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ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 2147–2154

Enzymatic resolution of methyl (1*RS*)-*N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate by Seaprose S

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Received 28 June 2007; accepted 6 September 2007

Abstract—An efficient biocatalytic process has been developed for the resolution of methyl (1*RS*)-*N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate *rac*-**1** by means of Seaprose S-mediated enantioselective hydrolysis to afford (1*S*)-**2** and (1*R*)-**1** in 87% and 93% isolated yield, 101% and 96% potency, and ee >99.8% and >99.5%, respectively.

1. Introduction

Biocatalysis has become a widely used tool in the pharmaceutical industry for the synthesis of chiral intermediates via asymmetric syntheses and kinetic resolutions, mediated most commonly by hydrolase, lyase, oxidoreductase, and transaminase enzymes.[1–8] Lipase-catalyzed transformations of carboxylic acids, alcohols, and amines, especially the enantioselective hydrolysis of esters and amides, and the stereoselective esterification of carboxylic acids and acylation of alcohols and amines, are among the most utilized biocatalytic methods for the preparation of enantiomerically pure alcohols, amines, amides, acids, and esters.[9–17] This derives from the early recognition of lipases as highly active and robust enzymes with broad substrate specificities, unique stereo-, chemo- and regio-selectivities, and the ability to perform a range of synthetically useful reactions under a variety of conditions, together with their decades-long industrial application in the pharmaceutical, fine-chemicals, and food sectors.[18–20]

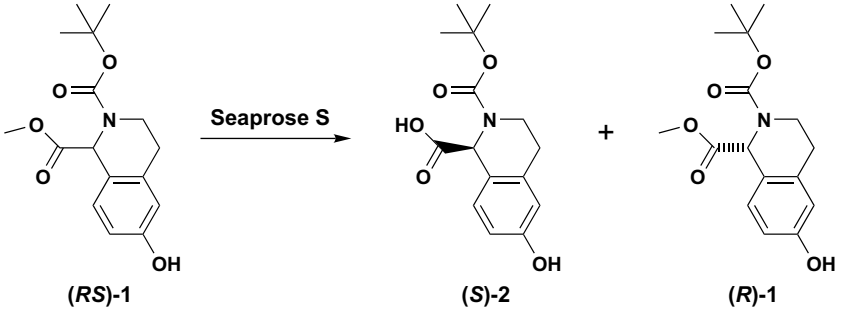

Figure 1. Biocatalytic hydrolytic resolution of methyl (1*RS*)-*N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate *rac*-**1**.

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0957-4166/$ - see front matter
doi:10.1016/j.tetasy.2007.09.011

Herein, we report the screening of enzymes for the enantioselective hydrolysis of racemic methyl *N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate **1**. The (*R*)-isomer is a key intermediate for the synthesis of potent agonists for a Liver X Receptor (LXR) discovery program.[21,22] We also report the screening of hydrolase biocatalysts for the resolution of (*RS*)-**1**, and the development of a highly enantioselective hydrolytic resolution process for the preparation of (1*S*)-**2** and (1*R*)-**1** (Fig. 1).

2. Results and discussion

About 140 native and immobilized hydrolase enzymes were screened for the enantioselective hydrolysis of (*RS*)-**1** at a substrate concentration of 1 g L^{-1} in aqueous buffer containing 1% v/v of DMSO (Tables 1–3), 303 K, 40 h. Only a few enzymes showed significant activity toward the substrate, most notably Protease P and Seaprose S (a more concentrated form of Protease P), which mediated highly (*S*)-selective hydrolysis with no detectable cleavage of the undesired (1*R*)-ester. Interestingly, the large majority of the lipase, protease, peptidase, and acylase biocatalysts appeared to be (*S*)-selective, while the esterase enzymes showed either no significant enantioselectivity or only slight (*R*)-preference. Biocatalyst screening for hydrolysis in monophasic aqueous–organic media (comprising 20%, 40%, 60% or 80% v/v of ethanol, propan-2-ol, butan-1-ol, acetonitrile, dimethoxymethane or THF in 50 mM phosphate buffer, pH 8.0), or transesterification in organic media (ethanol, propan-1-ol or butan-1-ol containing 0–10% v/v buffer, or acetonitrile, THF, methyl-THF, diethyl ether, MTBE, dichloromethane or toluene containing 0.1 M propan-1-ol and 0–10% v/v of buffer) were unsuccessful, with no significant reaction (<2%) being detected with any enzyme (native or immobilized) under any of the conditions tried. Similarly, hydrolysis in biphasic aqueous–organic media (buffer with an equal volume of *t*-butanol, *t*-amyl alcohol, methyl-tetrahydrofuran, 1,2-dimethoxyethane, diethyl ether, diisopropyl ether, methyl

Table 1. Lipase-mediated enantioselective hydrolysis of (*RS*)-**1**

Biocatalyst	[Biocatalyst] (g L^{-1})	Conversion (%)	Selectivity	ee_P (%)
Amano Lipase-AK	20	41	—	0
Amano Lipase-AP12	20	4	(*S*)	38
Amano Lipase-AY30	20	9	(*S*)	3
Amanop Lipase-D	20	10	(*S*)	9
Amano Lipase-F	20	16	(*S*)	5
Amano Lipase-FAP15	20	6	(*S*)	9
Amano Lipase-G	20	12	(*S*)	6
Amano Lipase-GC20	20	14	(*S*)	5
Amano Lipase-M	20	15	(*S*)	5
Amano Lipase-MAP10	20	17	(*S*)	4
Amano Lipase-N	20	9	(*S*)	10
Amano Lipase-PS	20	6	(*S*)	17
Amano Lipase-PS30	20	5	(*S*)	9
Amano Lipase-R	20	8	(*S*)	4
Biocatalysts Lipase-ANL	20	1	(*S*)	53
Biocatalysts Lipase-CCL	20	30	—	0
Biocatalysts Lipase-RJL	20	31	(*S*)	3
Boehringer Chirazyme-L3	20	17	(*S*)	3
Enzymatix Lipase-B1	20	2	(*S*)	14
Enzymatix Lipase-F5	20	50	—	0
Europa Lipase-4	20	32	—	0
Europa Lipase-13	20	35	—	0
Europa Lipase-14	20	28	—	0
Europa Lipase-21	20	45	—	0
Julich Lipase-RN	20	13	—	5
Julich Lipase-RO	20	20	(*S*)	5
Meito Sangyo Lipase-AL	20	19	—	0
Meito Sangyo Lipase-MY	20	18	—	0
Meito Sangyo Lipase-OF	20	39	—	0
Meito Sangyo Lipase-PL	20	47	—	0
Meito Sangyo Lipase-QLM	20	19	(*S*)	4
Meito Sangyo Lipase-SL	20	19	(*S*)	4
Meito Sangyo Lipase-TL	20	27	—	0
Meito Sangyo Lipase-UL	20	35	—	0
Sigma Lipase-CRL	20	30	—	0
Sigma Lipase-PPL	40	7	(*S*)	27
Sepracor Lipase-OF	20	53	—	1

Conditions: substrate [10 μL, 100 g L^{-1} of (*RS*)-**1** in DMSO] was added to a vigorously stirred solution/suspension of biocatalyst (1 mL, 20 or 40 g L^{-1} of biocatalyst in 0.1 M sodium phosphate, pH 8), and the mixture stirred at 400 rpm, 303 K, 40 h. The percentage conversion refers to the overall extent of hydrolysis. The selectivity refers to the configuration of the major hydrolysis product. ee values were determined by chiral HPLC.

Table 2. Esterase-mediated enantioselective hydrolysis of (*RS*)-**1**

Biocatalyst	[Biocatalyst] (g L^{-1})	Conversion (%)	Selectivity	ee_P (%)
NA				
Biocatalysts Lipomod-200	100	27	(*S*)	3
Boehringer Chirazyme-L2-c2	100	51	(*R*)	3
Boehringer Chirazyme-L5	100	43	(*R*)	3
Amano Lipase PS30/Accurel	100	38	—	0
Novo Lipolase-30T	100	80	—	0
Novo Lipozym-IM60	100	83	—	0
Novo Novozym-435	100	58	—	0
Fluka *B. thermoglucisdasus* Esterase	20	48	—	0
Fluka *B. stearothermophilus* Esterase	20	45	—	0
Fluka *C. lipolytica* Esterase	20	47	—	0
Fluka *M. miehei* Esterase	20	45	—	0
Fluka *R. oryzae* Esterase	20	36	—	0
Fluka *S. cerevisiae* Esterase	20	71	—	0
Fluka *S. diastatochromogenes* Esterase	20	64	—	0
Fluka *T. lanuginosus* Esterase	20	68	—	0
Julich Esterase-BS1	20	73	—	0
Julich Esterase-BS2	20	59	—	0
Julich Esterase-BS3	20	61	—	0
Julich Esterase-SD	20	58	—	0
Julich Esterase-PF	20	68	—	0
Immobilized Captopril Esterase	100	14	—	0

Conditions: substrate [10 μL, 100 g L^{-1} of (*RS*)-**1** in DMSO] was added to a vigorously stirred solution/suspension of biocatalyst (1 mL, 20 or 100 g L^{-1} of biocatalyst in 0.1 M sodium phosphate, pH 8), and the mixture stirred at 400 rpm, 303 K, 40 h. The percentage conversion refers to the overall extent of hydrolysis. The selectivity refers to the configuration of the major hydrolysis product. ee values were determined by chiral HPLC.

t-butyl ether, dichloromethane, hexane or heptane) gave very poor conversions (<5%) presumably due to unfavorable substrate partitioning and/or poor enzyme stability.

Further experiments were carried out to optimize Protease P and Seaprose S-mediated hydrolysis in aqueous milieu, with the focus on buffer type and concentration, substrate load, enzyme-to-substrate ratio, type and concentration of the cosolvent, and reaction temperature. Buffer screening over pH 5–9 showed that 0.1 M sodium phosphate, pH 7.5–8.0, gave the best results in terms of both conversion and enantioselectivity (Table 4). Thus, low rates and incomplete reactions were observed in the absence of buffer and when buffering with 50 mM phosphate and at pH values below 7.5, while high background (non-selective) hydrolysis and low ee values were incurred at pH values above 8.0. The effect of substrate loading over 1–5 g L^{-1}, with biocatalyst-to-substrate ratios of 5–20 for Protease P and 2.5–7.5 for Seaprose S was then examined (Table 5). It was found that a substrate input of 1 g L^{-1} with a Protease P load of 15–20 g L^{-1} or a Seaprose S load of 7.5 g L^{-1} consistently provided near-quantitative and highly enantiospecific hydrolysis of (1*S*)-**1**, with no detectable cleavage of (1*R*)-**1**. Although some experiments conducted with 2 and 5 g L^{-1} of substrate provided good conversions and enantioselectivities, reproducibility was lacking due to oiling-out and crusting of the substrate/product compromising conversions and selectivity, despite increasing the buffer strength to 0.2 M and/or the DMSO concentration to 15% v/v. The inclusion of cosolvents (ethanol, propan-2-ol, acetonitrile, acetone, tetrahydrofuran, and dimethoxymethane) at 5% or 10% v/v (in addition to 1% v/v of DMSO) led to drastic reductions in hydrolysis rates and conversions, together with significant drops in enantioselectivity (Table 6). Similarly, the optimal DMSO concentration was found to be 1–2% v/v, with higher levels leading to reductions in rate, conversion, and enantioselectivity. In an effort to circumvent the limitation of substrate load and improve its availability and reduce oiling-out, the hydrolysis of (1*RS*)-**1** was examined in the presence of Celite 561 and R633 filter-aids, microcrystalline and fibrous cellulose, and Amberlite XAD-4, 7, 8 and 16, and Diaion HP20, HP2MG resins, and using up to 10% v/v of DMSO as a cosolvent. However, there was no significant visible improvement of substrate dispersion in any case, with substrate crusting apparent after just one day, and the inclusion of adsorbent resulted in significantly decreased conversions (results not shown). With regards to reaction temperature, it was found that the results were optimal at about 293–303 K, with reaction rates dropping significantly below 303 K and resulting in incomplete conversions, while oiling-out of the substrate and rapid inactivation of the biocatalyst compromised selectivities and yields above 313 K. It should be noted that the corresponding ethyl, prop-1-yl and 2-hydroxyethyl esters (prepared in quantitative yields by stirring 0.2 M of (1*RS*)-**1**, in the corresponding alcohol with 5 mol % of ytterbium or scandium triflate as catalyst, 50 °C, 3 d) gave rather poor results, with severe oiling-out and low conversions [42–76% hydrolysis of (1*S*)-**1**] being observed.

Having optimized the reaction conditions (1 g L^{-1} of substrate, Seaprose S as catalyst, 2% v/v DMSO, 100 mM sodium phosphate, pH 8.0, rt), the work-up of the reaction mixture was then examined. Initial efforts at processing the reaction by way of adjusting the pH to 9–9.5 and extracting (1*R*)-**1** with MTBE, DCM, ethyl acetate or hexane, followed by acidification of the aqueous phase to pH

Table 3. Protease, peptidase and acylase-mediated enantioselective hydrolysis of (*RS*)-**1**

Biocatalyst	[Biocatalyst] (g L^{-1})	Conversion (%)	Selectivity	ee_P (%)
Amano Acylase	20	14	(*S*)	15
Amano D-Aminoacylase	20	3	(*S*)	2
Amino Acid Protease-A	20	0	—	0
Amano Acid Protease-II	20	1	—	0
Amano Protease-A	20	11	(*S*)	17
Amano Protease-M	20	6	(*S*)	8
Amano Protease-P (6K)	20	47	(*S*)	>99.8
Amano Seaprose S	20	50	(*S*)	>99.8
Amano Protease-S	20	2	(*S*)	3
Amano Newlase-F	20	0	—	0
Amano Peptidase-R	20	1	—	0
Amano Umamizyme	20	16	(*S*)	20
Julich Esterase-RO	20	0	—	0
Novo Flavorzyme-M6	20	3	(*S*)	4
Novo Neutrase-1	20	0	—	0
Novo Protames	20	1	—	0
Novo Semiacylase	20	1	—	0
Sigma PLE	20	5	(*R*)	3
Sigma Pig Acylase	20	1	—	0
Sigma a-Chymotrypsin	20	3	(*S*)	4
Sigma Subtilisin	20	15	(*S*)	22
Sigma Pronase-E	20	7	(*S*)	9
Sigma Proteinase-K	20	6	(*S*)	8
Sigma Trypsin	20	4	(*S*)	5
Sigma B. polymyxa Protease	20	3	(*S*)	3
Sigma Serratiopeptidase	20	2	—	0
Sigma A. saitoi Peptidase	20	1	—	0
Sigma Rhizopus Protease	20	2	—	0
Sigma S. caespitosus Protease	20	3	(*S*)	3
Sigma Bacillus Protease-N	20	1	—	0
Sigma Bacillus Proteinase	20	0	—	0
Fluka Ficin	40	1	—	0
Fluka Papain	40	1	—	0

Conditions: substrate [10 μL, 100 g L^{-1} of (*RS*)-**1** in DMSO] was added to a vigorously stirred solution/suspension of biocatalyst (1 mL, 20 or 40 g L^{-1} of biocatalyst in 0.1 M sodium phosphate, pH 8), and the mixture stirred at 400 rpm, 303 K, 40 h. The percentage conversion refers to the overall extent of hydrolysis. The selectivity refers to the configuration of the major hydrolysis product. ee values were determined by chiral HPLC.

2–2.5 and solvent extraction of (1*S*)-**2** were hindered by extensive emulsification during the extraction of the (*S*)-acid. This phenomenon was traced to the presence of the biocatalyst, and the use of temperature cycling, addition of salts and the application of different solvents (butan-1-ol, pentan-1-ol, methyltetrahydrofuran and pentan-3-one) failed to overcome emulsification. However, it was found that the simple addition of Celite filter aid during extraction of (1*S*)-**2** was sufficient to enable efficient recovery of the desired acid without significant emulsion issues. It should be mentioned that extraction with resins was examined as a more facile and cost-efficient alternative to solvent extraction—thus, Amberlite XAD16 was found to adsorb >98% of acid and ester from the reaction mixture acidified to pH 2–2.5, and both (1*R*)-**1** and (1*S*)-**2** were recovered in >95% yield upon back-extraction with MTBE. However, all attempts to separate the (*S*)-acid from the (*R*)-ester by extraction with base or pad-filtration through silica or alumina failed. High pH extractions were confounded by poor recoveries and the presence of polar impurities derived from the biocatalyst, resulting in severe contamination of the acid. On the other hand, very poor separations and/or recoveries of the acid, and less so the ester, were incurred with pad-filtration or direct elution of the Amberlite adsorbate. The application of alternative polystyrene/polyacrylate resins or ion exchangers, such as Amberlites XAD4, XAD7 and XAD8, Lewatits 1064 and 1163, Mitsubishi HP2MG and Ambersorb-359, although enabling the selective adsorption of (1*R*)-**1** at pH 9–9.5 in several cases, was precluded by difficulties encountered in recovering the adsorbed (*S*)-acid.

To assess the practicality of the developed resolution and work-up processes, the Seaprose S-mediated resolution was performed on a 2 g scale and the reaction mixture processed. Complete hydrolysis of (1*S*)-**1** was obtained after 3 d of reaction with a biocatalyst-to-substrate ratio of 8, with no detectable cleavage of the (1*R*)-**1**, and sequential extractions with MTBE provided crude (1*S*)-**2** with 94% recovery, 68% potency and ee >99.8%, and crude (1*R*)-**1** with 96% recovery, 91% potency and ee >99.5%. Polishing by way of trituration with aqueous acetic acid enabled a facile and effective purification of the acid, furnishing enantiopure (1*S*)-**2** in high potency (100–102%) and moderate recovery (87% mass recovery). Aqueous trituration with bicarbonate and acetic acid provided a significant, but not complete purification of (1*R*)-**1**, delivering enantiopure ester of moderate potency (95–96% potency) and good recovery (93% mass recovery).

Table 4. Effect of buffer type and pH on the Protease P-mediated (*S*)-enantioselective hydrolysis of (*RS*)-**1**

[Protease P] (g L^{-1})	[Substrate] (g L^{-1})	Buffer	[Buffer] (mM)	pH	Initial rate (mmol h^{-1} kg^{-1})	Conversion (%)	ee_P (%)
20	1.0	None	—	—	2.9	28	98.6
20	1.0	Sodium phosphate	50	7.5	6.6	44	99.2
20	1.0	Sodium phosphate	100	7.0	6.4	47	99.7
20	1.0	Sodium phosphate	100	7.5	8.1	50	>99.8
20	1.0	Sodium phosphate	100	8.0	8.9	50	>99.8
20	1.0	Sodium metaphosphate	100	7.0	6.9	41	>99.8
20	1.0	Sodium metaphosphate	100	7.5	7.6	49	>99.8
20	1.0	Sodium metaphosphate	100	8.0	7.8	52	99.1
20	1.0	Sodium metaphosphate	100	8.5	7.9	55	88.6
20	1.0	Sodium metaphosphate	100	9.0	8.8	63	79.3
20	1.0	Sodium bicarbonate	100	7.0	3.8	38	>99.8
20	1.0	Sodium bicarbonate	100	7.5	4.2	44	99.1
20	1.0	Sodium bicarbonate	100	8.0	4.7	47	98.0
20	1.0	Sodium bicarbonate	100	8.5	4.8	52	92.7
20	1.0	Tris	100	7.5	7.2	48	99.3
20	1.0	Tris	100	8.0	7.4	51	94.7
20	1.0	Tris	100	8.5	8.0	54	90.9
20	1.0	Triethanolamine	100	7.0	7.6	44	98.8
20	1.0	Triethanolamine	100	7.5	8.1	46	98.2
20	1.0	Triethanolamine	100	8.0	8.7	52	94.1

Conditions: substrate [10 μL, 100 g L^{-1} of (*RS*)-**1** in DMSO] was added to a vigorously stirred suspension of Protease P (1 mL, 20 g L^{-1} of biocatalyst in water, or 0.05 or 0.1 M buffer), and the mixture stirred at 400 rpm, 303 K, 40 h. Initial rates were measured at 1 h and are quoted as mmol of product formed per h per kg-enzyme. The percentage conversion refers to the overall extent of hydrolysis. ee values were determined by chiral HPLC.

Table 5. Effects of substrate, biocatalyst and DMSO loads on the Protease P and Seaprose S-mediated (*S*)-selective hydrolysis of (*RS*)-**1**

Biocatalyst	[Biocatalyst] (g L^{-1})	[Substrate] (g L^{-1})	[DMSO] (% v/v)	[Buffer] (mM)	Initial rate (mmol h^{-1} kg^{-1})	Conversion (%)	ee_P (%)
Protease P	5	1.0	2.0	100	7.4–8.0	37–39	99.1–99.5
Protease P	10	1.0	2.0	100	8.2–8.7	42–45	99.6–>99.8
Protease P	15	1.0	2.0	100	8.0–9.2	49–50	99.8–>99.8
Protease P	20	1.0	2.0	100	7.8–8.4	49–50	>99.8
Protease P	40	2.0	2.0	100	4.1–6.3	38–44	99.4–>99.8
Protease P	40	2.0	2.0	200	3.8–4.2	27–32	99.1 >99.8
Protease P	60	2.0	2.0	100	4.8–8.0	35–39	99.5–>99.8
Protease P	40	2.0	5.0	100	4.5–7.3	31–33	99.0–99.3
Protease P	40	2.0	10.0	100	3.8–8.2	27–36	99.2–99.7
Protease P	25	5.0	2.0	100	3.0–6.5	18–27	98.2–99.3
Protease P	50	5.0	2.0	100	5.9–7.8	22–25	99.4–99.8
Protease P	50	5.0	2.0	200	3.5–7.3	17–22	99.7–>99.8
Protease P	100	5.0	2.0	100	8.1	19	>99.8
Protease P	100	5.0	10.0	100	7.4	25	>99.8
Protease P	100	5.0	15.0	100	6.7	22	99.6
Seaprose S	5	1.0	2.0	100	8.5–9.1	40–42	99.7–>99.8
Seaprose S	7.5	1.0	2.0	100	9.1–9.5	49–50	>99.8
Seaprose S	10	1.0	2.0	100	8.8–9.8	50–51	99.4–>99.8
Seaprose S	5	2.0	2.0	100	4.8–7.5	28–34	99.1–99.5
Seaprose S	7.5	2.0	2.0	100	6.1–7.7	35–38	99.2–>99.8
Seaprose S	10	2.0	2.0	100	5.5–8.9	31–40	99.3–99.8
Seaprose S	15	2.0	2.0	100	5.7	36	98.4
Seaprose S	15	2.0	5.0	100	6.1	46	>99.8
Seaprose S	15	2.0	10.0	100	5.3	31	>99.8

Conditions: substrate [20–150 μL, 50–250 g L^{-1} of (*RS*)-**1** in DMSO] was added to a vigorously stirred suspension of Protease P or Seaprose S (1 mL, 5–100 g L^{-1} of biocatalyst in 0.1 or 0.2 M sodium phosphate, pH 8.0), and the mixture stirred at 400–600 rpm, 303 K, 40 h. Initial rates were measured at 1 h and are quoted as mmol of product formed per h per kg-enzyme. The percentage conversion refers to the overall extent of hydrolysis. ee values were determined by chiral HPLC.

3. Conclusion

The developed resolution process exhibits significant scale-up issues deriving from the low activity of the crude commercial biocatalyst, the presence of surface-active protein contaminants therein, and the low solubilities of the substrate and product. These lead to the requirement for a high biocatalyst-to-substrate ratio (8:1 w/w), a low sub-

Table 6. Effects of cosolvents on the Seaprose S-mediated (*S*)-selective hydrolysis of (*RS*)-**1**

[Biocat] (g L^{-1})	[Substrate] (g L^{-1})	[DMSO] (% v/v)	Cosolvent (% v/v)	[Buffer] (mM)	Initial rate (mmol h^{-1} kg^{-1})	Conversion (%)	ee_P (%)
8	1.0	1.0	—	100	9.6	50	>99.8
8	1.0	1.0	5% ethanol	100	2.9	22	>99.8
8	1.0	1.0	5% propan-2-ol	100	3.5	28	>99.8
8	1.0	1.0	5% acetonitrile	100	3.7	31	99.3
8	1.0	1.0	5% acetone	100	2.8	16	97.4
8	1.0	1.0	5% tetrahydrofuran	100	2.4	12	99.2
8	1.0	1.0	5% dimethoxymethane	100	2.1	18	98.6
8	1.0	1.0	10% ethanol	100	<0.2	<2	—
8	1.0	1.0	10% propan-2-ol	200	<0.2	3	—
8	1.0	1.0	10% acetonitrile	100	0.3	8	97.7
8	1.0	1.0	10% acetone	100	<0.2	<2	—
8	1.0	1.0	10% tetrahydrofuran	100	0.5	7	97.3
8	1.0	1.0	10% dimethoxymethane	100	0.3	5	96.9
8	1.0	2.0	—	100	8.4–8.8	49–50	>99.8
8	1.0	3.0	—	100	7.3–8.2	44–47	>99.8
8	1.0	4.0	—	100	6.2–6.7	39–41	99.2–99.5
8	1.0	5.0	—	100	5.3–6.0	34–42	98.8–99.3
8	1.0	6.0	—	100	4.2–5.5	30–38	98.2–99.4
8	1.0	8.0	—	100	4.0–5.1	29–34	99.2–>99.8
8	1.0	10.0	—	100	3.8–4.4	23–41	98.7–99.0

Conditions: substrate [10 μL, 100 g L^{-1} of (*RS*)-**1** in DMSO] was added to a vigorously stirred suspension of Seaprose S (1 mL, 8 g L^{-1} of biocatalyst in 0.1 or 0.2 M sodium phosphate, pH 8.0, containing 0–9% v/v of DMSO and 0–10% v/v of cosolvent), and the mixture stirred at 400–600 rpm, 303 K, 40 h. Initial rates were measured at 1 h and are quoted as mmol of product formed per h per kg-enzyme. The percentage conversion refers to the overall extent of hydrolysis. ee values were determined by chiral HPLC.

strate load (1 g L^{-1}), a long reaction time (3 d), and the need for high-volume solvent extraction procedures for the recovery of (1*S*)-**2** and (1*R*)-**1**, and make the process unsuitable for industrial implementation. However, it is envisaged that these issues can be overcome with the use of an immobilized, high-activity cloned biocatalyst, which could enable the resolution of high concentrations (>10 g L^{-1}) of substrate in monophasic or biphasic aqueous–organic media, and thus enable sufficiently high productivities and recovery efficiencies for economic scale-up. Efforts are currently underway to isolate and clone the biocatalyst and develop such a process. In addition, the deployment of in situ racemization of (1*S*)-**2** is also being investigated in order to enable conversions beyond 50%, although preliminary data indicate that thermal and acid/base-mediated approaches result in substantial degradation (hydrolysis, decarboxylation, and ring cleavage).

4. Experimental

4.1. General

Enzymes were purchased from Novozymes, Amano Enzyme Company, Biocatalysts, Boehringer, Julich, Meito Sangyo and Sigma–Aldrich. All other chemicals and solvents were purchased from Aldrich, Fluka and Sigma and were of AR quality or higher.

4.2. Preparation of racemic and enantiopure methyl *N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate[21]

Racemic methyl *N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate (*RS*)-**1** was prepared by the treatment of commercially available *N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylic acid with 1 equiv of $LiOH{\cdot}H_2O$ in THF for 40 min followed by the addition of 1 equiv of Me_2SO_4. The reaction mixture was heated at reflux for 3 h. After cooling the reaction vessel to room temperature, the THF was removed and the residue was diluted with CH_2Cl_2. The organic layer was washed with H_2O and brine, then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography with a gradient of 0–50% ethyl acetate in hexanes to yield (*RS*)-**1** as a pale, yellowish foam.

Enantiopure methyl *N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate isomers (*R*)-**1** and (*S*)-**1** were isolated by chiral preparative HPLC using chiralpak OD 20μ column (5 × 50 cm, eluting with 5% (EtOH/MeOH (50:50))/heptane, 50 mL/min flow rate). The retention times were 12.79 min for (*S*)-**1** and the 14.43 min for (*R*)-**1.** Characterization data was consistent with the data described below (Section 4.5). The stereochemistry assignment was made based on a crystal structure with LXRβ and a tetrahydroisoquinoline analog prepared from (*R*)-**1** (21).

4.3. Analytical methods

Reactions were followed by chiral RP-HPLC on a Shimadzu LC-10 system. Samples were diluted to 0.1–0.2 g L^{-1} with methanol containing 0.5% v/v of acetic acid and filtered (0.2 μm PTFE), prior to HPLC analysis. Samples were analyzed as follows: Chiralpak AS-RH (5 μm, 4.6 × 150 mm) column; elution with 0–20% v/v B over 15 min, then 20–25% v/v B over 15–45 min; solvent A was 8:2 v/v water–methanol containing 0.05% v/v TFA

and solvent B was 8:2 v/v acetonitrile–methanol containing 0.05% v/v TFA; 0.7 mL/min flow rate; ambient column temperature; 50 μL injection; 220 nm and 254 nm detection. The retention times were 9.5 min for the (*S*)-acid, 28.4 min for the (*S*)-ester, and 29.5 min for the (*R*)-ester. NMR spectra were recorded on a Bruker-300 or Jeol-400 spectrometers using deutero-chloroform as solvent. Optical rotations were recorded on a Perkin–Elmer 241 digital polarimeter at 20 °C using the sodium D line.

4.4. Screening of biocatalysts for the resolution of methyl (1*RS*)-*N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate (1*RS*)-1

4.4.1. Hydrolysis of (1*RS*)-1 in aqueous media. Substrate (10 μL, 100 g L^{-1} of *rac*-**1** in DMSO) was added to vigorously stirred biocatalyst solution/suspension (1 mL, 20–100 g L^{-1} of biocatalyst in 0.1 M sodium phosphate, pH 6, 7, 8 or 9), and the suspension stirred at 400 rpm, 303 K, 40 h.

4.4.2. Hydrolysis of (1*RS*)-1 in monophasic aqueous–organic media. Substrate (10 μL, 100 g L^{-1} of *rac*-**1** in DMSO), was added to vigorously stirred biocatalyst solution/suspension (1 mL, 20–100 g L^{-1} of biocatalyst in 0.1 M sodium phosphate, pH 8, containing 20–95% v/v of ethanol, propan-1-ol, propan-2-ol, butan-1-ol, acetonitrile, dimethoxymethane or THF), and the mixture stirred at 400 rpm, 303 K, 40 h.

4.4.3. Hydrolysis of (1*RS*)-1 in biphasic aqueous–organic media. Biocatalyst (0.5 mL, 10–50 g L^{-1} of biocatalyst in 0.1 M sodium phosphate, pH 8) was added to the substrate solution (0.5 mL, 2, 5 or 10 g L^{-1} of *rac*-**1** in *t*-butanol, *t*-amyl alcohol, methyl-THF, THP, 1,2-dimethoxyethane, DEE, DIPE, MTBE, DCM, hexane or heptane), and the mixture stirred at 500–600 rpm, rt, 40 h.

4.4.4. Transesterification of (1*RS*)-1 in organic media. Biocatalyst (10 or 50 mg) was added to substrate solution (0.5 mL, 5 or 10 g L^{-1} of *rac*-**1** in ethanol, propan-1-ol or butan-1-ol containing 0%, 2%, 5% or 10% v/v buffer, or in acetonitrile, THF, methyl-THF, DEE, MTBE, DCM or toluene containing 0.1 M propan-1-ol and 0%, 1%, 2%, 5% or 10% v/v of buffer), and the suspension stirred at 400 rpm, rt, 40 h.

4.5. Seaprose S-mediated enantioselective hydrolysis of methyl (1*RS*)-*N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate (1*RS*)-1

Seaprose S (16 g) was mixed with sodium phosphate buffer (20 mL, 0.1 M, pH 8.0) to form a paste, this was diluted with a buffer (1.92 L) and the resulting solution transferred to a 2 L glass bottle. The solution was stirred at 250 rpm with an overhead PTFE half-moon paddle stirrer, and DMSO (30 mL), followed by *rac*-**1** (2 g of ester dissolved in 30 mL of DMSO) added over 2 min, and stirring continued at room temperature for 3 days. Chiral HPLC analysis indicated complete hydrolysis of the (*S*)-ester without any detectable cleavage of the (*R*)-enantiomer. The pH of the reaction mixture was adjusted to 9.2–9.5 using 0.5 M sodium hydroxide, the mixture extracted with MTBE (2 × 1 L), and the organic extract evaporated at 25 °C to furnish the crude (*R*)-ester as a pale-brown viscous oil (1.06 g). This was triturated with aqueous sodium bicarbonate (100 mL, 25 mM) to give an off-white semi-solid, which was recovered and stirred with aqueous acetic acid (100 mL, 0.5% v/v) at rt, 1 h, then washed with water (3 × 10 mL), and dried under vacuum over Drierite, potassium hydroxide, and charcoal, at room temperature for 20 h to yield methyl (1*R*)-*N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylate as an off-white semi-solid: 0.87 g, 96% potency (HPLC), 93% yield, ee >99.5%; $[\alpha]_D^{20} = +18.2$ (*c* 0.2, methanol); LC–MS (ESI^+) *m*/*z* (%) = 308 ([M+H], 100); ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H, *t*Boc), 2.90–2.97 (m, 2H, 4-H), 3.28–3.34 (m, 2H, 3-H), 3.72 (s, 3H, OCH_3), 5.74 (s, 1H, 1-H), 6.47 (d, 1H, $J = 6.4$ Hz, 7-H), 6.88–7.04 (m, 2H, 5-H + 8-H), 9.35 (br s, 1H, OH) ppm; ^{13}C NMR ($CDCl_3$) δ 28.4, 28.6, 37.4, 52.6, 62.4, 79.8, 113.9, 115.7, 126.8, 127.0, 135.6, 154.9, 155.1, 171.0 ppm. The pH of the aqueous phase from the MTBE extraction was adjusted to 2.2–2.3 with aqueous sulfuric acid, and Celite (300 g) followed by MTBE (2 L) added, and the mixture filtered. The filter cake was washed with MTBE (2 × 1 L), the organic phase separated from the combined filtrates, the aqueous phase extracted with MTBE (2 × 1 L), and the combined organic phases evaporated at 25 °C, then dried under vacuum at 25 °C to furnish the crude (*S*)-acid as a pale-yellow solid (0.98 g). This was triturated with aqueous acetic acid (100 mL, 0.5% v/v), the suspension filtered, the filter cake washed with water (4 × 4 mL), then dried under vacuum over Drierite, potassium hydroxide and charcoal, at rt, 20 h to furnish (1*S*)-*N*-*t*Boc-6-hydroxy-3,4-dihydro-1*H*-isoquinoline-1-carboxylic acid as a soft white solid: 0.62 g, 101% potency (HPLC), 87% yield, ee >99.8%; mp 123–127 °C (decomp.); $[\alpha]_D^{20} = -19.2$ (*c* 0.2, methanol); LC–MS (ESI^+) *m*/*z* (%) = 294 ([M+H], 100), 316 ([M+Na], 68); ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*Boc), 2.92–2.96 (m, 2H, 4-H), 3.22–3.30 (m, 2H, 3-H), 5.78 (s, 1H, 1-H), 6.49 (d, 1H, $J = 6.2$ Hz, 7-H), 6.88–7.06 (m, 2H, 5-H + 8-H), 9.31 (br s, 1H, OH) ppm; ^{13}C NMR ($CDCl_3$) δ 28.7, 28.9, 37.6, 65.0, 80.3, 114.2, 116.1, 127.0, 127.5, 136.0, 155.2, 155.8, 171.3 ppm.

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