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Highly Enantioselective Synthesis of Arylaliphatic Tertiary Alcohols using Mutants of an Esterase from *Bacillus subtilis*

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Abstract: The kinetic resolution of a series of acetates of arylaliphatic tertiary alcohols was studied using recombinant esterase variants from *Bacillus subtilis* (BS2) expressed in *E. coli*. Highest enantioselectivities ($E > 100$) were achieved in the synthesis of 1,1,1-trifluoro-2-phenylbut-3-yn-2-ol and three *para*-substituted analogues using BS2 mutant G105A. With mutant E188D only two compounds were converted with $E > 100$. For a thiophene analogue or compounds with small variations in the aliphatic chain substantially lower conversions and/or enantioselectivity were observed, which also varied with the BS2 variant used. Thus, small changes in the substrate structure and point mutations in the esterase had a remarkable influence on both activity and enantioselectivity.

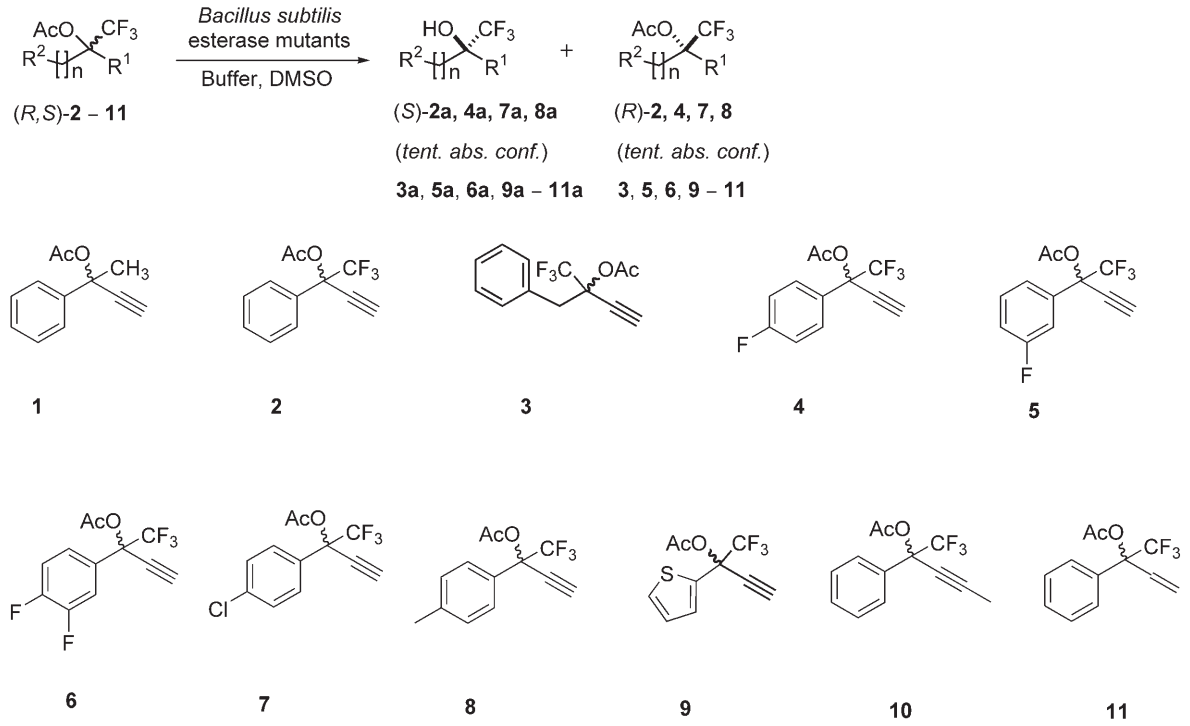
Keywords: enantioselectivity; enzyme catalysis; hydrolases; steric hindrance; tertiary alcohols

Introduction

Optically active tertiary alcohols are an important class of organic compounds with valuable applications in organic synthesis.[1,2] For instance, the tertiary α-acetylenic alcohols 2-phenylbut-3-yn-1-ol (**1a**) and 1,1,1-trifluoro-2-phenylbut-1-yn-3-ol (**2a**) were recently applied for the synthesis of A_{2A} receptor antagonists that were shown to be orally active in a mouse catalepsy model.[3] In addition, fluorine-containing optically active alcohols are also of great interest due to their potential use as ferroelectric liquid crystals and drugs.[4]

Carboxylester hydrolases (lipases, EC 3.1.1.3, esterases, EC 3.1.1.1) represent a class of versatile biocatalysts for the preparation of enantiopure compounds, especially of optically pure secondary alcohols and to a smaller extent also for the resolution of primary alcohols and carboxylic acids.[5] In contrast, tertiary alcohols (TAs) are not accepted as substrates by almost all carboxylester hydrolases due to their sterically demanding structure. We recently identified that a GGG(A)X-motif located in the active site region determines activity towards tertiary alcohols and that lipases or esterases with the much more common GX-motif are inactive.[6] The GGG(A)X-motif is located in the oxyanion hole pocket, which is required for the stabilisation of the oxyanion in the tetrahedral intermediate formed during the catalytic cycle of ester hydrolysis.[7] Unfortunately, the enantioselectivity of all GGG(A)X-hydrolases investigated so far has been very low. For instance, tertiary α-acetylenic acetate esters could only be resolved by *Candida rugosa* lipase with low enantioselectivity.[8,9]

For an esterase from *Bacillus subtilis* (BS2) expressed recombinantly in *E. coli*, we determined an $E = 3$ towards 2-phenylbut-3-yn-1-yl acetate (**1**) (Scheme 1).[6] In a molecular modelling study a mutant was predicted, that catalysed the hydrolysis of **1** at a 6-fold increased enantioselectivity ($E = 19$).[10] This value represented a notable increase but was still too low for preparative applications. One reason for the low enantioselectivity is a non-enzymatic and therefore not enantioselective autohydrolysis of **1** *via* an S_N1 mechanism.[8] This autohydrolysis could be suppressed by addition of the water-miscible co-solvent DMSO, resulting in an increased E value in the kinetic resolution of **1** ($E = 54$ at 20% v/v DMSO).[11] A further molecular modelling study identified another mutant with a substantially increased enantioselectivity. This BS2-mutant E188D hydrolysed **1** with an enantioselectivity of $E = 45$ (with 20% v/v DMSO)[11]. A valuable alternative to the addition of a cosolvent is the use of the trifluoromethyl analogue **2**, which is stable in aqueous solution due to the electron-withdrawing effect of the CF_3 group. This compound was resolved with excellent selectivity ($E > 100$) by both BS2 mutants.[11]



Scheme 1. Enzymatic kinetic resolution of arylaliphatic tertiary alcohol acetates catalysed by BS2 esterase mutants G105A and E188D.

In this paper, we have extended these studies to a series of the tertiary alcohol acetates **2**–**11** using both BS2 mutants G105A and E188D in order to identify more substrates for this kinetic resolution and to get further insights into the substrate range and selectivity of these esterases variants.

Results and Discussion

The tertiary alcohols **2a**–**11a** were synthesised by Grignard reactions from the corresponding ketones and ethynylmagnesium bromide.[8] Subsequent acetylation in the presence of dimethylaminopyridine yielded the corresponding acetates **2**–**11**. For all compounds except **10a** a method for the separation of their enantiomers by GC analysis could be established (see Experimental Section). Next, the acetates were subjected to analytical-scale kinetic resolutions using both BS2 esterase mutants (E188D or G105 A) at pH 7.5, 37 °C for 15 min in the presence of 10 % (G105A) or 20 % (E188D) DMSO (Scheme 1).

These experiments revealed that the activity and enantioselectivity of both mutants differed considerably with variation of the substrate structure (Table 1). Mutant G105A showed excellent enantioselectivity ($E > 100$) towards **2** and all *p*-substituted substrates **4**, **7** and **8**. In contrast, all other compounds were hardly converted at all or a substantial decrease in enantioselectivity (44 % conversion, but $E = 6$ for acetate **3**) took place. Introduction of a fluoro-substituent in the *m*-position (**5**) rather than the *p*-position (**4**) resulted in a considerably decreased activity and lower enantioselectivity and the double-substituted compound **6** was not converted at all. Also, the presence of a thiophene instead of a phenyl ring lowered the E value to only $E = 4$. Whereas mutant E188D showed similar activity and enantioselectivity as G105A in the resolution of **2**,[11] it was much less active and selective towards the series of acetates studied in this work. In contrast to G105A, only compound **4** was converted at high enantioselectivity, but the conversion was low. The thiophene derivative **9** was hydrolysed with high conversion (60 %) but at only moderate enantioselectivity ($E = 11$). Interestingly, the α-acetylenic group seems to be essential for catalysis as neither the methyl-substituted acetylenic acetate **10** nor the double bond derivative **11** were converted by both esterase mutants. This confirms previous reports using *Candida rugosa* lipase.[8,9]

Next, preparative 100 mg-scale resolutions of acetates **4**, **7** and **8** and a 500 mg-scale resolution of **2** were performed with mutant G105A in order to confirm the results from the analytical scale experiments (Table 2).

The absolute configuration of several products was inferred from the known enantiopreference of BS2 G105A towards the analogue substrate **1**.[6] Thus, for **2** ,**4** ,**7** and **8**, an (*S*)-preference of the enzyme was assumed. In the case of **3**, **5**, **6** and **9**–**11**, however, the

Table 1. Results of analytical scale kinetic resolutions of acetates **2–11**.

Substrate	Mutant	Units[a]	Enantiomeric excess [% ee_S][b]	[% ee_P][b]	Conversion [%][c]	E [ ][c]
2	G105A	12	94	96	49	>100
3	G105A	12	45	57	44	6
4	G105A	12	67	99	40	>100
5	G105A	12	7	83	8	12
6	G105A	12	n.d.[d]	n.d.	<1	n.d.
7	G105A	12	77	99	44	>100
8	G105A	20	57	>99	36	>100
9	G105 A	12	7	60	10	4
10	G105A	20	n.d.	32	4	n.d.
11	G105A	20	n.d.	n.d.	<1	n.d.
2	E188D	10	99	94	51	>100
3	E188D	10	n.d.	n.d.	<1	n.d.
4	E188D	10	19	99	16	>100
5	E188D	10	n.d.	n.d.	1	n.d.
6	E188D	10	n.d.	n.d.	<1	n.d.
7	E188D	10	4	99	4	n.d.
8	E188D	10	n.d.	n.d.	<1	n.d.
9	E188D	10	88	59	60	11
10	E188D	20	n.d.	n.d.	<1	n.d.
11	E188D	20	n.d.	n.d.	<1	n.d.

[a] The specific activity of BS2 G105A in the hydrolysis of pNPA was 0.8 U mg^{-1} lyophilisate and 0.3 U mg^{-1} for BS2 E188D.
[b] Determined by chiral GC analyses.
[c] Conversion after 15 min, E values were calculated from ee_S and ee_P.
[d] n.d.: not determined.

Table 2. Results of BS2 G105A-catalysed preparative scale kinetic resolutions of acetates **2**, **4**, **7** and **8**.

Substrate	Acetate [%][a]	[% ee_S][b]	Alcohol [%][a]	[% ee_P][b]	Conversion [%][c]	E [ ][c]
2	40	95	49	95	51	>100
4	39	80	21	>99	44	>100
7	47	>99	39	97	51	>100
8	43	97	48	>99	50	>100

[a] Isolated yield.
[b] Determined by chiral GC analysis.
[c] Conversion after 15 min, E values were calculated from ee_S and ee_P.

lower enantioselectivity and/or activity of the BS2 mutants did not allow a tentative assignment of the absolute configuration.

Molecular modelling was used to get an insight into the substrate specificities and enantioselectivities of the esterase mutants studied. Glycine 105 occupies a position close to the entrance of the active site of esterase BS2. A substitution by alanine decreases the available space for the larger substituent of the tetrahedral intermediate of the slower reacting enantiomer (Figure 1). Hence, this might explain the observed increase in enantioselectivity.[10] However, the effect of a substitution of glutamine 188 by aspartate cannot be explained by steric hindrance. Glu188 is neighbouring the catalytically active serine (Figure 1) and the increase in enantioselectivity for **1** and **2** compared to

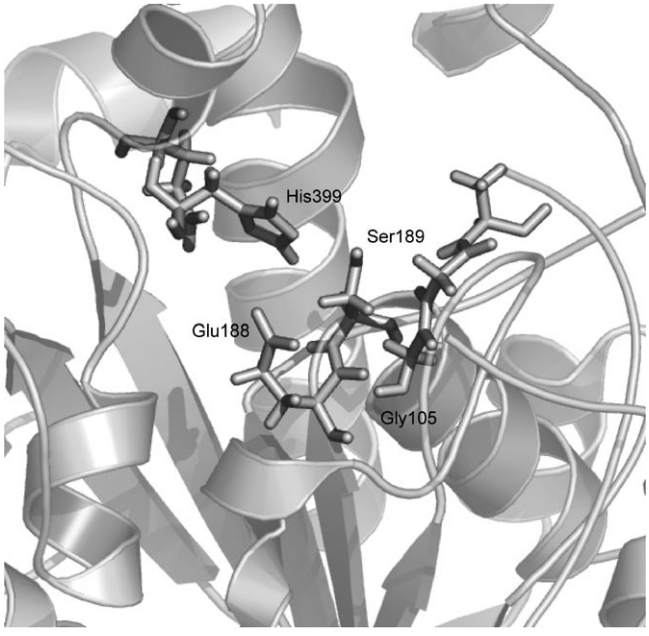


Figure 1. View of the active site of BS2 esterase. Ser189 and His399 from the catalytic site and the amino acids bearing the mutations Gly105 and Glu188 are highlighted.

the BS2 wild-type was attributed to an involvement of this amino acid in the hydrogen bonding network of the catalytic site during catalysis.[11] However, the model cannot explain why E188D did not convert substrates **3, 5**, **7** and **8** towards which G105A is still active.

Conclusions

In summary, we could show in this work that several acetates of α-acetylenic tertiary alcohol could be resolved by BS2 esterase mutants in excellent yields and optical purities. However, the substrate range of these esterases appears to be rather narrow, as small variations in the substrate structure resulted in considerable losses of enzyme activity and/or enantioselectivity.

Experimental Section

General Remarks

All chemicals were purchased from Fluka (Buchs, Switzerland), Sigma (Steinheim, Germany) and Merck (Darmstadt, Germany), unless stated otherwise. NMR spectroscopy experiments were performed on an ARX300 (300.13 MHz for ^{1}H and 75.5 MHz for ^{13}C), a Bruker Avance II (600.7 MHz for ^{1}H and 150.1 MHz for ^{13}C) or a Bruker Avance 600 device (600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C, Bruker, Karlsruhe, Germany), using the δ scale (ppm) for chemical shifts; ^{13}C NMR spectra were edited using DEPT techniques. Optical rotations were recorded on a Polarimat (Carl Zeiss, Jena). Mass spectra were recorded on a QP2010 GC-MS device (electron impact, 70 eV, Shimadzu, Japan).

Molecular Modelling

Molecular modelling of BS2 esterase was performed as described.[11]

General Procedure for the Synthesis of Tertiary Alcohols

Tertiary alcohols were prepared by adapting a previously reported procedure.[8] A solution of 30 mmol ketone (1.0 equiv.) in tetrahydrofuran (20 mL, THF) was added to a refluxing solution of ethynylmagnesium bromide (1.2 equivs.) in THF (70 mL). The mixture was refluxed until the ketone was consumed (control *via* thin layer chromatography). Water (20 mL) was added to the cooled reaction mixture and the product was extracted into diethyl ether (3× 100 mL), washed with saturated brine (100 mL) and evaporated under reduced pressure. The residue was distilled or purified by column chromatography.

(*R*,*S*)-1,1,1-Trifluoro-2-phenylbut-3-yn-2-ol (2a): Obtained as a colourless oil after bulb-to-bulb distillation; yield: 4.4 g (22 mmol, 76%). The spectroscopic data matched literature data.[11]

(*R*,*S*)-1,1,1-Trifluoro-2-benzylbut-3-yn-2-ol (3a): Obtained as a colourless oil after bulb-to-bulb distillation; yield: 3.9 g (18 mmol, 67%). ^{1}H NMR: δ=2.5 (1H, s, CCH), 2.6 (1H, s, OH), 3.1 (2H, dd. CH_2), 7.3 (5H, m, Ar-H); ^{13}C NMR: δ= 40.6 (s), 71.7 (q, J=32 Hz), 77.3 (s), 78.3 (s), 123.1 (q, J= 278 Hz), 127.6, 128.2, 131.1, 133.1; MS (EI): m/z=214 (M^+), 196, 156, 91, 65.

(*R*,*S*)-1,1,1-Trifluoro-2-(4′-fluorophenyl)-but-3-yn-2-ol (4a): Obtained as a colourless oil after bulb-to-bulb distillation; yield: 2.8 g (13 mmol, 50%). ^{1}H NMR: δ=2.8 (1H, s, CCH), 3.4 (1H, s, OH) 7.1, 7,7 (4H, m, Ar-H); ^{13}C NMR: δ=72.4 (q, J=33 Hz), 76.9 (s), 79.3 (s), 115.4 (d, J=21 Hz), 123.0 (q, J=284 Hz), 129.2, (d, J=8 Hz), 130.5 (s), 163.5 (d, J=249 Hz); MS (EI): m/z=218 (M^+), 201, 149, 123, 95, 53.

(*R*,*S*)-1,1,1-Trifluoro-2-(3′-fluorophenyl)-but-3-yn-2-ol (5a): Obtained as a yellowish oil after bulb-to-bulb distillation; yield: 4.5 g (21 mmol, 80%). ^{1}H NMR: δ=2.8 (1H, s, CH), 3.5 (1H, s, OH), 7.1 (1H, m, Ar-H), 7.5 (3H, m, Ar-H); ^{13}C NMR: δ=72.5 (q, J=33 Hz), 76.9 (s),79.0 (s), 114.5 (d, J=24 Hz), 116.7 (d, J=21 Hz), 122.9 (s), 123.0 (q, J= 286 Hz), 137.13 (d, J=8 Hz), 162.5 (d, J=247 Hz); MS (EI): m/z=218 (M^+), 200, 149, 123, 53.

(*R*,*S*)-1,1,1-Trifluoro-2-(3′,4′-difluorophenyl)-but-3-yn-2-ol (6a): Obtained as a colourless oil after bulb-to-bulb distillation; yield: 860 mg (3.6 mmol, 88%). ^{1}H NMR: δ=2.9 (1H, s, CH), 3.4 (1H, s, OH), 7.1, 7.6 (3H, m, Ar-H); ^{13}C NMR: δ=72.0 (q, J=34 Hz), 77.7 (s), 78.8 (s), 116.7 (d, J=14 Hz), 117.3 (d, J=12 Hz), 123.6 (s), 124.6 (d, J=285 Hz), 131.6 (s), 150.2 (dd, J=22 Hz, J=250 Hz), 151.4 (dd, J=16 Hz, J=254 Hz); MS (EI): m/z=236 (M^+), 219, 197, 187, 167.

(*R*,*S*)-1,1,1-Trifluoro-2-(4′-chlorophenyl)-but-3-yn-2-ol (7a): Obtained as a yellowish oil after bulb-to-bulb distillation; yield: 2.2 g (9.4 mmol, 39%). ^{1}H NMR: δ=2.8 (1H, s, CCH), 3.4 (1H, s, OH), 7.4, 7.7 (4H, m, Ar-H); ^{13}C NMR: δ=72.5, (q, J=32 Hz), 74.6 (s), 79.1 (s), 121.9 (q, J= 285 Hz), 128.3 (s), 128.6 (s), 133.2 (s), 135.9 (s); MS (EI): m/z=234 (M^+), 217, 195, 165, 53.

(*R,S*)-1,1,1-Trifluoro-2-(4′-methylphenyl)-but-3-yn-2-ol (8a): Obtained as a colourless oil after bulb-to-bulb distillation; yield: 720 mg (3.4 mmol 63%). ^{1}H NMR: δ=2.4 (3H, s, CH_3), 2.8 (1H, s, OH), 3.0 (1H, s, CCH), 7.2, 7.6 (5H, m, Ar-H); ^{13}C NMR: δ=21.2 (s), 72.7 (q, J=33 Hz), 77.0 (s), 79.6 (s), 123.2 (q, J=285 Hz), 125.9 (s), 129.0 (s), 131.6 (s), 139.7 (s); MS (EI): m/z=214 (M^+), 145, 115, 91, 53.

(*R*,*S*)-1,1,1-Trifluoro-2-thiophenbut-3-yn-2-ol (9a): Obtained as a colourless oil after bulb-to-bulb distillation; yield: 2.6 g (13 mmol, 47%). ^{1}H NMR: δ=2.8 (1H, s, OH), 3.3 (1H, s, CH), 7.0 [1H, m, C(CH)S], 7.4 [2H, m, SC(CH)]; ^{13}C NMR: δ=76.6 (s), 76.8 (q, J=285 Hz), 78.7 (s), 122.6 (q, J=285 Hz), 125.5 (s), 126.9 (s), 128.2 (s), 137.9 (s); MS (EI): m/z=206 (M^+), 189, 167, 137, 53.

(*R*,*S*)-1,1,1-Trifluoro-2-phenylpent-3-yn-2-ol (10a): Obtained as a colourless oil after bulb-to-bulb distillation; yield: 3.9 g (18 mmol, 63%). ^{1}H NMR: δ=2.0 (3H, s, CH_3), 2.9 (1H, s, OH), 7.4, 7.7 (5H, m, Ar-H); ^{13}C NMR: δ=3.7 (s), 72.9 (q, J=32 Hz), 75.4 (s), 85.3 (s), 123.4 (q, J= 285 Hz), 127.2 (s), 128.1 (s), 129.1 (s), 135.6 (s); MS (EI): m/z=214 (M^+), 195, 177, 164, 145, 67.

(*R*,*S*)-1,1,1-Trifluoro-2-phenylbut-3-en-2-ol (11a): Obtained as a colourless oil after column chromatography; yield: 4.0 g (19.8 mmol, 70%). ^{1}H NMR: δ=2.7 (1H, s, OH), 5.6 (2H, dd, CH_2), 6.5 (1H, dd, CH), 7.4, 7.6 (5H, m,

Ar-H); ^{13}C NMR: δ = 77.5 (q, J = 29 Hz), 118.3 (s), 124.7 (q, J = 283 Hz), 126.9 (s), 128.0 (s), 128.5 (s), 135.6 (s), 136.9 (s); MS (EI): m/z = 202 (M^+), 165, 133, 55.

General Procedure for the Synthesis of Tertiary Alcohol Acetates

To 4.5 mmol of the alcohol and dimethylaminopyridine (6 equivs.) in dry dichloromethane (60 mL), acetyl chloride (4 equivs.) was added dropwise and the solution was stirred overnight. The mixture was washed with 1 M HCl (3 × 100 mL) and distilled water (3 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4 before the solvent was removed under reduced pressure. For purification column chromatography was performed.

(*R*,*S*)-1,1,1-Trifluoro-2-phenylbut-3-yn-2-yl acetate (2): Obtained as a colourless oil after column chromatography; yield: 0.92 g (3.8 mmol, 82 %). The spectroscopic data matched literature data.[11]

(*R*,*S*)-1,1,1-Trifluoro-2-benzylbut-3-yn-2-yl acetate (3): Obtained as a colourless oil after column chromatography; yield: 652.3 mg (3.0 mmol, 24 %). ^{1}H NMR: δ = 2.1 (3 H, s, CH_3), 2.8 (1 H, s, CCH), 3.4 (2 H, dd, CH_2), 7.2 (5 H, m, Ar-H); ^{13}C NMR: δ = 21.5 (s), 39.7 (s), 75.0 (s), 75.2 (q, J = 31 Hz), 79.2 (s), 122.6 (q, J = 288 Hz), 127.6 (s), 128.1 (s), 130.9 (s), 132.8 (s), 167.6 (s); MS (EI): m/z = 214 ($M^+ - CH_3CO$), 196, 146, 91, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-(4′-fluorophenyl)-but-3-yn-2-yl acetate (4): Obtained as a yellow oil after column chromatography; yield: 1.0 g (3.8 mmol, 84 %). ^{1}H NMR: δ = 2.2 (3 H, s, CH_3), 2.9 (1 H, s, CCH), 7.1, 7.6 (4 H, m, Ar-H); ^{13}C NMR: δ = 21.3 (s), 74.9 (s), 76.3 (q, J = 34 Hz), 79.0 (s), 115.5 (d, J = 22 Hz), 122.1 (q, J = 284 Hz),128.3 (s), 129.0 (d, J = 8 Hz), 163.6 (d, J = 249 Hz), 166.8 (s); MS (EI): m/z = 260 (M^+), 217, 201, 189, 162, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-(3′-fluorophenyl)-but-3-yn-2-acetate (5): Obtained as a colourless oil after column chromatography; yield: 1.18 g (4.9 mmol, 97 %). ^{1}H NMR: δ = 2.1 (3 H, s, CH_3), 2.9 (1 H, s, CH), 7.1, 7.4 (4 H, m, Ar-H); ^{13}C NMR: δ = 21.2 (s), 74.7 (s), 76.2 (q, J = 32 Hz), 79.0 (s), 114.4 (d, J = 26 Hz), 117.0 (d, J = 21 Hz), 122.6 (s) 130.1 (d, J = 8 Hz), 182.7 (d, J = 247 Hz), 166.78; MS (EI): m/z = 245 ($M^+ - CH_3$), 225, 218, 201, 198, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-(3′,4′-difluorophenyl)-but-3-yn-2-yl acetate (6): Obtained as a colourless oil after column chromatography; yield: 326 mg (1.2 mmol, 63 %). ^{1}H NMR: δ = 2.2 (3 H, s, CH_3), 3.0 (1 H, s, CH), 7.2, 7.5 (3 H, m, Ar-H); ^{13}C NMR: δ = 21.1 (s), 74.4 (s), 76.2 (q, J = 29 Hz), 79.3 (s), 116.7 (d, J = 20 Hz), 117.5 (d, J = 18 Hz), 123.6 (s), 124.6 (q, J = 286 Hz), 129.4 (s), 150.3 (dd, J = 11 Hz, J = 256 Hz), 151.3 (dd, J = 8 Hz, J = 248 Hz), 166.7 (s). MS (EI): m/z = 278 (M^+), 243, 236, 219, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-(4′-chlorophenyl)-but-3-yn-2-yl acetate (7): Obtained as a yellow oil after column chromatography; yield: 1.0 g (3.6 mmol, 84 %). ^{1}H NMR: δ = 2.1 (3 H, s, CH_3), 2.9 (1 H, s, CCH), 7.4, 7.6 (4 H, m, Ar-H); ^{13}C NMR: δ = 21.1 (s), 74.7 (s), 76.6 (q, J = 32 Hz), 79.1 (s) 121.9 (q, J = 284), 128.3 (s), 128.8 (s), 130.9 (s), 136.1 (s), 166.7 (s); MS (EI): m/z = 276 (M^+), 233, 217, 198, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-(4′-methylphenyl)-but-3-yn-2-yl acetate (8): Obtained as a colourless oil after column chromatography; yield: 284 mg (1.1 mmol, 81 %). ^{1}H NMR: δ = 2.1 (3 H, s, CH_3), 2.3 (3 H, s, CH_3), 2.9 (1 H, s, CCH), 7.2, 7.5 (4 H, m, Ar-H); ^{13}C NMR: δ = 21.2 (s), 21.3 (s), 75.3 (s), 76.7 (q, J = 32 Hz), 78.6 (s), 122.1 (q, J = 284 Hz), 127.8 (s), 129.0 (s), 129.4 (s), 139.8 (s), 166.9 (s); MS (EI): m/z = 256 (M^+), 241, 213, 197, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-thiophenbut-3-yn-2-yl acetate (9): Obtained as a colourless oil after column chromatography; yield: 656 mg, 2.6 mmol (65 %) ^{1}H NMR: δ = 2.1 (3 H, s, CH_3), 2.9 (1 H, s, CCH), 7.0 [1 H, m, C(CH)S], 7.4 [2 H, m, SC(CH)]; ^{13}C NMR: δ = 21.3 (s), 73.9 (q, J = 34 Hz), 74.8 (s), 78.3 (s), 125.3 (q, J = 283 Hz), 126.9 (s), 128.0 (s), 129.2 (s), 135.3 (s), 166.8 (s); MS (EI): m/z = 248 (M^+), 228, 206, 189, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-phenylpent-3-yn-2-yl acetate (10): Obtained as a colourless oil after column chromatography; yield: 746 mg (2.9 mmol, 71 %). ^{1}H NMR: δ = 2.0 (3 H, s, CC-CH_3), 2.2 (3 H, s, CH_3), 2.9 (1 H, s, OH), 7.4, 7.6 (5 H, m, Ar-H); ^{13}C NMR: δ = 3.8 (s), 21.4 (s), 71.0 (s), 77.8 (q, J = 32), 87.3 (s), 122.4 (q, J = 284), 127.3 (s), 128.0 (s), 129.4 (s), 133.4 (s), 166.8 (s); MS (EI): m/z = 256 (M^+), 241, 213, 197, 43.

(*R*,*S*)-1,1,1-Trifluoro-2-Phenylbut-3-en-2-yl acetate (11): Obtained as a colourless oil after column chromatography; yield: 547 mg (2.3 mmol, 47 %). ^{1}H NMR: δ = 2.2 (3 H, s, CH_3), 5.6 (2 H, m, CH_2), 6.6 (1 H, dd, CH), 7.4 (5 H, m, Ar-H); ^{13}C NMR: δ = 21.9 (s), 83.2 (q, J = 32 Hz), 123.6 (q, J = 286 Hz), 126.5 (s), 127.2 (s), 128.6 (s), 129.2 (s), 130.9 (s), 134.2 (s), 167.6 (s); MS (EI): m/z = 244 (M^+), 202, 184, 165, 43.

General Procedure for Esterase-Catalyzed Small-Scale Resolutions

To a stirred solution of acetate (25 mM) in phosphate buffer (100 mM, pH 7.5) and the appropriate amount of cosolvent DMSO [10–20 % (v/v)], the appropriate amount of esterase solution was added to a total volume of 1 mL. The reaction mixture was stirred in a thermoshaker (Eppendorf, Hamburg, Germany) at 37 °C for 15 min. The reaction mixture was extracted twice with 400 μL dichloromethane, the combined organic layers were dried over anhydrous sodium sulphate and the organic solvent was removed under nitrogen. Enantioselectivity and conversion were calculated according to Chen et al.[12]

General Procedure for Esterase-Catalyzed Kinetic Resolution on a Preparative Scale

BS2 G105A lyophilisate with an activity of 0.8 U/mg (1500 U for **2** and 300 U for **4**, **7** and **8**, respectively) was added to a stirred solution of ester (2.3 mmol for **2**, 0.4 mmol for **4**, **7** and **8**) in 10% DMSO (v/v) and phosphate buffer (100 mM, pH 7.5) to a total volume of 100 mL for **2** and 20 mL for **4**, **7** and **8**, respectively. The reaction mixture was stirred for 15 min at 30 °C. The reaction mixture was extracted twice with 10 mL dichloromethane. The organic layers were combined and washed with brine (2 × 10 mL), water (2 × 10 mL) and dried over anhydrous $NaHSO_4$. The solvent was removed under reduced pressure. Purification was performed by column chromatography.

(−)-1,1,1-Trifluoro-2-phenyl-but-3-yn-2-ol (2a): Yield: 50 % (227 mg); 95 % *ee*; $[\alpha]_D^{21}$: −3.3° ($CHCl_3$, *c* 1.8).

(+)-1,1,1-Trifluoro-2-phenyl-but-3-yn-2-yl acetate (2): Yield: 40% (220 mg); 95% *ee*; $[\alpha]_D^{21}$: +32.6° ($CHCl_3$, *c* 1.2).

(−)-1,1,1-Trifluoro-2-(4′-fluorophenyl)-but-3-yn-2-ol (4a): Yield: 21% (18 mg); 99% *ee*; $[\alpha]_D^{21}$: −6.7° ($CHCl_3$, *c* 1.8).

(−)-1,1,1-Trifluoro-2-(4′-fluorophenyl)-but-3-yn-2-yl acetate (4): Yield: 39% (40 mg); 80% *ee*; $[\alpha]_D^{21}$: −2.9 ($CHCl_3$, *c* 1.4).

(+)-1,1,1-Trifluoro-2-(4′-chlorophenyl)-but-3-yn-2-ol (7a): Yield: 39% (36 mg); 97% *ee*; $[\alpha]_D^{21}$: +4.6° ($CHCl_3$, *c* 2.6).

(−)-1,1,1-Trifluoro-2-(4′-chlorophenyl)-but-3-yn-2-yl acetate (7): Yield: 47% (51 mg); >99% *ee*; $[\alpha]_D^{21}$: −35.7° ($CHCl_3$, *c* 1.1).

(+)-1,1,1-Trifluoro-2-(4′-methylphenyl)-but-3-yn-2-ol (8a): Yield: 48% (41 mg); >99% *ee*; $[\alpha]_D^{21}$: +27.6° ($CHCl_3$, *c* 2.5).

(−)-1,1,1-Trifluoro-2-(4′-methylphenyl)-but-3-yn-2-yl acetate (8): Yield: 43% (44 mg); 97% *ee*; $[\alpha]_D^{21}$: −1.6° ($CHCl_3$, *c* 1.5).

Production of BS2 Esterase Mutants

BS2 esterase mutants G105A and E188D were produced as described[11] by expression in *E. coli* using a rhamnose induction system. The esterase mutants were isolated by cell disruption and the supernatant was directly used for biocatalysis. Esterase activity was determined spectrophotometrically by hydrolysis of *p*-nitrophenol acetate (pNPA, 10 mM in DMSO) in sodium phosphate buffer (100 mM, pH 7.5). *p*-Nitrophenol released was quantified at 410 nm ($\varepsilon = 15 \times 10^3\,M^{-1}cm^{-1}$). One Unit (U) of activity was defined as the amount of enzyme releasing 1 μmol *p*-nitrophenol per min under assay conditions.

Chiral GC Analyses

GC analyses were carried with chiral columns. Hydrodex®-β-3B [heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin)] was used on a GC-MS QP2010 (Shimadzu, Japan, Column A) and on a GC-14 A gas chromatograph (Shimadzu, Tokyo, Japan, Column C), the column heptakis-(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin, provided by Prof. König (University of Hamburg, Germany) was used on a GC-14 A gas chromatograph (Shimadzu, Tokyo, Japan, column B). Details are given in Table 3.

The absolute configuration of **2**, **4**, **7**, and **8** was inferred from the known enantiopreference of BS2 G105 A towards the similar substrate 3-phenylbut-1-yn-3-yl acetate.[10]

Table 3. Details of chiral GC analyses.

Compound	T_{column} [°C][a]	Column	Retention time [min]	
2a	P1	A	29.6 (*S*)	31.1 (*R*)
2	P1	A	15.2 (*S*)	15.8 (*R*)
3a	120	B	11.8	14.2
3	110	C	17.3	18.8
4a	P1	A	31.1 (*R*)	36.0 (*S*)
4	P1	A	15.2 (*S*)	16.1 (*R*)
5a	P1	A	33.7	37.4
5	P1	A	16.0	17.3
6a	P1	A	34.7	40.1
6	P1	A	13.8	14.7
7a	P2	A	28.0 (*S*)	31.9 (*R*)
7	P2	A	14.3 (*R*)	15.0 (*S*)
8a	P1	A	35.1 (*R*)	49.4 (*S*)
8	P1	A	21.5 (*S*)	22.5 (*R*)
9a	P3	C	35.9	37.3
9	P3	C	16.3	17.5
10a	P1	C	30.3	31.3
10	-	-	n.d.	n.d.
11a	100	C	14.5	15.1
11	100	C	7.9	8.7

[a] P1: temperature program: 15 min 100 °C – 4 °C min^{-1} – 120 °C; P2: temperature program: 15 min 125 °C – 4 °C min^{-1} – 145 °C; P3: temperature program: 15 min 90 °C – 4 °C min^{-1} – 115 °C.

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