised through the *Burkholderia cepacia* lipase-catalysed acylation of the primary hydroxy group of *N*-Boc-protected 1-(3-hydroxypropyl)-6,7-bis(methyloxy)-1,2,3,4-tetrahydroisoquinoline (±)-**3** and the enantioselective hydrolysis of the corresponding *O*-decanoate (±)-**4** [R = $(CH_2)_8Me$] with a remote, four-atom distant stereogenic centre. High enantioselectivities were observed for the (*S*)-selective O-acylation with vinyl decanoate in the presence of Et_3N and Na_2SO_4 in *t*-BuOMe at 45 °C (*E* = 68), and for the (*S*)-selective hydrolysis with H_2O in *t*-BuOMe at 45 °C (*E* = 52). The enzymatic resolutions, performed in two steps, afforded the key alcohol and ester enantiomers with high enantiomeric excesses (ee ⩾ 94%). Ring-closure reactions of alcohol enantiomers (+)-**3** and (−)-**3** with thionyl chloride afforded the desired crispine A enantiomers (+)-**1** and (−)-**1** (ee ⩾ 95%).

1. Introduction

The plant *Carduus crispus* has long been used in Chinese folk medicine for the treatment of colds, stomach problems and rheumatism. 8,9-Bis(methyloxy)-1,2,3,5,6,10b-hexa-hydropyrrolo[2,1-*a*]isoquinoline **1** (crispine A) and another isoquinoline alkaloid **2**, containing a guanidyl group (crispine E) (Scheme 1), isolated in 2002 from *C. crispus* by Zhao and co-workers,[1] display high biological activity against SKOV3, KB and HeLa human cancer cell lines.

In recent years, extensive investigations have been carried out on the chemistry of tetrahydroisoquinoline alkaloids,[2] in view of their potential pharmaceutical activity. Crispine A was obtained by synthesis[3] before its isolation from *C. crispus*, and both racemic[4] and enantiopure[2c,5] forms have been prepared. Crispine E, a basic constituent of *C. crispus*, has also been studied because of its promising pharmaceutical activity.[1,6] Itoh and co-workers reported the synthesis of (−)-crispine E by using chiral auxiliaries,[5b] and the enantioselective synthesis of the antipode (+)-crispine E, via asymmetric transfer hydrogenation, used as a key step in the synthesis, has recently been described.[7] Almost all of the approaches are based on an asymmetric synthetic route; only Turner and co-workers reported a chemo-enzymatic synthesis of (+)-crispine A through the monoamine oxidase-catalysed deracemisation of racemic crispine A.[5d,8]

Our aim was to develop new enzymatic strategies for the synthesis of enantiopure *N*-Boc-protected 1-(3-hydroxypropyl)-6,7-dimethoxy)-1,2,3,4-tetrahydroisoquinoline (+)-**3** and (−)-**3**, key intermediates for the preparation of enantiomeric crispine A and E (Scheme 1). In spite of the low to moderate enantioselectivities described in the literature for the lipase-catalysed asymmetric acylations of primary alcohols,[9] and especially those with a remote stereogenic centre,[10] we first planned acylation of racemic *N*-Boc-protected 1-(3-hydroxypropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (±)-**3** in an organic medium. Results on the lipase-catalysed hydrolysis of O-acylated primary alcohols with a remote stereogenic centre[9a,10e,11] suggested the possibility of the lipase-catalysed enantioselective hydrolysis of racemic *N*-Boc-protected 1-(3-acyloxypropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (±)-**4**.

2. Results and discussion

2.1. Synthesis of (±)-3 and (±)-4

Racemic (±)-**3** was prepared in a four-step process, according to known literature methods.[12] First, β-(3,4-dimethoxyphenyl)ethylamine was reacted with γ-butyrolactone, and the resulting amide was subjected to Bischler–Napieralski cyclisation. Next, the sodium borohydride reduction of the ring-closed 1-(3-hydroxypropyl)-6,7-dimethoxy-3,4-dihydroisoquinoline afforded 1-(3-hydroxypropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline. Finally, *N*-Boc protection with di-*tert*-butyl dicarbonate furnished the desired (±)-**3**. Acylation[10c] of (±)-**3** with acetic anhydride gave the corresponding (±)-**4**, R = Me, while with decanoyl chloride resulted in (±)-**4**, R = $(CH_2)_8Me$ (see Section 4).

* Corresponding author. Tel.: +36 62 54 5564; fax: +36 62 54 5705.
E-mail address: fulop@pharm.u-szeged.hu (F. Fülöp).

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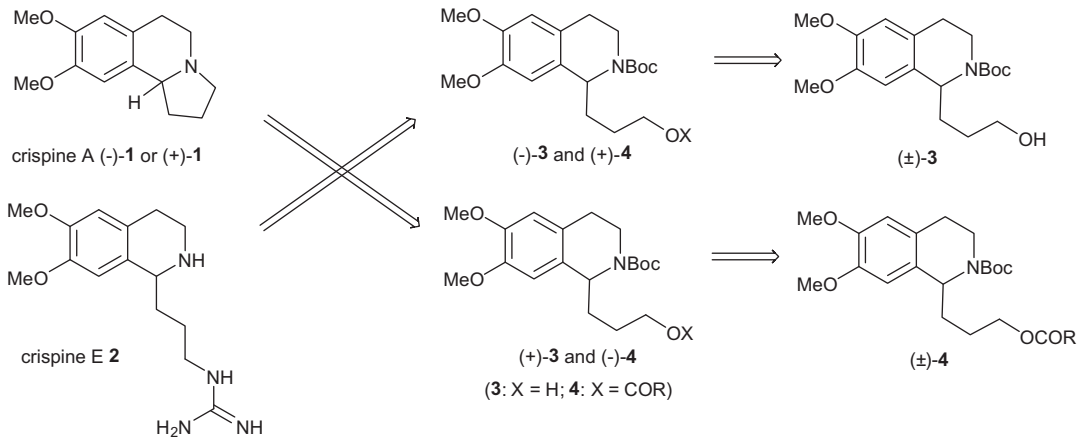
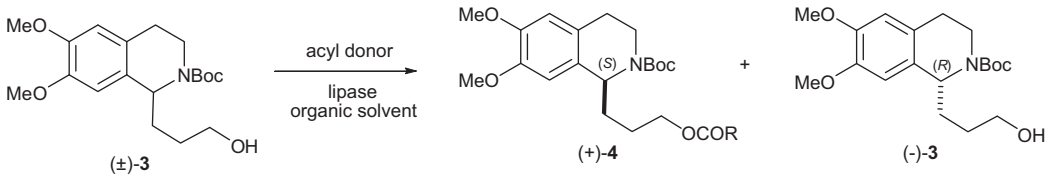


Scheme 1. Planned synthesis of enantiopure **3** and **4**.



Scheme 2. Enzymatic acylation of (±)-**3**.

Table 1
Conversion, enantiomeric excesses (ee) and enantioselectivities (*E*) of the acylation of (±)-**3**[a]

Entry	Enzyme	Acyl donor (equiv)	Solvent	*t* (h)	ee_s[b] (%)	ee_p[b] (%)	Conversion (%)	*E*
1	CAL-A[c]	$MeCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	24	No reaction			
2	CAL-B	$MeCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	1	*rac*	*rac*	43	1
3	Lipase AK[c]	$MeCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	24	No reaction			
4	Lipase AY[c]	$MeCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	24	No reaction			
5	PPL	$MeCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	24	No reaction			
6	Lipase PS[c]	$MeCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	1	4	22	15	1.6
7	Lipase PS[c]	$MeCH{=}CHCO_2Me$ (1.1)	*i*Pr_2O	1	8	35	19	2.2
8	Lipase PS[c]	$Me(CH_2)_2CO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	1	12	71	14	7
9	lipase PS[c]	*t*-$BuCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	1	No reaction			
10	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	1	9	85	10	13
11	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (1.1)	*t*-BuOMe	1	10	95	10	43
12	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (1.1)[+Et_3N + Na_2SO_4]	*t*-BuOMe	1	15	95	14	45
13	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (3)[+Et_3N + Na_2SO_4]	*t*-BuOMe	1	19	96	17	59
14	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (4)[+Et_3N + Na_2SO_4]	*t*-BuOMe	1	33	95	26	53
15	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (1.1)	*n*-Hexane	1	26	88	23	20
16	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (1.1)	Toluene	1	5	67	7	5
17	Lipase PS[c]	$Me(CH_2)_8CO_2CH{=}CH_2$ (1.1)	MeCN	1	No reaction			
18	lipase PS[c]	$Me(CH_2)_{10}CO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	1	8	86	7	14
19	Lipase PS-IM	$MeCO_2CH{=}CH_2$ (1.1)	*i*Pr_2O	1	7	14	33	1.4
20	Lipase PS-IM	$Me(CH_2)_8CO_2CH{=}CH_2$ (1.1)	*t*-BuOMe	1	26	92	22	30

[a] 0.0125 M substrate, 30 mg mL^{-1} enzyme, 1 mL of organic solvent, acyl donor, 45 °C.
[b] According to HPLC (see Section 4).
[c] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

2.2. Enzymatic acylation of (±)-3

The enantioselectivities of the lipase-catalysed resolutions of primary alcohols are well known to be considerably lower then those of secondary alcohols. Few articles have dealt with the asymmetric acylation of primary alcohols with an even more remotely located stereocentre. The lipases most commonly used in those reactions were lipase PS (from *Burkholderia cepacia*, formerly called *Pseudomonas cepacia* lipase) and lipase AK (from *Pseudomonas fluorescens*).[9,10]

Due to the earlier results on the lipase-catalysed enantioselective (*E* >200) acylation of alcohols with the stereogenic centre at a distance of two atoms from the reaction centre,[10b–e] we attempted the acetylation of (±)-**3** with 1.1 equiv of vinyl acetate in the presence of lipase PS [containing 20% (w/w) lipase adsorbed on Celite in the presence of sucrose] in *i*Pr_2O at 45 °C (Scheme 2, R = Me).

However, poor levels of enantioselectivity (*E* <2) were observed (Table 1, entry 6). Accordingly, preliminary experiments were needed to determine the optimum conditions for gram-scale resolution. The effects of enzyme, acyl donor, solvent, temperature and additive on the enantioselectivity were investigated (Table 1). Amongst the lipases tested, CAL-A (*Candida antarctica* lipase A) (entry 1), lipase AK (entry 3), lipase AY (from *Candida rugosa*) (entry 4)

and PPL (porcine pancreatic lipase) (entry 5) did not catalyse the reaction (no product was detected after 24 h). Acylation was very fast with CAL-B (*Candida antarctica* lipase B) (~43% conversion after 1 h), but no enantioselectivity was observed (entry 2). Lipase PS-IM (from *Burkholderia cepacia*) gave an even faster reaction (33% conversion after 1 h) than with lipase PS, but again with low enantioselectivity (E <2) (entry 19).

When the reaction temperature was lowered (from 45 to 30 °C, and then to 2 °C), the conversion decreased slightly (data not shown), but no beneficial effect on the enantioselectivity was observed (E <2). Accordingly, subsequent experiments were planned at 45 °C.

In an effort to enhance the enantioselectivity, vinyl acetate was replaced by other vinyl esters [vinyl butyrate (entry 8), vinyl pivalate (entry 9), vinyl decanoate (entry 10), vinyl laurate (entry 18) and *i*-propenyl acetate (entry 7)]. The best enantioselectivities (E ~14) were observed for the esters with longer carbon chains, and vinyl decanoate was therefore chosen as the acyl donor for further studies.

When catalytic amounts of Et_3N and Na_2SO_4 were added to the reaction mixture, a slight increase in reaction rate was observed (entry 12 vs entry 11). The reaction rate was further enhanced when the lipase PS-catalysed acylation was performed with 3 or even 4, instead of 1.1 equiv of vinyl decanoate (entries 12–14).

In order to improve the enantioselectivity, we also tested several solvents, such as *t*-BuOMe, *n*-hexane, toluene and acetonitrile (MeCN) (entries 11 and 15–17). Overall, *n*-hexane ensured a slightly better combination of enantioselectivity and reaction rate (entry 15) than did *t*-BuOMe (entry 11), but with regard to the development of more environmentally friendly routes to enantiopure products, *t*-BuOMe was chosen as a green solvent for further optimisation and gram-scale reactions.

Reactions with 50 or 70 mg mL^{-1} instead of 30 mg mL^{-1} of lipase PS in *t*-BuOMe in the presence of 1.1 equiv of vinyl decanoate and catalytic amounts of Et_3N and Na_2SO_4 at 45 °C exhibited slightly higher reaction rates (after 1 h, conversions of 26% with 30 mg mL^{-1}; 28% with 50 mg mL^{-1} and 32% with 70 mg mL^{-1}). However, there was basically no influence on the enantioselectivity (E ~50) and for reasons of economy, 30 mg mL^{-1} lipase PS was finally chosen for the gram-scale resolution of (±)-**3**.

In view of the results of the optimisation study, we decided to perform the gram-scale resolution of (±)-**3** in the presence of 30 mg mL^{-1} lipase PS, with 4 equiv of vinyl decanoate and catalytic amounts of Et_3N and Na_2SO_4, in *t*-BuOMe, at 45 °C in two steps. We planned to stop the acylation at about 40% conversion (ee acylated product ~95%), and then perform the subsequent reaction until about 60% conversion (ee unreacted alcohol ~95%). The results are shown in Table 3 and the Section 4.

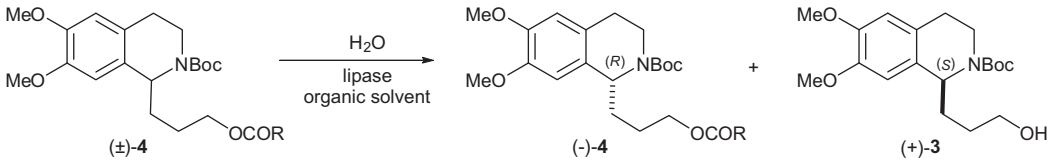
2.3. Enzymatic hydrolysis of (±)-4

The previous results on the lipase-catalysed deacylation of O-acylated primary alcohols,[9a,10e,11,13] prompted us to start our experiments on the hydrolysis of racemic *N*-Boc-protected 1-(3-acetyloxypropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline with lipase PS as catalyst and H_2O as the nucleophile (Scheme 3, R = Me).

Since a slow reaction with relatively low enantioselectivity (E ~5) was observed when the lipase PS-catalysed reaction was performed in *t*-BuOMe (Table 2, entry 1), we conducted preliminary screening experiments with enzymes (30 mg mL^{-1}) including CAL-A, CAL-B, lipase AY and PPL. Practically no reaction was observed for the majority of the lipases tested (entries 4–6) even after 3 weeks; the only exception was CAL-A, which catalysed the hydrolysis with a comparable reaction rate to that with lipase PS, but with a lower E value (entry 3).

Our earlier observations on the enzymatic hydrolysis of *N*,*O*-diacetyl derivatives of cyclic 1,3-amino alcohols[10c] led us to use EtOH instead of H_2O as a nucleophile for the lipase PS-catalysed hydrolysis, but the reaction became slower and no beneficial change in E (Table 2, entry 2) was observed.

The size of the acyl moiety had a marked effect on both the enantioselectivity and the reaction rate. For example, significantly higher enantioselectivity and a faster reaction were observed for



Scheme 3. Enzymatic hydrolysis of (±)-**4**.

Table 2
Conversion, enantiomeric excess (ee) and enantioselectivity (E) of the hydrolysis of (±)-**4**[a]

Entry	Enzyme	R	Nucleophile (equiv)	Solvent	t (h)	ee_s[b] (%)	ee_p[b] (%)	Conversion (%)	E
1	Lipase PS[c]	Me	H_2O (0.5)	*t*-BuOMe	50	11	65	14	5.2
2	Lipase PS[c]	Me	EtOH (*t*-BuOMe:EtOH 24:1)	*t*-BuOMe	50	3	63	5	4.5
3	CAL-A[c]	Me	H_2O (0.5)	*t*-BuOMe	50	3	26	10	1.8
4	CAL-B	Me	H_2O (0.5)	*t*-BuOMe	50	*rac*	*rac*	50	1
5	Lipase AY[c]	Me	H_2O (0.5)	*t*-BuOMe	500	No reaction			
6	PPL	Me	H_2O (0.5)	*t*-BuOMe	500	No reaction			
7	Lipase PS[c]	$(CH_2)_8Me$	H_2O (1)	*t*-BuOMe	120	32	96	25	67
8	Lipase PS[c]	$(CH_2)_8Me$	H_2O (1)	iPr_2O	120	24	84	22	15
9	Lipase PS[c]	$(CH_2)_8Me$	H_2O (1)	*n*-hexane	120	50	88	36	27
10	Lipase PS[c]	$(CH_2)_8Me$	H_2O (0)	*t*-BuOMe	120	20	96	17	60
11	Lipase PS[c]	$(CH_2)_8Me$	H_2O (4)	*t*-BuOMe	120	28	96	23	64
12	Lipase PS-IM	$(CH_2)_8Me$	H_2O (1)	*t*-BuOMe	120	54	77	41	13
13	CAL-B	$(CH_2)_8Me$	H_2O (1)	*t*-BuOMe	50	*rac*	*rac*	71	1
14	Lipase AY[c]	$(CH_2)_8Me$	H_2O (1)	*t*-BuOMe	120	10	72	12	7
15	Lipase AK[c]	$(CH_2)_8Me$	H_2O (1)	*t*-BuOMe	120	2	45	4	3

[a] 0.0125 M substrate, 30 mg mL^{-1} enzyme, 1 mL of *t*-BuOMe, 45 °C.
[b] According to HPLC (see Section 4).
[c] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

the hydrolysis of the corresponding decanoate [R = $(CH_2)_8Me$] than for the acetate (R = Me) (Table 2, entry 7 vs entry 1).

In an attempt to increase the enantioselectivity further, lipase PS-IM, CAL-B, lipase AY and lipase AK were also tested for the hydrolysis of (±)-**4** [R = $(CH_2)_8Me$]. Although all of these lipases demonstrated similar enantioselectivities (Table 2, entries 12–15), none of them proved to be a better catalyst than lipase PS (E ~65).

In order to determine the effects of H_2O in the reaction medium on the enzymatic activity, (±)-**4** [R = $(CH_2)_8Me$] was hydrolysed in the presence of different amounts of water (Table 2, entries 7, 10 and 11). Neither the enantioselectivity nor the conversion was affected when 1 or 4 equiv of water were used. Furthermore, the hydrolysis took place with comparable E, but was slower without the addition of any H_2O (Table 2, entry 10) (the H_2O present in the enzyme preparation or in the solvent used was sufficient for the hydrolysis).

Solvents such as iPr_2O and n-hexane (Table 2, entries 8 and 9) were also tested for the lipase PS (30 mg mL^{-1}) catalysed hydrolysis of (±)-**4** [R = $(CH_2)_8Me$], but none of them ensured a better combination of E or reaction rate than with t-BuOMe (Table 2, entry 7).

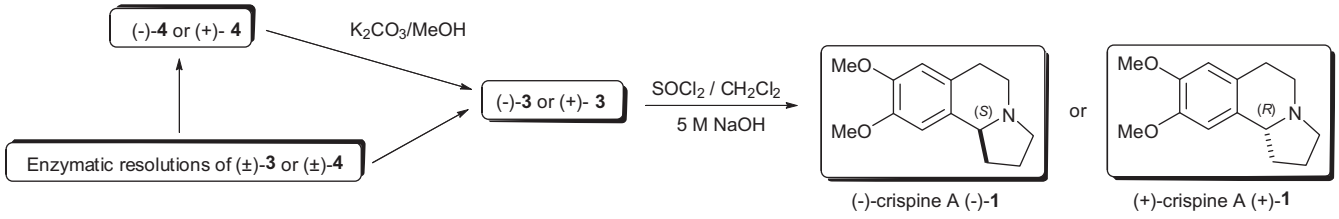
When the reaction was performed with 75 mg mL^{-1} instead of 30 mg mL^{-1} of lipase PS in t-BuOMe in the presence of 1 equiv of H_2O at 45 °C, both the reactivity and the enantioselectivity increased slightly (after 9 days: conversion = 39%, E = 60 for 30 mg mL^{-1}, and conversion = 44%, E = 71 for 75 mg mL^{-1} enzyme). However, for reasons of economy, 30 mg mL^{-1} lipase PS was chosen for the preparative-scale resolutions of (±)-**4** [R = $(CH_2)_8Me$].

The results of the above experiments suggested the preparative-scale resolution of (±)-**4** [R = $(CH_2)_8Me$] in the presence of lipase PS (30 mg mL^{-1}), with 1 equiv of H_2O, in t-BuOMe, at 45 °C, in two steps. The results are presented in Table 3 and in the Section 4.

Table 3
Lipase PS[a]-catalysed preparative-scale resolutions of (±)-**3** and (±)-**4** [R = $(CH_2)_8Me$][a]

	Time (h)	Conversion on workup (%)	Enantiomer	Yield (%)	Isomer	ee[b] (%)	$[\alpha]_D^{25}$ ($CHCl_3$)
(±)-**3**	1	43	(*S*)-**4** [R = $(CH_2)_8Me$]	31	(*S*)	94	+52 (*c* 0.2)
	3.5	64	(*R*)-**3**	31	(*R*)	95	-60 (*c* 0.5)
(±)-**4** [R = $(CH_2)_8Me$]	54	24	(*S*)-**3**	21	(*S*)	94	+59 (*c* 0.265)
	192	60	(*R*)-**4** [R = $(CH_2)_8Me$]	28	(*R*)	96	-58 (*c* 0.305)

[a] 30 mg mL^{-1} lipase PS [containing 20% (w/w) lipase adsorbed on Celite in the presence of sucrose], t-BuOMe, at 45 °C; 4 equiv of vinyl decanoate for the acylation of (±)-**3** and 1 equiv of water for the hydrolysis of (±)-**4** [R = $(CH_2)_8Me$].
[b] According to HPLC (see Section 4).



Scheme 4. Syntheses of (−)-**1** and (+)-**1**.

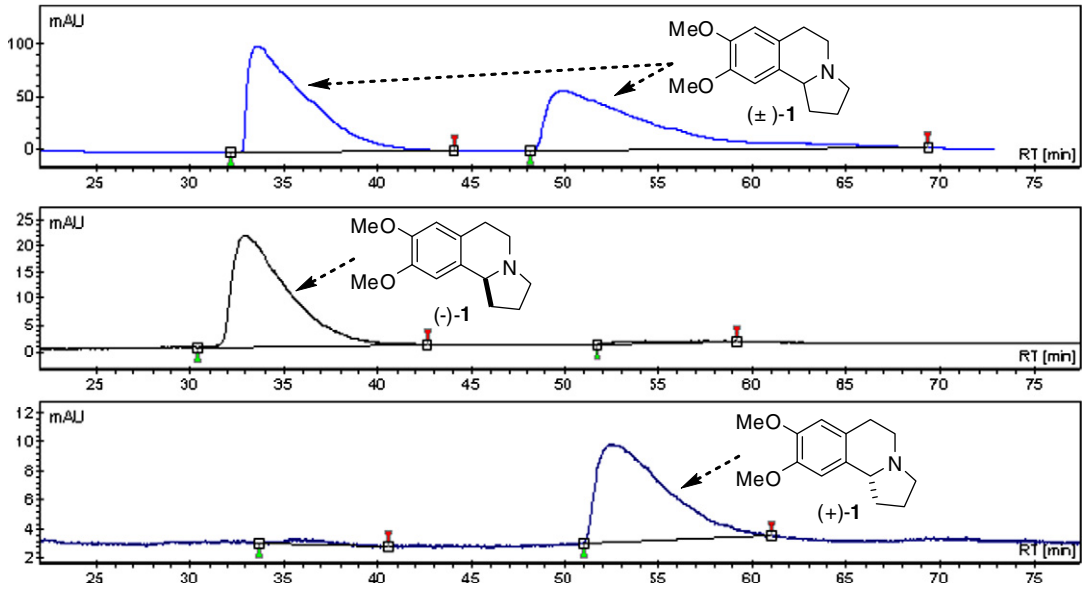


Figure 1. Enantioseparation of (±)-**1** [Chiralcel OD-H column, eluent: n-hexane: (Et_2NH 0.1% v/v):iPA (95:5), flow rate: 0.5 mL min^{-1}, detection at 260 nm].

2.4. Synthesis of (−)-1 and (+)-1

The O-acylated enantiomers (+)-**4** [R = $(CH_2)_8Me$] and (−)-**4** [R = $(CH_2)_8Me$] were hydrolysed to the corresponding alcohols (+)-**3** and (−)-**3** in K_2CO_3/MeOH, at room temperature, without a loss in enantiopurity. The enantiopure (+)-**3** and (−)-**3** were then converted to the corresponding enantiopure crispine A (Scheme 4).

In contrast with the literature cyclisation procedure,[2c] (+)-**3** and (−)-**3** did not need deprotection. Instead they underwent cyclisation in the presence of thionyl chloride to furnish (+)-**1** and (−)-**1** (ee ⩾ 94%) in 1 step in relatively high yields (Section 4).

It should be mentioned that (+)-**3** and (−)-**3** can also serve as starting enantiomers for the synthesis of enantiomerically pure crispine E. As an example, Itoh and co-workers[5b] described the synthesis of (−)-crispine E (−)-**2** from aminoalcohol (+)-**3** through a Mitsunobu reaction, followed by removal of the Boc groups.

In order to determine the exact ee values for the crispine A enantiomers prepared, a new direct HPLC method was devised (Fig. 1).

2.5. Absolute configurations

The stereochemistry of the crispine A enantiomers was confirmed by comparing the α values with the literature data[1,5a] (see Section 4). Thus, both the lipase PS-catalysed acylation of (±)-**3** and the hydrolysis of (±)-**4** [R = $(CH_2)_8Me$] displayed (*S*)-selectivity.

3. Conclusions

A new total synthesis of crispine A enantiomers has been developed via the *Burkholderia cepacia* lipase-catalysed acylation of the primary hydroxy group of *N*-Boc-protected 1-(3-hydroxypropyl)-6,7-bis(methyloxy)-1,2,3,4-tetrahydroisoquinoline (±)-**3** or enantioselective hydrolysis of the corresponding O-decanoate (±)-**4** [R = $(CH_2)_8Me$], with a remote, four-atom distant stereogenic centre. The (*S*)-selective acylation of (±)-**3** was performed in two steps with vinyl decanoate (0.5 equiv), using lipase PS in *t*-BuOMe, at 45 °C (*E* = 68), while the lipase PS-catalysed *S*-selective two-step hydrolysis of (±)-**4** [R = $(CH_2)_8Me$] required 1 equiv of H_2O as nucleophile, and the reaction was performed in *t*-BuOMe, at 45 °C (*E* = 52). The enantiomers of **3** and **4** [R = $(CH_2)_8Me$] (ee ⩾ 94%) were isolated in good yields (⩾21%), with separation by column chromatography. Ester enantiomers (+)-**4** [R = $(CH_2)_8Me$] and (−)-**4** [R = $(CH_2)_8Me$] were hydrolysed to the corresponding alcohols (+)-**3** and (−)-**3** in K_2CO_3/MeOH without a loss of enantiopurity. A direct ring-closing reaction of (+)-**3** and (−)-**3** without deprotection resulted in the desired enantiomers of crispine A (ee ⩾ 95%).

It should be mentioned that, although crispine A enantiomers have previously been prepared by asymmetric routes, to the best of our knowledge they have not yet been obtained via a strategy involving an enzyme-catalysed kinetic resolution. In parallel, a new HPLC method has been developed for the enantioseparation of racemic crispine A on a Chiralcel OD-H column.

4. Experimental

4.1. Materials and methods

Lipase PS-IM (from *Burkholderia cepacia,* immobilised on diatomaceous earth) was a gift from Amano Enzyme Europe Ltd, Lipase AK (*Pseudomonas fluorescens*) was from Amano Pharmaceuticals, Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin), lipase PS (*Pseudomonas cepacia*) and PPL (porcine pancreas lipase type II) were from Sigma, and Chyrazyme L-5 (lipase A from *Candida antarctica*) was from Novo Nordisk. Before use, lipase PS, lipase AK, lipase AY and CAL-A (5 g) were dissolved in a tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g). The lipase preparation thus obtained contained 20% (w/w) lipase. The solvents were of the highest analytical grade.

In a typical small-scale experiment, (±)-**3** or (±)-**4** (0.025 M solution) in an organic solvent (1 mL) was added to the enzyme tested (30, 50 or 75 mg mL^{-1}). The acyl donor (1.1, 3 or 4 equiv) or H_2O (0.5, 1 or 4 equiv) was added, followed in some cases by catalytic amounts of Et_3N and Na_2SO_4. The mixture was shaken at 2, 30, 45 or 60 °C.

The ee values for the amino alcohol, amino ester and crispine A enantiomers produced were determined by HPLC [Chiralpak IA column, eluent: *n*-hexane:*i*PA (85:15), flow rate: 0.5 mL min^{-1}, detection at 260 nm]; retention times (min) for (−)-**3**: 25.61, for (+)-**3**: 41.61, for (−)-**4** [R = $(CH_2)_8Me$]: 12.43 and for (+)-**4** [R = $(CH_2)_8Me$]: 15.6, and [Chiralcel OD-H column, eluent: *n*-hexane (Et_2NH 0.1% v/v):*i*PA (95:5), flow rate: 0.5 mL min^{-1}, detection at 260 nm] for (−)-**1**: 33.61 and for (+)-**1**: 49.84.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. The results of elemental analyses (CHNS) corresponded closely (within ±0.3%) with the calculated data in all cases.

4.2. Synthesis of (±)-4 [R = $(CH_2)_8Me$]

Alcohol (±)-**3** (500 mg, 1.42 mmol) was dissolved in $CHCl_3$ (50 mL). Decanoyl chloride (297.5 mg, 1.56 mmol) was then added. The solution was stirred at room temperature for overnight. After evaporation, the residue was chromatographed on silica. Elution with AcOEt:hexane (1:5) afforded (±)-**4** [R = $(CH_2)_8Me$] as a light-yellow oil (589 mg, 82%). The ^1H NMR (400 MHz, $CDCl_3$, 50 °C, TMS) spectroscopic data for (±)-**4** [R = $(CH_2)_8Me$]: δ (ppm) = 0.91 (3H, t, *J* = 6.4 Hz, CH_2CH_3), 1.3 (16H, br s), 1.51 (9H, s, $C(CH_3)_3$), 1.61–1.84 (4H, m, $CH_2CH_2CH_2OH$), 2.62–2.66 (1H, m, $ArCH_2$), 2.81–2.9 (1H, m, $ArCH_2$), 3.2 (1H, br s, CH_2N), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.11–4.19 (3H, m, CH_2OCOCH_2 and CH_2N overlapping), 5.1 (1H, br s, CHN), 6.61 (2H, s, Ar). Anal. Calcld for $C_{29}H_{47}NO_6$: C, 68.88; H, 9.37; N, 2.77. Found: C, 68.59; H, 9.44; N, 2.60.

4.3. Gram-scale resolution of (±)-3

Racemic **3** (500 mg, 1.42 mmol) was dissolved in *t*-BuOMe (40 mL). Lipase PS (1.2 g, 30 mg mL^{-1}), vinyl decanoate (0.65 mL, 2.84 mmol), Et_3N (14.37 mg, 0.142 mmol) and Na_2SO_4 (catalytic amount) were added and the mixture was shaken in an incubator shaker at 45 °C for 1 h. The enzyme was filtered off at 43% conversion (ee_p = 94%), and the solvent was evaporated off. The residue was chromatographed on silica, with elution with *n*-hexane:EtOAc (5:1) affording the ester (*S*)-**4** [R = $(CH_2)_8Me$] {223 mg, 31%; $[\alpha]_D^{25} = +52$ (*c* 0.2, $CHCl_3$); a light-yellow oil; ee = 94%} and the enantiomerically enriched alcohol **3** (244 mg, 0.69 mmol; ee = 70%). In order to obtain **3** with high ee, the alcohol ($ee_{\mathbf{3}}$ = 70%) (244 mg) was subjected to a second enzymatic resolution in *t*-BuOMe (40 mL), using lipase PS (400 mg, 10 mg mL^{-1}), vinyl decanoate (0.65 mL, 2.84 mmol), Et_3N (7.2 mg) and a catalytic amount of Na_2SO_4 at 45 °C. The reaction was stopped after 3.5 h (ee_S = 95%). After a second column chromatography, the desired (*R*)-**3** {156 mg, 31%; $[\alpha]_D^{25} = -60$ (*c* 0.5, $CHCl_3$); a light-yellow oil, ee = 95%} was obtained.

The ^1H NMR (400 MHz, $CDCl_3$, 50 °C, TMS) spectroscopic data for (*R*)-**3**: δ (ppm) = 1.51 [9H, s, $C(CH_3)_3$], 1.66–1.87 (4H, m, $CH_2CH_2CH_2OH$), 2.66 (1H, dt, *J* = 15.6, 3.7 Hz, $ArCH_2$), 2.81–2.89

(1H, m, ArCH_2), 3.2 (1H, br s, CH_2N), 3.72–3.78 (2H, m, CH_2OH), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.15 (1H, br s, CH_2N), 5.15 (1H, br s, CHN), 6.6 (1H, s, Ar), 6.64 (1H, s, Ar). Anal. Calcd for $C_{19}H_{29}NO_5$: C, 64.93; H, 8.32; N, 3.99. Found: C, 64.82; H, 8.03; N, 4.21.

The ^{1}H NMR (400 MHz, $CDCl_3$, 50 °C, TMS) spectroscopic data for (*S*)-**4** [R = $(CH_2)_8$Me] were similar to those for (±)-**4** [R = $(CH_2)_8$Me]. Analysis: found for (*S*)-**4** [R = $(CH_2)_8$Me]: C, 68.88; H, 9.37; N, 2.77.

4.4. Gram-scale resolution of (±)-4 [R = $(CH_2)_8$Me]

Racemic **4** [R = $(CH_2)_8$Me] (400 mg, 0.79 mmol) was dissolved in *t*-BuOMe (40 mL). Lipase PS (1.2 g, 30 mg mL^{-1}) and water (14 μL, 0.79 mmol) were added and the mixture was shaken at 45 °C for 54 h. The enzyme was filtered off at 24% conversion (ee_p = 95%). Column chromatography under the above-mentioned conditions afforded (*S*)-**3** [59 mg, 21%; $[\alpha]_D^{25} = +59$ (*c* 0.265, $CHCl_3$); a light-yellow oil; ee = 95%] and enantiomerically enriched **4** (259 mg, 0.51 mmol; ee = 30%). The ester (ee_4 = 30%) was subjected to a second enzymatic hydrolysis in *t*-BuOMe (40 mL) with lipase PS (300 mg) and water (11 μL, 0.61 mmol), at 45 °C. The reaction was stopped after 192 h (ee_s = 96%). By using the separation procedure described above, the desired (*R*)-**4** [R = $(CH_2)_8$Me] {110 mg, 28%); $[\alpha]_D^{25} = -58$ (*c* 0.305, $CHCl_3$); a light-yellow oil, ee = 96%} was obtained.

The ^{1}H NMR (400 MHz, $CDCl_3$, 50 °C, TMS) spectroscopic data for (*S*)-**3** were similar to those for (*R*)-**3**. Analysis: found for (*S*)-**3**: C, 64.77; H, 8.28; N, 4.11.

The ^{1}H NMR (400 MHz, $CDCl_3$, 50 °C, TMS) spectroscopic data for (*R*)-**4** [R = $(CH_2)_8$Me] were similar to those for (*S*)-**4** [R = $(CH_2)_8$Me] and (±)-**4** [R = $(CH_2)_8$Me]. Analysis: found for (*R*)-**4** [R = $(CH_2)_8$Me]: C, 68.71; H, 9.26; N, 2.62.

4.5. Deacylation of (*S*)-4 [R = $(CH_2)_8$Me] and (*R*)-4 [R = $(CH_2)_8$Me]

A mixture of (*S*)-**4** [R = $(CH_2)_8$Me] (210 mg, 0.415 mmol) and K_2CO_3 (0.14 g, 0.96 mmol) in MeOH (15 mL) was refluxed for 8 h. After evaporation, the residue was dissolved in H_2O (20 mL) and extracted with Et_2O. The organic phase was dried (Na_2SO_4), filtered and evaporated. The product (*S*)-**3** {[0.13 g, 89%; $[\alpha]_D^{25} = +60$ (*c* 0.31, $CHCl_3$); ee = 94%} was obtained as a light-yellow oil. Similarly, (*R*)-**4** [R = $(CH_2)_8$Me] (0.14 g, 0.96 mmol) afforded (*R*)-**3** {[0.082 g, 84%; $[\alpha]_D^{25} = -60$ (*c* 0.2, $CHCl_3$); ee = 96%}.

4.6. Synthesis of crispine A enantiomers (*R*)-1 and (*S*)-1

Alcohol (−)-**3** or (+)-**3** (100 mg, 0.28 mmol) was dissolved in CH_2Cl_2 (7 mL). Thionyl chloride (100 mg, 0.84 mmol) was added. After stirring for 2 h at 40 °C, the reaction mixture was cooled to below 20 °C and a solution of NaOH (5 N) was added until pH ⩾9. After a further stirring for 1 h at room temperature, the reaction was quenched with 10 mL water. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and evaporated. The oily residue was purified by column chromatography, with $CHCl_3$:MeOH (9:1) as eluent. The product was crystallised from Et_2O as the desired crispine A enantiomer (*R*)-**1** {55 mg (83%); mp 87–89 °C, lit.[1] mp 88–89 °C; lit.[5i] mp 86–88 °C; $[\alpha]_D^{25} = +82$ (*c* 0.255, MeOH), lit.[5e] $[\alpha]_D^{25} = +43.9$ (MeOH), lit.[1] $[\alpha]_D^{25} = +91.0$ (MeOH), $[\alpha]_D^{25} = +60$ (*c* 0.255, $CHCl_3$), lit.[5a] $[\alpha]_D^{23} = +100.4$ (*c* 1, $CHCl_3$), lit.[5i] $[\alpha]_D^{23} = +96.9$ (*c* 1.1, $CHCl_3$), ee = 95%} or (*S*)-**1** {52 mg, 78 %; mp 87–89 °C, lit.[1,5e] mp 88–89 °C, lit.[5i] mp 86–88 °C; $[\alpha]_D^{25} = -61$ (*c* 0.28, $CHCl_3$), lit.[5i] $[\alpha]_D^{23} = -95$ (*c* 1.3, $CHCl_3$), ee = 96%}, as a colourless solid.

The ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS) spectroscopic data for (*R*)-**1** were similar to those for (*S*)-**1**: δ (ppm) = 1.69–1.95 (3H, m, CH_2CH_2CH_2N), 2.56–2.58 (1H, m, CH_2CH_2N), 2.56–2.65 (2H, m, CH_2N), 2.69–2.77 (1H, m, ArCH_2), 3.05–3.22 (3H, m, CH_2N and ArCH_2 overlapping), 3.41–3.43 (1H, m, CHN), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.6 (1H, s, Ar), 6.64 (1H, s, Ar). Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found for (*R*)-**1**: C, 71.88; H, 8.29; N, 6.09. Found for (*S*)-**1**: C, 72.00; H, 8.28; N, 6.02.

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