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Burkholderia cepacia lipase and activated β-lactams in β-dipeptide and β-amino amide synthesis

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ABSTRACT

The work describes fluorine-activated and *N*-Boc-activated β-lactams as acyl donors to *N*-nucleophiles in the presence of *Burkholderia cepacia* lipase (lipase PS-D). Fluorine activation at the β-lactam ring causes the ring to open in high enantioselectivity and allows the preparation of β-dipeptides and β-amino amides. In the case of *N*-Boc-activation, the chemical ring opening is significant. β-Dipeptide formation can then be considerably enhanced by the presence of lipase PS-D and/or by temperature.

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1. Introduction

β-Lactam (2-azetidinone) rings are essential parts of β-lactam antibiotics. The ring makes the drug bactericidal due to the inactivation of a bacterial transpeptidase enzyme (essential for bacterial growth). The inactivation is caused by the N1–C2 opening of the lactam ring when the active-site serine hydroxyl reacts with the ring.[1] In addition to this, the enantiomers of β-lactams are versatile intermediates in various types of synthetic products, for example, as acylating agents for coupling reactions with *O*- and *N*-nucleophiles.[2,3] Accordingly, considerable interest has been focused on the asymmetric synthesis and regioselective ring opening of β-lactams.[2–5] For the chemical ring opening with free alcohols and α-amino esters, the work of Palomo et al. have shown the importance of using N-activated β-lactams in the presence of NaN_3 or KCN so that the ring-opening rate is promoted under neutral conditions.[2,3]

Lipases are serine hydrolases and used as versatile biocatalysts for the production of enantiopure compounds. The facts that lipases readily work in organic solvents, are economical and catalyze a wide variety of reactions other than the hydrolysis of triglycerides, have made many new synthetic applications possible. We have previously used *Candida antarctica* lipase A (CAL-A) for the preparation of β-dipeptides through the N-acylation of a β-amino ester with the 2,2,2-trifluoroethyl ester of another N-protected β-amino acid.[6] The enantioselective N1–C2 opening of a β-lactam ring with alcohols in the presence of *Burkholderia cepacia* lipase (lipase PS adsorbed on Celite[7] or commercial lipase PS-D) represents another application.[8,9] It is generally accepted that lipases do not split amide bonds. However, β-lactams lack the resonance stabilization typical to normal peptide bonds, and this explains why the active-site serine of lipases can accept β-lactams as acyl donors in the formation of the so-called acyl-enzyme intermediate (step 1, Scheme 1). This intermediate subsequently reacts with the nucleophiles present. This ability has been widely used and thoroughly reviewed for the kinetic resolution of β-lactams through hydrolysis with *C. antarctica* lipase B (CAL-B) in organic solvents.[10,11]

β-Amino acid residues are present in many natural compounds and synthetic peptidomimetics in the forms of β-amino amides and β-peptides.[2,3,12,13] This motivated us to start studies of β-dipeptide formation based on the use of ring and/or N-activated β-lactams **1**–**3** as acyl donors to β-amino esters (Fig. 1). Fluorine as an isostere to hydrogen was used to activate the ring in compounds **1** and **2**. N-Activation was accomplished by Boc-protection, as Boc is a common N-protecting group in peptide chemistry. The work was started with the lipase PS-D-catalyzed ring-opening studies using the amonolysis and aminolysis of *rac*-**1a** as novel applications of lipase catalysis (Scheme 1). Lipase PS-D was a natural choice as a catalyst because it was previously applicable in the alcoholysis of *rac*-**1a** in dry organic solvents and exposed the lactam ring to hydrolysis much less than CAL-B.[8] In dipeptide synthesis, *tert*-butyl esters rather than the corresponding *n*-alkyl esters were used in order to prevent the previously observed lipase PS-D-catalyzed interesterification-type reaction.[9a] In mechanistic terms, the two acyl donors (a β-lactam and a *n*-alkyl ester) of interesterification form their own acyl-enzyme intermediates with the serine hydroxyl of the lipase (step 1, Scheme 1). The *n*-alcohol released from the *n*-alkyl ester can then serve as a nucleophile to react with the β-lactam-based acyl-enzyme intermediate and lead to the formation of the ester as a side product. Attention was paid to minimize the enzymatic hydrolysis of the β-lactam ring by the water in the

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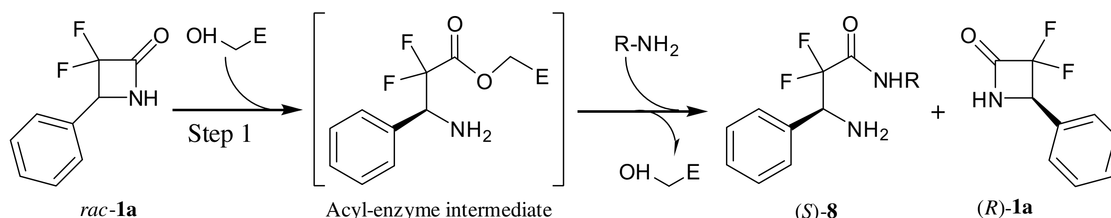


Scheme 1. Mechanism for the lipase PS-D-catalyzed kinetic resolution of *rac*-**1** with amines RNH_2 (R = H, ^{i}Pr, ^{i}Bu or *n*-Bu).

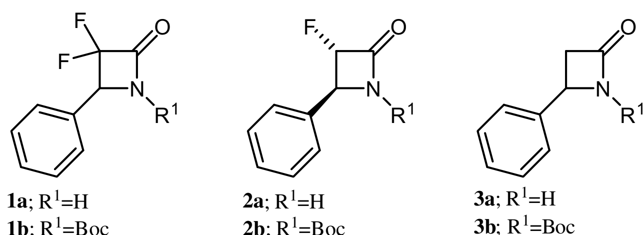


Figure 1. Studied racemic and enantiopure β-lactams.

enzyme preparation and the formation of the amino acid as a side product.

2. Results and discussion

2.1. Synthesis of compounds 1–7

Compounds *rac*-**1a**–**3a** and their enantiomers (ee = 99%) were prepared by known methods from the corresponding N-hydroxymethylated β-lactams and vinyl butanoate using chemoenzymatic pathways.[9a,14] *N*-Boc protections with Boc_2O in dichloromethane furnished (3*R*,4*R*)-**2b** and the enantiomers of **3b**. Attempts to prepare the enantiomers of **1b** failed due to the lability caused by the high activation stage. Enantiopure *tert*-butyl esters (*R*)-**6** and (*S*)-**7** were obtained combining literature methods. Thus, the lipase-catalyzed kinetic resolution of the corresponding racemic ethyl ester[15] and β-lactam[9b] first afforded (*R*)-**4** and (*S*)-**5**. The compounds were hydrolyzed to the corresponding acids followed by transformations into (*R*)-**6** and (*S*)-**7** in AcO^{t}Bu in the presence of a catalytic amount of $HClO_4$ (Scheme 2).[16]

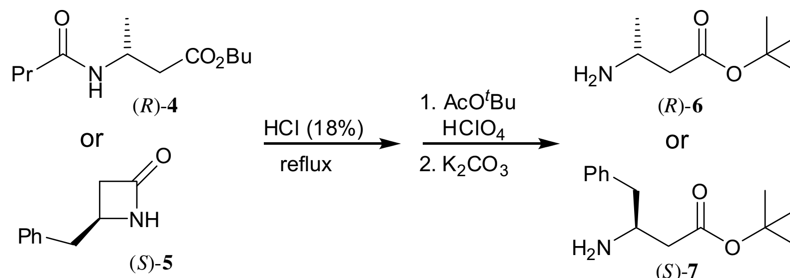
2.2. Lipase PS-D-catalyzed amonolysis and aminolysis of *rac*-1a

The reaction of *rac*-**1a** as a novel acyl donor for ammonia and various primary amines was first studied in the presence of lipase PS-D in *tert*-butyl methyl ether (TBME) (Scheme 1). The results are shown in Table 1. Highly enantioselective amonolysis in TBME saturated with ammonia took place, giving the enantiomers (*R*)-**1a** and (*S*)-**8** (R = H) in enantiopure forms at 50% conversion (entry 1). The hydrolysis of the lactam ring did not play a role as long as TBME was initially saturated with ammonia. It is worth noting that amonolysis became slow when ammonia was continuously bubbled into the reaction mixture. Since ammonia easily evaporates and special equipments were not available to control its concentration, we focused our attention on aminolysis studies. In the case of aminolysis, water in the enzymatic system clearly competed with the added isopropylamine when equimolar concentrations of the amine to the β-lactam were used (entries 2–4). However, both the unreacted (*R*)-**1a** and the amide (*S*)-**8** (R = ^{i}Pr) formed were enantiopure at 50% conversion, indicating excellent enantioselectivity for both hydrolysis and aminolysis. The proportion of the hydrolysis (the value in the brackets) was clearly reduced when the amount of lipase was increased although more water in the enzyme preparation came to the system at the same time (entries 2–4). This must mean that the enzyme content affects the aminolysis (favoring aminolysis) more than the hydrolysis rate. The proportion of hydrolysis (12%, entry 4), as determined by the GC method, is in accordance with the isolated yield of (*S*)-**8** (32% as calculated from the racemate, the theoretical yield at 50% conversion being 38%). The proportion of hydrolysis was further reduced when the amount of the amine was increased from 1 to 2 equiv (entries 4 and 5). At the same time, a decrease in reactivity was detected. The reaction in diisopropyl ether (DIPE) was most

Table 1
Lipase PS-D-catalyzed amonolysis and aminolysis of *rac*-**1a** (0.05 M) in TBME

Entry	Lipase PS-D (mg mL^{-1})	Nucleophile (equiv)	Time (h)	*C* (*x*)[a] (%)	ee$^{(R)\text{-}\mathbf{1a}}$ (%)	ee$^{(S)\text{-}\mathbf{8}}$ (%)
1	30	NH_3 (satd)	7	50 (0)	>99	>99
2	20	^{i}PrNH$_2$ (1)	10	49 (31)	87	>99
3	30	^{i}PrNH$_2$ (1)	7	50 (12)	>99	>99
4	40	^{i}PrNH$_2$ (1)	5	50 (12)	>99	>99
5	40	^{i}PrNH$_2$ (2)	5	42 (4)	68	97
6	40	^{i}PrNH$_2$ (2)[b]	2	50 (3)	87	96
7	30	^{i}BuNH$_2$ (1)	7	48 (—)[c]	89	97
8	30	*n*-BuNH$_2$ (1)	7	37 (—)[c]	46	80

[a] Proportion of hydrolysis from the observed conversion.
[b] Reaction in DIPE.
[c] Proportion of hydrolysis not determined.



Scheme 2. Preparation of *tert*-butyl esters.

effective (entry 6). The reactions of *rac*-**1a** with isobutylamine and *n*-butylamine were less enantioselective (entries 7 and 8). In these last-mentioned cases, the proportions of hydrolyses were not quantified.

2.3. β-Dipeptide synthesis

The next effort concerned β-dipeptide synthesis in DIPE. Enantiopure β-lactams **1**–**3** served as acyl donors to enantiopure amino esters **6** and **7** (Scheme 3). When enantiopure substrates are used, the resulting dipeptide is a pure stereoisomer, as shown in Table 2. We considered it important to look for new enzymatic methods for the preparation of β-peptides as so far, only two methods exist: the already mentioned CAL-A-catalyzed N-acylation of a β-amino ester[6] and N-acylation (and hydrolysis) with the previously found β-peptidyl aminopeptidases from *Sphingosinicella* strains and *Ochrobacterum anthropi*.[13] The drawback of the first method is that the size of the substituents at the acyl donor (2,2,2-trifluoroethyl ester) is limited. This can be easily explained on the basis of the previously published 3D-structure of CAL-A, where the acyl binding pocket is shown to be a deep and narrow tunnel.[17] The benefits of lipase catalysis, on the other hand, are the good availability of commercial and relatively inexpensive lipase preparations, and the fact that the normal peptide bond formed is not cleaved by lipases.

In accordance with the excellent (*S*)-enantioselectivity toward *rac*-**1a** (Table 1) and with the observed (*R*)-selectivity in the N-acylation of β-amino esters,[9a,15,18] (*S*)-**1a** was quantitatively transformed into the β-dipeptide **9** (entry 1) in the presence of (*R*)-**6** and lipase PS-D in DIPE (Table 2). Due to the (*S*)-selective ring opening, (*R*)-**1a** gave only traces of chemically formed dipeptide under the same conditions (entry 2). The incorporation of (3*S*,4*S*)-**2a** (the faster reacting enantiomer according to the previous methanolysis[9a]) and (*R*)-**6** afforded a gum-like dipeptide with 51% chemical yield (entry 3). At this point, the β-lactam was totally consumed by the cooperative enzymatic actions of the dipeptide formation and the hydrolysis of the lactam ring. For characterization, the formed dipeptide was transformed into the *N*-Boc-protected **10**. On the other hand, the unactivated (*R*)-**3a** showed no reaction with (*R*)-**6** under the enzymatic reaction conditions (entry 5), indicating the need to activate β-lactams for lipase-catalyzed aminolysis. When β-lactams were *N*-Boc-protected, chemical ring opening became highly significant. Accordingly, even the less reactive enantiomers (3*R*,4*R*)-**2b** and (*S*)-**7** could be effectively transformed into the dipeptide without lipase PS-D (entry 4). Similarly dipeptide **13** was chemically prepared from (*S*)-**3b** and (*S*)-**7** (entry 8).

As the *N*-Boc-protection exposes the β-lactam ring to chemical ring opening, it became necessary to study the enzymatic versus chemical β-dipeptide formation in more detail. Accordingly, the reaction of (*R*)-**3b** with (*R*)-**6** was studied in the presence (filled signs) and in the absence (open signs) of lipase PS-D in DIPE at different temperatures (Fig. 2). The progression curves for the disappearance of (*R*)-**3b** are shown in Figure 2. Increasing the temperature from 23 °C to 57 °C had a significant effect on the chemical reactivities; the β-lactam (Δ) completely reacted to form **12** in five days at 57 °C. In accordance with the curve (○), the preparative scale formation of **12** reached only 50% conversion after six days at room temperature (Table 2, entry 6). Since the enzymatic

Table 2
Reactions of β-lactams (0.05 M) with β-amino esters (0.1 M) in DIPE in the presence and in the absence of lipase PS-D (40 mg mL^{-1})

Entry	β-Lactam[a]	β-Amino ester[a]	Time (h)	β-Dipeptide	*C* (%)	Yield (%)	$[\alpha]_D^{22}$ (10^{-1} deg cm^2 g^{-1})
1	(*S*)-**1a**	(*R*)-**6**	15	**9**	100	96	+28.8 (*c* 1.00, $CHCl_3$)
2	(*R*)-**1a**	(*R*)-**6**	24	—	7[b]	—	—
3	(3*S*,4*S*)-**2a**	(*R*)-**6**	18	**10**[c]	100	51	+4.2 (*c* 0.75, $CHCl_3$)[c]
4	(3*R*,4*R*)-**2b**	(*S*)-**7**	24	**11**[d]	100	97	+8.7 (*c* 1.00, $CHCl_3$)
5	(*R*)-**3a**	(*R*)-**6**	66	—	0	—	—
6	(*R*)-**3b**	(*R*)-**6**	144	**12**[d]	50	47	+33.0 (*c* 1.05, $CHCl_3$)
7	(*R*)-**3b**	(*R*)-**6**	48	**12**[e]	93	82	+32.4 (*c* 1.05, $CHCl_3$)
8	(*S*)-**3b**	(*S*)-**7**	144	**13**[d]	69	65	−15.0 (*c* 1.00, $CHCl_3$)

[a] Ee 99% or higher.
[b] Peptide formation corresponds to 7% conversion with and without lipase PS-D.
[c] For the *N*-Boc protected **10**.
[d] No enzyme added.
[e] Temperature 57 °C; enzyme content 60 mg mL^{-1}.

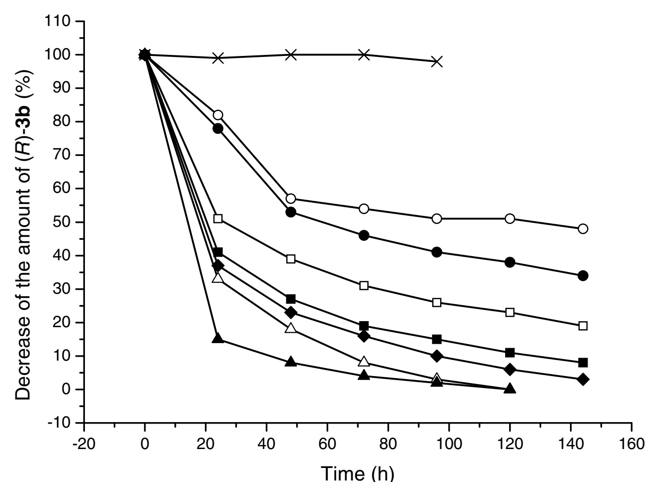
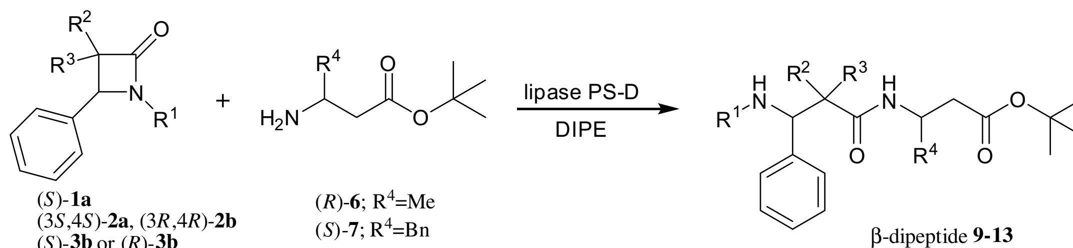


Figure 2. Progression curves for the disappearance of (*R*)-**3b** in its reaction with (*R*)-**6** in DIPE in the absence [(○) at 23 °C, (□) at 47 °C and (Δ) at 57 °C] and in the presence [(●) at 23 °C and (■) at 47 °C, 40 mg mL^{-1}; (◆) at 47 °C and (▲) at 57 °C, 60 mg mL^{-1}] of lipase PS-D; (×) stability of (*R*)-**3b** in DIPE in the presence of lipase PS-D (40 mg mL^{-1}) at 23 °C.



Scheme 3. Lipase PS-D-catalyzed synthesis of β-dipeptides in DIPE.

reaction is always accompanied by extensive chemical reaction, temperature effects on enzymatic reactivities stay largely unsolved in the present work. However, it is clear that the presence of lipase PS-D greatly favors the conversion attained after a certain time. Thus, 92% of (*R*)-**3b** (▲) had reacted after two days in the presence of the enzyme (60 mg mL^{-1}) at 57 °C, while the same conversion was attained chemically in three days in the absence of lipase PS-D (Δ). This is in accordance with the preparative scale synthesis of **12** in the presence of lipase PS-D (Table 2, entry 7). As shown for the reaction at 47 °C, increase in the enzyme content from none (□) through 40 mg mL^{-1} (■) to 60 mg mL^{-1} (♦) also gave reactivity enhancements. Thus, the reaction without the enzyme consumed 70% of (*R*)-**3b** in four days, while only 48 and 40 h were required to reach the same conversion in the presence of 40 and 60 mg mL^{-1} of the enzyme, respectively. When (*R*)-**3b** (×) together with lipase PS-D (40 mg mL^{-1}) was incubated in DIPE without an added nucleophile, the β-lactam concentration was unchanged, indicating stability of the lactam ring against hydrolysis by the water in the enzyme preparation. KCN and NaN_3 were previously observed to enhance the ring opening of β-lactams under neutral conditions.[2,3] It occurred to us that lipase PS-D catalysis in the place of KCN or NaN_3 also turned the ring opening more effective.

3. Conclusion

We have shown that fluorine activation facilitates the enantioselective lipase-catalyzed β-dipeptide and β-amino amide formation in novel reactions where β-lactams serve as acyl donors to *N*-nucleophiles in lipase PS-D-catalyzed transformations under non-aqueous conditions. As a consequence, a novel lipase-catalyzed β-dipeptide formation is possible, provided that ring-activation, N-activation or both are used. Accordingly, the capacity to open the ring progressively decreases, while going from difluorinated **1a** through monofluorinated **2a** and finally to unreactive **3a**. Interestingly, *N*-Boc activation exposes the β-lactam ring to strong chemical ring opening with *N*-nucleophiles, and the process can be considerably enhanced by the presence of lipase PS-D and/or by increasing the temperature.

4. Experimental

4.1. Materials and methods

TBME (water content 25 ppm according to Karl Fischer titration) and DIPE (water content 28 ppm) were of the highest analytical grade and were stored over molecular sieves (4 Å). Lipase PS-D from *B. cepacia* was purchased from Amano Europe, England. Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063–0.200 μm). TLC was carried out with Merck Kieselgel $60F_{254}$ sheets. Unless stated otherwise, all enzymatic reactions were performed at room temperature (23 °C). Melting points were measured on a Sanyo instrument at a heating rate of 2 °C. Optical rotations were determined with a Perkin–Elmer polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

The NMR spectra were recorded on a Bruker 500 spectrometer with tetramethylsilane (TMS) as an internal standard for ^1H and ^{13}C NMRs, and with 2,2,2-trifluoroethanol as an internal standard for the ^{19}F NMR. ^1H–^1H COSY, ^1H–^{13}C HQSC, and ^1H–^{13}C HMBC spectra were used for the assignment of the chemical shifts when necessary. Mass spectra were taken on a VG 7070E mass spectrometer.

In a typical small-scale experiment, lipase PS-D was added to one of the β-lactams (0.05 M) in a TBME or DIPE in the presence of ammonia, an amine, or a β-amino ester (0.05–0.1 M). The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by chiral HPLC on a CHIRACEL-OD column (0.46 × 25 cm) in the case of β-dipeptides and chiral GC on a Chrompack CP-Chirasil-DEX CB column in the case of amonolysis and aminolysis of *rac*-**1**.

4.2. Preparation of (3*R*,4*R*)-2b and the enantiomers of 3b

4-Dimethylaminopyridine (DMAP, 2 mg, 0.02 mmol) was added to a solution of (3*R*,4*R*)-**2a** (28 mg, 0.17 mmol, ee = 99%) and Boc_2O (74 mg, 0.34 mmol) in dry dichloromethane (1 mL). After 55 min, the solvent was evaporated off, and the residue was purified on silica gel eluting with ethyl acetate in petroleum ether (1:30, v/v) to afford (3*R*,4*R*)-**2b** as a solid product (36 mg, 0.14 mmol, mp 96–97 °C, $[\alpha]_D^{22} = -76.0$ (*c* 0.50, $CHCl_3$)) in 80% yield; HRMS: M^+ found (M^+ calculated for $C_{14}H_{16}FNO_3$) 265.112500 (265.111422); MS: *m*/*z* (relative intensity) 265 (0.02), 192 (15), 164 (3), 122 (100), 96 (3), 77 (3); ^1H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 5.00 (dd, *J* = 13.0 Hz, 2.0 Hz, 1H), 5.25 (dd, *J* = 53.3 Hz, 1.8 Hz, 1H), 7.30–7.44 (m, 5H).

(*R*)-**3b** $[\alpha]_D^{22} = +114.0$ (*c* 0.48, $CHCl_3$) was prepared from (*R*)-**3a** (ee = 99%) in 97% yield and (*S*)-**3b** $[\alpha]_D^{22} = -115.0$ (*c* 0.48, $CHCl_3$) from (*S*)-**3a** (ee = 99%) in 86% yield as described above; HRMS: M^+ found (M^+ calculated for $C_{14}H_{17}NO_3$) 247.121500 (247.120844); MS: *m*/*z* (relative intensity) 247 (3), 192 (3), 174 (5), 132 (32), 104 (100), 77 (10); ^1H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 2.92 (dd, *J* = 16.0 Hz, 3.0 Hz, 1H), 3.43 (dd, *J* = 16.0 Hz, 6.0 Hz, 1H), 4.92 (dd, *J* = 6.0, 3.5 Hz, 1H), 7.27–7.40 (m, 5H).

4.3. Preparation of (*R*)-6 and (*S*)-7

A solution of (*R*)-**4** (1.11 g, 4.85 mmol, ee >99%) in aqueous HCl (18%) was refluxed for 16 h before the solvent was evaporated off, affording the crude acid which was used without further purification. Next, $HClO_4$ (70%, 7.28 mmol, 631 μL) was added to the suspension of the acid in AcO^tBu (12 mL). The reaction mixture was stirred at room temperature for 24 h, yielding (*R*)-**6** (270 mg, 1.70 mmol, ee > 99%, $[\alpha]_D^{22} = -24.8$ (*c* 1.00, $CHCl_3$)) in 35% yield over the two steps. (*R*)-**6** was further purified on silica gel eluting with methanol (5% v/v) in dichloromethane as an eluent. ^1H NMR (500 MHz, $CDCl_3$) δ 1.11 (d, *J* = 6.5 Hz, 3H), 1.46 (s, 9H), 1.68 (br s, 2H), 2.21 (dd, *J* = 15.5, 8.0 Hz, 1H), 2.33 (dd, *J* = 15.5, 4.5 Hz, 1H), 3.34 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 23.38, 28.13, 44.19, 45.48, 80.50, 171.82.

(*S*)-**7** $[\alpha]_D^{22} = +3.1$ (*c* 1.00, $CHCl_3$) was prepared from (*S*)-**5** (ee = 99%) in 61% yield with mp 30–31 °C; MS: *m*/*z* (relative intensity) 236 (M^++1, 0.22), 191 (8), 144 (29), 120 (37), 88 (100); ^1H NMR (500 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.93 (br s, 2H), 2.28 (dd, *J* = 16.0, 8.5 Hz, 1H), 2.43 (dd, *J* = 16.0, 4.0 Hz, 1H), 2.64 (dd, *J* = 13.0, 8.0 Hz, 1H), 2.77 (dd, *J* = 13.0, 5.5 Hz, 1H), 3.45 (m, 1H), 7.20–7.33 (m, 5H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.15, 42.67, 43.52, 49.77, 80.79, 126.52, 128.56, 129.35, 138.54, 171.79.

4.4. Preparative-scale kinetic resolution of *rac*-1a with isopropylamine

Compound *rac*-**1a** (200 mg, 1.09 mmol) and lipase PS-D (880 mg) were added to the solution of $^iPrNH_2$ (64 mg, 1.09 mmol) in TBME (22 mL). The reaction was stopped by filtering off the enzyme at 50% conversion after 6 h. The solvent was evaporated off and the residue was purified on silica gel eluting with 40% ethyl acetate in petroleum ether to afford (*R*)-**1a** [93 mg, yield 46%, $ee^{(R)\text{-}\mathbf{1a}}$ >99%] and (*S*)-**8** (R = ^iPr) as a solid product [83 mg, 32%, $ee^{(S)\text{-}\mathbf{8}}$ >99%, $[\alpha]_D^{22} = +4.2$ (*c* 1.00, $CHCl_3$)], mp 91–92 °C; HRMS: M^+ found (M^+ calculated for $C_{12}H_{16}F_2N_2O$) 242.122700 (242.123070);

MS: m/z (relative intensity) 242 (0.24), 222 (15), 164 (5), 146 (6), 106 (100), 79 (13), 43 (6); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (d, 3H, J = 6.5 Hz, CH_3), 1.08 (d, 3H, J = 6.5 Hz, CH_3), 2.00 (br s, 2H, NH_2), 3.97 (m, 1H, CH $(CH_3)_2$), 4.59 (t, 1H, J = 13.5 Hz, C$H$$NH_2$), 6.04 (br s, 1H, N$H$CO), 7.31–7.38 (m, 5 arom. H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 22.07 (CH_3), 22.19 (CH_3), 41.62 ($CH(CH_3)_2$), 57.87 (t, J = 23.9 Hz, $CHNH_2$), 116.35 (t, J = 258.3 Hz, CF_2), 128.03, 128.50, 128.54, 136.26 (arom. C), 162.61 (t, J = 28.9 Hz, CO); ^{19}F NMR (471 MHz, $CDCl_3$) δ −116.39 (dd, J = 247.0, 14.1 Hz), −117.38 (dd, J = 247.0, 11.8 Hz).

4.5. Preparation of β-dipeptide 9

Lipase PS-D (40 mg mL^{-1}) was added to a solution of (*S*)-**1a** (16 mg, 0.08 mmol, ee = 99%) and (*R*)-**6** (24 mg, 0.15 mmol, ee = 99%) in dry DIPE (1.5 mL). The reaction was stopped after 15 h by filtering off the enzyme. The solid part was washed with dichloromethane (4 mL) and the filtrate was concentrated under vacuum. The residue was purified on silica gel eluting with ethyl acetate in petroleum ether (1:1, v/v) to afford dipeptide **9** {25 mg, 0.07 mmol, mp 100–101 °C, $[\alpha]_D^{22} = +28.8$ (c 1.00, $CHCl_3$)} in 96% yield; HRMS: M$^+$ found (M$^+$ calculated for $C_{17}H_{24}F_2N_2O_3$) 342.174300 (342.175499); MS: m/z (relative intensity) 342 (0.7), 285 (4), 191 (7), 106 (100); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13 (d, J = 6.5 Hz, 3H, CH_3CH), 1.42 (s, 9H, $(CH_3)_3$), 2.12 (dd, J = 15.8 Hz, 5.5 Hz, 1H, CH_2), 2.27 (dd, J = 15.8 Hz, 4.5 Hz, 1H, CH_2), 4.19 (m, 1H, CH), 4.60 (m, 1H, CH), 7.05 (d, J = 8.0 Hz, 1H, CONH), 7.32–7.40 (m, 5H).

4.6. Preparation of β-dipeptide 10

Lipase PS-D (40 mg mL^{-1}) was added to the solution of (3*S*,4*S*)-**2a** (30 mg, 0.18 mmol, ee = 99%) and (*R*)-**6** (58 mg, 0.36 mmol, ee = 99%) in dry DIPE (3.6 mL). After 18 h, the reaction was worked up as above to afford the gum-like dipeptide **10** (30 mg, 0.09 mmol) in 51% yield. In order to facilitate the characterization, the free amino group of **10** was protected in the quantitative yield in the presence of Boc_2O (2 equiv) and NEt_3 (2.5 equiv) in dry dichloromethane. The data for *N*-Boc-protected **10** are as follows. $[\alpha]_D^{22} = +4.2$ (c 0.75, $CHCl_3$); HRMS: M$^+$ found (M$^+$ calculated for $C_{22}H_{33}FN_2O_5$) 424.236800 (424.237351); MS: m/z (relative intensity) 424 (0.5), 295 (32), 206 (43), 191 (24), 150 (81), 106 (77), 57 (100); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (d, J = 7.0 Hz, 3H), 1.38 (s, 9H), 1.43 (s, 9H), 1.66 (m, 1H), 2.10 (dd, J = 15.5 Hz, 4.0 Hz, 1H), 4.09 (m, 1H), 5.22 (d, J = 51.5 Hz, 1H), 5.26 (m, 1H), 7.27–7.33 (m, 5H).

4.7. Non-enzymatic preparation of β-dipeptide 11

(*S*)-**7** (38 mg, 0.16 mmol, ee = 99%) was added to a solution of (3*R*,4*R*)-**2b** (21 mg, 0.08 mmol, ee = 99%) in dry DIPE (1.6 mL). The reaction was worked up after 24 h as above except that ethyl acetate in petroleum ether (1:8, v/v) was used as an eluent to afford solid **11** {39 mg, 0.08 mmol, mp 136–137 °C, $[\alpha]_D^{22} = +8.7$ (c 1.00, $CHCl_3$)} in 97% yield; HRMS: M$^+$ found (M$^+$ calculated for $C_{28}H_{37}FN_2O_5$) 500.270000 (500.268651); MS: m/z (relative intensity) 500 (1), 409 (11), 297 (35), 191 (46), 106 (55), 88 (86), 57 (100); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (s, 18H), 1.64 (m, 1H), 2.04 (dd, J = 16.0 Hz, 4.0 Hz, 1H), 2.74 (m, 2H), 4.21 (m, 1H), 5.19 (d, J = 50.1 Hz, 1H), 5.23 (m, 1H), 7.08–7.27 (m, 10H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 28.11, 28.32, 37.23, 39.32, 46.37, 55.98 (d, J = 17.6 Hz), 80.15, 81.34, 92.75 (d, J = 194.9 Hz), 126.70, 127.93, 128.15, 128.40, 128.55, 129.17, 136.10, 137.35, 154.67, 165.77 (d, J = 18.9 Hz), 170.55.

4.8. Preparation of β-dipeptide 12

Lipase PS-D (60 mg mL^{-1}) was added to a solution of (*R*)-**3b** (31 mg, 0.13 mmol, ee = 99%) and (*R*)-**6** (40 mg, 0.25 mmol, ee = 99%) in dry DIPE (2.5 mL) and was stirred at 57 °C for 48 h before the work-up as above, except that ethyl acetate in petroleum ether (1:3, v/v) was used as an eluent to afford dipeptide **12** {43 mg, 0.11 mmol, mp 140–141 °C, $[\alpha]_D^{22} = +32.4$ (c 1.05, $CHCl_3$)} in 82% yield; HRMS: M$^+$ found (M$^+$ calculated for $C_{22}H_{34}N_2O_5$) 406.247800 (406.246773); MS: m/z (relative intensity) 406 (8), 294 (65), 249 (36), 119 (62), 106 (74), 57 (100); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (d, J = 6.5 Hz, 3H), 1.42 (s, 18H), 2.28 (d, J = 5.5 Hz, 2H), 2.54 (dd, J = 14.0 Hz, 5.5 Hz, 1H), 2.68 (m, 1H), 4.19 (m, 1H), 5.01 (m, 1H), 6.03 (d, J = 8.0 Hz, 1H), 6.26 (br s, 1H), 7.20–7.35 (m, 5H).

4.9. Non-enzymatic preparation of β-dipeptide 13

Solution of (*S*)-**3b** (25 mg, 0.10 mmol, ee = 99%) and (*S*)-**7** (47 mg, 0.20 mmol, ee = 99%) in dry DIPE (2.0 mL) was stirred for 144 h, before the work-up as above, except that ethyl acetate in petroleum ether (1:4, v/v) was used as an eluent to afford dipeptide **13** (31 mg, 0.07 mmol, mp 139–140 °C, $[\alpha]_D^{22} = -15.0$ (c 1.00, $CHCl_3$)) in 65% yield; HRMS: M$^+$ found (M$^+$ calculated for $C_{28}H_{38}N_2O_5$) 482.278300 (482.278073); MS: m/z (relative intensity) 482 (4), 409 (13), 353 (15), 279 (20), 192 (60), 88 (100); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.43 (s, 9H), 2.17 (dd, J = 16.0 Hz, 5.5 Hz, 1H), 2.26 (dd, J = 16.0 Hz, 5.5 Hz, 1H), 2.53 (m, 2H), 2.69 (m, 2H), 4.33 (m, 1H), 5.04 (m, 1H), 6.98–7.33 (m, 10H).

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