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Enantioselective enzymatic acylation of 1-(3′-bromophenyl)ethylamine

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Abstract—An efficient biocatalytic process has been developed for the resolution of 1-(3′-bromophenyl)ethylamine (*RS*)-**1** by way of enantioselective lipase-mediated (*R*)-selective acylation with ethyl 2-methoxyacetate to afford (*S*)-amine (*S*)-**1** and (*R*)-2″-methoxyacetamide ((*R*)-**2**) in 91–95% and 90–92% isolated yield, respectively, and both with >99% ee.

1. Introduction

Enantiopure amines are widely used in the pharmaceutical, agrochemical and fine chemical industries as synthetic intermediates, chiral auxiliaries, catalysts and resolving agents.[1–8] Amongst the methodologies that have been developed for the industrial production of enantiopure amines, lipase-mediated enantioselective acylation has been increasingly utilized, despite many other available methodologies, such as hydroamination, hydrosilylation, asymmetric hydrogenation and diastereomeric salt crystallization, transaminase-mediated reductive amination or amine oxidase or deaminase-mediated resolution.[9–12]

Lipases have long been recognized as highly active and stable enzymes endowed with broad substrate ranges together with unique stereo-, chemo- and regio-selectivities and the robustness to operate efficiently in a wide variety of solvent, pH and temperature conditions. These attributes have made lipases outstanding catalysts for synthetic biotransformations that involve the carboxyl group, such as (thio)esterification, transesterification, perhydrolysis and aminolysis, wherein the water is replaced by an alcohol, thiol, hydroperoxide or amine.[13–15] The principle of lipase-catalyzed aminolysis of carboxylic esters was first described in 1984 by Inada et al.[16] in a seminal paper that contributed greatly by initiating the synthetic application of lipases in organic media.[17–19] Following this publication, numerous reports of the enantioselective lipase-mediated acylation of amines appeared in the 1990s and the field has since gained significant industrial academic and significance, as described in a number of recent reviews.[20–29]

Herein we report the highly enantioselective CALB/Novozym 435- and amano lipase PS-catalyzed acylation of 1-(3′-bromophenyl)ethylamine (*RS*)-**1** with ethyl 2-methoxyacetate as the acyl donor, to furnish the (*S*)-amine (*S*)-**1** and (*R*)-2″-methoxyacetamide (*R*)-**2** in high yield (90–96%) and high enantiopurity (ee >99%). The enantiomers of **1** and **2** were required with enantiopurities >99.5% as intermediates in the synthesis of IB kinase (IKK) modulators and other pharmaceuticals.[30–39]

2. Results and discussion

Initially, a range of hydrolases were evaluated (Table 1) at the room-temperature enantioselective N-acylation of (*RS*)-**1** with vinyl acetate, isopropenyl acetate and ethyl acetate as acyl donors and methyl *t*-butyl ether (MTBE) as solvent, with the focus on obtaining highly enantioenriched (ee >99%) amide in high yield (>45% of starting material) (Scheme 1). While vinyl acetate proved to be too reactive and resulted in high levels (12–34%) of non-selective non-enzymatic acetylation as well as the formation of several side products, isopropenyl acetate and ethyl acetate furnished fairly clean enzymatic N-acylations without significant background reactions. Although a number protease, acylase, esterase and amidase biocatalysts did provide significant (*R*)- or (*S*)-enantioselectivity, conversions and/or selectivities were generally low, with overall conversions

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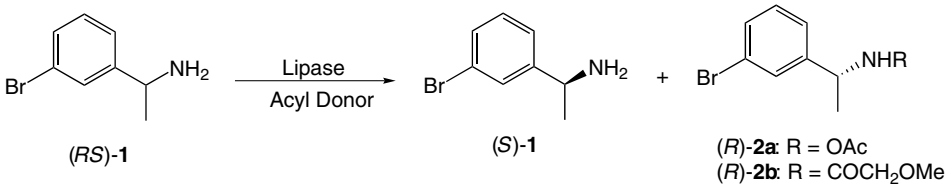
Table 1. Hydrolase-mediated enantioselective N-acylation of (*RS*)-**1** with isopropenyl acetate

Biocatalyst	[Biocatalyst] (mg/mL)	Conversion (%, HPLC)	Amide product	ee (amide) (%, HPLC)
Amano Acylase	20	4	(*R*)-**2**	40
Amano D-Aminoacylase	20	12	(*R*)-**2**	23
Amino Acid Protease-A	20	1	(*R*)-**2**	6
Amano Acid Protease-II	20	1	(*S*)-**2**	71
Amano Protease-A	20	6	(*R*)-**2**	1
Amano Protease-M	20	5	(*S*)-**2**	1
Amano Protease-P (6K)	20	3	(*S*)-**2**	4
Amano Seaprose S	20	2	(*R*)-**2**	14
Amano Protease-S	20	2	(*R*)-**2**	2
Amano Newlase-F	20	2	(*S*)-**2**	1
Amano Peptidase-R	20	2	(*S*)-**2**	9
Amano Umamizyme	20	4	(*RS*)-**2**	0
Julich Esterase-RO	20	1	(*RS*)-**2**	0
Novo Flavorzyme-M6	20	1	(*S*)-**2**	21
Novo Neutrase-1	20	1	(*S*)-**2**	12
Novo Protames	20	4	(*S*)-**2**	33
Novo Semiacylase	20	10	(*S*)-**2**	1
Sigma PLE	20	2	(*R*)-**2**	2
Sigma Pig Acylase	20	2	(*S*)-**2**	2
Subtilisin-Celite R633	25	6	(*S*)-**2**	7
Alcalase-Celite R633	25	0	(*RS*)-**2**	0

Reactions were conducted with 50 mM (*RS*)-**1**, 100 mM (2 mol equiv) isopropenyl acetate and 125 mg/mL 4 Å molecular sieves in dry MTBE, using 20 mg/mL (free-form) or 25 mg/mL (immobilized) biocatalyst, with stirring at 400 rpm at 40 °C for 20 h. Conversions and enantioselectivities were determined by HPLC.



Scheme 1.

remaining below 15%, even after 3 days of incubation at 40 °C. In contrast, several lipases (Table 2) were found to effect the enantioselective N-acylation with isopropenyl acetate as the acyl donor, notably amano lipase-AK, Europa Lipase-14, Meito Sangyo Lipase-TL, Boehringer Chirazyme-L2, Amano Lipase-PS30 on Accurel and Novozym-435, all of which showed (*R*)-selectivity, whilst Boehringer Chirazyme-L5 was the only lipase that showed the opposite (*S*)-selectivity. The best results were obtained with Chirazyme-L2 and Novozym-435, which provided close to 100% conversion of (*R*)-**1** with very high enantioselectivity (>99.8% ee). In the case of ethyl acetate, Europa Lipase-14, Meito Sangyo Lipase-TL, Boehringer Chirazyme-L2-c2, Amano Lipase PS30/Accurel, Amano Lipase PS30 in sol–gel, Novo Novozym-435 and CALB on Celite provided (*R*)-selective N-acylation, albeit with lower selectivities (Table 3). The best results were obtained with Chirazyme-L2, Novozym-435 and CALB on Celite, which furnished (*R*)-**2a** with 95–97% ee at 51–54% conversion.

Despite the good initial results with isopropenyl acetate, subsequent experiments with this acyl donor gave poor conversions and enantioselectivities, due to low enzymatic acylation activity and/or extensive (up to 60%) by-product formation with certain batches of isopropenyl acetate. In view of the poor reproducibility of isopropenyl acetate reactions, and the lower cost of ethyl acetate and its reduced propensity towards side reactions, resolution with the latter was studied under different solvent conditions with the aim of improving the ee of (*R*)-**2a** to over 99% (Table 3). Although hexane and dichloromethane did provide (*R*)-**2a** with >99.8% ee, the conversions were very low (<10%), and further modifications of the reaction conditions (solvent, additives, biocatalyst load, acyl donor-to-substrate ratio and reaction temperature) did not afford the desired combination of high enantiopurity for (*S*)-**1** and (*R*)-**2a** together with a high conversion. Thus, although highly enantiopure (*S*)-**1** could be obtained via Chirazyme-L2 and Novozym-435-mediated enantioselective acylation of 0.5 M (*RS*)-**1** in neat ethyl acetate, these conditions afforded conversions over 60% resulting in a substantial loss of enantiopurity in (*R*)-**2a** as well as the formation of hydrophobic by-products. On the other hand, (*R*)-**2a** with ee above 99.8% was obtained by running reactions to low conversions, for example, 700 g/L (3.5 M) substrate and 451 g/L (5.1 M, 46 mol % excess) ethyl acetate, with 70 g/L Novozym-435, provided 18% of (*R*)-**2a** with 95% ee after 20 h, but at the expense of very low ee for (*S*)-**1**.

Table 2. Lipase-mediated enantioselective N-acylation of (*RS*)-**1** with isopropenyl acetate

Lipase biocatalyst	[Biocatalyst] (mg/mL)	Conversion (%, HPLC)	Amide product	ee (amide) (%, HPLC)
Amano Lipase-A	25	0	(*R*)-**2**	0
Amano Lipase-AK	25	40	(*R*)-**2**	92
Amano Lipase-AP12	25	0	(*R*)-**2**	0
Amano Lipase-AY30	25	0	(*R*)-**2**	0
Amano Lipase-D	25	1	(*R*)-**2**	>99.8
Amano Lipase-F	25	0	(*R*)-**2**	0
Amano Lipase-FAP15	25	0	(*R*)-**2**	0
Amano Lipase-G	25	0	(*R*)-**2**	0
Amano Lipase-GC20	25	0	(*R*)-**2**	0
Amano Lipase-M	25	1	(*R*)-**2**	>99.8
Amano Lipase-MAP10	25	1	(*R*)-**2**	>99.8
Amano Lipase-N	25	1	(*R*)-**2**	>99.8
Amano Lipase-PS	25	5	(*R*)-**2**	>99.8
Amano Lipase-PS30	25	9	(*R*)-**2**	>99.8
Amano Lipase-R	25	0	(*R*)-**2**	0
Biocatalysts Lipase-ANL	25	0	(*R*)-**2**	0
Biocatalysts Lipase-CCL	25	1	(*R*)-**2**	>99.8
Biocatalysts Lipase-RJL	25	0	(*R*)-**2**	0
Boehringer Chirazyme-L3	25	0	(*R*)-**2**	0
Enzymatix Lipase-B1	25	14	(*R*)-**2**	84
Enzymatix Lipase-F5	25	2	(*R*)-**2**	>99.8
Europa Lipase-4	25	30	(*R*)-**2**	90
Europa Lipase-13	25	16	(*R*)-**2**	92
Europa Lipase-14	25	50	(*R*)-**2**	92
Europa Lipase-21	25	33	(*R*)-**2**	91
Julich Lipase-RN	25	0	(*R*)-**2**	0
Julich Lipase-RO	25	0	(*R*)-**2**	0
Meito Sangyo Lipase-AL	25	9	(*R*)-**2**	84
Meito Sangyo Lipase-MY	25	1	(*R*)-**2**	>99.8
Meito Sangyo Lipase-OF	25	10	(*R*)-**2**	70
Meito Sangyo Lipase-PL	25	13	(*R*)-**2**	73
Meito Sangyo Lipase-QLM	25	0	(*R*)-**2**	0
Meito Sangyo Lipase-SL	25	21	(*R*)-**2**	88
Meito Sangyo Lipase-TL	25	50	(*R*)-**2**	93
Meito Sangyo Lipase-UL	25	2	(*R*)-**2**	>99.8
Sigma Lipase-CRL	25	1	(*R*)-**2**	>99.8
Sigma Lipase-PPL	25	3	(*R*)-**2**	45
Sepracor Lipase-OF	25	2	(*R*)-**2**	>99.8
Biocatalysts Lipomod-200	100	2	(*R*)-**2**	>99.8
Boehringer Chirazyme-L2-c2	100	49	(*R*)-**2**	>99.8
Boehringer Chirazyme-L5	100	11	(*S*)-**2**	−23
Amano Lipase PS30 on Accurel	100	41	(*R*)-**2**	96
Novo Lipolase-30T	100	7	(*R*)-**2**	40
Novo Lipozym-IM60	100	6	(*R*)-**2**	77
Novo Novozym-435	100	50	(*R*)-**2**	>99.8

Reactions were conducted with 50 mM (*RS*)-**1**, 100 mM (2 mol equiv) isopropenyl acetate and 125 mg/mL 4 Å molecular sieves in dry MTBE, using 25 mg/mL (free-form) or 100 mg/mL (immobilized) biocatalyst, with stirring at 400 rpm at 40 °C for 20 h. Conversions and enantioselectivities were determined by HPLC.

In view of the suboptimal results obtained with vinyl, isopropenyl and ethyl acetates, a variety of other acyl donors were screened for the enantioselective (N)-acylation using Novozym 435 as catalyst (Table 4). Of the acyl donors tested, diethyl oxalate and ethyl methoxyacetate were notable in furnishing highly enantiopure (>99% ee) (*S*)-**1** and (*R*)-**2** with 0.6 mol equiv of the acyl donor at close to complete (50%) the conversion with very little background reaction (<1%). Ethyl 2-methoxyacetate was selected over the former acyl donor since the formation of mono- and bis-oxamides complicated work-up and recovery procedures. It should be noted that methyl 2-methoxyacetate was also tested but found to be unsuitable as an acyl donor due to significant (>5%) non-selective background reaction with (*RS*)-**1**, and low enzyme activity, presumably due to inactivation of the enzyme by methanol released during the reaction. A screening of solvents for the N-acylation of (*RS*)-**1** with ethyl 2-methoxyacetate revealed MTBE as the best solvent, which despite promoting extensive precipitation of the corresponding (*R*)-2-methoxyacetamide product gave almost complete conversion of (*R*)-**1** and high enantiopurities (>99% ee) for both (*S*)-**1** and (*R*)-**2b** at substrate loads up to 251 g/L (Table 5). Diethyl ether and hexane were also promising solvents, although furnishing lower enantiopurities (93–99%) for the desired (*S*)-amine, while also incurring conversions above 50%. Incomplete reactions were observed with dichloromethane and acetonitrile as solvents.

Table 3. Lipase-mediated enantioselective N-acylation of (*RS*)-**1** with ethyl acetate

Biocatalyst	[Biocatalyst] (mg/mL)	Reaction medium (v/v)	Conversion (%, HPLC)	Amide product	ee (amide) (%, HPLC)
Europa Lipase-14	25	Neat EtOAc	30	(*R*)-**2**	66
Meito Sangyo Lipase-TL	25	Neat EtOAc	38	(*R*)-**2**	59
Boehringer Chirazyme-L2-c2	100	Neat EtOAc	50	(*R*)-**2**	76
Amano Lipase PS30/Accurel	100	Neat EtOAc	20	(*R*)-**2**	50
Amano Lipase PS30 in Sol–gel	100	Neat EtOAc	17	(*R*)-**2**	5
Novo Novozym-435	100	Neat EtOAc	51	(*R*)-**2**	75
CALB on Celite	100	Neat EtOAc	53	(*R*)-**2**	70
Europa Lipase-14	25	Neat EtOAc + 4AMS	28	(*R*)-**2**	92
Meito Sangyo Lipase-TL	25	Neat EtOAc + 4AMS	36	(*R*)-**2**	90
Boehringer Chirazyme-L2-c2	100	Neat EtOAc + 4AMS	53	(*R*)-**2**	87
Amano Lipase PS30/Accurel	100	Neat EtOAc + 4AMS	25	(*R*)-**2**	93
Amano Lipase PS30 in Sol–gel	100	Neat EtOAc + 4AMS	26	(*R*)-**2**	50
Novo Novozym-435	100	Neat EtOAc + 4AMS	52	(*R*)-**2**	88
CALB on Celite	100	Neat EtOAc + 4AMS	54	(*R*)-**2**	85
Europa Lipase-14	25	1:9 EtOAc–MTBE	16	(*R*)-**2**	98
Meito Sangyo Lipase-TL	25	1:9 EtOAc–MTBE	18	(*R*)-**2**	95
Boehringer Chirazyme-L2-c2	100	1:9 EtOAc–MTBE	52	(*R*)-**2**	97
Amano Lipase PS30/Accurel	100	1:9 EtOAc–MTBE	12	(*R*)-**2**	98
Amano Lipase PS30 in Sol–gel	100	1:9 EtOAc–MTBE	3	(*R*)-**2**	51
Novo Novozym-435	100	1:9 EtOAc–MTBE	51	(*R*)-**2**	97
CALB on Celite	100	1:9 EtOAc–MTBE	54	(*R*)-**2**	95
Europa Lipase-14	25	1:9 EtOAc–DCM	2	(*R*)-**2**	>99.8
Meito Sangyo Lipase-TL	25	1:9 EtOAc–DCM	2	(*R*)-**2**	>99.8
Boehringer Chirazyme-L2-c2	100	1:9 EtOAc–DCM	10	(*R*)-**2**	>99.8
Amano Lipase PS30/Accurel	100	1:9 EtOAc–DCM	1	(*R*)-**2**	70
Amano Lipase PS30 in Sol–gel	100	1:9 EtOAc–DCM	0	(*R*)-**2**	0
Novo Novozym-435	100	1:9 EtOAc–DCM	18	(*R*)-**2**	98
CALB on Celite	100	1:9 EtOAc–DCM	18	(*R*)-**2**	98
Europa Lipase-14	25	1:9 EtOAc–hexane	10	(*R*)-**2**	89
Meito Sangyo Lipase-TL	25	1:9 EtOAc–hexane	5	(*R*)-**2**	94
Boehringer Chirazyme-L2-c2	100	1:9 EtOAc–hexane	27	(*R*)-**2**	97
Amano Lipase PS30/Accurel	100	1:9 EtOAc–hexane	9	(*R*)-**2**	88
Amano Lipase PS30 in Sol–gel	100	1:9 EtOAc–hexane	6	(*R*)-**2**	>99.8
Novo Novozym-435	100	1:9 EtOAc–hexane	24	(*R*)-**2**	95
CALB on Celite	100	1:9 EtOAc–hexane	27	(*R*)-**2**	96

Reactions were conducted with 50 mM (*RS*)-**1**, with or without 125 mg/mL 4 Å molecular sieves in the appropriate solvent mixture, using 25 mg/mL (free-form) or 100 mg/mL (immobilized) biocatalyst, with stirring at 400 rpm at 40 °C for 20 h. Conversions and enantioselectivities were determined by HPLC.

Table 4. Novozym 435-mediated enantioselective N-acylation of (*RS*)-**1**

Acyl donor	Conversion (%, HPLC)	Amide product	ee (amine) (%, HPLC)	ee (amide) (%, HPLC)
Propyl acetate	18	(*S*)-**2**	7	>99.8
Prop-2-yl acetate	22	(*R*)-**2**	13	>99.8
Butyl acetate	16	(*R*)-**2**	5	>99.8
Triacetin	40	(*R*)-**2**	60	93
Propyl butyrate	13	(*R*)-**2**	0	95
Ethyl valerate	24	(*R*)-**2**	14	51
Ethyl (*S*)-lactate	39	(*R*)-**2**	51	81
Ethyl glycolate	41	(*R*)-**2**	34	>99.8
Diethyl oxalate	48	(*R*)-**2**	94	>99.8
Ethyl trichloroacetate	13	(*S*)-**2**	−4	23
Ethyl levulinate	6	(*S*)-**2**	−7	>99.8
Ethyl 2-methoxyacetate	49	(*R*)-**2**	99.2	99.1

Reactions were conducted with 250 mM (*RS*)-**1** and 150 mM (0.6 mol equiv) of acyl donor in dry MTBE, using 10 mg/mL Novozym 435, with stirring at 400 rpm at 40 °C for 20 h. Conversions and enantioselectivities were determined by HPLC.

Two preparative-scale resolutions of (*RS*)-**1** were performed at a 0.19 and 0.25 molar scale using Novozym 435 as catalyst, ethyl 2-methoxyacetate as acyl donor and MTBE as solvent. Both reactions were run close to completion with >99% conversion of (*R*)-amine and, respectively, provided 23.7 and 29.7 g (92% and 90% isolated yield) of (*R*)-**2b** as a white crystalline solid with >99% potency and >99.8% ee, and 17.6 and 22.1 g (95% and 91% isolated yield) of (*S*)-**1** as a light yellow/orange oil with 100% and 100% potency and 99.8% and 99.2% ee.

Candida antarctica lipase B (CALB) was also immobilized on surface-treated Celite R633 (as described in Section 3) and the immobilized enzyme evaluated in the resolution process as an alternative to Novozym 435. The resolution of 2.5 mmol of racemic amine at ca. 140 g/L in MTBE with 0.6 mol equiv of ethyl 2-methoxyacetate and a 10% w/w load (with respect to amine substrate) of CALB, provided (*S*)-**1** and (*R*)-**2b** in 95% and 96% isolated yield, 100% and 100% potency and 99.3% and >99.8% ee, respectively, with an overall conversion of >49% after 2 d. Thus, the in-house prepared immobilized catalyst appeared to be comparable

Table 5. Novozym 435-mediated enantioselective N-acylation of (*RS*)-**1**

Solvent	[Substrate] (M)	[Acyl donor] (M)	Conversion (%, HPLC)	ee (*S*)-1 (%, HPLC)	ee (*R*)-2 (%, HPLC)
Methyl *t*-butyl ether	0.63	0.38	59	99	>99.8
Methyl *t*-butyl ether	1.26	0.77	56	99	>99.8
Diethyl ether	0.63	0.38	62	96	>99.8
Diethyl ether	1.26	0.77	63	99	>99.8
Dichloromethane	0.63	0.38	33	36	>99.8
Dichloromethane	1.26	0.77	43	54	>99.8
Hexane	0.63	0.38	47	65	>99.8
Hexane	1.26	0.77	56	93	>99.8
Acetonitrile	0.70	0.44	44	69	>99.8

Reactions were conducted in dry solvent using 0.6 mol equiv of ethyl 2-methoxyacetate, with 10% w/w of Novozym 435 with respect to amine substrate, with stirring at 400 rpm at 40 °C for 20 h. Conversions and enantioselectivities were determined by HPLC. Precipitation of (*R*)-2-methoxyamide was observed in the case of MTBE, diethyl ether and hexane, while no precipitation was observed for dichloromethane or acetonitrile.

to or better than Novozym 435, and may provide a more economic alternative to the commercial catalyst.

A small-scale resolution of (*RS*)-**1** was also effected with Amano Lipase PS30 immobilized on Accurel polypropylene as catalyst. The resolution of 2.5 mmol of racemic amine at ca. 140 g/L in MTBE with 0.6 mol equiv of ethyl 2-methoxyacetate and a 40% w/w load (with respect to amine substrate) of lipase, provided (*S*)-**1** and (*R*)-**2b** in 91% and 91% isolated yield, 100% and >99% potency and 98.4% and >99.8% ee, respectively, with an overall conversion of >49% after 2 d. Thus, Amano Lipase PS30-Accurel appeared to be a good alternative to CALB/Novozym-435, although it is required at 40% w/w of substrate load as opposed to 10% w/w for the latter biocatalysts.

3. Experimental

3.1. General

Immobilized *Candida antartica* lipase B (Novozym 435) was purchased from Novozymes, Davis, CA. Other lipases, esterases and proteases (Tables 1–3) were purchased from Amano Enzyme Company, Biocatalysts, Boehringer, Julich, Meito Sangyo and Sigma–Aldrich. Racemic and enantiopure amine **1** was provided by Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Lawrenceville. Ethyl 2-methoxyacetyate and all other chemicals and solvents were purchased from Sigma–Aldrich.

3.2. Analytical methods

Reactions were followed by chiral RP-HPLC on Shimadzu LC-10 systems: For amide analysis, samples were diluted to 0.2–0.5 mM with methanol and filtered (0.2 μm PTFE), prior to HPLC analysis as follows: Daicel Chiralpak AS-RH (5 μm, 0.46 × 15 cm) column; elution with 30% v/v B over 0–10 min, then 30–60% v/v B over 10–20 min; solvent A was 8:2 water–methanol containing 0.05% TFA and solvent B was 8:2 acetonitrile–methanol containing 0.05% TFA; 0.7 mL/min flow rate; ambient column temperature; 20 μL injection; 210 and 220 nm detection; retention times were 9.6 min for (*R*)-acetamide, 11.2 min for (*S*)-acetamide, 13.9 min for (*R*)-2-methoxyacetamide and 16.7 min for (*S*)-methoxyacetamide. For chiral amine analysis, samples were diluted to 10 mM with methanol, an aliquot (20 μL) treated with a mixture of water (40 μL), sodium bicarbonate (8 μL, 1 M) and Marfey's reagent (40 μL, 2% w/w in 1:1 acetonitrile–methanol) at 300 rpm, 40 °C, 1 h. The solution was quenched with hydrochloric acid (88 μL, 1 M), diluted to 0.2–0.5 mM with 1:1 acetonitrile–water (0.98 mL) and filtered (PTFE, 0.45 μm) prior to HPLC analysis as follows: Phenomenex Synergi Max-RPA (4 μm, 0.2 × 5 cm) column; elution with 10–100% B over 0–8 min, then hold at 100% B over 8–10 min; solvent A was 8:2 water–methanol containing 0.05% TFA and the solvent B was 8:2 acetonitrile–methanol containing 0.05% TFA; 0.6 mL/min flow rate; 25 °C column temperature; 20 μL injection; 340 and 350 nm detection; the retention times were for 7.6 min for (*R*)-amine and 8.0 min for (*S*)-amine. NMR spectra were recorded on a Bruker-300 or Jeol-400 spectrometers using deuterochloroform as solvent. Optical rotations were recorded on a Perkin–Elmer 241 digital polarimeter at 20 °C using the sodium D line.

3.3. Screening of hydrolases for the enantioselective acylation of (*RS*)-1

3.3.1. Immobilization of subtilisin and alcalase. Subtilisin solution (1 mL, 50 mg/mL in 0.1 M potassium phosphate, pH 7.0, containing 0.1 M sodium sulfate) was mixed with Celite R633 (1 g) and the damp powder dried in air at rt for 24 h. The immobilizate was obtained as a free-flowing powder (yield 1.08 g). For Alcalase, the enzyme solution (0.8 mL) was mixed with a potassium phosphate stock (0.1 mL, 1 M, pH 7.0) and sodium sulfate stock (0.1 mL, 1 M). The solution was then mixed with Celite R633 (1 g) and the damp powder dried in air at rt for 24 h. The immobilizate was obtained as a free-flowing powder, yield 1.16 g.

3.3.2. Enantioselective acylation of (*RS*)-1. In a typical reaction, a mixture of (*RS*)-**1** (50–500 mM), acyl donor (0.3–1.0 M or neat), biocatalyst (10, 25 or 100 mg/mL, or indicated amount) with or without molecular sieves (100–150 mg/mL) in a 2 mL glass vial was incubated at 40 °C, with stirring at approximately 400 rpm for the indicated time period.

3.4. Immobilized amano lipase PS30-mediated resolution of (*RS*)-1

Amano lipase PS-30 was immobilized on Accurel polypropylene as described earlier.[39] Amano PS 30-Accurel (0.2 g, 40% w/w of substrate input) was added to a mixture of (*RS*)-**1** (0.5 g, 2.5 mmol) and ethyl 2-methoxyacetate (0.18 g, 1.5 mmol, 0.6 mol equiv) and MTBE (3 mL) in a 6 mL vial, and the mixture stirred at rt for 2 days. After 2 days, the reaction mixture [showing extensive precipitation of (*R*)-**2b**] was diluted with methanol (ca. 1.5 mL) to dissolve the amide precipitate, then filtered to remove the biocatalyst and washed with 3:1 MTBE–methanol. The organic phases were combined and rotary evaporated at 40 °C to furnish a light-orange semi-solid. This was suspended in water (3 mL) and titrated with aqueous sulfuric acid (1 M) to pH 2.5. The suspension was stirred at rt for 0.5 h, and then filtered. The cake was suspended in water (2 mL) and the pH adjusted to 2.5 with sulfuric acid (0.1 M). The suspension was filtered, after which the cake was washed with aqueous sulfuric acid (2 × 2 mL, 10 mM), then water (3 × 2 mL), and the cake filter dried in air at rt for 1 h, then dried under vacuum over Drierite at rt, 24 h, to give (*R*)-**2b** as an off-white crystalline solid: 0.31 g, 99% potency (HPLC), 91% yield, ee >99.8%; $[\alpha]_D^{20} = +20.2$ (*c* 2.7, methanol); LC–MS and NMR data are identical to those of compound isolated in Section 3.6.1. The aqueous acidic extracts were pooled and extracted with MTBE (3 × 3 mL), the pH of the aqueous phase adjusted with sodium hydroxide (1 M) to pH 9.75, the resulting emulsion extracted with MTBE (3 × 3 mL), the organic layer washed with brine (3 × 3 mL) then dried over anhydrous magnesium sulfate. Filtration and evaporation at 50 °C provided (*S*)-**1** as a pale yellow oil: 0.23 g, 100% potency (HPLC), 91% yield, ee 98.4%; $[\alpha]_D^{20} = -23.9$ (*c* 2.8, methanol); LC–MS and NMR data identical to those of compound isolated in Section 3.6.1.

3.5. Immobilized CALB-mediated resolution of (*RS*)-1

3.5.1. Immobilization of CALB on Celite R633. 3-Glycidoxypropyltrimethoxysilane (0.69 g, 2.1 mmol) in THF (2 mL) was added to a mixture of poly(ethylene glycol)-*bl*-poly(propylene glycol)-*bl*-poly(ethylene glycol) (1 g, 0.53 mmol, MW = 1900), zirconium(IV) chloride (20 mg) and THF (4 mL), and the solution stirred at 400 rpm at rt for 15 min, after which the temperature raised to 70 °C over 1 h and stirring continued at reflux for 20 h. A portion of the solution (5.0 mL, ca. 1.25 g of solids) was diluted with ethanol (6 mL) and applied onto Celite R-633 (10 g, powdered). The wet powder was dried in air at rt for 5 h, then cured at 120 °C for 20 h to give ca. 11.3 g of modified Celite R633. A solution of CALB (0.6 g in 4 mL of ice-cold 0.2 M sodium phosphate, pH 7.0, containing 10 mM each of calcium and magnesium acetates) was coated onto the modified Celite R633 (4 g), the wet powder was dried in air at rt for 5 h, then under vacuum, over Drierite at rt for 20 h. This yielded 4.90 g of immobilized CALB (ca. 12.2% w/w CALB loading) as a free-flowing off-white powder. The specific activity of the immobilizate was 5.3 times that of lyophilized CALB in the resolution of (*RS*)-**1**.

3.5.2. CALB-Celite-mediated resolution of (*RS*)-1. CALB-Celite (0.41 g, 10% w/w of substrate input) was added to a mixture of (*RS*)-**1** (0.50 g, 2.5 mmol), ethyl 2-methoxyacetate (0.18 g, 1.5 mmol, 0.6 mol equiv) and MTBE (3 mL) in a 6 mL vial, after which the mixture stirred at rt for 2 days. The reaction mixture [showing some precipitation of (*R*)-**2b**] was diluted with ethanol (ca. 2.0 mL) to dissolve the 2-methoxyacetamide precipitate, filtered, and the biocatalyst washed with 2:3 ethanol–MTBE. The combined organic phases were evaporated at 40 °C to yield a light-orange semi-solid, which was suspended in water (3 mL) and titrated with aqueous sulfuric acid (1 M) to pH 2.5. The resulting suspension was stirred at rt for 0.5 h, and then filtered. The filter cake was suspended in water (2 mL). The pH was adjusted to 2.5 with sulfuric acid (0.1 M) and the mixture then stirred at 200 rpm at rt for 0.5 h, after which it was filtered. The cake was washed with aqueous sulfuric acid (2 × 2 mL, 10 mM), then water (3 × 2 mL), filter dried in air at rt for 1 h, then dried under vacuum over Drierite at rt for 18 h, to give (*R*)-**2b** as an off-white crystalline solid: 0.33 g, 101% potency (HPLC), 96% yield, ee >99.8%; $[\alpha]_D^{20} = +18.5$ (*c* 2.0, methanol); LC–MS and NMR data identical to those of compound isolated in Section 3.6.1. The aqueous extracts were pooled and extracted with MTBE (3 × 3 mL), the pH of the aqueous phase adjusted to 9.75 with sodium hydroxide (1 M), and the resulting emulsion extracted with MTBE (3 × 3 mL). The MTBE extract was washed with brine (3 × 3 mL), dried over anhydrous magnesium sulfate, filtered, then evaporated at 50 °C to furnish (*S*)-**1** as a pale yellow oil: 0.24 g, 102% potency (HPLC), 95% yield, ee 99.3%; $[\alpha]_D^{20} = -25.6$ (*c* 2.1, methanol); LC–MS and NMR data identical to those of compound isolated in Section 3.6.1.

3.6. Preparative-scale Novozym 435-mediated resolution of (*RS*)-1

3.6.1. Reaction 1. (*RS*)-**1** (38 g, 190 mmol), ethyl 2-methoxyacetate (13.5 g, 114 mmol, 0.6 mol equiv, 20 mol % excess) and MTBE (115 mL) were added in sequence to a 250 mL PTFE flask, followed by Novozym-435 (3.8 g, 10% w/w of substrate input), and the flask sealed and shaken at 200 rpm, 35 °C, 42 h. The reaction mixture [showing extensive precipitation of (*R*)-**2b**] was diluted with methanol (50 mL) to dissolve the precipitate formed, filtered and the biocatalyst washed with 3:1 MTBE–methanol. The combined organic phases were evaporated at 40 °C to give a sticky light-orange solid, which was suspended in water (150 mL) and titrated with aqueous sulfuric acid (2 M) to pH 2.5, and the resulting suspension filtered. The filter cake was suspended in water (50 mL) and the pH adjusted to 2.5 with sulfuric acid (2 M), after which the suspension filtered. The filter cake was washed with aqueous sulfuric acid (50 mL, 10 mM), then water (3 × 50 mL) and the cake filter dried in air at rt for 1 h, then dried under vacuum over Drierite at rt for 24 h, to give (*R*)-**2b** as an off-white crystalline solid: 23.7 g, 99% potency (HPLC), 92% yield, ee >99.8%; mp 131–135 °C (decomp); $[\alpha]_D^{20} = +21.5$ (*c* 3.0, methanol); LC–MS (ESI^+) *m*/*z* (%) = 273 ([M+H], 100), 295 ([M+Na], 27); ^1H NMR ($CDCl_3$) δ 1.53 (d, 3H, 2-H), 3.35 (s, 3H, CH_3), 3.62 (s, 2H, CH_2O), 5.02 (q, 1H, 1-H), 7.29 (d, 1H, 6′-H), 7.33

(dd, 1H, 5′-H), 7.45 (d, 1H, 4′-H), 7.51 (s, 1H, 2′-H), 8.33 (br d, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 22.6, 51.0, 57.2, 73.4, 123.1, 126.8, 130.5, 130.9, 132.3, 147.2, 170.4 ppm. The aqueous acidic extracts were pooled and extracted with MTBE (3 × 50 mL). The pH of the aqueous phase was adjusted with sodium hydroxide (5 M) to pH 9.75. The resulting emulsion was extracted with MTBE (3 × 50 mL) and the combined MTBE extracts washed with brine (2 × 25 mL) then dried over anhydrous magnesium sulfate. Evaporation at 50 °C provided the (*S*)-**1** as a pale-yellow oil: 17.6 g, 100% potency (HPLC), 95% yield, ee 99.8%; $[\alpha]_D^{20} = -24.7$ (*c* 2.9, methanol); LC–MS (ESI^+) *m/z* (%) = 201 ([M+H], 100); ^{1}H NMR ($CDCl_3$) δ 1.31 (d, 3H, 2-H), 1.53 (br s, 2H, NH_2), 4.11 (q, 1H, 1-H), 7.25 (d, 1H, 6′-H), 7.31 (dd, 1H, 5′-H), 7.41 (d, 1H, 4′-H), 7.47 (s, 1H, 2′-H) ppm; ^{13}C NMR ($CDCl_3$) δ 26.2, 51.5, 124.3, 126.3, 129.1, 129.5, 132.4, 146.5 ppm.

3.6.2. Reaction 2. (*RS*)-**1** (50 g, 250 mmol), ethyl 2-methoxyacetate (17.7 g, 150 mmol, 0.6 mol equiv, 20 mol % excess) and MTBE (ca. 150 mL) were added in sequence to a 250 mL PTFE flask, followed by Novozym-435 (5 g, 10% w/w of substrate input). The flask was sealed, and shaken at 200 rpm at 37 °C for 20 h. The reaction mixture [showing some precipitation of (*R*)-**2b**] was filtered through a coarse sieve to recover the immobilized enzyme, the latter washed with MTBE (3 × 10 mL), and the combined solution left at rt for 0.5 h to initiate the crystallization of (*R*)-**2b**. The solution was stirred with cooling in ice for 1 h and then filtered. The filter cake was washed with ice-cold MTBE (2 × 20 mL) and then filter dried at rt for 1 h. The filter cake was then washed with aqueous sulfuric acid (50 mM, 3 × 50 mL) and water (3 × 50 mL), after which it was filter dried at rt to give the first crop of (*R*)-**2** as a white crystalline solid: 22.3 g, >99% potency (HPLC), 68% yield, >99.8% ee; $[\alpha]_D^{20} = +19.7$ (*c* 2.9, methanol); LC–MS and NMR data identical to those of compound isolated in Section 3.6.1. The filtrate was evaporated at 40 °C to yield an orange semi-solid, which was titrated with aqueous sulfuric acid (1 M) to pH 2.0 and the resulting suspension filtered and the second crop of (*R*)-**2b** filter dried at rt. The aqueous filtrate was extracted with MTBE (4 × 50 mL), then titrated with aqueous sodium hydroxide (10% w/w) to pH 10.0. The solution was saturated with sodium chloride, and then extracted with MTBE (4 × 60 mL). The combined MTBE extracts were washed with brine (2 × 50 mL), dried over anhydrous magnesium sulfate and then evaporated at 50 °C to yield (*S*)-**1** as a light orange oil: 22.1 g, 105% potency (HPLC), 91% yield, 99.2% ee; $[\alpha]_D^{20} = -26.9$ (*c* 3.0, methanol); LC–MS and NMR data identical to those of compound isolated in Section 3.6.1. The MTBE extracts resulting from the washing of the acidified aqueous solution were combined with the second crop of (*R*)-**2b**. The resulting suspension was evaporated to dryness at 40 °C and the resulting solid stirred with 3:1 hexane–MTBE (2 × 50 mL) then filtered, after which the solid was filter dried at rt, then extracted with aqueous sulfuric acid (50 mM, 3 × 20 mL) then water (3 × 20 mL), then filter dried at rt to give a second crop of (*R*)-**2** as a white crystalline solid: 7.4 g, >99% potency (HPLC), 22% yield, >99.8% ee; $[\alpha]_D^{20} = +20.1$ (*c* 3.0, methanol). The overall isolated yields were: (*S*)-**1** (22.1 g, 91%), (*R*)-**2b** (29.7 g, 90%).

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