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Enzymatic kinetic resolution of Morita-Baylis-Hillman acetates

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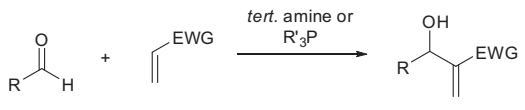
ABSTRACT

Racemic Morita-Baylis-Hillman adducts derived from the reaction of acrylonitrile with benzaldehyde, cinnamaldehyde and hydrocinnamaldehyde have been successfully resolved by means of enzymatic kinetic resolution. The (+)-alcohol products were isolated with 94–97% ee after lipase-mediated enantioselective hydrolysis of the corresponding acetates. Mosher's double derivatisation protocol was applied to these isolated products and the absolute configuration of the alcohols was found to be (*S*) for all three substrates.

1. Introduction

The Morita-Baylis-Hillman reaction is an important carbon–carbon bond forming reaction that leads to highly functionalised adducts containing a newly formed stereogenic centre.[1] The reaction, which may be catalysed by tertiary amines or phosphines, typically occurs between a carbon electrophile, for example an aldehyde, and the α-position of an activated alkene (Scheme 1).[2]

Enantiopure Morita-Baylis-Hillman adducts may be obtained in one of two ways: by employing an enantioselective version of the Morita-Baylis-Hillman reaction,[3] or by resolution of the racemic adduct. A number of different biocatalytic methods for the resolution of Morita-Baylis-Hillman adducts have been applied. These methods include: lipase-catalysed hydrolysis of Morita-Baylis-Hillman acetates,[4] lipase-catalysed transesterification of Morita-Baylis-Hillman alcohols in the presence of an acyl donor,[5] lipase-catalysed hydrolysis of Morita-Baylis-Hillman esters (where EWG = CO_2R),[6] resolution by yeast-catalysed reduction of the alkene,[7] and resolution by nitrile-hydrolysing organisms for acrylonitrile-derived Morita-Baylis-Hillman adducts.[8]



Scheme 1. Morita-Baylis-Hillman reaction.

Our interest in nitrile-hydrolysing enzymes[9] led us to the synthesis of Morita-Baylis-Hillman adducts derived from acrylonitrile, with the intention of using nitrilases or nitrile hydratases for resolution of the new stereogenic centre. After resolution, these highly functionalised, enantiopure compounds could be used in the synthesis of a diverse range of products.[10] Initial results using nitrile-hydrolysing enzymes were disappointing and thus we turned our attention instead to lipases. Previous lipase-resolution studies on Morita-Baylis-Hillman adducts have generally found most success with aliphatic substrates,[4b,c,5] thus we set out to resolve an aromatic substrate, together with closely related rigid and more flexible homologues to try and establish a preferred substrate profile for the selected lipases.

2. Results and discussion

The reaction of aldehyde **1** and acrylonitrile **2** in the presence of DABCO **3** as catalyst gave rise to the desired Morita-Baylis-Hillman adducts **4** (Scheme 2). Acetylation of these adducts under standard conditions gave rise to Morita-Baylis-Hillman acetates **5**, the starting compounds for lipase hydrolysis studies.

We had access to approximately 100 different lipase preparations and hydrolysis of each of the three substrates was tested in the presence of all enzymes in a phosphate buffer at pH 7.0 (Scheme 3). These reactions were performed on a very small scale and monitored by TLC. In cases where hydrolysis was observed, the reaction was repeated and analysed by chiral HPLC to determine the reaction enantioselectivity.

Selected results for the enzymatic hydrolysis reactions are shown in Table. 1. The enantiomeric ratio (*E*),[11] which gives an indication of the selectivity of an irreversible kinetic resolution independent of conversion, was used to rank the performance of

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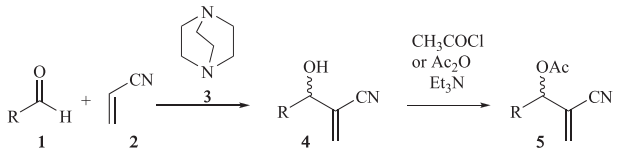
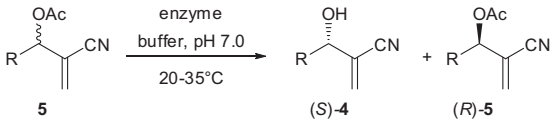


a R= Ph
b R= PhCH=CH-
c R= $PhCH_2CH_2$-

Scheme 2. Preparation of Morita-Baylis-Hillman adducts **4** and acetates **5**.



a R= Ph
b R= PhCH=CH-
c R= $PhCH_2CH_2$-

Scheme 3. Enzymatic hydrolysis of Morita-Baylis-Hillman acetates **5**.

the different enzymes. Successful enzymatic kinetic resolutions were possible for all three of the substrates tested, with cinnamaldehyde derivative **5b** giving the best overall resolution with a lipase from *Pseudomonas fluorescens* (Sigma-Aldrich cat. no. 95608) (entry 7) and Amano AK lipase (entry 8). These two enzymes also performed well in the resolution of benzaldehyde derivative **5a** (entries 4 and 6). Other enzymes able to resolve both **5a** and **5b** were Lipo Max CXT (entries 1 and 10) and Lipozyme® CALB L (*Candida antarctica* lipase B) (entries 5 and 9). The lipase from *Pseudomonas cepacia* (Sigma-Aldrich cat. no. 62309) and the Amano lipase from *P. fluorescens* (Sigma-Aldrich cat. no. 534730) both performed well for the resolution of substrate **5a** (entries 2 and 3), but showed no selectivity at all in the case of substrate **5b** (entries 11 and 12). We were surprised at the lack of selectivity of the Amano lipase from *P. fluorescens* (entry 12) for substrate **5b** considering the fact that the Amano AK lipase (entry 8) demonstrated excellent selectivity.

Compound **5c** proved to be the most challenging of the three substrates, with far fewer enzymes being capable of stereoselective hydrolysis of this compound. Two enzymes were identified that gave reasonable E values for this substrate, Novozym® 435 (entry 13) and Lipozyme® CALB L (entry 14), both of which are preparations of *C. antarctica* lipase B. Lipozyme® CALB L proved to be the only enzyme preparation that was able to selectively hydrolyse all three substrates.

In order to determine the absolute configuration of the alcohol product formed in each case, scaled-up resolution reactions were carried out so that sufficient enantiopure alcohol and acetate could be obtained to carry out Mosher's double derivatisation protocol for assignment of absolute configuration.[12] Results from these experiments are shown in Table 2. Larger scale resolutions of substrate **5a** were carried out twice using Amano lipase from *P. fluorescens*, once to optimise the isolation of enantiopure alcohol, and a second time to optimise the isolation of enantiopure acetate. Alcohol (+)-**4a** was isolated with an ee of 94% in 46% yield (entry 1), while the second reaction gave acetate (−)-**5a** in 99% ee and 73% yield (entry 2). For the resolution of substrate **5b**, the first reaction was performed using Amano AK lipase and the alcohol product (+)-**4b** was isolated with an ee of 97% in 42% yield (entry 3), while acetate (−)-**5b** was isolated in 69% yield and an ee of 92% using Lipozyme® CALB L (entry 4). The final substrate **5c** was also resolved using Lipozyme® CALB L and the alcohol (+)-**4c** was obtained with a 95% ee in a yield of 44% (entry 5). Unfortunately, due to limited quantities of enzyme we were unable to isolate enantiopure acetate (−)-**5c**. Optical rotation measurements were performed on the three isolated alcohols and the sign of rotation for all three was found to be (+), while a (−) rotation was observed for the residual acetates. For enantiopure acetates (−)-**5a** and (−)-**5b**, hydrolysis to the corresponding alcohol was performed prior to confirmation of absolute configuration by Mosher's double derivatisation protocol.

Analysis of the ^{1}H NMR spectra of the (*S*)- and (*R*)-Mosher ester derivatives for alcohol products (+)-**4a**, (+)-**4b** and (+)-**4c** allowed us to determine that the (*S*)-alcohol was obtained from all three of the enzymatic hydrolysis reactions. Figure 1A depicts the Mosher ester derivatives of (*S*)-**4a–c** in the preferred conformation. Considering the case of (*S*)-Mosher derivative **6b** as an example, Fig. 1A clearly shows that the ^{1}H NMR signals of the cinnamyl group should be shielded by the Mosher moiety's phenyl group. In contrast, for the (*R*)-Mosher derivative **7b**, the same cinnamyl group protons should be relatively deshielded. The reverse is true for the methylene protons of the acrylonitrile group, which should be shielded by the Mosher group's phenyl ring in **7b**, and relatively deshielded in **6b**. These predicted changes in chemical shift were in fact observed in the ^{1}H NMR spectra, in full agreement with the (*S*)-assignment for compounds (+)-**4a–c**.

Relevant ^{1}H NMR shifts for the Mosher ester derivatives of the isolated alcohols are shown in Table 3. For all three products, it

Table 1
Results of enzymatic hydrolysis of Morita-Baylis-Hillman acetates **5a–c**

Entry	Substrate	Enzyme	Conv. (%)[a]	Time (h)	ee_s (%)[b]	ee_p (%)[c]	E[d]
1	**5a**	Lipo Max CXT from *P. alcaligenes* (Genencor International)	45	4	75	94	72
2	**5a**	Lipase from *P. cepacia* (62309)	51	19	94	90	65
3	**5a**	Amano lipase from *P. fluorescens* (534730)	46	34	79	92	58
4	**5a**	Lipase from *P. fluorescens* (95608)	22	24	26	94	44
5	**5a**	Lipozyme® CALB L (Novozymes)	29	26	37	93	40
6	**5a**	Amano AK lipase (lot 0351202)	48	96	80	88	36
7	**5b**	Lipase from *P. fluorescens* (95608)	51	72	96	93	100
8	**5b**	Amano AK lipase (lot 0351202)	40	48	61	96	100
9	**5b**	Lipozyme® CALB L (Novozymes)	20	3	24	97	70
10	**5b**	Lipo Max CXT from *P. alcaligenes* (Genencor International)	24	3	29	93	36
11	**5b**	Lipase from *P. cepacia* (62309)	23	5	6	20	2
12	**5b**	Amano lipase from *P. fluorescens* (534730)	34	24	17	34	2
13	**5c**	Novozym® 435	17	17	19	96	54
14	**5c**	Lipozyme® CALB L (Novozymes)	29	43	38	94	51

[a] conversion (%) = $ee_s/(ee_s + ee_p) \times 100$ (from chiral HPLC);
[b] ee_s = %ee of (*R*)-acetate.
[c] ee_p = %ee of (*S*)-alcohol product.
[d] E (enantiomeric ratio) = $\{\ln[1 - c(1 + ee_p)]\}/\{\ln[1 - c(1 - ee_p)]\}$.

Table 2
Results from scaled-up resolution reactions

Entry	Resolved Compound	Reaction time (d)	Conv (%)	Yield (%)	ee (%)	$[\alpha]_D$ (*c* 0.5, MeOH)
1	(+)-**4a**	2	36	46	94	+68.4
2	(−)-**5a**	14	54	73	99	−27.8
3	(+)-**4b**	1	26	42	97	+50.4
4	(−)-**5b**	14	53	69	92	−58.8
5	(+)-**4c**	3	21	44	95	+31.6

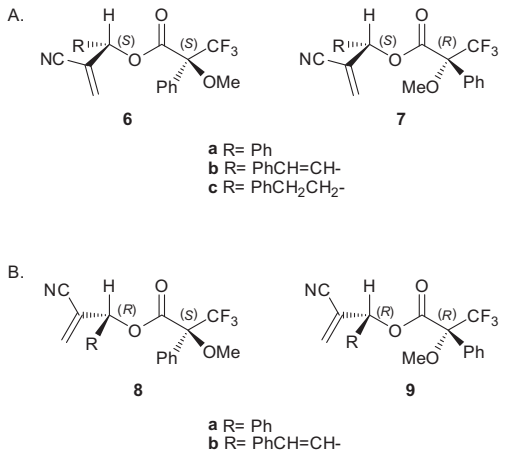


Figure 1. (*S*)- and (*R*)-Mosher ester derivatives: **A**. **6a–c** and **7a–c**; **B**. **8a–b** and **9a–b**.

Table 3
^{1}H NMR data for Mosher ester derivatives **6a–c** and **7a–c**

Alcohol	Group	(*S*)-Mosher derivative **6** δ (ppm)	(*R*)-Mosher derivative **7** δ (ppm)
(+)-**4a**	Acrylonitrile H	5.98	5.93
	Acrylonitrile H	6.13	6.06
(+)-**4b**	Acrylonitrile H	6.14	6.01
	Acrylonitrile H	6.16	6.08
	α-Cinnamyl H	6.74	6.85
(+)-**4c**	Acrylonitrile H	6.07	5.96
	Acrylonitrile H	6.14	6.08
	Benzylic CH_2	2.60–2.47	2.71–2.61

is clear that the chemical shifts for the acrylonitrile protons are shielded in the (*R*)-Mosher derivative, which corresponds to the alcohols all being of (*S*)-configuration. In agreement with this assignment is the fact that for the (*S*)-Mosher derivative, shielding is observed in the chemical shifts of the α-cinnamyl proton in **6b** and the benzylic protons in **6c**. The aromatic protons of compound **6a** were not used in this exercise, as it was not possible to clearly distinguish between the two sets of phenyl ring signals. The ^{13}C NMR spectra for compounds **6a–c** and **7a–c** clearly showed the expected C-F coupling, with $^1J_{C\text{-}F}$ values of 289 Hz and $^2J_{C\text{-}F}$ values of 28 Hz being observed. As confirmation of these results, the hydrolysis products of unreacted esters (−)-**5a** and (−)-**5b** were reacted to give (*S*)-Mosher derivatives **8a–b** and (*R*)-Mosher derivatives **9a–b** (Fig. 1B). Analysis of the ^{1}H NMR data of these products showed the configuration at the secondary alcohol to be (*R*), as expected.

It should be noted that a few of the enzymes tested preferentially gave the (*R*)-alcohol product. Of these, only hydrolysis of substrate **5b** by two enzymes gave a result selective enough to be practically relevant. The first enzyme was Lipopan F BG, a commercial enzyme used in the baking industry that is a lipase derived from *Fusarium* sp. This gave (*R*)-**4b** with 90% ee at 27% conversion, corresponding to an E value of 27. The second enzyme was found to be Alcalase® 2.4 L FG, which is in fact a protease (subtilisin) from *Bacillus licheniformis*. This second result is not surprising, as subtilisin generally displays the opposite enantiopreference to most lipases.[13]

To the best of our knowledge, there are no previous reports of the enzymatic kinetic resolution of substrates **5b** and **5c**, while alcohols **4b** and **4c** have not previously been reported in the literature in enantiopure form. Kinetic resolution of compound **5a** by enzymatic hydrolysis has previously been reported by Basavaiah using crude pig liver acetone powder, but a very poor ee of 60% was obtained. He recorded that the sign of the optical rotation of the scalemic alcohol was (+), but did not determine the absolute configuration of the major enantiomer.[4c] Bornsheuer et al. attempted resolution of **5a** by *P. cepacia* lipase-catalysed transesterification in the presence of acyl donors but could achieve only 9% conversion and 76% ee of (+)-product after 33 days.[5a] A non-enzymatic method for resolution of **4a** has been reported where an ee of 93% and yield of 13% of **4a** were obtained, but the absolute configuration of the product was not determined.[14] Thus, our method represents the first practical resolution method reported for obtaining **4a** in enantiopure form.

We compared our findings of the (*S*)-alcohols displaying (+) optical rotations with closely related examples in the literature. A non-enzymatic resolution of the Morita-Baylis-Hillman adduct prepared from 2-methoxybenzaldehyde and acrylonitrile was performed and the authors reported a (+) optical rotation for the (*R*)-alcohol, which because of a change in Cahn-Ingold-Prelog priority on introduction of the 2-methoxy group, is in agreement with our findings.[15] Similarly, enzymatic kinetic resolution of the Morita-Baylis-Hillman adduct from acetaldehyde and acrylonitrile gave rise to the (*S*)-alcohol product, which displayed a (+) optical rotation.[4b] Vasconcellos et al., however, reported obtaining the (*R*)-alcohol product when they resolved the Morita-Baylis-Hillman adduct of 4-nitrobenzaldehyde and acrylonitrile and the sign of the optical rotation measured was (+).[4b]

3. Conclusions

In the enzyme screening experiments, benzaldehyde derivative **5a** was found to be resolvable by the largest number of enzymes. However, the best enantioselectivity was observed for the rigid cinnamyl homologue **5b**. The more flexible hydrocinnamyl derivative **5c** proved to be most difficult to resolve, with fewer enzymes being able to discriminate between the two enantiomers. One enzyme, Lipozyme® CALB L, although not the best-performing enzyme for each of the individual substrates, was able to successfully resolve all three substrates. Scaled-up resolution of substrate **5a** was carried out using Amano lipase from *P. fluorescens* and alcohol (*S*)-(+)-**4a** was isolated in 46% yield and with 94% ee. Substrate **5b** was resolved using Amano AK lipase and alcohol (*S*)-(+)-**4b** was isolated in 42%, with an ee of 97%. Resolution of the final substrate **5c** was achieved using Lipozyme® CALB L and alcohol (*S*)-(+)-**4c** was obtained in 44% yield and 95% ee.

4. Experimental

4.1. General

All reagents and solvents were obtained from commercial suppliers. All solvents used for chromatographic separation were distilled before use. HPLC analytical reagents were used without purification. Reactions were monitored using TLC (thin layer chromatography) on aluminium-backed Merck silica gel 60 F_{254} plates. Separation of compounds by column chromatography was performed on normal silica gel (particle size 063–0.200 mm) or flash silica gel (particle size 0.040–0.063) purchased from Merck. Small scale enzymatic reactions were done on an Esco provocell ™ microplate shaker. Infra-red spectra were recorded using a Bruker Tensor 27 single channel infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on either a Bruker AVANCE 300 MHz, Bruker AVANCE 400 MHz or on a Bruker AVANCE III 500 MHz spectrometer. Spectra were recorded in deuterated chloroform, unless otherwise stated. The chemical shift values for all spectra obtained are reported in parts per million and referenced against the internal standard, TMS, which occurs at zero parts per million. Coupling constants are given in Hertz. High resolution mass spectra were recorded on a Waters Synapt G1 or G2 mass spectrometer using an ESI positive source and a cone voltage of 15 V. The enzymes used were purchased from Sigma–Aldrich, Enzymes SA or Amano, or were gifts from Novozymes. Enzyme reactions were monitored using chiral HPLC on a Dionex Ultimate 3000 instrument equipped with a photodiode array detector. The mobile phase used consisted of isopropyl alcohol (IPA) and hexane. Detection of the eluted analytes was achieved using a TSP variable wavelength UV detector at 215 nm. All calculations were based on peak area. The optical rotations of the enantiopure compounds were recorded on a Jasco P-2000 polarimeter.

4.2. Preparation of Morita-Baylis-Hillman adducts 4a–c

4.2.1. Synthesis of (±)-2-[(hydroxyphenyl)methyl]acrylonitrile 4a

A mixture of benzaldehyde (10.45 g, 0.098 mol), acrylonitrile (40 mL, 0.608 mol) and DABCO (10.9 g, 0.098 mol) were stirred at 0 °C for 19 h. After completion, ethyl acetate and water were added to the reaction mixture. The organic layer was separated, dried over $MgSO_4$, and purified by column chromatography (40% ethyl acetate/hexane) to afford the desired product **4**) as a colourless oil (14.81 g, 95%). R_f = 0.56 (40% ethyl acetate/hexane); IR (neat, cm^{-1}) 3421, 3032, 2229, 1453; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.34 (m, 5H), 6.09 (d, J = 1.5 Hz, 1H), 6.01 (d, J = 1.2 Hz, 1H), 5.28–5.26 (m, 1H), 2.69 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.2, 130.3, 128.70, 128.6, 126.5, 126.0, 117.0, 73.7. Chiral HPLC: Lux 3 μm cellulose-2 (250 × 4.6 mm); mobile phase, hexane: IPA (96:4); flow rate = 1 mL/min); t_R = 21.35 min, t_R = 23.57 min.

4.2.2. Synthesis of (±)-(*E*)-3-hydroxy-2-methylene-5-phenyl-4-pentenenitrile 4b

A mixture of *trans*-cinnamaldehyde (10.5 g, 0.008 mol), acrylonitrile (15.67 mL, 0.239 mol), DABCO (17.834 g, 0.159 mol), and phenol (7.5 mL, 0.079 mol) were stirred at 25 °C for 24 h. Extractive workup and column chromatographic purification (20% ethyl acetate/ hexane) afforded **4b** as a colourless oil (11.5 g, 80%). R_f = 0.52 (40% ethyl acetate/hexane); IR (neat, cm^{-1}) 3422, 3028, 2228, 1495, 1449; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.23 (m, 5H), 6.73 (d, J = 15.8 Hz, 1H), 6.19 (dd, J = 15.9, 6.9 Hz, 1H), 6.11–6.07 (m, 1H), 6.04–5.99 (m, 1H), 4.90 (d, J = 6.7 Hz, 1H), 2.52 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.6, 133.8, 130.0, 128.7, 128.5, 126.9, 126.6, 125.5, 117.0, 72.9.

Chiral HPLC: Lux 5 μm cellulose-1 (250 × 4.60 mm); mobile phase, hexane/IPA (90:10); flow rate = 1 mL/min; t_R = 18.80 min, t_R = 24.50 min.

4.2.3. Synthesis of (±)-3-hydroxy-2-methylene-5-phenylpentanenitrile 4c

The procedure used to synthesise **4a** was used to synthesise **4c** except that the reaction time was changed to 6 d. Purification by column chromatography gave **4c** as a colourless oil (9.0 g, 65%). R_f = 0.6 (40% ethyl acetate/hexane); IR (neat, cm^{-1}) 3420, 3027, 2227, 1496, 1454; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.10 (m, 5H), 5.95–5.89 (m, 2H), 4.21–4.12 (m, 1H), 3.21 (d, J = 4.4 Hz, 1H), 2.79–2.59 (m, 2H), 2.06–1.85 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.8, 130.4, 128.5, 128.4, 126.6, 126.1, 117.1, 71.3, 37.0, 31.2. Chiral HPLC: Lux 3 μm cellulose-2 (250 × 4.6 mm); mobile phase, hexane/IPA (96:4); flow rate = 1 mL/min; t_R = 17.77 - min, t_R = 19.45 min.

4.3. Preparation of Morita-Baylis-Hillman acetates 5a–c

4.3.1. Synthesis of (±)-2-cyano-1-phenylallyl acetate 5a

At first, Et_3N (3 mL, 0.040 mol), Ac_2O (2 mL, 0.040 mol), and DMAP (24.28 mg, 0.199 mmol) were added to a stirred solution of **4a** (3.16 g, 0.020 mol) in dichloromethane (25 mL). The resulting mixture was stirred at 25 °C for 30 min. An aqueous saturated solution of $NaHCO_3$ was added to the reaction mixture in a separation funnel; and shaken. The organic layer was dried using $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (20% ethyl acetate/hexane) to afford **5a** as a colourless oil (3.56 g, 89%). R_f = 0.61 (30% ethyl acetate/hexane). IR (neat, cm^{-1}) 3035, 2229, 1745, 1371, 1217; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42–7.32 (m, 5H), 6.33–6.31 (m, 1H), 6.03–6.00 (m, 1H), 5.98–5.96 (m, 1H), 2.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 135.7, 132.0, 129.2, 128.9, 126.9, 123.2, 116.2, 74.3, 20.8. HRMS *m/z* calcd for $C_{12}H_{11}NO_2Na$ [M+Na$^+$]: 224.0687, found: 224.0681. Chiral HPLC: Chiralpak AD-H (250 × 4.6 mm); mobile phase, hexane /IPA (96:4); flow rate = 1 mL/min); t_R = 8.26 min, t_R = 8.86 min.

4.3.2. Synthesis of (±)-(*E*)-4-cyano-1-phenylpenta-1,4-dien-3-yl acetate 5b

To a solution of **4b** (2.02 g, 0.011 mol) in dry THF were added Et_3N (12 mL, 0.087 mol) and AcCl (4 mL, 0.087 mol) at 0 °C for 20 min. The compound of interest was extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The crude extract was purified by column chromatography (20% EtOAc/ hexane) to afford **5b** (2.01 g, 82%) as a colourless oil. R_f = 0.68 (40% EtOAc/hexane); IR (neat, cm^{-1}) 3028, 2228, 1741, 1496, 1217; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.24 (m, 5H), 6.76 (d, J = 15.9 Hz, 1H), 6.17 (dd, J = 15.9, 7.3 Hz, 1H), 6.06 (dd, J = 2.5, 0.9 Hz, 2H), 5.96–5.91 (m, 1H), 2.14 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 135.7, 135.3, 132.2, 128.8, 128.7, 126.9, 122.5, 122.3, 116.2, 73.4, 21.0. HRMS *m/z* calcd for $C_{14}H_{13}NO_2Na$ [M+Na$^+$]: 250.0844, found: 250.0824. Chiral HPLC: Lux 5 μm cellulose-1 (250 × 4.60 mm); mobile phase, hexane: IPA (90:10); flow rate = 1 mL/min; t_R = 9.82 min, t_R = 10.69 min.

4.3.3. Synthesis of (±)-2-cyano-5-phenyl-pent-1-ene-yl acetate 5c

The same procedure used to synthesise **5a** was used to synthesise **5c.** This afforded **5c** as a colourless oil (4.8 g, 91%). R_f = 0.54 (20% ethyl acetate/hexane); IR (neat, cm^{-1}) 2937, 2227, 1742, 1454, 1371, 1220; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.12 (m, 5H), 6.02–5.98 (m, 1H), 5.95–5.91 (m, 1H), 5.30–5.21 (m, 1H), 2.65 (t, J = 7.8 Hz, 2H), 2.22–1.98 (m, 2H) overlapping 2.06 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 140.1, 132.9, 128.6,

128.3, 126.3, 122.5, 116.1, 72.6, 34.4, 31.1, 20.8. HRMS *m/z* calcd for $C_{14}H_{15}NO_2Na$ [M+Na$^+$]: 252.1000, found: 252.0985. Chiral HPLC: Lux 5 μm amylose-2 (250 × 4.6 mm); mobile phase, Hexane: IPA (96:4); flow rate = 1 mL/min, t_R = 9.45 min, t_R = 13.08 min.

4.4. Enzymatic hydrolysis reactions of 5a–c

4.4.1. General procedure for enzymatic hydrolysis screening reactions

The substrate (7 mg) was added to pH 7.00 phosphate buffer (1 mL) containing enzyme (7 mg). The reaction mixture was put on an orbital shaker at the specified temperature and monitored using TLC and chiral HPLC.

4.4.2. Enzymatic hydrolysis of (±)-2-cyano-1-phenylallyl acetate 5a

4.4.2.1. Isolation of (+)-4a. Compound **5a** (1.00 g) was added to a mixture containing Amano lipase from *P. fluorescens* (534730) (1.00 g) in phosphate buffer (50 mL) at pH 7.00. The resulting mixture was stirred for 44 h at 30 °C and the product was extracted using ethyl acetate. Purification by column chromatography (10% ethyl acetate/hexane) afforded as colourless oils (+)-**4a** [180 mg, 46%, ee = 94%; $[\alpha]_D$ = +68.4 (*c* 0.5, MeOH)] and scalemic (−)-**5a** (404 mg, ee = 53%).

4.4.2.2. Isolation of (−)-5a. Compound **5a** (1.50 g) was reacted under the conditions described in 4.4.2.1 for 14 d, after which column chromatography (10% ethyl acetate/hexane) afforded (−)-**5a** [544 mg, 73%, ee = 99%; $[\alpha]_D$ = −27.8 (*c* 0.5, MeOH)] and (+)-**4a** (655 mg, ee = 84%).

4.4.3. Enzymatic hydrolysis of (±)-(*E*)-4-cyano-1-phenylpenta-1,4-dien-3-yl acetate 5b

4.4.3.1. Isolation of (+)-4b. Compound **5b** (0.60 g) was added to a mixture containing Amano AK lipase (lot 0351202) (0.60 g) in phosphate buffer (50 mL) at pH 7.00. The resulting mixture was stirred for 24 h at 25 °C and the product was extracted using ethyl acetate. Purification by column chromatography (20% ethyl acetate/hexane) afforded as colourless oils (+)-**4b** [100 mg, 42%, ee = 97%; $[\alpha]_D$ = +50.4 (*c* 0.5, MeOH)] and scalemic (−)-**5b** (336 mg, ee = 33%).

4.4.3.2. Isolation of (−)-5b. Compound **5b** (1.17 g) was reacted under the conditions descried in 4.4.3.1, except the enzyme used was Lipozyme® CALB L (Novozymes). After 14 days, column chromatography (20% ethyl acetate/hexane) afforded as colourless oils (−)-**5b** [330 mg, 69%, ee = 92%; $[\alpha]_D$ = −58.8 (*c* 0.5, MeOH)] and (+)-**4b** (465 mg, ee = 81%).

4.4.4. Enzymatic hydrolysis of (±)-2-cyano-5-phenyl-pent-1-ene-yl acetate 5c

Compound **5c** (0.72 g) was added to a mixture containing Lipozyme® CALB L (Novozymes) (0.72 g) in phosphate buffer (50 mL) at pH 7.00. The resulting mixture was stirred for 64 h at 35 °C and the product was extracted using ethyl acetate. Purification by column chromatography (20% ethyl acetate/hexane) afforded as colourless oils (+)-**4c** [130 mg, 44%, ee = 95%; $[\alpha]_D$ = +31.6 (*c* 0.5, MeOH)] and scalemic (−)-**5c** (440 mg, ee = 25%).

4.5. General method for the preparation of Mosher derivatives

(+)- or (−)-Alcohol (1 equiv), and either (*R*)-MTPA or (*S*)-MTPA (3.2 equiv), DCC (2 equiv) and DMAP (0.39 equiv) were added to a 25 mL round bottom flask containing dichloromethane (3 mL). The mixture was stirred at room temperature for 30 min. Water was added to the reaction mixture in a separation funnel and shaken. The dichloromethane layer was separated and dried over Na_2SO_4 and subjected to flash column chromatography on silica gel using ethyl acetate/ hexane as eluent.

4.5.1. Preparation (*S*)-[(*S*)-2-cyano-1-phenylallyl]3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 6a

(*S*)-[(*S*)-2-Cyano-1-phenylallyl]3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **6a** (230 mg, 80%) was isolated as a colourless oil. R_f = 0.64 (30% ethyl acetate/hexane); IR (neat, cm^{-1}) 2952, 2230, 1753, 1453, 1167; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.30 (m, 9H), 7.24–7.20 (m, 1H), 6.49 (s, 1H), 6.13 (d, *J* = 1.2 Hz, 1H), 5.98 (d, *J* = 1.5 Hz, 1H), 3.60–3.57 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 134.1, 133.2, 131.5, 129.8, 129.6, 129.0, 128.5, 127.2 (br s), 127.1, 123.1 (q, $J_{C\text{-}F}$ = 289 Hz), 122.2, 116.0, 84.7 (q, $J_{C\text{-}F}$ = 28 Hz), 76.3, 55.9. HRMS *m/z* calcd for $C_{20}H_{16}F_3NO_3Na$ [M+Na$^+$]: 398.0980, found: 398.0985.

4.5.2. Preparation of (*S*)-[(*S*,*E*)-4-cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 6b

(*S*)-[(*S*,*E*)-4-Cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **6b** was isolated as a colourless oil (80 mg, 82%). R_f = 0.75 (40% ethyl acetate/hexane); IR (neat, cm^{-1}) 2951, 2224, 1753, 1452, 1167; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53–7.49 (m, 2H), 7.44–7.29 (m, 8H), 6.74 (d, *J* = 15.7 Hz, 1H), 6.16 (br s, 1H), 6.15–6.13 (m, 2H), 6.07 (dd, *J* = 15.8, 7.4 Hz, 1H), 3.62–3.61 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 137.2, 134.9, 133.3, 131.7, 129.8, 129.1, 128.8, 128.5, 127.3 (br s), 127.0, 123.2 (q, $J_{C\text{-}F}$ = 289 Hz), 121.3, 120.70, 116.0, 84.8 (q, $J_{C\text{-}F}$ = 28 Hz), 75.3, 55.9. HRMS *m/z* calcd for $C_{22}H_{18}F_3NO_3Na$ [M+Na$^+$]: 424.1136, found: 424.1135.

4.5.3. Preparation of (*S*)-[(*S*)-2-cyano-5-phenylpent-1-en-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 6c

(*S*)-[(*S*)-2-Cyano-5-phenylpent-1-en-3-yl]3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **6c** was isolated as a colourless oil (56 mg, 82%). R_f = 0.59 (20% ethyl acetate /hexane); IR (neat, cm^{-1}) 3030, 2229, 1752, 1453, 1167; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58–7.48 (m, 2H), 7.46–7.38 (m, 3H), 7.32–7.18 (m, 3H), 7.08–7.02 (m, 2H), 6.14 (s, 1H), 6.07 (d, *J* = 1 Hz, 1H), 5.43 (dd, *J* = 8.4, 5.2 Hz, 1H), 3.60–3.54 (m, 3H), 2.60–2.47 (m, 2H), 2.30–2.15 (m, 1H), 2.11–1.97 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 139.6, 134.6, 131.7, 129.9, 128.7, 128.6, 128.3, 127.2 (br s), 126.5, 123.2 (q, $J_{C\text{-}F}$ = 290 Hz), 121.5, 115.7, 84.6 (q, $J_{C\text{-}F}$ = 28 Hz), 74.9, 55.7, 34.3, 30.6. HRMS *m/z* calcd for $C_{22}H_{20}F_3NO_3Na$ [M+Na$^+$]: 426.1293, found: 426.1285.

4.5.4. Preparation of (*R*)-[(*S*)-2-cyano-1-phenylallyl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 7a

(*R*)-[(*S*)-2-Cyano-1-phenylallyl]3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **7a** was isolated as a colourless oil (133 mg, 82%). R_f = 0.64 (30% ethyl acetate/hexane); IR (neat, cm^{-1}) 2951, 2230, 1753, 1452, 1167; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.30 (m, 10H), 6.56–6.52 (m, 1H), 6.06 (d, *J* = 1.0 Hz, 1H), 5.93 (d, *J* = 1.4 Hz, 1H), 3.47 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 134.3, 132.1, 131.7, 129.8, 129.1, 128.5, 127.4, 127.3 (br s), 123.2 (q, $J_{C\text{-}F}$ = 289 Hz), 122.0, 115.7, 84.7 (q, $J_{C\text{-}F}$ = 28 Hz), 75.9, 55.6. HRMS *m/z* calcd for $C_{20}H_{16}$ F_3NO_3Na [M+Na$^+$]: 398.0980, found: 398.0969.

4.5.5. Preparation of (*R*)-[(*S*,*E*)-4-cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropan-oate 7b

(*R*)-[(*S*,*E*)-4-Cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropan-oate **7b** was isolated as a colourless oil (58 mg, 84%). R_f = 0.75 (40% ethyl acetate/hexane); IR (neat, cm^{-1}) 2950, 2219, 1752, 1452, 1167; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54–7.50 (m, 2H), 7.45–7.30 (m, 8H), 6.85 (d,

J = 15.1 Hz, 1H), 6.23–6.13 (m, 2H), 6.08 (d, J = 1 Hz, 1H), 6.01 (d, J = 1 Hz, 1H), 3.57–3.55 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 137.5, 134.9, 132.7, 131.7, 129.9, 129.2, 128.8, 128.6, 127.3 (br s), 127.1, 123.2 (q, $J_{C\text{-}F}$ = 289 Hz), 121.2, 121.0, 115.7, 84.7 (q, $J_{C\text{-}F}$ = 28 Hz), 75.2, 55.60. HRMS m/z calcd for $C_{22}H_{18}F_3NO_3Na$ [M +Na$^+$]: 424.1136, found: 424.1145.

4.5.6. Preparation of (*R*)-[(*S*)-2-cyano-5-phenylpent-1-en-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 7c

(*R*)-[(*S*)-2-Cyano-5-phenylpent-1-en-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **7c** was isolated as a colourless oil (192 mg, 81%). R_f = 0.59 (20% ethyl acetate/hexane); IR (neat, cm^{-1}) 3030, 2230, 1752, 1452, 1168; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56–7.48 (m, 2H), 7.47–7.38 (m, 3H), 7.34–7.18 (m, 3H), 7.16–7.10 (m, 2H), 6.08 (s, 1H), 5.96 (d, J = 1 Hz, 1H), 5.50–5.43 (m, 1H), 3.58–3.53 (m, 3H), 2.71–2.61 (m, 2H), 2.32–2.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 139.5, 134.0, 131.4, 129.9, 128.7, 128.6, 128.3, 127.3 (br s), 126.6, 123.2 (q, $J_{C\text{-}F}$ = 289 Hz), 121.3, 115.5, 84.7 (q, $J_{C\text{-}F}$ = 28 Hz), 74.7, 55.6, 34.4, 30.9. HRMS m/z calcd for $C_{22}H_{20}F_3NO_3Na$ [M+Na$^+$]: 426.1293, found: 426.1268.

4.5.7. Preparation of (*S*)-[(*R*)-2-cyano-1-phenylallyl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 8a

(*S*)-[(*R*)-2-Cyano-1-phenylallyl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **8a** was isolated as a colourless oil (155 mg, 74%). R_f = 0.64 (30% ethyl acetate/hexane). IR (neat, cm^{-1}) 2951, 2230, 1753, 1452, 1167; ^{1}H NMR (300 MHz, CDCl3) δ 7.45–7.32 (m, 10H), 6.55–6.53 (m, 1H), 6.07 (d, J = 1.1 Hz, 1H), 5.94 (d, J = 1.5 Hz, 1H), 3.48–3.46 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.2, 134.3, 132.1, 131.7, 129.9, 129.1, 128.5, 127.4, 127.3 (br s), 123.2 (q, $J_{C\text{-}F}$ = 289 Hz), 122.0, 115.7, 84.6 (q, $J_{C\text{-}F}$ = 28 Hz), 75.9, 55.6. HRMS m/z calcd for $C_{20}H_{20}F_3N_2O_3$ [M+NH$_4^+$]: 393.1421, found 393.1429.

4.5.8. Preparation of (*S*)-[(*R,E*)-4-cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropan-oate 8b

(*S*)-[(*R,E*)-4-Cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropan-oate **8b** was isolated as a colourless oil (122 mg, 71%). R_f = 0.75 (40% ethyl acetate/hexane). IR (neat, cm^{-1}) 2950, 2229, 1751, 1451, 1166; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 8.0 Hz, 3H), 7.47–7.31 (m, 7H), 6.85 (d, J = 14.5 Hz, 1H), 6.25–6.12 (m, 2H), 6.09–6.08 (m, 1H), 6.01 (d, J = 1 Hz, 1H), 3.57–3.54 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 137.5, 134.9, 132.6, 131.7, 129.9, 129.2, 128.8, 128.6, 127.3 (br s), 127.0, 123.2 (q, $J_{C\text{-}F}$ = 289 Hz), 121.2, 121.0, 115.7, 84.7 (q, $J_{C\text{-}F}$ = 28 Hz), 75.2, 55.6. HRMS m/z calcd for $C_{22}H_{18}F_3NO_3Na$ [M +Na$^+$]: 424.1136, found 424.1155.

4.5.9. Preparation of (*R*)-[(*R*)-2-cyano-1-phenylallyl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 9a

(*R*)-[(*R*)-2-Cyano-1-phenylallyl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate **9a** was isolated as a colourless oil (130 mg, 76%). R_f = 0.64 (30% ethyl acetate/hexane). IR (neat, cm^{-1}) 2951, 2230, 1753, 1452, 1167; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.30 (m, 9H), 7.24–7.20 (m, 1H), 6.50 (s, 1H), 6.13 (d, J = 1.2 Hz, 1H), 5.99–5.97 (m, 1H), 3.61–3.58 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.2, 134.1, 133.1, 131.6, 129.8, 129.6, 129.0, 128.5, 127.3 (br s), 127.2, 123.2, (q, $J_{C\text{-}F}$ = 289 Hz), 122.3, 116.0, 84.8 (q, $J_{C\text{-}F}$, 28 Hz), 76.4, 55.8. HRMS m/z calcd for $C_{20}H_{20}F_3N_2O_3$ [M+NH$_4^+$]: 393.1421, found 393.1412.

4.5.10. Preparation of (*R*)-[(*R,E*)-4-cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 9b

(*R*)-[(*R,E*)-4-cyano-1-phenylpenta-1,4-dien-3-yl] 3,3,3-trifluoro-2-methoxy-2-phenyl-propanoate **9b** was isolated as a colourless oil (131 mg, 73%). R_f = 0.75 (40% ethyl acetate/hexane). IR (neat, cm^{-1}) 2951, 2230, 1751, 1450, 1167; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54–7.48 (m, 2H), 7.42–7.31 (m, 8H), 6.74 (d, J = 15.6 Hz, 1H), 6.18–6.12 (m, 3H), 3.63–3.60 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 137.2, 134.9, 133.3, 131.8, 129.8, 129.1, 128.8, 128.5, 127.3 (br s), 127.0, 123.2 (q, $J_{C\text{-}F}$ = 289 Hz), 121.3, 120.8, 115.9, 84.8 (q, $J_{C\text{-}F}$ = 28 Hz), 75.3, 55.8. HRMS m/z calcd for $C_{22}H_{18}F_3NO_3Na$ [M+Na$^+$]: 424.1136, found 424.1110.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetasy.2017.08.001.

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