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The effect of catechin derivatives on the enantioselectivity of lipase-catalyzed hydrolyses of alkynol benzoate esters

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Abstract—Polyphenols, such as (+)-catechin and pyrogallol could be used to enhance stereochemical control in the lipase-catalyzed hydrolysis of alkynol benzoate esters, leading to increased enantioselectivities in the kinetic resolution of alkynols with lipase Amano AH.

1. Introduction

Optically active alkynols are important chiral building blocks for numerous natural products and drugs.[1–7] Several chemical and enzymatic methods to obtain optically active alkynols have been developed including: resolution of a diastereomeric ether,[8] chemical[9,10] and enzymatic[11] asymmetric reductions of the corresponding ketones, asymmetric transesterification of racemic alcohols,[12] and asymmetric hydrolysis of the corresponding esters using biocatalysts.[13]

Lipases are widely used as environmentally benign catalysts in organic synthesis. They have broad substrate specificities and exhibit high stereoselectivities. Although many reported examples afford excellent enantioselectivities, the efficiency of general lipase-catalyzed kinetic resolutions is not satisfactory for practical use and therefore, several methods that increase the enantioselectivities of lipase-catalyzed reactions have been developed. These include treatment of a lipase with an organic solvent[14] and with 2-propanol,[15] addition of an alkaloid,[16] amino alcohols,[17] DMSO,[18] crown-[19] and thiacrown[20] ethers, cyclodextrin,[21] PEG,[22] and metal ions.[23] However, despite the many successful reported examples, many unsuccessful examples of kinetic resolution still exist. Therefore, new methods to increase the enantioselecitivity of lipase-catalyzed reactions, which could make biocatalytic procedures standard for the syntheses of enantiopure compounds, are still awaited.

We report herein that catechin derivatives can act as effective additives to control lipase-catalyzed hydrolysis reactions. Benzoate derivatives of alkynols were used as substrates and the effect of catechin derivatives on their kinetic resolution with lipase Amano AH (*Pseudomonas* sp.) was studied.

2. Results and discussion

2.1. Hydrolysis of 2-benzoyloxy-3-hexyne 1b

Hydrolysis of the benzoate ester **1b** with *Pseudomonas* lipase Amano AH in a phosphate buffer (67 mM, pH 7.0) afforded the corresponding (*R*)-alcohol and (*S*)-ester. The absolute configuration of these products was determined by preparation of the Mosher ester derivatives as described below. As a result, the e.e. of the alcohol and the ester were found to be 69 and 41%, respectively. Since the selectivity (*E* value[24]) was found to be only 8 and was not satisfactory for practical use. Thus, it was necessary to modify the reaction in order to improve the selectivity. Selective inhibitors have often been used to increase low selectivity of enzymatic reactions. For example, alkaloid[16] and amino alcohol additives[17] have been used for lipase-catalyzed reactions, and allyl alcohol,[25] methyl vinyl ketone[26] and chloroacetate,[26] were used for yeast-catalyzed reduction. We chose catechins since they are known to be potential inhibitors for urokinase,[27] a hydrolytic enzyme. We expected that the catechin would inhibit the lipase-catalyzed reaction, hopefully enantioselec-

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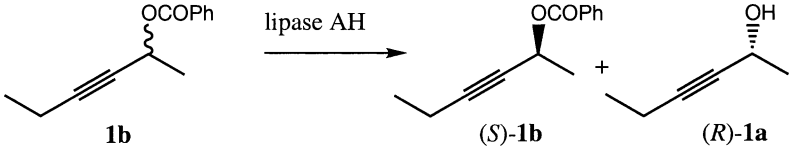
tively, and lead to increased selectivity. Therefore, (+)-catechin was added to the reaction mixture to observe its effect on the lipase-catalyzed hydrolysis of **1b**. As a result, after 96 h, >99% e.e. of **1a** was obtained at the conversion of 22.8%. Thus, excellent selectivity (E >260) was achieved (Scheme 1).

Because the catechin increased the enantioselectivity of the lipase-catalyzed hydrolysis in practice and since catechin is classified as part of the polyphenol group, other catechin derivatives, including simple polyphenols, were tested for their ability to increase the enantioselectivities of the hydrolysis using lipase AH and the results are summarized in Table 1 and Scheme 2.

In addition to (+)-catechin, all the other catechin derivatives tested enhanced the stereoselectivity of the lipase-catalyzed reaction markedly. The ability to improve the stereoselectivity of lipase-catalyzed hydrolysis was found to be universal for catechin derivatives. (+)-Catechin is much more effective than (−)-catechin, although the latter still has sufficient ability to increase the enantioselectivity of the enzymatic reaction. Epicatechin, gallocatechin, and epigallocatechin are also effective. In addition, other polyphenols such as naringenin, quercetin, and myricetin are effective to a greater or lesser degree. The effect of polyphenon-100, which is an extract of green tea, is not remarkable: since polyphenone-100 has less than 17% catechin content, the additive would not be effective. Nevertheless, the fact that a broad range of catechin derivatives increases the enantioselectivity of the enzymatic reaction is noteworthy. All the catechin derivatives may inhibit hydrolysis, decrease the reaction rate, and increase the enantioselectivity. Catechins in general are therefore effective additives that increase the enantioselectivity of the reaction. This is the first reported example of stereochemical control of enzymatic reaction using catechin derivatives.

Next, we tested the effect of simple phenols on the stereoselectivity of the reaction. To our surprise, these



Without additive	37.4% conversion	41.4% ee	69.4% ee	E = 8
In the presence of (+)-catechin	22.8% conversion	29.3% ee	>99% ee	E >260

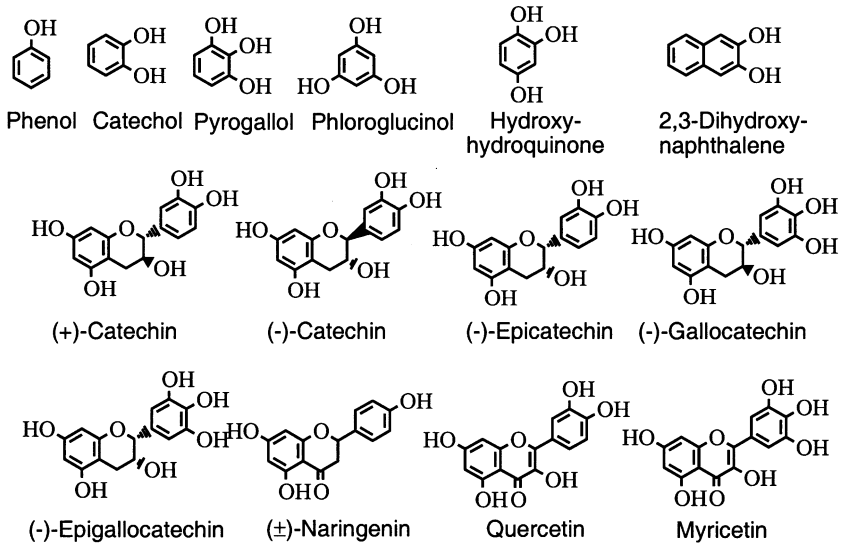
[substrate] = 6.0 mM, [lipase] = 20 mg, phosphate buffer (pH 7.0, 67 mM) = 2 ml, 30 °C, 96 h

Scheme 1.

Table 1. Lipase-catalyzed hydrolysis of **1b** in the presence of catechin derivative[a]

Additive[b]	Time/days	Ester (% e.e.)	Alcohol (% e.e.)	Conv. (%)	E value
None	4	41.4	69.4	37.4	8
None	7	53.0	6.7	88.7	2
(+)-Catechin	7	79.8	>99	44.5	>490
(−)-Catechin	7	51.9	98.3	34.0	200
Catechin	7	52.4	>99	34.6	>340
Epicatechin	7	67.6	98.2	40.8	220
Gallocatechin	7	72.1	>99	42.1	>430
Epigallocatechin	7	70.8	>99	41.5	>420
Naringenin	7	75.8	96.5	44.0	130
Quercetin	7	71.3	84.8	45.7	26
Myricetin	7	71.0	98.6	41.9	300
Polyphenon-100[d]	7	64.1	64.6	49.8	9
Phenol	4	19.1	49.0	28.1	4
Phenol[c]	4	37.7	>99	27.6	>290
Catechol	7	13.8	27.5	33.4	2
Catechol[c]	7	72.6	>99	42.3	>430
Pyrogallol	7	59.8	>99	37.7	>370
Phloroglucinol	3	54.4	90.1	37.7	33
Hydroxyhydroquinone	7	24.2	98.6	19.7	180
2,3-Dihydroxynaphthalene	7	18.5	98.1	15.9	120

[a] Reaction conditions : [**1b**] = 6.0 mM, [lipase Amano AH] = 20 mg, [solvent: 67 mM phosphate buffer pH 7.0] = 2 ml, reaction temperature = 30°C.
[b] 1.7 mM.
[c] 8.5 mM.
[d] Green tea extract, total catechin contents >80 wt%, consisting of: (−)-epicatechin 8.2%, (−)-epigallocatechin 8.5%, (−)-epigallocatechin gallate 52.5%, epicatechin gallate 5.6%, (−)-gallocatechin gallate 6.1%, (−)-catechin gallate 2.3%.



Scheme 2. Catechins.

phenol derivatives were also found to be very effective inhibitors. Phenols decrease the reactivity of the lipase and increase its enantioselectivity. In particular, pyrogallol is the most effective additive of those screened. To test if these additives acted as organic solvents, several organic solvents were used as additives in the lipase-catalyzed hydrolysis (Table 2). Organic solvents often change the stereoselecitivity of a lipase-catalyzed reaction.[14,18,28] However, the use of organic solvents as additives did not affect the reaction rates nor the enantioselectivities of the reactions at a concentration where catechin was observed to increase the selectivity. Thus, polyphenol compounds are active in controlling the stereoselectivity of the lipase-catalyzed reaction and an organic solvent effect is not operating in these reactions.

Although the precise mechanism for selective inhibition is not clear at present, it is possible that the catechin is incorporated into the lipase. It is known that organic molecules can be incorporated into an enzyme. For instance, after crystals of γ-chymotrypsin have been suspended in hexane, the crystals contained seven hexane molecules and two of them are found near the active site.[29] Also, the structure of a solvent used in lipase-catalyzed transesterification has been known to affect the enantioselectivity of the reaction.[28] Thus, organic compounds can penetrate an enzyme structure and could affect the rate and selectivity of the enzyme. Catechin has been reported to inhibit cyclin kinase[30] and xanthin oxidase.[31] In the case of urokinase, a hydrolytic enzyme, one of the catechins, epigallocatechin gallate, was incorporated, and inhibited the enzymatic reaction.[27]

Thus, catechins could be incorporated in the lipase and inhibit (though not completely) the reaction of the lipase with the ester. In the presence of catechins, the (*S*)-ester is thought to be difficult to be incorporated rather than the enantiomer or the hydrolysis of the incorporated substrate is limited to the (*R*)-ester. We propose that this method is a novel and useful method to increase the selectivity of the enzymatic reaction.

2.2. Use of catechins as additives in the kinetic resolution of racemic esters

The benzoates **2b**–**14b** were used as substrates in the kinetic resolution with lipase Amano AH and the effect of catechin was examined. The results are listed in Table 3 and Scheme 3. Selectivities of the enzymatic hydrolysis of **2b**–**4b** are high without any additives and

Table 2. Effect of organic compounds to lipase-catalyzed hydrolysis of **1b**[a]

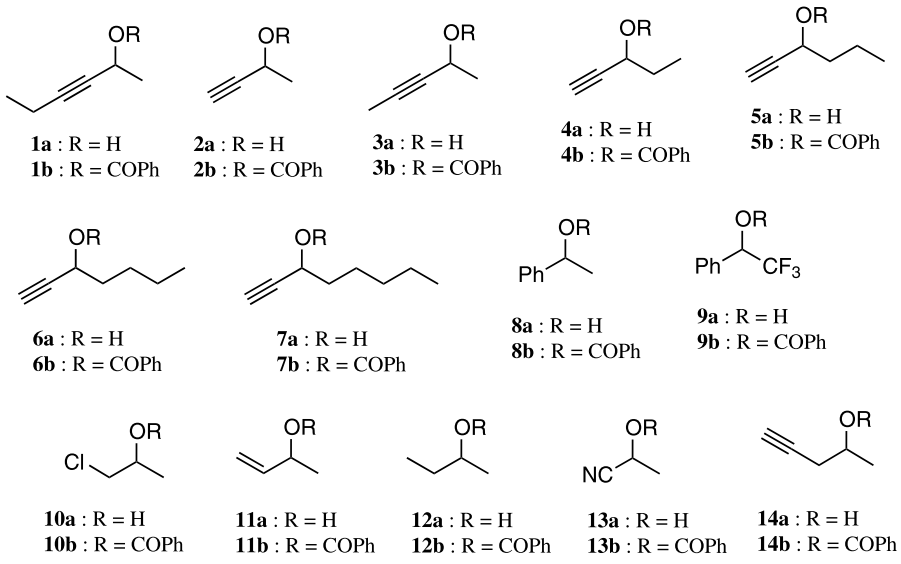
Additive	Ester (% e.e.)	Alcohol (% e.e.)	Conv. (%)	*E* value
MeOH	29.8	55.6	34.9	5
EtOH	29.1	80.2	26.6	12
Cyclohexane	40.1	72.1	35.7	9
THF	27.1	82.4	24.8	14
Dioxane	26.3	52.0	33.6	4
CH_3CN	34.7	72.0	32.5	9
iPr_2O	28.3	65.2	30.3	6
t-BuOMe	38.2	68.8	35.7	8
Et_2O	25.0	37.7	39.9	3
Acetone	39.3	53.5	42.4	5
Benzene	54.4	75.3	41.9	12
Toluene	23.4	77.7	23.2	10
CH_2Cl_2	36.1	82.8	30.4	15
$CHCl_3$	38.0	86.7	30.5	20

[a] Reaction conditions: [**1b**] = 6.0 mM, [lipase] = 20 mg, [additive] = 8.5 mM, [solvent; 67 mM phosphate buffer pH 7.0] = 2 ml, reaction temp. 30°C for 100 h.

Table 3. Lipase-catalyzed hydrolysis of benzoates[a]

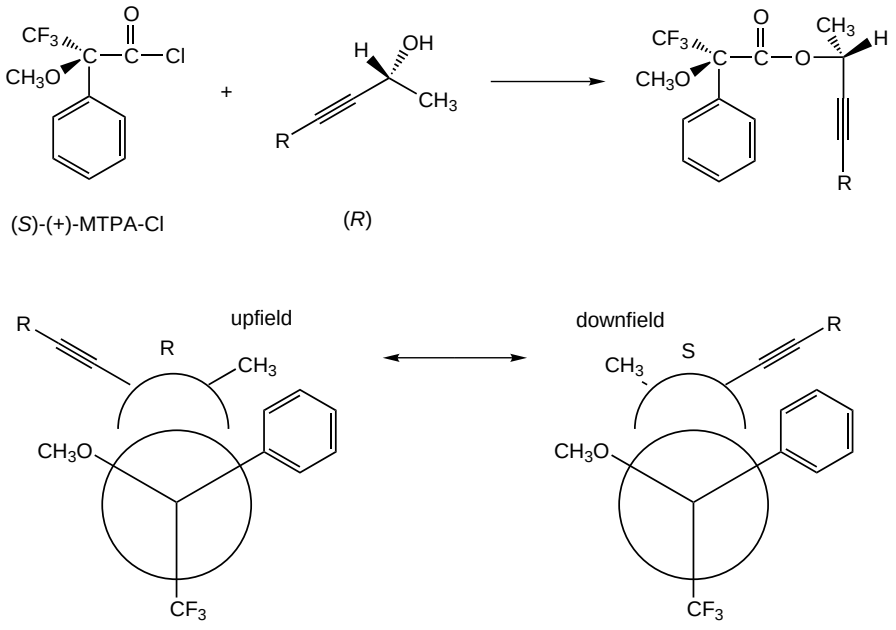
Substrate	(+)-Catechin (mM)	Time (days)	Ester (% ee)	Alcohol (% ee)	Conv. (%)	*E* value
1b	0	4	41.4(*S*)	69.4(*R*)[b]	37.4	8
1b	8.4	4	29.3(*S*)	>99(*R*)[b]	22.8	>260
2b	0	2	95.1(*S*)	97.6(*R*)[c,37]	49.4	310
2b	8.4	2	93.4(*S*)	97.5(*R*)[c,37]	48.9	300
3b	0	3	46.3(*S*)	>99(*R*)[b]	31.8	>300
3b	8.4	3	37.7(*S*)	>99(*R*)[b]	27.5	>290
4b	0	2	65.2	>99	39.7	>390
4b	17	2	76.2	>99	43.5	>460
5b	0	4	6.5	13.5	32.3	1.4
5b	17	4	27.2	90.0	23.7	25
6b	0	4	8.7(*R*)	25.9(*S*)	25.2	1.9
6b	17	6	7.4(*S*)	59.5(*R*)	11.0	4.2
7b	0	6	3.1	16.6	15.8	1.4
7b	17	6	8.4	76.1	9.9	8.0
8b	0	10	55.2(*R*)	48.6 (*S*)[c,33]	53.2	4.9
8b	17	10	5.3(*S*)	>99(*R*)[c,33]	5.2	>210
9b	0	10	77.8(*R*)	58.5(*S*)[c,38]	57.1	8.7
9b	17	10	5.5(*R*)	>99(*S*)[c,38]	5.3	>210
10b	0	2	60.4	71.2	45.9	11
10b	17	2	36.1	94.6	27.1	51
11b	0	4	19.1	38.6	33.1	2.7
11b	17	4	14.6	35.6	29.1	2.4
12b	0	0.17	6.4	25.5	20.1	1.8
12b	17	0.17	5.1	43.1	10.6	2.6
13b	0	0.25	30.5	33.4	47.7	2.7
13b	17	0.25	31.8	33.1	49.0	2.7
14b	0	4	9.3	53.3	14.8	3.6
14b	17	4	5.9	56.8	9.3	3.8

[a] Reaction conditions: [substrate] = 6.0 mM, [lipase Amano AH] = 20 mg, [solvent: 67 mM phosphate buffer pH 7.0] = 2 ml, reaction temperature = 30°C.
[b] Absolute configuration was determined by comparing its specific rotational value with that reported.
[c] Absolute configuration was determined by GC analysis by comparing with the authentic sample.



Scheme 3. Substrates.

additives did not change the selectivities. Enantioselectivities increased when **5b**–**10b** were used as substrates. Thus, catechin is more effective with substrates, which have acetylene-, phenyl-, and chloro-substituents. Lipase-catalyzed kinetic resolution of 1-phenylethanol using either hydrolysis[32] of the acetate or esterification with vinyl acetate[33] are known to be efficient and high *E*-values have been reported. On the contrary, kinetic resolution of the benzoate with lipase AH proceeds with low stereoselectivity and the addition of catechin



Scheme 4.

increases the stereoselectivity. The selectivity of the hydrolysis reactions of substrates **11b**–**14b** were not affected by the additive. Although **14b** has an acetylene unit, catechin did not increase the enantioselectivity of the reaction. For stereochemical control by catechin, it is necessary for the substrate to have an acetylenic unit directly connected to the hydroxyl group. Nevertheless, substrate specificity of the catechin-derived stereochemical control is broad enough to find useful applications in organic synthesis.

2.3. Absolute configuration of 2-benzoyloxy-3-hexyn-2-ol 1a

The absolute configuration of the alcohol product from the hydrolysis of **1b** was determined by derivation to the Mosher derivative. Mosher esters derived from (S)-(+)-MTPA-Cl and (*R*)- or (*S*)-isomer would give configurations such as those shown in Scheme 4.[34] In this configuration, the methyl group (CH-C$\underline{H}_3$) in the (*R*)-isomer should exhibit an upfield chemical shift relative to that of the (*S*)-isomer in the NMR spectrum. The esters of the (*R*)-isomers of **2a** and **3a**, the absolute configurations of which were already known through comparison of the specific rotation value with the literature data, exhibited the expected chemical shifts (Table 4). Since the methyl group of the Mosher ester of the product **1a** of the hydrolysis of **1b** appeared at higher field than its antipode, the configuration of the hydrolysis product was assigned as *R*.

3. Experimental

3.1. General

Instruments: Gas chromatographic analyses for the determination of e.e. of chiral alcohols were performed using a Shimadzu GC-14A Gas Chromatograph with a Shimadzu C-R6A Chromatopac equipped with a chiral GC-column (Chiraldex G-TA (Tokyo Kasei Kogyo Co., Ltd) or CP-Chirasil DEX-CB (Chrompack)). HPLC analyses were performed using a Hitachi 655 Liquid Chromatograph with a Hitachi D-2500 Chromato-Integrator and a Spectrophotometer 852 III equipped with a chiral HPLC-column (Chiralcel OD or Chiralpak AD (Daicel Chemical Ind., Ltd.)). ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-200 spectrometer. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. IR spectra were obtained from a Jasco FT/IR-5300. Elemental analyses were carried out with a Yanako MT-5 Elemental Analyzer. Melting points were measured using a Yanagimoto micromelting point apparatus and are uncorrected.

Materials: Lipase Amano AH was supplied by Amano Pharmaceutical Co., Ltd. Propargyl alcohol derivatives were purchased from Tokyo Kasei Kogyo Co., Ltd.

Table 4. ^{1}H NMR chemical shift of Mosher esters[a]

Mosher ester	-C$\underline{H}_3$(methyl)	C-$\underline{H}$(acetylene)	C-$\underline{H}$(methyn)
(±)-**2a**	1.53 d	2.48 d	5.64 m
	1.59 d	2.54 d	
(*R*)-**2a**	1.53 d	2.53 d	5.64 m
(±)-**3a**	1.48 d	1.80 d	5.62 m
	1.54 d	1.84 d	
(*R*)-**3a**	1.48 d	1.84 d	5.63 m
(±)-**1a**	1.49 d	2.20 m	5.62 m
	1.55 d		
Product **1a**[b]	1.49 d	2.22 m	5.65 m

[a] See Section 3.
[b] The hydrolyzed product of the enzymatic reaction.

Other materials were purchased from Nacalai Tesque, Inc, Tokyo Kasei Kogyo Co, Ltd, Wako Pure Chemical Industries, Ltd, and Aldrich Chemical Co, Inc.

3.1.1. 3-Benzoyloxy-1-butyne 2b. Benzoyl chloride (2.0 g, 14 mmol) was added to a solution of **2a** (1.0 g, 14 mmol) in pyridine (5 mL) at 0–5°C. The resulting mixture was stirred at room temperature for 2 h. Brine (30 mL) was added and ether (30 mL) was added to the mixture. The organic phase was separated and washed with 5% aqueous HCl, saturated aqueous brine, saturated aqueous $NaHCO_3$, and saturated aqueous brine, successively. The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified with silica gel column chromatography using hexane–ethyl acetate (10:1) as eluent to give white powder (2.1 g, 83%). Mp 44.9–45.1°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.63 (3H, d, $J=6.8$ Hz), 2.49 (1H, d, $J=2.2$ Hz), 5.68 (1H, m), 7.43 (2H, m), 7.53 (1H, m) and 8.06 (1H, m); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 165.3, 133.1, 129.7, 128.3, 82.1, 73.0, 60.5, and 21.3; IR (NaCl) $\nu=2114$ and 1713 cm^{-1}. Anal. calcd for $C_{11}H_{10}O_2$: C, 75.84; H, 5.79. Found: C, 75.79; H, 5.73%.

3.1.2. 2-Benzoyloxy-3-pentyne 3b. Prepared using the procedure described above. Yield: 1.1 g (97%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.58 (3H, d, $J=6.8$ Hz), 1.84 (3H, d, $J=2.0$ Hz), 5.65 (1H, m), 7.49 (3H, m) and 8.06 (2H, m). 50 MHz ^{13}C NMR ($CDCl_3$) δ (ppm) 162.0, 133.0, 130.1, 129.7, 128.3, 106.1, 81.2, 77.8, 61.4, 21.8 and 3.6. IR (NaCl) $\nu=2255$ and 1721 cm^{-1}. Anal. calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.27; H, 6.50%.

3.1.3. 2-Benzoyloxy-3-hexyne 1b. Prepared using the procedure described above. Yield: 0.58 g (87%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.13 (3H, t, $J=7.5$ Hz), 1.58 (3H, d, $J=6.8$ Hz), 2.22 (2H, m), 5.68 (1H, m), 7.43 (2H, m), 7.55 (1H, m) and 8.06 (2H, m); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 165.4, 132.9, 130.1, 129.6, 128.2, 86.8, 77.9, 61.3, 21.8, 13.6 and 12.3. IR (NaCl) $\nu=2245$ and 1723 cm^{-1}. Anal. calcd for $C_{13}H_{14}O_2$: C, 77.20; H, 6.98. Found: C, 77.33; H, 7.10%.

3.1.4. 3-Benzoyloxy-1-pentyne 4b. Prepared using the procedure described above. Yield 1.1 g (96%). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.12 (3H, t, $J=7.4$ Hz), 1.96 (2H, m), 2.50 (1H, d, $J=2.2$ Hz), 5.57 (1H, m), 7.46 (2H, m), 7.58 (1H, m) and 8.09 (2H, m); IR (NaCl) $\nu=2124$ and 1725 cm^{-1}. Anal. calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.47; H, 6.49%.

3.1.5. 3-Benzoyloxy-1-hexyne 5b. Prepared using the procedure described above. Yield 0.92 g (89%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 0.99 (3H, t, $J=7.3$ Hz), 1.59 (2H, m), 1.92 (2H, m), 2.48 (1H, d, $J=2.2$ Hz), 5.61 (1H, m), 7.45 (2H, m), 7.56 (1H, m) and 8.06 (2H, m); IR (NaCl) $\nu=2124$ and 1725 cm^{-1}. Anal. calcd for $C_{13}H_{14}O_2$: C, 77.20 H, 6.98. Found: C, 76.94; H, 7.00%.

3.1.6. 3-Benzoyloxy-1-heptyne 6b. Prepared using the procedure described above. Yield 0.71 g (73%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 0.86 (3H, t, $J=7.1$ Hz), 1.39 (4H, m), 1.83 (2H, m), 2.41 (1H, d, $J=2.2$ Hz), 5.52 (1H, m), 7.45 (3H, m) and 8.00 (2H, m); IR (NaCl) $\nu=2124$ and 1725 cm^{-1}. Anal. calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46. Found: C, 77.78; H, 7.53%.

3.1.7. 3-Benzoyloxy-1-octyne 7b. Prepared using the procedure described above. Yield 0.80 g (87%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 0.90 (3H, brt, $J=6.8$ Hz), 1.34 (4H, m), 1.55 (2H, m), 1.90 (2H, m), 2.48 (1H, d, $J=2.2$ Hz), 5.60 (1H, m), 7.48 (3H, m) and 8.08 (2H, m); IR (NaCl) $\nu=2122$ and 1725 cm^{-1}. Anal. calcd for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.42; H, 8.03%.

3.1.8. 1-Benzoyloxy-1-phenylethane 8b. Prepared using the procedure described above. Yield 0.87 g (94%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.67 (3H, d, $J=6.6$ Hz), 6.14 (1H, m), 7.43 (8H, m) and 8.08 (2H, m); IR (NaCl) $\nu=1719$ cm^{-1}. Anal. calcd for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24. Found: C, 79.50; H, 6.23%.

3.1.9. 1-Benzoyloxy-1-phenyl-2,2,2-trifluoroethane 9b. Prepared using the procedure described above. Yield 0.28 g (88%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 6.37 (1H, m), 7.52 (8H, m) and 8.13 (2H, m); IR (NaCl) $\nu=1740$ cm^{-1}. Anal. calcd for $C_{15}H_{11}O_2F_3$: C, 64.29; H, 3.96. Found: C, 64.37; H, 4.01%.

3.1.10. 1-Chloro-2-benzoyloxypropane 10b. Prepared using the procedure described above. Yield 0.83 g (79%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.47 (3H, d, $J=6.4$ Hz), 3.72 (2H, d, $J=5.2$ Hz), 5.36 (1H, m), 7.46 (2H, m), 7.58 (1H, m) and 8.07 (2H, m); IR (NaCl) $\nu=1721$ cm^{-1}. Anal. calcd for $C_{10}H_{11}ClO_2$: C, 60.46; H, 5.58. Found: C, 60.32; H, 5.44%.

3.1.11. 2-Benzoyloxy-3-butene 11b. Prepared using the procedure described above. Yield 1.1 g (89%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.46 (3H, d, $J=6.6$ Hz), 5.19 (1H, m), 5.34 (1H, m), 5.61 (1H, m), 5.97 (1H, m), 7.50 (3H, m) and 8.07 (2H, m); IR (NaCl) $\nu=1719$ cm^{-1}. Anal. calcd for $C_{11}H_{10}O_2$: C, 74.98; H, 6.86. Found: C, 74.77; H, 6.90%.

3.1.12. 2-Benzoyloxybutane 12b. Prepared using the procedure described above. Yield 1.1 g (94%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 0.98 (3H, t, $J=7.5$ Hz), 1.34 (3H, d, $J=6.6$ Hz), 1.69 (2H, m), 5.10 (1H, m), 7.49 (3H, m) and 8.05 (2H, m); IR (NaCl) $\nu=1717$ cm^{-1}. Anal. calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.09; H, 7.94%.

3.1.13. 2-Benzoyloxy-1-butyronitrile 13b. Prepared using the procedure described above. Yield 1.2 g (96%); ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.78 (3H, d, $J=7.0$ Hz), 5.65 (1H, m), 7.48 (2H, m), 7.62 (1H, m) and 8.05 (1H, m); IR (NaCl) $\nu=1732$ cm^{-1}. Anal. calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18. Found: C, 68.50; H, 5.15%.

3.1.14. 4-Benzoyloxy-1-pentyne 14b. Prepared using the procedure described above. Yield 1.0 g (90%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.47 (3H, d, $J=6.2$ Hz), 2.04 (1H, t, $J=2.7$ Hz), 2.60 (2H, m), 5.26 (1H, m), 7.44 (2H, m), 7.56 (1H, m) and 8.06 (2H, m); IR (NaCl) $\nu=2124$ and 1719 cm^{-1}. Anal. calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.39; H, 6.44%.

3.1.15. Enzymatic hydrolysis of 3b. Preparation of mixture of (*R*)-**3a** and (*S*)-**3b**. A mixture of **3b** (0.46 g, 2.4 mmol), Amano AH (4.0 g) and (+)-catechin (1.0 g, 3.4 mmol) in phosphate buffer (67 mM, pH = 7.0, 400 mL) was stirred at 30°C for 6 days. The reaction mixture was extracted three times with ether. The organic phase was collected and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was distilled under reduced pressure to afford optically active **3a** as a pale yellow oil (36 mg, 18%, 99% e.e.). The resulting distillation residue was purified by silica gel column chromatography using hexane–ethyl acetate (10:1) as eluent to give optically active **3b** as pale yellow oil (215 mg, 47%, 89% e.e.). Compound **3a**: $[\alpha]_D^{25}=42.7$ (*c* 0.91, Et_2O) (lit.)[35] (*R*)-**3a** $[\alpha]_D^{25}=+20$ (*c* 0.72, $CHCl_3$). Compound **3b**: yield $[\alpha]_D^{25}=-20.7$ (*c* 1.2, $CHCl_3$).

3.1.16. Enzymatic hydrolysis of 1b. Preparation of mixture of (*R*)-**1a** and (*S*)-**1b**: **1a**: yield 53 mg (23%, 98% e.e.). $[\alpha]_D^{25}=+27.2$ (*c* 0.58, Et_2O). Compound **1b**: yield 231 mg (48%, 95% e.e.), $[\alpha]_D^{25}=-20.1$ (*c* 1.1, $CHCl_3$).

3.1.17. Enzymatic Hydrolysis of 6b. Preparation of mixture of (*R*)-**6a** and (*S*)-**6b**: (*R*)-**6a**: yield 22 mg (8.2%, 46% e.e.) $[\alpha]_D^{25}=+14.2$ (*c* 1.0, Et_2O) (lit.)[36] (*S*)-**6a** (14% e.e.) $[\alpha]_D^{23}=-3.2$ (*c* 2.29, Et_2O). (*S*)-**6b**: yield 180 mg (35%, 21% e.e.).

3.2. Preparation of Mosher ester (MTPA(α-methoxy-α-trifluoromethylphenylacetyl) derivatives)[34]

(*S*)-(+)-MTPA-Cl (100 mg, 0.40 mmol) was added to a solution of the alcohol (0.1 mmol) and pyridine (0.1 mL) in carbon tetrachloride (1.0 mL) at 0–5°C. The resulting mixture was stirred at room temperature for 2 h. Brine (30 mL) was added and ether (30 mL) was added to the mixture. The organic phase was separated and washed with 5% aqueous HCl, saturated aqueous brine, saturated aqueous $NaHCO_3$, and saturated aqueous brine, successively. The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The ^{1}H NMR spectrum of this residue was then obtained.

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