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Enantiomers of adrenaline-type amino alcohols by *Burkholderia cepacia* lipase-catalyzed asymmetric acylation

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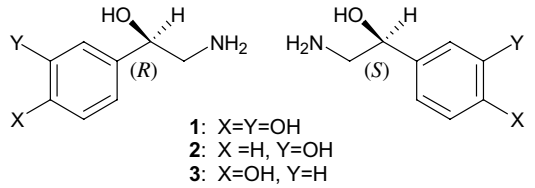
Abstract—*Burkholderia cepacia* lipase-catalyzed acylation with butanoic anhydride was used for the preparation of pharmaceutically important norphenylephrine and octopamine enantiomers, all in 98% ee. Reactivity of the phenolic OH groups and easy racemization, especially in the case of octopamine, complicated optimization. For norphenylephrine, chemical *N*-acylation was fast and allowed the subsequent enzymatic benzylic acylation in situ. The preparation of the octopamine enantiomers became possible by using the *N*-Fmoc protected substrate and by the *Candida antarctica* lipase B-catalyzed deprotection of the OH groups before the *N*-deprotection was performed.

1. Introduction

(*R*)-(−)-Noradrenaline (*R*)-**1** is a natural catecholamine, which acts as an α-adrenoceptor agonist (Scheme 1, X = Y = OH).[1,2] The compound also shows activity at β-receptors, structural factors determining the activity in such a way that α-activity is dramatically decreased and β-activity increased when the compound is *N*-substituted and the substituent is larger than methyl. Importantly, 2-amino-1-phenylethanol analogues exert their pharmacological activity through the stereoisomer, which corresponds to the absolute configuration of (*R*)-(−)-noradrenaline. In such a stereostructure, the three important groups (the charged nitrogen, the phenyl group and the benzylic OH) are in the most favourable orientation for interaction with a receptor. The task of the catecholic *meta*- and *para*-OH groups of 2-amino-1-phenylethanol agonists has been shown to be key to receptor activation rather than to affect the binding process.[3] When the biological activity of chiral molecules is studied both the enantiomers in an enantiopure form are needed.



Scheme 1.

Synthetic methods to enantiopure adrenergic 2-amino-1-phenylethanols include the transformations of optically active cyanohydrins and β-azido alcohols.[4–6] In these cases, chemical, chemoenzymatic and purely biocatalytic methods have afforded the target molecules. Resolution of 2-amino-1-phenylethanols has also been used. Thus, octopamine, as its salts with optically active acids, has been resolved by fractional crystallization,[7] while the lipase-catalyzed kinetic resolution has served as one of the most fascinating methods in the case of various ring-substituted 2-amino-1-phenylethanols.[8–11] We previously used *Burkholderia cepacia* lipase (lipase PS)-catalyzed asymmetric acylation of various 2-amino-1-phenylethanols with an acid anhydride in toluene/THF (3:1) as a highly effective method to pure enantiomers.[10] The addition of THF was necessary in order to improve the solubility of 2-amino-1-phenylethanols while the ring substituents were typically unreactive under the reaction conditions. Herein we report the preparation of both the enantiomers of norphenylephrine **2** (X = H, Y = OH; Scheme 1) and octopamine **3** (X = OH, Y = H) with phenolic OH groups using the

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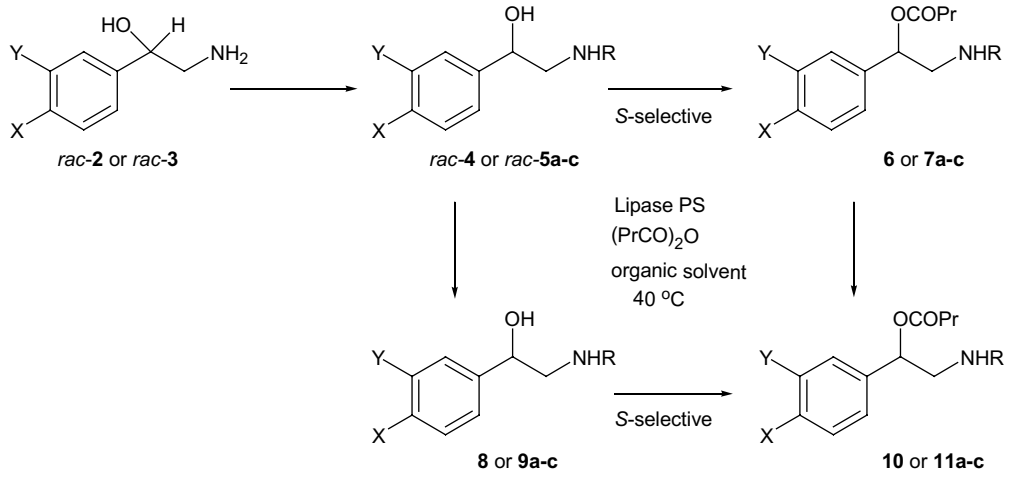


Scheme 2. Reagents and conditions: norphenylephrine derivatives: **4** and **6** (X = H, Y = OH, R = COPr); **8** and **10** (X = H, Y = OCOPr, R = COPr). Octopamine derivatives: **5** and **7** (X = OH, Y = H); **9** and **11** (X = OCOPr, Y = H) with **a** (R = COPr), **b** (R = Boc) and **c** (R = Fmoc).

lipase PS-catalyzed acylation with butanoic anhydride. The behaviour of the enantiomers for agonist promoted human α_{2A}-adrenoceptor activation has already been published although the details for the preparation of the enantiomers were left to wait a proper optimization.[3] Difficulties arose from two facts. First, substrates **2** and **3** are trifunctional, with all functional groups tending to react under acylation conditions (Scheme 2). A more serious problem was connected to the easy racemization of **3**, in particular. Thus, attention had to be paid to protecting groups, which allow deprotection under conditions where the amino alcohol is stereochemically stable.

2. Results and discussion

2.1. Asymmetric acylation of norphenylephrine and octopamine derivatives

Chemo- and/or regioselectivities for the kinetic resolution of polyfunctional compounds are possible advantages of lipase catalysis. In the best cases, these selectivities can save time and chemicals by reducing the number of synthetic steps because separate protection/deprotection steps become unnecessary. Based on this reasoning, the lipase PS-catalyzed acylation of various 2-amino-1-phenylethanols with an acid anhydride in toluene/THF (3:1) was previously studied.[10] In this method, highly efficient chemical *N*-acylation was followed by highly enantioselective enzymatic *O*-acylation in situ. Accordingly, enzymatic chemoselectivity had no possibility in playing a role in the studied kinetic resolutions. Herein, compounds **2** and **4** and **3** and **5a–c** were subjected to the lipase PS-catalyzed acylation with butanoic anhydride in toluene/THF (3:1). The phenolic and benzylic OH groups were both susceptible to enzymatic acylation and the various acylation products were all detected by the chiral GC method. As shown for the acylations of **4** and **5c** (Figs. 1 and 2) as examples for general trends, the lipase showed relatively high chemoselectivity towards the benzylic OH, leading to just low amounts of **8** and **9c** (♦) and also to those of **10** and **11c**

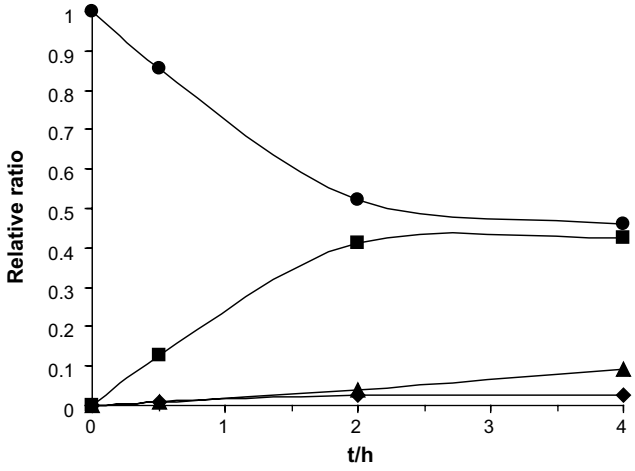


Figure 1. Progression curves for the lipase PS-catalyzed reaction between **4** and butanoic anhydride in toluene THF (3:1) at 40 °C; the disappearance of **4** (●) and the appearances of **6** (■), **8** (◆) and **10** (▲).

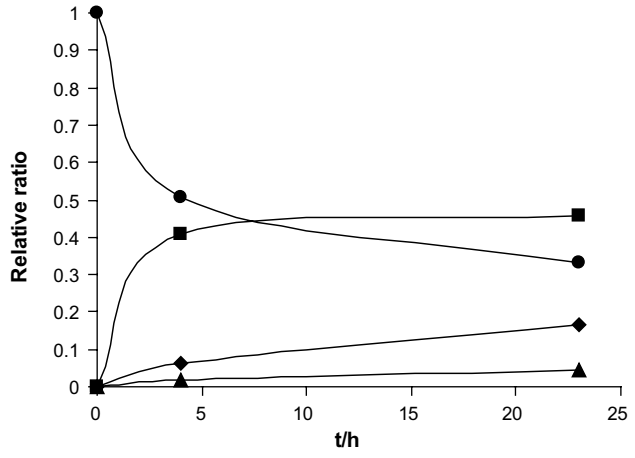


Figure 2. Progression curves for the lipase PS-catalyzed reaction between **5c** and butanoic anhydride in toluene THF (3:1) at 40 °C; the disappearance of **5c** (●) and the appearances of **7c** (■), **9c** (◆) and **11c** (▲).

(▲). Except for negligible benzylic acylation of **4** (2.5% in 16 h), other chemical reactions were not detected.

The lipase-catalyzed benzylic acylation of **4** and **5a–c** and that of **8** and **9a–c** proceeded in a highly *S*-selective manner while the acylation of the phenolic OH was not enantioselective (Scheme 2). Accordingly, after long enough reaction times, **8** and **9a–c** could be obtained as pure (*R*)-enantiomers after the (*S*)-enantiomers had further reacted in the formation of (*S*)-**10** and (*S*)-**11a–c**, respectively. The phenolic acylation of (*S*)-**6** and (*S*)-**7a–c** was another route to the respective (*S*)-**10** and (*S*)-**11a–c**. However, due to the high chemo- and enantioselectivity of the present reactions towards benzylic acylation, it is advantageous to stop the reaction as soon as the unreacted substrate enantiomer has had time enough to become enantiomerically pure. This reasoning holds well for the kinetic resolution of *rac*-**2** through *rac*-**4** in the presence of lipase PS preparation and butanoic anhydride in toluene/THF (3:1). The unreacted (*R*)-**4** (ee = 97%) and the produced (*S*)-**6** (ee = 93%) were obtained at 55% conversion (row 1, Table 1), allowing the straightforward gram-scale preparation of the enantiomers of **2** as described later. It is noteworthy that the conversion of the reaction always means the total reaction.

The lipase PS-catalyzed acylation of *rac*-**3** through *rac*-**5a** with butanoic anhydride in organic solvents was more complicated and the reactions tended to stop at conversions shown in Table 1 (entries 2 and 3) with ee values, which were not acceptable for pharmaceutical studies. With acid anhydrides as acyl donors, large amounts of acid were introduced into the reaction mixture during the course of the reaction. However, the negative effect of butanoic acid was at least partly ruled out by replacing the anhydride with 2,2,2-trifluoroethyl butanoate and by observing that the reaction in *tert*-alcohols then similarly stopped (entry 3 compared to entries 4 or 5). The amount of acid seemed to affect enantiopurities throughout (entries 3–5). Racemization can be understood through the resonance stabilized kinoidic structure of the substrate.

In order to prepare the enantiomers of **3**, a separate *N*-protection step was introduced through the synthesis of *rac*-**5b** and *rac*-**5c**. For the lipase PS-catalyzed acylation of the *N*-Boc-protected amino alcohol **5b** in toluene/THF (3:1), long reaction time (142 h to reach 50% conversion) gave a reason to turn to the *N*-Fmoc protected amino alcohol **5c** (entries 6 and 7, respectively; Table 1). As a more important reason, the stereochemical stability of the enantiomers under the subsequent deprotection steps favoured the Fmoc protection. In order to get the unreacted (*R*)-**5c** in an enantiopure form, there was a need to conduct the reaction considerably over 50% conversion. As a consequence, the reaction mixture contained considerable amounts of (*R*)-**9c** in addition to (*R*)-**5c** and (*S*)-**7c** (Fig. 2). The amount of (*S*)-**11c** always remained negligible.

2.2. Deprotection and enantiopurity

Due to easy racemization, conditions used for the removal of the *N*-protective groups (PrCO, Boc and Fmoc) of the present compounds are critical for enantiopurity. For this purpose, (*R*)-**4** and (*R*)-**5a–c** were subjected to various conditions, which are typically used in order to remove the protecting groups (Table 2).

Table 1. Lipase PS (50 mg/mL)-catalyzed acylation of **4** and **5a–c** (0.05 M) with butanoic anhydride (0.2 M) at 40 °C

Entry	Substrate	Solvent	Time (h)	Conversion (%)	Product	$ee_{4\text{ or }5}/ee_{6\text{ or }7}$ (%)
1	**4**[a]	Toluene/THF (3:1)	4	55	**6**	97/93
2	**5a**	Toluene/THF (3:1)	4	49	**7a**	88/90
3	**5a**	*tert*-Amyl alcohol	5	29	**7a**	*rac*/*rac*
4	**5a**[b]	*tert*-Amyl alcohol	3	15	**7a**	17/>98
5	**5a**[b]	*tert*-Butyl alcohol	24	15	**7a**	18/>98
6	**5b**[c]	Toluene/THF (3:1)	142	50	**7b**	>98/>98
7	**5c**[c]	Toluene/THF (3:1)	4	49	**7c**	64/93

[a] Butanoic anhydride 0.3 M.
[b] 2,2,2-Trifluoroethyl butanoate as an acyl donor.
[c] Butanoic anhydride 0.1 M.

Table 2. Effect of *N*-deprotection on enantiopurity

Entry	Compound	Method	$ee_{(4\text{ or }5a–c)}$ (%)	$ee_{(2\text{ or }3)}$ (%)
1	(*R*)-**4**	H_2SO_4 (0.4–1 M)/MeOH	>98	>98
2	(*R*)-**4**	(1) HCl (0.54 M)/MeOH, (2) NH_3	>98	>98
3	(*R*)-**5a**	(1) HCl (0.54 M)/MeOH, (2) NH_3	>98	*rac*
4	(*R*)-**5b**	(1) HCl (0.54 M)/MeOH, (2) NH_3	>98	*rac*
5	(*R*)-**5b**	BF_3/Et_2O	>98	*rac*
6	(*R*)-**5b**	HCl (1 M)/AcOH	>98	*rac*
7	(*R*)-**5b**	Iodotrimethylsilane	>98	No reaction
8	(*R*)-**5c**	5% Piperidine/THF	>98	>98

Table 3. Screening of lipases (75 mg/mL) and solvents for the alcoholysis of (*S*)-**7c** (0.05 M) at 40 °C

Entry	Enzyme	Solvent	Nucleophile	*t* (h)	Yield[a] (%)	ee_{5c} (%)
1	CAL-B	Toluene/THF (3:1)	OctOH (0.15 M)	96	20	98
2	CRL	Toluene/THF (3:1)	OctOH (0.15 M)	96	0	—
3	Lipase PS	Toluene/THF (3:1)	OctOH (0.15 M)	96	16	98
4	CAL-B	THF	OctOH (0.15 M)	70	6	98
5	CAL-B	H_2O [5% (v/v)]/THF	OctOH (0.15 M)	70	0	—
6	CAL-B	EtOH	EtOH	96	20	98
7	CAL-B	Toluene/THF (3:1)	2-PrOH (0.3 M)	94	68	98
8	CAL-B	THF	2-PrOH (10 M)	94	45	98
9	CAL-B	Toluene/THF (1:1)	2-PrOH (6.5 M)	94	40	98

[a] Relative proportion.

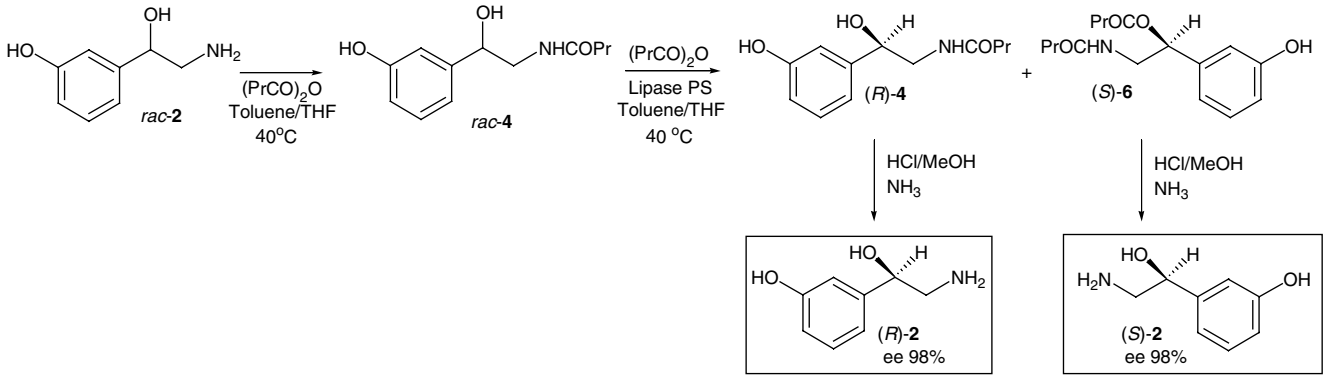
Hydrolysis or methanolysis in the presence of HCl is commonly used for splitting amide bonds. The method worked well in the case of compound (*R*)-**4** (entries 1 and 2). However, (*R*)-**5a** and (*R*)-**5b** were unable to withstand acidic reaction conditions, thus leading to total racemization (entries 3–6). An explanation is the resonance stabilization through the kinoidic intermediate of octopamine-like compounds under acidic conditions. Fmoc is typically removed under basic conditions. Accordingly, the deprotection of (*R*)-**5c** easily proceeded using 5% piperidine in THF, leading to (*R*)-**3** without any racemization (entry 8).

Removal of an ester group from (*S*)-**7c** with saponification using NaOH or K_2CO_3 caused racemization. This was disappointing because the removal of the Fmoc had proceeded at the same time. The only feasible way for the deprotection proved to be enzymatic alcoholysis. For this purpose, three lipases were first screened for the octanolysis of (*S*)-**7c** in toluene/THF (3:1) (entries 1–3, Table 3). *Candida rugosa* lipase (CRL)-catalyzed alcoholysis did not proceed at all while lipase PS and CAL-B gave slow reactions. No improvement was seen when the composition of the solvent was changed (entries 4 and 5). The reaction in neat ethanol was also slow (entry 6). Finally, 2-propanolysis in toluene/THF (3:1) was exploited in order to split the ester bond from **7c**. Phenolic and benzylic esters reacted under the same conditions with the phenolic ester bond being more alert to enzymatic alcoholysis than the benzylic one.

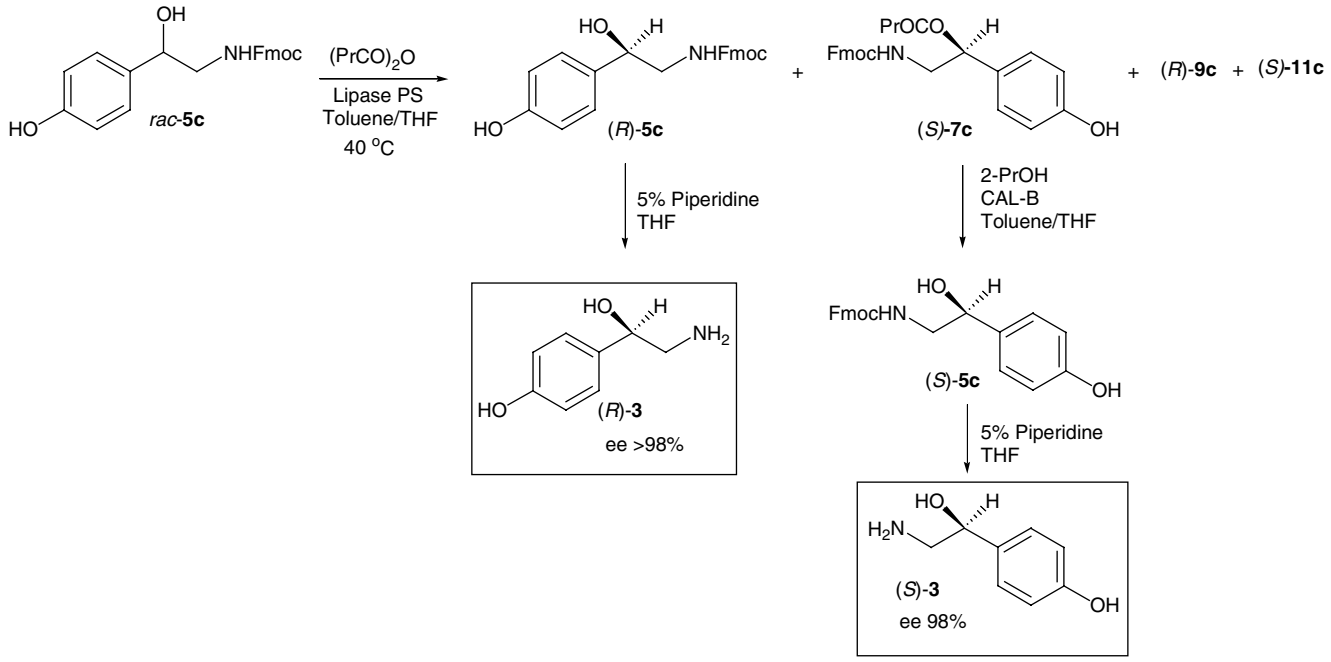
2.3. Gram-scale preparation of the enantiomers of 2 and 3

The results in Tables 1 and 2 clearly indicate that the previously developed acylation method with an acid anhydride in toluene/THF (3:1) well suits the kinetic resolution of *rac*-**2** with butanoic anhydride and the lipase PS preparation. A gram-scale resolution was performed as described in the Experimental section (Scheme 3). The enantiopure compounds (*R*)-**4** and (*S*)-**6** were isolated close to quantitative chemical yields. Deprotection with HCl/MeOH followed by NH_3-bubbling proceeded well without racemization in the formation of enantiopure (*R*)-**2** and (*S*)-**2**.

The gram-scale kinetic resolution of *rac*-**5c** was performed as described in the Section 4 (Scheme 4). The lipase PS-catalyzed acylation led to the resolved mixture containing the unreacted (*R*)-**5c** and the produced (*S*)-**7c**, (*R*)-**9c** and (*S*)-**11c** at 67% conversion. The products were all separated by column chromatography. (*R*)-**5c** and (*R*)-**9c**, on one hand, and (*S*)-**7c** and (*S*)-**11c**, on the other hand, can be joined if desired to produce (*R*)- and (*S*)-**3**, respectively. Herein, the ester group of (*S*)-**7c** was first removed by CAL-B-catalyzed 2-propanolysis in toluene/THF. The reaction was slower than when performed on a small scale and 90% conversion was reached in 16 days. Long reaction times are evidently due to the equilibrium nature of the enzymatic alcoholysis. Afterwards, the Fmoc-group of (*R*)-**5c** and (*S*)-**5c** was removed by piperidine treatment leading to enantiopure (*R*)-**3** and (*S*)-**3**, respectively.



Scheme 3.



Scheme 4.

3. Conclusions

The acylations of *N*-protected norphenylephrine and octopamine derivatives **4** and **5a**–**c** with butanoic anhydride were studied in the presence of the lipase PS preparation in toluene/THF (Scheme 2, Table 1). The non-enantioselective acylations at the phenolic OH groups were observed with both substrates although chemoselectivity strongly favoured the highly enantioselective benzylic acylation. The enantiomers of norphenylephrine **2** were prepared starting with the chemical *N*-acylation of *rac*-**2** and continuing with enzymatic acylation in situ (Scheme 3). The deprotection of the *N*,*O*-diacylated (*S*)-product **6** and that of the unreacted *N*-acylated *R*-enantiomer **4** was accomplished by refluxing in HCl/MeOH and finally by bubbling NH_3. It was not possible to use the same method for the enzymatic kinetic resolution of *rac*-**3** because the reactions had a tendency to stop at early conversions with the enantiomers becoming racemized under the deprotection conditions. Instead, the *N*-Fmoc-protected substrate was used for lipase PS-catalyzed acylation with butanoic anhydride (Scheme 4). The resolution step resulted in 98% ee at 67% conversion for the two main enantiomers (*R*)-**5c** and (*S*)-**7c**. It occurred that in order to prevent racemization, the *O*-deprotection through 2-propanolysis by CAL-B had to be done before the *N*-Fmoc group was removed by the piperidine treatment.

4. Experimental

4.1. Materials and methods

Lipase from *B. cepacia* (previously *Pseudomonas cepacia* lipase; lipase PS) was purchased from Amano Pharmaceutical Co., Ltd (Nagoya, Japan). Chirazyme L2 (Novozym 435, CAL-B) was a product from Roche. Lipase from *C. rugosa* (CRL) was obtained from Sigma. Before use, lipase PS was adsorbed on Celite® in the presence of sucrose and Tris–HCl buffer (20 mM, pH 7.9). The enzyme preparation thus obtained contained 38% (w/w) of the lipase and Celite® and 23% (w/w) of sucrose.[12] Norphenylephrine and octopamine hydrochlorides, 9-fluorenylchloroformate (FmocCl), di-*tert*-butyl dicarbonate (Boc_2O) and butanoic anhydride were purchased from Aldrich. Solvents used were of the highest analytical grade and obtained from Merck or Lab Scan Ltd.

Norphenylephrine *rac*-**2** was prepared by bubbling ammonia through its hydrochloride solution in chloroform/ethanol (3:1). HCl was removed from commercial octopamine hydrochloride using Amberlite IRA-401 anion exchange resin in methanol. *N*-Boc-protected **5b** was prepared from **3** with di-*tert*-butyl dicarbonate and triethylamine in methanol. *N*-Fmoc-protected **5c** was obtained using 9-fluorenylchloroformate and diisopropyl amine in water/dioxane. Amides **4** and **5a** were obtained as a result of the reactions of **2** and **3** with butanoic anhydride while the amides in this solution were used in the enzymatic *O*-acylations.

In a typical lipase-catalyzed acylation reaction, butanoic anhydride (0.2 or 0.1 M) or 2,2,2-trifluoroethyl butanoate (0.2 M) was added into one of the amino alcohol derivatives **4** or **5a**–**c** (0.05 M) in an organic solvent (2 mL) and the enzyme preparation (50 mg/mL) was added to start the enzymatic reaction at 40 °C. The progress of the reactions was followed by taking samples (100 μL) at intervals, filtering off the enzyme and analyzing the sample by GC on a Chrompack CP-Chirasil-L-valine capillary column after the derivatization of the free alcohol groups with propionic anhydride. The

absolute configurations of octopamine enantiomers **3** were determined by comparing the specific rotations to those in the literature.[7] In the case of norphenylephrine enantiomers **2**, the (*S*)-enantiomer was expected to react as in the case of octopamine and other 2-amino-1-phenylethanols studied in our previous works.[8–10] ^{1}H NMR spectra were recorded on a Bruker 200 or 400 spectrometer, with tetramethylsilane as an internal standard. MS-spectra were recorded on a VG analytical 7070E instrument equipped with VAXstation 3100 M76 computer. Optical rotations were measured using a Jasco DIP-360 polarimeter, the $[\alpha]_D$ values being in units of 10^{-1} deg cm^2 g^{-1}.

4.2. Gram-scale resolution of *rac*-2

rac-**2** (1.03 g, 6.7 mmol) was dissolved in toluene/THF ((3:1), 142 mL) after which butanoic anhydride (4.6 mL, 28 mmol) was added. After the formation of *rac*-**4** (5 min), the enzyme preparation (7.1 g) was added and the reaction allowed to proceed to 52% conversion in 3 h at 40 °C. The enzyme was filtered off and the solvent evaporated. Purification by column chromatography [silica, ethyl acetate/hexane (7:3)] yielded the unreacted (*R*)-**4** {0.692 g, 3.1 mmol, ee >98%, $[\alpha]_D^{20} = +5.0$ (*c* 2.0, MeOH)]} and the produced (*S*)-**6** 0.821 g, 2.8 mmol, ee 98%, $[\alpha]_D^{20} = +46.8$ (*c* 1.0, MeOH)]. The proportions of **8** and **10** were negligible and the products not separated.

(*R*)-**4**: ^{1}H NMR ($CDCl_3$, 25 °C): δ (ppm) 0.83 (t, 3H, $J = 7.4$, CH_3CH_2), 1.49 (m, 2H, $J = 7.4$, $CH_3CH_2CH_2$), 2.05 (t, 2H, $CH_3CH_2CH_2CO$), 3.05 and 3.27 (m, 2H, $CHCH_2NHCOPr$), 5.37 (d, 1H, $CHCH_2NHCOPr$), 6.63 (dd, 1H, C_6H_4), 6.73 (d, 1H, $J = 7.8$, C_6H_4), 6.76 (s, 1H, C_6H_4), 7.10 (t, 1H, $J = 7.8$, C_6H_4), 7.83 (t, 1H, CH_2NHCO) and 9.29 (s, 1H, ArO*H*). ^{13}C NMR ($CDCl_3$, 25 °C): (ppm) 13.7, 18.7, 37.3, 46.9, 71.5, 112.9, 113.9, 116.6, 128.9, 145.4, 157.2 and 172.3. Mass spectrum M^+ (calculated for $C_{12}H_{17}NO_3$) = 223.1212 (223.1208).

(*S*)-**6**: ^{1}H NMR ($CDCl_3$, 25 °C): δ (ppm) 0.84 (tt, 2×3H, $J = 7.4$, CH_3CH_2), 1.43–1.59 (qq, 2×2H, $J = 7.4$, $CH_3CH_2CH_2$), 2.05 and 2.31 (tt, 2×2H, $J = 8.5$, $CH_3CH_2CH_2CO$), 3.27–3.41 (m, 2H, $CHCH_2NHCOPr$), 5.66 (dd, 1H, $J = 4.2$, $CHCH_2NHCOPr$), 6.69 (t, 3H, C_6H_4), 7.15 (t, 1H, C_6H_4), 7.99 (t, 1H, CH_2NHCO) and 9.46 (s, 1H, ArO*H*). ^{13}C NMR ($CDCl_3$, 25 °C): δ (ppm) 13.9, 14.0, 18.4, 19.1, 36.0, 37.7, 44.2, 74.0, 113.4, 115.3, 117.1, 129.9, 140.5, 157.8, 172.5 and 172.7. Mass spectrum M^+ (calculated for $C_{16}H_{23}NO_4$) = 293.1636 (293.1627).

4.3. Deprotection of (*R*)-4 and (*S*)-6

(*R*)-**4** (0.420 g, 1.9 mmol) or (*S*)-**6** (0.600 g, 2.0 mmol) was dissolved in HCl/methanol (10 mL) and the mixture refluxed overnight. Methanol was evaporated, the residue dissolved in chloroform/ethanol (3:1) and ammonia bubbled through the solution. The immediately formed white precipitate (NH_4Cl) was filtered off. The solution was evaporated and the residue purified by column chromatography (silica, methanol/dichloromethane (1:1)). Enantiopure (*R*)-**2** {0.280 g, 1.3 mmol, ee 98%, $[\alpha]_D^{20} = -1.7$ (*c* 5.8, MeOH)} or (*S*)-**2** {0.300 g, 1.0 mmol, ee 98%, $[\alpha]_D^{20} = +1.7$ (*c* 5.6, MeOH)} was obtained.

(*R*)-**2**: ^{1}H NMR (DMSO, 25 °C): δ (ppm) 2.75 (m, 2H, CH_2NH_2), 3.10 (s, 1H, CHO*H*), 4.25 (dd, 1H, $CH(OH)CH_2$), 6.51–6.65 (m, 3H, C_6H_4) and 7.01 (t, 1H, C_6H_4). ^{13}C NMR (DMSO, 25 °C): δ (ppm) 47.1, 71.4, 115.9, 119.7, 123.8, 129.5, 146.2 and 150.8. Mass spectrum M^+ (calculated for $C_8H_{11}NO_2$) = 153.0792 (153.0790).

4.4. Gram-scale resolution of *rac*-5c

Lipase PS preparation (5.35 g) was added into the solution of *rac*-**5c** (2.00 g, 5.32 mmol) and butanoic anhydride (1.75 mL, 10.7 mmol) in toluene/THF [107 mL, (3:1)] at 40 °C. After 24 h, the enzyme was filtered off at 67% conversion. Purification by column chromatography (silica, ethylacetate/hexane (1:1)) yielded the unreacted (*R*)-**5c** {0.71 g, 1.89 mmol, ee 96%, $[\alpha]_D^{20} = +4.5$ (*c* 1.0, MeOH)} and the produced (*S*)-**7c** {0.79g, 1.77 mmol, ee 98%, $[\alpha]_D^{20} = +47.5$ (*c* 1.0, MeOH)}, (*R*)-**9c** {[0.26 g, 0.59 mmol, ee >98%, $[\alpha]_D^{20} = +9.7$ (*c* 1.0, MeOH)} and (*S*)-**11c** {0.52 g, 1.00 mmol, ee >98%, $[\alpha]_D^{20} = +31.0$ (*c* 1.0, MeOH)}.

(*R*)-**5c**: ^{1}H NMR (DMSO, 25 °C): 3.09 (m, 2H, $CHCH_2NHFmoc$), 4.23 (m, 2H, $NHCO_2CH_2CH$), 4.49 (d, 1H, CHO*H*), 5.23 (t, 1H, $CH(OH)CH_2$), 6.68 (d, 2H, $J = 8.3$, C_6H_4), 7.10 (d, 2H, $J = 8.3$, C_6H_4), 7.28–7.42 (tt, 2×2H, Fmoc), 7.85 (dd, 2×2H, Fmoc) and 9.27 (s, 1H, ArO*H*). ^{13}C NMR (DMSO, 25 °C): 48.7, 70.5, 71.7, 109.6, 114.7, 120.0, 121.3, 127.1, 127.2, 128.9, 134.0, 137.4, 138.3, 142.6, 143.0, 156.4 and 157.9. Mass spectrum M^+ (calculated for $C_{23}H_{21}NO_4$) = 375.1481 (375.1471).

(*S*)-**7c**: ^{1}H NMR (DMSO, 25 °C): 0.84 (t, 3H, CH_3CH_2, $J = 7.4$), 1.52 (m, 2H, $CH_3CH_2CH_2$, $J = 7.4$), 2.22 (m, 2H, $CH_3CH_2CH_2CO$), 3.37 (m, 2H, $CHCH_2NHFmoc$), 4.23 (d, 1H, $NHCO_2CH_2CH$), 4.26 (m, 2H, $NHCO_2CH_2CH$), 5.68 (dd, 1H, $CH_2CHOCOPr$), 6.68 (d, 2H, $J = 8.37$, C_6H_4), 7.11 (d, 2H, $J = 8.37$, C_6H_4), 7.31 (t, 2H, Fmoc), 7.41 (t, 2H, Fmoc), 7.55 (m, 1H, CH_2NHCO), 7.65 (m, 2H, Fmoc), 7.88 (d, 2H, Fmoc) and 9.52 (s, 1H, ArO*H*). ^{13}C NMR (DMSO, 25 °C): 13.8, 18.4, 36.1, 45.8, 47.1, 65.9, 74.0, 115.6, 120.6, 125.6, 127.5, 128.1, 128.3, 129.1, 141.2, 144.3, 156.7, 157.7 and 172.6. Mass spectrum M^+ (calculated for $C_{27}H_{27}NO_5$) = 445.1898 (445.1889).

(*R*)-**9c**: ^{1}H NMR (DMSO, 25 °C): 0.87 (t, 3H, $J = 7.1$, CH_3CH_2), 1.65 (m, 2H, $J = 7.1$, $CH_3CH_2CH_2$), 2.25 (m, 2H, $CH_3CH_2CH_2CO$), 3.37 (m, 2H, $CHCH_2NHFmoc$), 4.20 (m, 2H, $NHCO_2CH_2CH$), 4.23 (d, 1H, $NHCO_2CH_2CH$), 5.50 (d, 1H, $CH(OH)CH_2$), 7.10 (d, 2H, C_6H_4), 7.30–7.43 (m, 6H, C_6H_4 and Fmoc), 7.61 (t, 1H, CH_2NHCO), 7.65 (d, 2H, Fmoc) and 7.88 (d, 2H, Fmoc). ^{13}C NMR (DMSO, 25 °C): 13.3, 17.9, 32.2, 45.8, 48.3, 59.5, 71.4, 107.5, 117.7, 120.0,

127.5, 136.7, 138.8, 143.9, 149.4 and 171.7. Mass spectrum M^+ (calculated for $C_{27}H_{27}NO_5$) = 445.1897 (445.1889).

(*S*)-**11c**: ^{1}H NMR (DMSO, 25 °C): 0.84 (m, 2 × 3H, J = 7.1, CH_3CH$_2$), 1.51 and 1.63 (m, 2 × 2H, J = 7.1, CH$_3$CH_2CH$_2$), 2.36 (m, 2 × 2H, J = 7.1, CH$_3$CH$_2$CH_2-CO), 3.34 (m, 2H, CHCH_2NHFmoc), 4.20 (m, 2H, NHCO$_2$CH_2CH), 4.23 (d, 1H, NHCO$_2$CH$_2$CH), 5.77 (m, 1H, CH$_2$CHOCOPr), 7.10 (d, 2H, C$_6H_4$), 7.30–7.43 (m, 6H, C$_6H_4$ and Fmoc), 7.65 (d, 2H, Fmoc) and 7.88 (d, 2H, Fmoc). ^{13}C NMR (DMSO, 25 °C): 13.8, 18.3, 35.7, 36.0, 45.8, 47.1, 66.0, 73.6, 120.6, 122.3, 125.6, 127.5, 136.3, 141.2, 156.6, 172.1 and 172.6. Mass spectrum M^+ (calculated for $C_{31}H_{33}NO_6$) = 515.2329 (515.2308).

4.5. Deprotection of (*R*)-5c and (*S*)-7c

Compound (*S*)-**7c** (0.79 g, 1.77 mmol, ee 98%) and 2-propanol (0.83 mL) were dissolved in 35.3 mL of toluene/THF (3:1) followed by the addition of CAL-B (2.65 g, 75 mg/mL). The mixture was shaken at 40 °C for 16 days in order to reach 90% conversion. The enzyme was filtered off and the solvent evaporated. Purification by column chromatography [silica, ethylacetate/hexane (1:1)] followed by crystallization from hexane yielded (*S*)-**5c** {0.36 g, 0.95 mmol, ee >98%, $[\alpha]_D^{20}$ −4.9 (*c* 1.0, MeOH)}.

One of the compounds (*R*)-**5c** (0.50 g, 1.34 mmol, ee 96%) or (*S*)-**5c** (0.30 g, 0.80 mmol, ee 98%) was dissolved in 5% piperidine in THF and the solution stirred for 2 h. Purification by column chromatography [silica, dichloromethane/methanol (1:1)] yielded (*R*)-**3** {0.32 g, 2.1 mmol, ee >98%, $[\alpha]_D^{20} = -31.6$ (*c* 1.0, MeOH), lit.[7] $[\alpha]_D^{25} = -37.4$ (*c* 1.0, H_2O)} or (*S*)-**3** {0.12 g, 0.80 mmol, ee 98% and $[\alpha]_D^{20} = +35.4$ (*c* 1.0, MeOH), lit.[7] $[\alpha]_D^{25} = +37.2$ (*c* 1.0, H_2O)}.

(*R*)-**3**: ^{1}H NMR (DMSO, 25 °C): 2.64–2.86 (m, 2H, CHCH_2NH$_2$), 3.18 (s, 1H, CHOH), 4.59 (m, 1H, CH(OH)CH$_2$), 6.77 (d, 2H, J = 8.5, C$_6H_4$) and 7.17 (d, 2H, J = 8.5, C$_6H_4$). ^{13}C NMR (DMSO, 25 °C): δ (ppm) 49.0, 71.8, 120.5, 121.9, 127.6, 129.4, 137.9 and 149.8. Mass spectrum M^+ (calculated for $C_8H_{11}NO_2$) = 153.0787 (153.0790).

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