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Probing the specificity of the S_1', leaving group, site of subtilisin *Bacillus lentus* using an enzyme-catalyzed transesterification reaction

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Abstract

Subtilisin *Bacillus lentus* catalyzes transesterifications between *N*-acetyl-L-phenylalanine vinyl ester and a wide range of alcohols. Reaction yields are high when primary alcohols are used, and quantitative with methanol. With chiral alcohols, the reaction is enantioselective, and the stereoselectivity is reversed on going from open chain secondary alcohols to β-branched primary alcohols. A model is proposed to account for this change in absolute configuration preference. © 1998 Elsevier Science Ltd. All rights reserved.

Hydrolase-catalyzed transesterifications of alcohols using vinyl or isopropenyl acetate as the acyl donor are widely used to resolve racemic alcohols and to effect stereoselective acylation of prochiral and meso diols.[1] While lipases have enjoyed wide success in this regard, serine proteases have had more limited applications, with the greatest successes to date being reported for the regioselective acylation of carbohydrates,[2] nucleosides,[3] alkaloids[4] and steroids.[5] Subtilisins are amongst the most stable, inexpensive and ubiquitous of serine proteases. However, in view of their ease of availability and low cost, they appear underused as preparative catalysts. Accordingly, we have initiated a program to extend the synthetic potential of subtilisins. In this paper, we evaluate the spectrum of applicability of subtilisin *Bacillus lentus* (SBL) in transesterification reactions, and probe the specificity of the S_1'[6] site of SBL in such reactions. The prospects in this regard were encouraging, since kinetic studies[7] on subtilisin-catalyzed transesterifications between vinyl acetate and various alcohols, and related effects of solvent on enzyme specificity, have been reported.[8] Also, recent studies have documented some of the potential of subtilisins in organic synthesis.[9]

Initially, we examined catalysis by SBL of the reactions between vinyl and isopropenyl acetate and a range of alcohols. However, these reactions were too slow to be of preparative value. We presumed that this was the result of the low affinity of SBL for small acyl groups and that better results might be achieved with larger acyl groups that would interact more favorably with S_1. The recent report of the preparation

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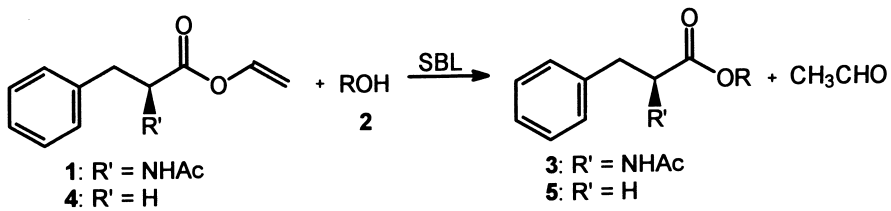
of *N*-acetyl-L-phenylalanine vinyl ester[10] (**1**) pointed to a solution to the small acyl group problem, since the benzyl side chain of **1** would target the S_1 pocket of SBL, thereby improving acyl enzyme formation. The specificity of **1** for S_1 would also eliminate competition between the S_1 and S_1' binding sites for the incoming nucleophile, thereby permitting an unequivocal study of the spatial restrictions of the S_1' pocket. The vinyl ester **1** was prepared in reasonable (51%) yield and excellent (>99%) enantiomeric excess *via* the palladium acetate-catalyzed coupling[11] of vinyl acetate with *N*-acetyl-L-phenylalanine.

The transesterification reaction investigated is shown in Scheme 1. SBL was immobilized in a potassium chloride matrix (KCl–SBL).[12] The KCl–SBL preparation was more active than SBL lyophilized from phosphate buffer alone, and enzyme activity was higher when KCl–SBL was lyophilized from phosphate buffer than when lyophilized from Tris.[13] KCl–SBL was most active in *tert*-butanol and acetonitrile. The activity of KCl–SBL was also surveyed in isopropyl ether, *tert*-butyl methyl ether, cyclohexane and octane with **1** as the substrate, but in none of these solvents did the yields of the reaction approach those attained in *tert*-butanol and acetonitrile.



Scheme 1.

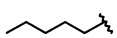
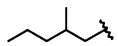
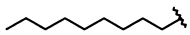
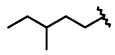
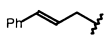
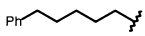
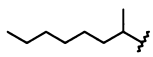
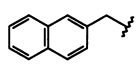
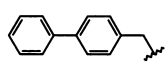
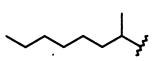
A spectrum of primary, secondary, achiral and chiral alcohols (**2**) were surveyed. In general, reactions were carried out at 50°C for 24 hours to allow comparisons of the yields obtained. All reactions were verified as being enzyme-catalyzed by running blank reactions containing the same reactants in the absence of SBL. In no case was a background reaction observed.[14] The results of the alcohol screen are recorded in Table 1.

It can be seen that the S_1' site of SBL exhibits a broad structural tolerance for primary alcohol nucleophiles, with the highest yields being obtained for those with the least steric bulk. In fact, the reaction with the smallest alcohol, methanol (**2a**), was essentially quantitative. There appears to be little restriction on the steric bulk accommodated by S_1' as even the largest nucleophile of the series, 4-biphenylmethanol (**2h**), gave excellent (75%) conversion to **3h**. However, alkyl chain branching is somewhat deleterious, with 2- (**2k**) and 3-methylpentanol (**2l**) being lower yielding (58 and 55% respectively) than *n*-pentanol (**2b**, 86%). Furthermore, for the β-branched achiral alcohol, cyclohexylmethanol (**2i**), the yield (66%) is lower than with benzyl alcohol (**2d**, 78%), to which it is structurally very similar, or with the other benzylic alcohols **2g** (70%) and **2h** (75%). From this it would appear that the nature of the β-branch is also important. Branching at an sp^3 hybridized center engenders more steric hindrance than at an sp^2 hybridized center. Reactions with the secondary alcohols **2m**, **2n** and **2o** gave lower yields (37%, 51%, 20% respectively) even when the standard reaction time was increased threefold, indicating that branching at an α-carbon is even less well tolerated by S_1'.

The stereoselectivity of the S_1' pocket towards racemic alcohols was also explored. In the reactions with the racemic primary alcohols **2j**, **2k** and **2l**, some stereoselectivity is manifest in the products **3j**, **3k** and **3l** respectively. However, when secondary alcohols are used, the stereoselectivity is much greater, with products of very high d.e. obtained in the reactions with (±)-2-octanol (**2o**, >99% d.e.), and (±)-1-phenylethanol (**2m**, 95%). Interestingly, the reaction with (±)-*endo*-norborneol (**2q**) proceeded to give the ester **3q** with good d.e. (82%) while the (±)-*exo*-isomer (**2p**) yielded **3p** of only 25% d.e.

Due to the expense of **1** it was decided to investigate the activity of SBL with the less costly, and

Table 1
Results of the alcohol screen in Scheme 1 reactions

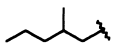
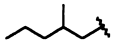
Acyl Donor	Alcohol (**2**)	R	Product (% yield)	Acyl Donor	Alcohol (±**2**)	R	Product (% yield, % d.e.)	Abs. config.
1	**a**	Me	**3a** (98)[a]	**1**	**j**		**3j** (53, 30)	*R*
1	**b**		**3b** (86)	**1**	**k**		**3k** (58, 26)	*R*
1	**c**		**3c** (64)	**1**	**l**		**3l** (55, 21)	*S*
1	**d**		**3d** (78)	**1**	**m**		**3m** (37, 95)[b]	*S*
1	**e**		**3e** (85)	**1**	**n**		**3n** (51, 46)[b]	*S*
1	**f**		**3f** (77)	**1**	**o**		**3o** (20, >99)[b]	*S*
1	**g**		**3g** (70)	**1**	**p**		**3p** (50, 25)[b]	*R*
1	**h**		**3h** (75)	**1**	**q**		**3q** (56, 82)[b]	*R*
1	**i**		**3i** (66)	**4**	**j**		**5j** (52,50[c])	*R*
4	**a**	Me	**5a** (94)[a]	**4**	**m**		**5m** (45, 90[c])[b]	*S*
				4	**o**		**5o** (31, >99[c])[b]	*S*

All reactions were carried out in *t*-BuOH at 50 °C for 24 hr. except: [a]MeCN, 20 °C, 24 hr.; [b] *t*-BuOH, 50 °C, 72 hr.; [c]number quoted is an e.e. value. Cyclohexanol was also used as a nucleophile, with NMR analysis indicating a 46% yield, but the product proved inseparable from the starting material.

readily synthesized[11] achiral analog, 3-phenylpropionic acid vinyl ester (**4**). When **4** was used as the acyl donor with a representative selection of racemic alcohols, the reaction yields and e.e.s were similar to those obtained with **1**, thus demonstrating that the activity of the enzyme was not significantly altered by changing the acyl donor. In fact, an increase in e.e. (from 30% to 50%) is observed for the primary alcohol used, 2-phenylpropanol (**2j**).

It is possible to increase the enantioselectivity of an enzyme-catalyzed reaction by lowering the temperature at which it is carried out.[15] Consequently, we investigated this approach as a means of increasing the d.e. of the products of reactions between **1** and the β-branched primary alcohols **2j** and **2k**. The results are shown in Table 2. Excellent yields were obtained when using acetonitrile as the solvent, although in the series of reactions with **2k** this was accompanied by a loss in enantioselectivity at 4°C. Cooling of this reaction to −20°C retained the high yield and restored the d.e. to the level of the initial screen. Reactions carried out in *tert*-butanol showed a modest increase in the d.e. of the products,

Table 2
Temperature studies on Scheme 1 reactions using racemic primary alcohols

Acyl Donor	Alcohol (±2) R	Temp. °C	Time, hr	Solvent	Product (% yield, % d.e)
1	**j** 	20	48	*t*-BuOH	**3j** (26, 35)
1	**j** 	4	120	*t*-BuOH	**3j** (15, 40)
1	**j** 	4	48	MeCN	**3j** (99, 37)
1	**k** 	4	24	MeCN	**3k** (91, 4)
1	**k** 	-20	40	MeCN	**3k** (82, 20)

although this was at the expense of yield, probably due to reduced substrate concentration, resulting from freezing of the medium.

Absolute configurations of the products of the enzyme-catalyzed reactions were determined on diastereoisomerically pure samples of **3m**, **3n** and **3o** that were chemically prepared from *N*-acetyl-L-phenylalanine and enantiomerically pure **2m**, **2n** and **2o**. Absolute configurations of the enzyme product esters **3m**, **3n** and **3o** were then determined by HPLC coinjection or by NMR analysis of samples spiked with the reference compounds. When enantiomerically pure alcohols were not available for the synthesis of reference samples, absolute configurations were obtained by hydrolysis of the product ester and comparison of the optical rotation of the alcohol obtained with the literature reference standards.[16] In the case of 3-methyl-1-pentanol (**2l**) the alcohol was oxidized to the known acid[17] in order to assign the absolute configuration. From the results, a distinct enantioselectivity pattern for the S_1' pocket was discovered. For open chain secondary alcohols, a preference for the *S*-enantiomer was evident, while a reversal of selectivity, favoring the *R*-enantiomer, was apparent for β-branched primary alcohols. In the case of the single γ-branched primary alcohol used (**2l**), the selectivity reversed once more, with the product ester **3l** incorporating an alcohol moiety with the *S*-configuration.

The way lipases control enantioselectivity of transesterifications with chiral alcohols[18] has been extensively studied and, based on the size and orientation of the substituents at the stereocenter, empirical rules have been proposed to predict which enantiomer of the alcohol will react preferentially. A model to explain why subtilisin Carlsberg is specific for the *S*-enantiomer of secondary alcohols has also been formulated[7a] and X-ray studies have shown the active sites of subtilisin Carlsberg and lipases to be approximate mirror images of one another[19] in accord with the opposite enantioselectivity of these enzymes for secondary alcohols.

Building on these Kazlauskas[18g] and Klibanov[7a] model proposals, we find that the reversals in stereospecificity of the S_1' pocket encountered in our SBL-catalyzed transesterification reactions with open chain alcohols can be rationalized by the models depicted in Fig. 1. Figure 1(a) is the Klibanov subtilisin model and also represents the orientation of substituents in the SBL active site when the nucleophile is a secondary alcohol. Figure 1(b) represents the SBL picture for β-branched primary alcohols and is similar to the *Pseudomonas cepacia* lipase model proposed by Kazlauskas. Figure 1(c) shows that SBL will be specific for the *S*-enantiomer of 3-methyl-1-pentanol (**2l**), as observed, if the orientation of the alkyl groups remain the same as in Fig. 1(b). It is also interesting to note that although

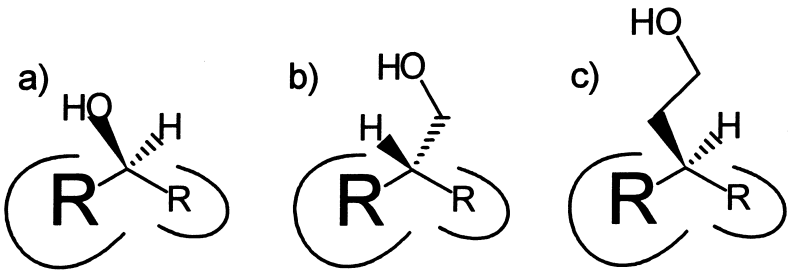


Fig. 1. Model to predict and explain the specificity of SBL for (a) secondary alcohols (b) β-branched primary alcohols (c) ɣ-branched primary alcohols. The size of 'R' denotes the relative size of the substituents

SBL is highly specific for the *S*-enantiomer of open chain secondary alcohols, it has a preference for the *R*-enantiomers of the cyclic norborneols (**2p** and **2q**).

In conclusion, our experiments show that, with a specific acyl donor, SBL can be a very useful transesterification catalyst in organic solvents. While we cannot quantify the degree of improvement conferred by using **1** in place of vinyl acetate, we estimate that the rate increase with *N*-acetyl-L-phenylalanine vinyl ester as the acyl donor is >100 fold. There was no detectable yield after 24 hours in attempted transesterifications using vinyl acetate under the Table 1 conditions. SBL exhibits broad specificity in the S_1' pocket and can be used to catalyze transesterifications of vinyl esters such as **1** and **4** with a wide range of alcohols in good to excellent yields. The enantioselectivity of the S_1' pocket is excellent for secondary alcohols, but diminishes the further the stereocenter is removed from the OH-group of a chiral acyclic alcohol nucleophile. Furthermore, the enantiomeric preferences of these SBL-catalyzed reactions are predictable using simple models of the type formulated for other hydrolases. While much more needs to be done before SBL can achieve the breadth of applicability enjoyed by lipases in transesterifications, the current results represent substantial progress. Further improvements in preparative SBL applications are currently being sought, including increasing the enzyme's activity and tailoring its specificity by chemical modification of SBL mutants.[20]

1. Experimental section

Subtilisin *Bacillus lentus* (SBL) was obtained as a crude solution from Genencor International Inc. and was purified according to a previously published procedure.[20c] Vinyl acetate was distilled before use. All alcohols used as substrates were used as received from Aldrich or Lancaster. *t*-BuOH was dried by distillation from Mg turnings and I_2. Melting points were determined using an Electrothermal IA9000 series digital melting point apparatus, and are uncorrected. Optical rotation data were obtained using a Perkin–Elmer 243B polarimeter. Products of enzyme-catalyzed reactions were identified by their ^{1}H NMR spectra, run in $CDCl_3$ using a Varian Gemini 200 MHz NMR spectrometer, and HRMS data, acquired using a Micromass 70-250S (double focussing) mass spectrometer for EI spectra, and a Micromass ZAB-SE for FAB spectra (where indicated). ^{13}C NMR spectra were obtained at 50.3 MHz using the Varian Gemini NMR spectrometer. Diastereoisomeric and enantiomeric excesses were determined by HPLC, using a Chiralcel OD column and a hexane–isopropanol eluent system (60:1–12:1 range, flow rate 1mL/min), or by ^{1}H NMR spectroscopy using a Varian Unity 500 MHz NMR spectrometer. Values quoted are judged to be accurate to ±2%.

1.1. N-*Acetyl-L-phenylalanine vinyl ester (**1**)*

Using the method of Lobell and Schneider,[11] a mixture of *N*-acetyl-L-phenylalanine (1.012 g, 4.883 mmol), $Pd(OAc)_2$ (219 mg, 0.2 eq), KOH (27 mg, 0.1 eq) and 45 mL vinyl acetate (100 eq) was stirred for 24 h at 20°C under N_2. The reaction mixture was then added to Et_2O (300 mL), stirred for 10 min and filtered. After evaporation of the filtrate, the crude product was purified by flash chromatography using a step gradient (25:75 EtOAc:hexanes followed by 50:50 EtOAc:hexanes) to give *N*-acetyl-L-phenylalanine vinyl ester (**1**) (581 mg, 51%, >99% e.e.), which was recrystallized from EtOAc:hexanes. M.p. 90.0–91.0°C [lit.[10] 56.0–58.0°C]; $[\alpha]_D^{25}$=+32.7 (c 1.05, $CHCl_3$); ν_{max} (KBr) 3318, 1770, 1753 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34–7.07 (6H, m), 6.11 (1H, br d, *J* 7.7 Hz), 5.00–4.91 (2H, m), 4.66 (1H, dd, *J* 6.2, 1.9 Hz), 3.25–3.08 (2H, m), 1.98 (3H, s); ^{13}C NMR ($CDCl_3$) δ 169.8, 168.8, 140.6, 135.4, 129.0, 128.4, 127.0, 99.0, 52.9, 37.4, 22.8; HRMS calcd for $C_{13}H_{16}NO_3$ ($M+1^+$) 234.113019. Found: 234.112903.

1.2. *3-Phenylpropionic acid vinyl ester (**4**)*[21]

Following the method described for **1** above, but using 3-phenylpropionic acid (209 mg, 1.39 mmol), $Pd(OAc)_2$ (62 mg, 0.2 eq), KOH (8 mg, 0.1 eq) and vinyl acetate (13 mL, 100 eq). The crude product was purified by flash chromatography using a step gradient (hexanes followed by 5:95 EtOAc:hexanes) to yield 3-phenylpropionic acid vinyl ester (**4**) in 70% yield. ν_{max} (neat) 1756 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37–7.20 (6H, m), 4.90 (1H, dd, *J* 14.1, 1.6 Hz), 4.59 (1H, dd, *J* 6.4, 1.6 Hz), 3.02 (2H, t, *J* 7.8 Hz), 2.74 (2H, t, *J* 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 170.3, 141.6, 140.6, 129.0, 128.8, 126.9, 98.2, 36.1, 31.2; HRMS calcd for $C_{11}H_{12}O_2$ (M^+) 176.083730. Found: 176.083816.

1.3. *Preparation of SBL in a KCl matrix*[12]

SBL (52.5 mg) was dissolved in 50 mM phosphate buffer pH 8.4 (30 mL). KCl (10.78 g) was added to the solution and dissolved with swirling. The resulting solution was flash frozen in liquid N_2 and lyophilized for 48 h. The dry mixture was stored in a dessicator at −20°C prior to use.

1.4. *A general method for SBL-catalyzed transesterifications*

KCl–SBL powder (438.2 mg), *N*-acetyl-L-phenylalanine vinyl ester (**1**) (9.8 mg, 42 μmol) and benzyl alcohol (**2d**) (45 μL, 434 μmol) were mixed in a glass vial under N_2. Dry *t*-BuOH (2 mL) was added and the contents stirred at 50°C for 24 h under nitrogen. The reaction was terminated by filtration through Celite, evaporated, and the residue purified by flash column chromatography (EtOAc:hexanes 25:75 followed by 50:50 EtOAc:hexanes) to yield *N*-acetyl-L-phenylalanine benzyl ester (**3d**) (10.0 mg, 78%, >99% e.e.) as white crystals, m.p. 76.0–78.0°C. $[\alpha]_D^{27}$=+17.8 (c 0.82, $CHCl_3$); ^1H NMR δ 7.41–7.19 (8H, m), 6.98 (2H, m), 5.91 (2H, br d, *J* 7.4 Hz) 5.15, (2H, m), 4.92 (1H, m), 3.12 (2H, d, *J* 6.0 Hz), 1.98 (3H, s); HRMS calcd for $C_{18}H_{19}NO_3$ (M^+) 297.136494. Found: 297.136298.The following compounds were prepared using the above procedure, except where stated.

1.5. N-*Acetyl-L-phenylalanine methyl ester (**3a**)*[22,23]

From **1** (10.3 mg, 44.2 μmol) and **2a** (20 μL, 494 μmol). Reaction performed in MeCN at room temperature. White crystals (9.6 mg, 98%, >99% e.e.), m.p. 84.5–86.0°C [lit.[23] 86.0–87.0°C].

$[\alpha]_D^{25}$=+105.6 (c 0.46, $CHCl_3$) [lit.[23] $[\alpha]_D^{25}$=+101.5 (c 1.00, $CHCl_3$)]; ^{1}H NMR δ 7.31–7.23 (3H, m), 7.12–7.05 (2H, m), 5.90 (1H, br d, *J* 5.9 Hz), 4.88 (1H, app q, *J* 5.9 Hz), 3.73 (3H, s), 3.12 (2H, m), 1.98 (3H, s); HRMS calcd for $C_{12}H_{15}NO_3$ (M^+) 221.105194. Found: 221.104113.

1.6. N-*Acetyl-L-phenylalanine pentyl ester (**3b**)*

From **1**, (10.7 mg, 45.9 μmol) and **2b** (50 μL, 460 μmol). Colorless oil (11.2 mg, 86%, >99% e.e.). $[\alpha]_D^{26}$=+59.7 (c 0.71, $CHCl_3$); ^{1}H NMR δ 7.30–7.22 (3H, m), 7.15–7.07 (2H, m), 5.90 (1H, d, *J* 6.0 Hz), 4.88 (1H, app q, *J* 6.0 Hz), 4.10 (2H, dt, *J* 3.0, 6.0 Hz), 3.13 (2H, d, *J* 6.0 Hz), 1.99 (3H, s), 1.60 (2H, m), 1.31 (4H, m), 0.91 (3H, t, *J* 7.0 Hz); HRMS calcd for $C_{16}H_{23}NO_3$ (M^+) 277.167794. Found: 277.166817.

1.7. N-*Acetyl-L-phenylalanine nonyl ester (**3c**)*

From **1**, (9.9 mg, 42.4 μmol) and **2c** (75 μL, 430 μmol). Colorless oil (9.0 mg, 64%, >99% e.e.). $[\alpha]_D^{29}$=+38.2 (c 0.67, $CHCl_3$); ^{1}H NMR δ 7.32–7.22 (3H, m), 7.12–7.07 (2H, m), 5.92 (1H, br d, *J* 7.2 Hz), 4.87 (1H, m), 4.09 (2H, dt, *J* 2.5, 6.6 Hz), 3.12 (2H, d, *J* 6.0 Hz), 1.99 (3H, s), 1.59 (2H, m), 1.27 (10H, m) 0.88 (3H, t, *J* 6.4 Hz); HRMS calcd for $C_{20}H_{32}NO_3$ ($M+1^+$) 334.238219. Found: 334.237586.

1.8. N-*Acetyl-L-phenylalanine cinnamyl ester (**3e**)*

From **1**, (10.5 mg, 45.0 μmol) and **2e** (60 mg, 447 μmol). White crystals (12.3 mg, 85%, >99% e.e.), m.p. 91.0–93.0°C. $[\alpha]_D^{25}$=+4.9 (c 0.88, $CHCl_3$); ^{1}H NMR δ 7.41–7.20 (8H, m), 7.13–7.08 (2H, m), 6.64 (1H, d, *J* 16.0 Hz), 6.21 (1H, dt, *J* 16.0, 6.8 Hz), 5.93 (1H, br d, *J* 8.0 Hz), 4.92 (1H, m), 4.77 (2H, br d, *J* 6.6 Hz), 3.14 (2H, d, *J* 5.8 Hz) 2.00 (3H, s); HRMS calcd for $C_{20}H_{21}NO_3$ (M^+) 323.152118. Found: 323.152144.

1.9. N-*Acetyl-L-phenylalanine 5-phenylpentyl ester (**3f**)*

From **1**, (10.6 mg, 45.5 μmol) and **2f** (77 μL, 457 μmol). Colorless oil (12.7 mg, 77%, >99% e.e.). $[\alpha]_D^{26}$=+47.1 (c 0.81, $CHCl_3$); ^{1}H NMR δ 7.43–7.05 (10H, m), 5.91 (1H, br d, *J* 7.2 Hz), 4.87 (1H, m), 4.08 (2H, dt, *J* 6.9, 2.6 Hz), 3.10 (2H, d, *J* 6.9 Hz), 2.61 (2H, t, *J* 7.2 Hz), 1.97 (3H, s), 1.63 (4H, m), 1.33 (2H, m); HRMS calcd for $C_{22}H_{27}NO_3$ (M^+) 353.199094. Found: 353.199583.

1.10. N-*Acetyl-L-phenylalanine 2-naphthalenemethyl ester (**3g**)*

From **1**, (10.6 mg, 45.5 μmol) and **2g** (73.4 mg, 464 μmol). White crystals (11.0 mg, 70%, >99% e.e.), m.p. 122.0–123.5°C. $[\alpha]_D^{25}$=+5.8 (c 0.53, $CHCl_3$); ^{1}H NMR δ 7.87–7.78 (4H, m), 7.52 (2H, m), 7.41 (1H, dd, *J* 8.6, 1.6 Hz), 7.17 (3H, m), 6.98 (2H, m), 5.92 (1H, br d, *J* 7.6 Hz), 5.35 (1H, d, *J* 12.2 Hz), 5.26 (1H, d, *J* 12.2 Hz), 4.95 (1H, m), 3.12 (2H, d, *J* 5.8 Hz), 1.99 (3H, s); HRMS calcd for $C_{22}H_{21}NO_3$ (M^+) 347.152144. Found: 347.151309.

1.11. N-*Acetyl-L-phenylalanine 4-biphenylmethyl ester (**3h**)*

From **1**, (10.3 mg, 44.2 μmol) and **2h** (80.6 mg, 437 μmol). White crystals (12.3 mg, 75%, >99% e.e.), m.p. 123.5–125.0°C. $[\alpha]_D^{26}$=−3.4 (c 0.44, $CHCl_3$); ^{1}H NMR δ 7.62–7.57 (4H, m), 7.51–7.34 (4H, m),

7.26–7.20 (4H, m), 7.02–6.95 (2H, m), 5.90 (1H, br d, *J* 8.0 Hz), 4.94 (1H, m), 3.14 (2H, d, *J* 6.0 Hz), 1.99 (3H, s); HRMS calcd for $C_{24}H_{23}NO_3$ (M^+) 373.167794. Found: 373.166087.

1.12. N-*Acetyl-L-phenylalanine cyclohexylmethyl ester (**3i**)*

From **1**, (11.6 mg, 49.8 μmol) and **2i** (61 μL, 498 μmol). White crystals (10.1 mg, 66%, >99% e.e.), m.p. 80.0–82.0°C. $[\alpha]_D^{26}$=+51.4 (c 0.84, $CHCl_3$); ^{1}H NMR δ 7.35–7.22 (3H, m), 7.16–7.07 (2H, m), 5.91 (1H, br d, *J* 7.2 Hz), 4.90 (1H, m), 3.91 (2H, m), 3.12 (2H, d, *J* 5.8 Hz), 2.00 (3H, s), 1.80–1.55 (6H, br m), 1.35–1.13 (3H, br m), 1.07–0.82 (2H, br m); HRMS calcd for $C_{18}H_{26}NO_3$ ($M+H^+$) 304.191269. Found: 304.191834.

1.13. N-*Acetyl-L-phenylalanine 2-phenyl-1-propyl ester (**3j**)*

From **1**, (10.1 mg, 43.3 μmol) and (±)-**2j** (60 μL, 429 μmol). Colorless oil (7.5 mg, 53%, 30% d.e.). ^{1}H NMR δ 7.37–7.18 (8H, m), 7.01–6.83 (2H, m), 5.87 (1H, br d, *J* 8.0 Hz), 4.84 (1H, m), 4.18 (2H, m), 3.08–2.94 (3H, m), 1.95 (3H, s), 1.28 (3H, d, *J* 7.2 Hz); HRMS calcd for $C_{20}H_{23}NO_3$ ($M+1^+$) 326.175619. Found: 326.177031.

1.14. N-*Acetyl-L-phenylalanine 2-methyl-1-pentyl ester (**3k**)*

From **1**, (10.3 mg, 44.2 μmol) and (±)-**2k** (52 μL, 419 μmol). Colorless oil (7.5 mg, 58%, 26% d.e.). ^{1}H NMR δ 7.29–7.22 (3H, m), 7.12–7.07 (2H, m), 5.91 (1H, br d, *J* 7.3 Hz), 4.89 (1H, m), 4.01–3.84 (2H, m), 3.11 (2H, d, *J* 5.8 Hz), 1.98 (3H, s), 1.74 (1H, m), 1.30 (4H, m), 0.89 (6H, m); HRMS calcd for $C_{17}H_{25}NO_3$ (M^+) 291.183444. Found: 291.183350.

1.15. N-*Acetyl-L-phenylalanine 3-methyl-1-pentyl ester (**3l**)*

From **1**, (10.3 mg, 44.2 μmol) and (±)-**2l** (52 μL, 429 μmol). Colorless oil (7.1 mg, 55%, 21% d.e.). ^{1}H NMR δ 7.30–7.20 (3H, m), 7.14–7.05 (2H, m), 5.90 (1H, br d, *J* 8.1 Hz), 4.88 (1H, m), 4.13 (2H, m), 3.12 (2H, app d, *J* 6.1 Hz), 1.99 (3H, s), 1.70–1.10 (5H, br m), 0.87 (6H, m); HRMS calcd for $C_{17}H_{25}NO_3$ (M^+) 291.183444. Found: 291.184308.

1.16. N-*Acetyl-L-phenylalanine 1-phenylethyl ester (**3m**)*[24]

From **1**, (9.9 mg, 42.4 μmol) and (±)-**2m** (52 μL, 431 μmol). Stirred at 50°C for 72 h. Colorless oil (4.9 mg, 37%, 95% d.e.). $[\alpha]_D^{22}$=+4.9 (c 0.49, $CHCl_3$) [lit.[24,25] $[\alpha]_D^{25}$=−31.4 (c 0.5, $CHCl_3$)]; ^{1}H NMR δ 7.38–7.25 (7H, m), 7.17–7.11 (2H, m), 6.80 (1H, m), 5.90 (2H, m), 3.10 (2H, m), 1.96 (3H, s), 1.57 (3H, d, *J* 6.6 Hz); HRMS calcd for $C_{19}H_{21}NO_3$ (M^+) 311.152144. Found: 311.153015.

1.17. N-*Acetyl-L-phenylalanine 2-butyl ester (**3n**)*[24]

From **1**, (10.3 mg, 44.2 μmol) and (±)-**2n** (40 μL, 436 μmol). Stirred at 50°C for 72 h. Colorless oil (5.9 mg, 51%, 46% d.e.). ^{1}H NMR δ 7.32–7.22 (3H, m), 7.18–7.09 (2H, m), 5.90 (1H, br), 4.85 (2H, m), 3.11 (2H, m), 1.99 (s, minor diastereoisomer), 1.97 (s, major diastereoisomer), 1.56 (2H, m), 1.20 (3H, m), 0.88 (3H, m); HRMS calcd for $C_{15}H_{21}NO_3$ (M^+) 263.152144. Found: 263.153343.

1.18. N-*Acetyl-L-phenylalanine 2-octyl ester (**3o**)*[24]

From **1**, (10.5 mg, 45.0 μmol) and (±)-**2o** (68 μL, 427 μmol). Stirred at 50°C for 72 h. Colorless oil (2.9 mg, 20%, >99% d.e.). $[\alpha]_D^{25}$=+47.1 (c 0.48, $CHCl_3$) [lit.[24] $[\alpha]_D^{22}$=+53.3 (c 0.80, $CHCl_3$)]; ^{1}H NMR δ 7.29–7.23 (3H, m), 7.15–7.10 (2H, m), 5.90 (1H, br d, *J* 7.2 Hz), 4.86 (2H, m), 3.11 (2H, app d, *J* 5.8 Hz), 1.98 (3H, s), 1.49 (2H, m), 1.26 (8H, br s), 1.18 (3H, d, *J* 6.4 Hz), 0.89 (3H, t, *J* 6.3 Hz); HRMS calcd for $C_{19}H_{29}NO_3$ (M^+) 319.214744. Found: 319.215998.

1.19. N-*Acetyl-L-phenylalanine* exo-*norbornyl ester (**3p**)*

From **1**, (10.1 mg, 43.3 μmol) and (±)-**2p** (48.9 mg, 435 μmol). Stirred at 50°C for 72 h. Colorless oil (6.6 mg, 50%, 25% d.e.). ^{1}H NMR δ 7.29–7.22 (3H, m), 7.11–7.07 (2H, m), 6.21 (1H, br d, *J* 7.4 Hz), 4.79 (1H, m), 4.57 (1H, m), 3.06 (2H, m), 2.24 (2H, m), 1.94 (3H, s), 1.62 (1H, m), 1.42 (3H, m), 1.06 (2H, m); HRMS calcd for $C_{18}H_{23}NO_3$ (M^+) 301.167794. Found: 301.167151.

1.20. N-*Acetyl-L-phenylalanine* endo-*norbornyl ester (**3q**)*

From **1**, (10.1 mg, 43.3 μmol) and (±)-**2q** (51.1 mg, 455 μmol). Stirred at 50°C for 72 h. Colorless oil (7.4 mg, 56%, 82% d.e.). ^{1}H NMR δ 7.30–7.21 (3H, m), 7.13–7.08 (2H, m), 6.15 (1H, m), 4.88 (2H, m), 3.07 (2H, m), 2.40 (1H, m), 2.18 (1H, m), 1.97 (3H, s), 1.96 (1H, m), 1.56 (2H, m), 1.31(4H, m), 0.90 (1H, m); HRMS calcd for $C_{18}H_{23}NO_3$ (M^+) 301.167794. Found: 301.168322.

*1.21. 3-Phenylpropionic acid methyl ester (**5a**)*[26]

From **4**, (10.2 mg, 57.9 μmol) and **2a** (24 μL, 593 μmol). Reaction performed in MeCN at room temperature. Colorless oil (8.9 mg, 94%). ^{1}H NMR δ 7.32–7.15 (5H, m), 3.67 (3H, s), 2.96 (2H, t, *J* 7.4 Hz), 2.62 (2H, t, *J* 7.4 Hz); HRMS calcd for $C_{10}H_{12}O_2$ (M^+) 164.083730. Found: 164.084406.

*1.22. 3-Phenylpropionic acid 2-phenylpropyl ester (**5j**)*

From **4**, (9.9 mg, 56.3 μmol) and (±)-**2j** (79 μL, 565 μmol). Colorless oil (7.9 mg, 52%, 50% e.e.). ^{1}H NMR δ 7.35–7.14 (10H, m), 4.18 (2H, m), 3.06 (1H, m), 2.90 (2H, t, *J* 7.6 Hz), 2.59 (2H, t, *J* 7.4 Hz), 1.25 (3H, d, *J* 6.0 Hz); HRMS (+FAB) calcd for $C_{18}H_{21}O_2$ ($M+H^+$) 269.1542. Found: 269.1573.

*1.23. 3-Phenylpropionic acid 1-phenylethyl ester (**5m**)*[24]

From **4**, (14.1 mg, 80.0 μmol) and (±)-**2m** (112 μL, 802 μmol). Stirred at 50°C for 72 h. Colorless oil (8.6 mg, 45%, 90% e.e.). $[\alpha]_D^{25}$=−39.0 (c 0.39, $CHCl_3$) [lit.[24] $[\alpha]_D^{22}$=−47.9 (c 1.70, $CHCl_3$)]; ^{1}H NMR δ 7.36–7.15 (10H, m), 4.89 (1H, q, *J* 7.5 Hz), 2.96 (2H, t, *J* 7.6 Hz), 2.59 (2H, t, *J* 7.6 Hz), 1.26 (3H, d, *J* 7.2 Hz); HRMS calcd for $C_{17}H_{18}O_2$ (M^+) 254.130680. Found: 254.131134.

*1.24. 3-Phenylpropionic acid 2-octyl ester (**5o**)*[24,27]

From **4**, (10.5 mg, 59.6 μmol) and (±)-**2o** (95 μL, 597 μmol). Stirred at 50°C for 72 h. Colorless oil (4.9 mg, 31%, >99% e.e.). $[\alpha]_D^{25}$=+7.9 (c 0.47, $CHCl_3$) [lit.[24] $[\alpha]_D^{22}$=+12.1 (c 0.80, $CHCl_3$)]; ^{1}H NMR δ 7.33–7.19 (5H, m), 4.89 (1H, m), 2.95 (2H, t, *J* 7.4 Hz), 2.61 (2H, t, *J* 7.4 Hz), 1.48 (2H, m), 1.25

(8H, br s), 1.17 (3H, d, *J* 6.4 Hz), 0.88 (3H, t, *J* 6.4 Hz); HRMS (+FAB) calcd for $C_{17}H_{27}O_2$ ($M+H^+$) 263.2011. Found: 263.1980.

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