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Application of a modified Mosher's method for the determination of enantiomeric ratio and absolute configuration at C-3 of chiral 1,3-dihydroxy ketones

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ABSTRACT

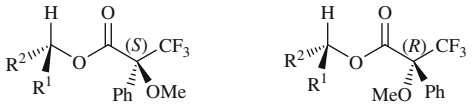
The enantiomeric ratio and absolute configuration of products of the transketolase reaction are typically determined by comparison of the specific rotation or derivatisation and HPLC or GC. A Mosher's ester method has been developed via ester formation at the primary alcohol C-1 which can be used to determine the stereoselectivity of the reaction, as well as the absolute configuration of the product at C-3.

1. Introduction

Several methods are available for determining the absolute stereochemistries of compounds including comparison of specific rotation data to reported values, chiral HPLC and GC with correlation to known or synthesised compounds,[1] X-ray crystallography, and NMR spectroscopy via the use of lanthanide shift reagents[2] or chiral derivatising agents.[3,4] The latter approach, in particular the application of the Mosher's method using 2-methoxy-2-(trifluoromethyl) phenylacetic acid (MTPA) has been extensively used:[4–6] it should be noted that it has been reliably utilised for determining the absolute configuration of secondary alcohols and primary amines.[5,6] For example, chiral secondary alcohols have typically been coupled in two separate, analogous experiments using each enantiomer of the Mosher's acid. The chemical shift differences of the ester protons at R^1 and R^2 (Fig. 1) were then calculated ($\Delta\delta = \delta_{S\text{-MTPA}} - \delta_{R\text{-MTPA}}$) and assigned. Protons at R^1 in the (*R*)-MTPA diastereoisomer were observed upfield relative to the (*S*)-MTPA ester due to the diamagnetic effect of the aromatic ring.[4]

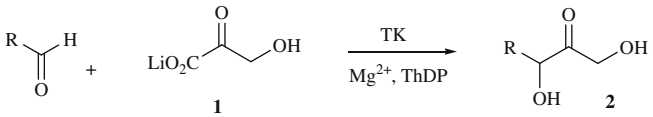
α,α′-Dihydroxy ketone functionalities are found in several biologically important compounds, and can be used as synthons in further structural elaboration to a range of compounds including ketosugars and aminodiols.[7–10] The enzyme transketolase (TK) (EC 2.2.1.1) has been shown to catalyse in vitro the condensation of hydroxypyruvate (Li-HPA, **1**) with a range of aldehydes to stereoselectively generate α,α′-dihydroxy ketones **2** (Scheme 1); a biomimetic route to these compounds has also been reported.[8–13] In addition, asymmetric routes to such chiral dihydroxyacetones have been established using Ender's chiral auxiliary methodology.[14]

Several assays have been described which detect α,α′-dihydroxy ketones in biocatalytic conversions including spectrophotometric and colorimetric methods.[11a,15] However, chiral assays that both establish the enantiomeric purity and determine the absolute stereochemistry of these structural motifs are most important. For TK-generated products this has predominantly been carried out by comparison of the specific rotation, and enantiomeric purities have frequently not been determined. Recently, an acetate derivatisation and chiral HPLC and GC method has been reported, together with Ender's SAMP methodology to confirm absolute stereochemistries.[12] The use of chiral auxiliaries and a six-step procedure to determine absolute stereochemistries however is time consuming, and although suitable for a range of compounds, the reaction fails to accommodate derivatives when R is Ar. A more convenient and



(*S*)-diastereoisomer (*R*)-diastereoisomer

Figure 1. Two Mosher conformers showing the (*S*)-MTPA and (*R*)-MTPA derivatives.



Scheme 1. TK-catalysed reaction with cofactors Mg^{2+} and thiamine diphosphate (ThDP) to generate α,α′-dihydroxy ketones.

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rapid approach would be the application of the modified Mosher's method for determining both the enantiomeric purity and absolute stereochemistry at the C-3 carbinol stereocentre. To date, a modified Mosher's method has been applied to 1,3-diol motifs via derivatisation as di-MTPA esters which was successfully used with acyclic *syn*-diols and cyclic diols, however irregularities in the $\Delta\delta$ of acyclic *anti*-1,3-diols made this method not applicable in all cases.[16] Interestingly, the modified Mosher's method has been used to determine the absolute configuration of primary alcohols with chiral methyl groups at C-2: chemical shifts of the oxymethylene protons in each MTPA ester (attached at C-1) were noted, and a larger chemical shift difference was observed in the (2*S*,2′*S*)- and (2*R*,2′*R*)-isomers compared to the (2*S*,2′*R*)- and (2*R*,2′*S*)-isomers.[17] This was attributed to restricted rotation along the C2–C3 bond. Due to the presence of the ketone at C-2 in **2,** and the conformational restriction this may confer, application of the modified Mosher's method, via coupling at C-1, to α,α′-dihydroxy ketones was investigated to establish whether it could be used to determine the absolute configuration and ee at C-3.

2. Results and discussion

The absolute stereochemistry and enantiomeric purity of (3*S*)-1,3-dihydroxypentan-2-one **2a** have been established previously,[12] and so were used in the initial studies for the attachment of MTPA at the primary hydroxyl position. The diastereotopic protons H_a and H_b in **2a** are normally observed at 4.59 and 4.70 ppm, and may be shifted in each MTPA ester as described previously for C-2 methyl primary alcohol systems.[17] Compound **2a** was prepared using wild-type (WT)-TK.[12] Coupling of **2a** to either (*R*)- or (*S*)-MTPA was performed under standard conditions (Scheme 2), and after purification to remove the remaining starting materials, urea side products and di-MTPA esters which were formed in low yields, the MTPA esters **4a** and **5a** were analysed by ^{1}H NMR spectroscopy in $CDCl_3$. The ^{1}H NMR signals for diastereotopic protons H_a and H_b in the (*R*)-MTPA ester **4a** for the major isomer (2*R*,3′*S*) were at δ_H 5.05 ppm ($\Delta\delta_H^{2R,3'S}$ 0) and for the minor isomer were at δ_H 4.93 and 5.17 ppm ($\Delta\delta_H^{2R,3'R}$ 0.24) (Fig. 2, spectrum **A**). For the (*S*)-MTPA ester **5a** the major isomer (2*S*,3′*S*) signals for H_a and H_b were at δ_H 4.93 ppm and 5.17 ppm ($\Delta\delta_H^{2S,3'S}$ 0.24) and the minor isomer (2*S*,3′*R*) signals were at δ_H 5.05 ppm ($\Delta\delta_H^{2S,3'R}$ is 0) (Fig. 2, spectrum **B**). Averaging of the two ee values calculated from the integration of H_aH_b signals in spectra **A** and **B** gave an ee of 55% which was comparable to that of 58% determined by GC methods.[12]

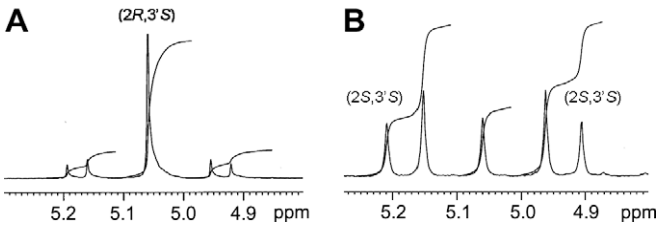
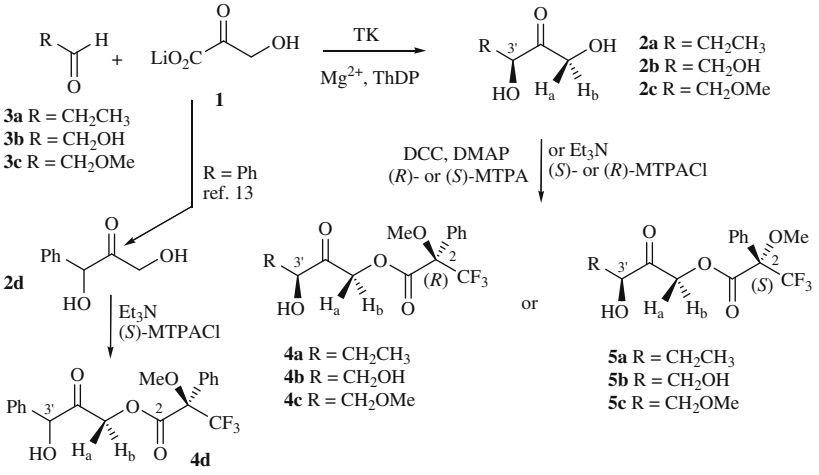


Figure 2. ^{1}H NMR signals for diastereotopic protons H_a and H_b in the (*R*)-MTPA ester **4a** (spectrum **A**) and (*S*)-MTPA ester **5a** (spectrum **B**).

As with the work of Kobayashi et al. on chiral primary alcohols with methyl groups at C-2, the oxymethylene proton separation varied and $\Delta\delta_H^{2R,3'R}$ and $\Delta\delta_H^{2S,3'S} > \Delta\delta_H^{2R,3'S}$ and $\Delta\delta_H^{2S,3'R}$. The ee could be determined to within 5% of the value from GC analysis. α,α′-Dihydroxyketones with reported absolute configurations were then used to investigate the validity of the method.

First, commercially available L-erythrulose **2b**, prepared from **1** and glycolaldehyde **3b** using TK, was converted into the corresponding (*R*)- and (*S*)-MTPA esters using MTPA chloride. The ^{1}H NMR analysis revealed that H_a and H_b in the (*R*)-MTPA ester **4b** (major isomer (2*R*,3′*S*)) were at δ_H 5.11 and 5.19 ppm ($\Delta\delta_H^{2R,3'S}$ is 0.08) and negligible signals for the minor isomer were observed. For the (*S*)-MTPA ester **5b**, the major isomer (2*S*,3′*S*) signals for H_a and H_b were observed at δ_H 5.05 and 5.23 ppm ($\Delta\delta_H^{2S,3'S}$ is 0.18) while no minor isomer was detected. Integration of the signals for H_a and H_b as before indicated L-erythrulose to be of >95% ee. Again for the oxymethylene protons $\Delta\delta_H^{2S,3'S} > \Delta\delta_H^{2R,3'S}$ enabling, by formation of both MTPA esters, confirmation of absolute stereochemistry as well as the enantiomeric purity.

2-Methoxy ethanal **3c** has been described as an aldehyde acceptor in the TK reaction with spinach TK, where an ee of 60% was observed (*S*-major isomer).[18] This transformation was performed using WT *Escherichia coli* TK to validate Mosher's method for stereochemical determination. Compound **2c** was formed in 30% yield and coupling to the MTPA chlorides gave **4c** and **5c** with H_a and H_b for the major isomers at 5.13 and 5.17 ppm (2*R*,3′*S*) and 5.02 and 5.29 ppm (2*S*,3′*S*), respectively. Again $\Delta\delta_H^{2S,3'S}$ (0.27) > $\Delta\delta_H^{2R,3'S}$ (0.04) (Table 1), confirming the application of the method, and integration of H_a and H_b for both esters **4c** and **5c** as before gave an average value for the ee of 57%, which was comparable to that reported for spinach-TK.[18] The synthesis of the 1,3-dihydroxy-1-phenylpropan-2-one **2d** has been described previously and the MTPA method was also explored with this aromatic analogue to



Scheme 2. TK-catalysed reactions using **3** to generate ketones **2** and the formation of MTPA esters **4** and **5**.

Table 1
Chemical shifts for MTPA esters and absolute stereochemistries

R	MTPA ester	$\Delta\delta_H$ H_aH_b (major isomer)	ee[a] of **2**	Lit. ee
CH_2CH_3	**4a** (*R*)	0 (2*R*,3′*S*)	55% **2a**	58% (*S*)[12]
CH_2CH_3	**5a** (*S*)	0.24 (2*S*,3′*S*)		
CH_2OH	**4b** (*R*)	0.08 (2*R*,3′*S*)	>95% **2b**	>98% (*S*)[12]
CH_2OH	**5b** (*S*)	0.18 (2*S*,3′*S*)		
CH_2OMe	**4c** (*R*)	0.04 (2*R*,3′*S*)	57% **2c**	60% (*S*)[18]
CH_2OMe	**5c** (*S*)	0.27 (2*S*,3′*S*)		

[a] Determined from the integration of ^{1}H NMR signals for H_a and H_b from both the (*R*)- and (*S*)-MTPA esters.

establish whether the separation of H_a and H_b was similar to that for the aliphatic series.[13,19] Compound **2d** was prepared using the biomimetic TK reaction described previously[13] and was converted to the mono-MTPA diastereoisomeric esters **4d** using (*S*)-MTPA chloride. Protons H_a and H_b were observed as before and by analogy to the assignment for the aliphatic series, $\Delta\delta_H^{2R,3'R}$ was 0.31 while $\Delta\delta_H^{2S,3'R}$ was 0.05, highlighting the potential application of this approach with aromatic analogues.

3. Conclusion

The stereoselectivity of *E. coli* WT-TK using 2-methoxy ethanal as a substrate was comparable to that observed with spinach TK, and was consistent with previous reports indicating a preference for formation of the (*S*)-isomer in the biotransformation. In addition, by using (*R*)- and (*S*)-MTPA we have demonstrated that the ees of α,α′-dihydroxyketones can routinely be determined, to within 5% of values determined using GC or HPLC method, as well as the absolute stereochemistries. The method can be used with a range of aliphatic α,α′-dihydroxyketones **2** with lipophilic and polar R groups. Determination of the absolute stereochemistries is more convenient than the multistep chiral auxiliary procedures described previously, and is also applicable to a wider range of compounds including aromatic systems (R is Ar). This is important for future work using chemical or biocatalytic routes to α,α′-dihydroxyketones.

4. Experimental

4.1. General information

Unless otherwise noted, solvents and reagents were of reagent grade from commercial suppliers (Sigma–Aldrich) and were used without further purification. Dry CH_2Cl_2 was obtained using anhydrous alumina columns.[20] All moisture-sensitive reactions were performed under a nitrogen or argon atmosphere using oven-dried glassware. Reactions were monitored by TLC on Kieselgel 60 F_{254} plates with detection by UV, potassium permanganate and phosphomolybdic acid stains. Flash column chromatography was carried out using silica gel (particle size 40–63 μm). ^{1}H NMR and ^{13}C NMR spectra were recorded at the field indicated using Bruker AMX300 MHz and Avance 500 and 600 MHz machines. Coupling constants are measured in hertz (Hz) and unless otherwise specified, NMR spectra were recorded at 298 K. Mass spectra were recorded on a LTQ Orbitrap XL. Infrared spectra were recorded on a Shamadzu FTIR-8700 infrared spectrophotometer. Optical rotations were recorded on an Optical Activity Limited PolAAR2000 polarimeter at 589 nm, quoted in deg cm^2 g^{-1} and concn (*c*) in g/100 mL.

Lithium hydroxypyruvate was synthesised as described previously.[21] (3*S*)-1,3-Dihydroxypentan-2-one **2a** (58% ee) was prepared as described previously using WT *E. coli* TK,[12] and L-erythrulose **2b** was commercially available.

4.2. (2*R*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-hydroxy-2′-oxo-pentyl ester 4a

The reaction was carried out under anhydrous conditions. To a stirred solution of **2a** (0.030 g, 0.25 mmol) in CH_2Cl_2 (5 mL) were added DCC (0.057 g, 0.28 mmol) and DMAP (0.012 g, 0.02 mmol) in CH_2Cl_2 (10 mL) at 0 °C and the reaction mixture was stirred for 2 min. (*S*)-MTPA (0.030 g, 0.08 mmol) was added and the reaction mixture was stirred for 30 min, after which further (*S*)-MTPA (0.020 g, 0.08 mmol) was added and the mixture was stirred for 48 h at rt. The solvent was removed in vacuo, and the crude product was dry loaded onto silica gel and purified using flash chromatography (EtOAc/hexane, 3:2) to afford **5a** as a colourless oil (0.035 g, 59%), (2*R*,3′*S*)-55% ee (from the integrations of H_a and H_b ^{1}H NMR signals from **4a** and **5a**). R_f = 0.45 (EtOAc/hexane, 3:2); $[\alpha]_D^{20} = +13.3$ (*c* 0.15, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3429br s, 2925s, 1712s; ^{1}H NMR (500 MHz; $CDCl_3$) δ 1.00 (3H, m, C*H*$_3$), 1.65 (1H, m, C*H*HCH$_3$) 1.89 (1H, m, CH*H*CH$_3$), 2.86 (1H, d, *J* 6.0, O*H*), 3.64 (3H, s, OMe), 4.29 (1H, m, C*H*OH), 4.93 (0.26H, d, *J* 17.0, C*H*HO (2*R*,3′*R*)), 5.05 (1.48H, s, CH_2O (2*R*,3′*S*)), 5.17 (0.26H, d, *J* 17.0, CH*H*O (2*R*,3′*R*)), 7.54 (3H, m, Ph), 7.61 (2H, m, Ph); ^{13}C NMR (125 MHz; $CDCl_3$) δ 8.8 (*C*H$_3$), 27.2 (*C*H$_2$CH$_3$), 55.8 (O*C*H$_3$), 67.2, 76.2, 123.2 (q, J_{CF} 283, *C*F$_3$), 127.6, 128.6, 129.9, 131.8, 166.3 (*C*=O ester), 204.2 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ −72.2; *m/z* (FTMS) [M+NH_4] calcd for $C_{15}H_{21}F_3O_5N$, 352.1366; found 352.1371.

4.3. (2*S*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-hydroxy-2′-oxo-pentyl ester 5a

The same procedure was used as that described above for **4a** using (*S*)-MTPA to give **5a** as a colourless oil (0.030 g, 57%) (2*S*,3′*S*)-55% ee (from integrations of H_a and H_b ^{1}H NMR signals from **4a** and **5a**). R_f = 0.45 (EtOAc/hexane, 3:2); $[\alpha]_D^{20} = -37.0$ (*c* 0.1, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.00 (3H, t, *J* 7.4, C*H*$_3$), 1.69 (1H, m, C*H*HCH$_3$) 1.89 (1H, m, CH*H*CH$_3$), 2.87 (1H, d, *J* 5.3, O*H*), 3.64 (3H, s, OMe), 4.29 (1H, m, C*H*OH), 4.93 (0.82H, d, *J* 17.0, C*H*HO (2*S*,3′*S*)), 5.05 (0.36H, s, CH_2O (2*S*,3′*R*)), 5.17 (0.82H, d, *J* 17.0, CH*H*O (2*S*,3′*S*)), 7.54 (3H, m, Ph), 7.61 (2H, m, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ 8.7 and 8.8 (*C*H$_3$), 27.2, 55.8 (O*C*H$_3$), 67.2, 76.2, 123.2 (q, J_{CF} 283, *C*F$_3$), 127.6, 128.5, 128.7, 129.9, 166.2 (*C*=O ester), 204.2 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ −72.2.

4.4. (2*R*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′,4′-dihydroxy-2′-oxo-butyl ester 4b

The reaction was carried out under anhydrous conditions. To a stirred solution of **2b** (0.030 g, 0.25 mmol) in CH_2Cl_2 (5 mL) were added triethylamine (34 μL, 0.25 mmol) and (*S*)-MTPA chloride (34 μL, 0.18 mmol) in CH_2Cl_2 (2 mL) and the reaction mixture was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (EtOAc) to afford **4b** as a colourless oil (0.054 g, 63%), (2*R*,3′*S*) >95% ee (from integrations of H_a and H_b ^{1}H NMR signals of **4b** and **5b**). R_f = 0.45 (EtOAc); $[\alpha]_D^{20} = +10.2$ (*c* 0.4, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3429br s, 2925s, 1712s; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.64 (3H, s, OMe), 3.89 (1H, dd, *J* 10.6 and 3.8, C*H*HOH), 3.95 (1H, dd, *J* 10.6 and 3.8, C*H*HOH), 4.35 (1H, dd, *J* 3.8 and 3.8, C*H*OH), 5.11 (1H, d, *J* 17.0, C*H*HO (2*R*,3′*S*)), 5.19 (1H, d, *J* 17.0, CH*H*O (2*R*,3′*S*)), 7.43 (3H, m, Ph), 7.62 (2H, m, Ph), no (2*R*,3′*R* isomer detected); ^{13}C NMR (75 MHz; $CDCl_3$) δ 55.9 (O*C*H$_3$), 63.5, 68.0, 76.4, 84.6 (q, J_{CF} 28, *C*CF$_3$), 123.1 (q, J_{CF} 288, *C*F$_3$), 127.5, 128.6, 129.9, 131.7, 166.5 (*C*=O ester), 203.3 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ −72.2; *m/z* (FTMS) [M+NH_4] calcd for $C_{14}H_{19}F_3O_6N$, 354.1159; found 354.1162.

4.5. (2*S*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′,4′-dihydroxy-2′-oxo-butyl ester 5b

The same procedure was used as that described above for **4b** using (*R*)-MTPA chloride to give **5b** as a colourless oil (0.044 g, 51%) (2*S*,3′*S*) >95% ee (from integrations of H_a and H_b ^{1}H NMR signals of **4b** and **5b**). R_f = 0.45 (EtOAc); $[\alpha]_D^{20} = -14.4$ (*c* 0.5, $CHCl_3$), ^{1}H NMR (500 MHz; $CDCl_3$) δ 3.63 (3H, s, OMe), 3.92 (2H, m, CH_2OH), 4.35 (1H, dd, *J* 4.0 and 3.8, C*H*OH), 5.05 (1H, d, *J* 17.0, C*H*HO (2*S*,3′*S*)), 5.23 (1H, d, *J* 17.0, CH*H*O (2*S*,3′*S*)), 7.43 (3H, m, Ph), 7.62 (2H, m, Ph), no (2*S*,3′*R* isomer detected); ^{13}C NMR (125 MHz; $CDCl_3$) δ 55.8 (OCH_3), 63.5, 67.9, 76.3, 84.6 (q, J_{CF} 28, *C*CF_3), 123.1 (q, J_{CF} 288, *C*F_3), 127.5, 128.5, 129.9, 131.7, 166.4 (*C*=O ester), 203.1 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ −72.2.

4.6. 1, 3-Dihydroxy-4-methoxy-butan-2-one 2c

ThDP (22 mg, 48 μmol) and $MgCl_2{\cdot}6H_2O$ (39 mg, 180 μmol) were dissolved in H_2O (10 mL) and the pH was adjusted to 7 with 0.1 M NaOH. To this stirred solution, at 25 °C, was added WT-TK clarified lysate (2 mL)[11c,12] and the mixture was stirred for 20 min. In another flask, **1** (110 mg, 1 mmol) and **3c** (74 mg, 1 mmol) were dissolved in H_2O (8 mL) and the pH was adjusted to 7 with 0.1 M NaOH. Following the 20-min enzyme/cofactor pre-incubation, the **1**/**3c** mixture was added to the enzyme solution and the mixture was stirred at 25 °C for 24 h. During this time, the pH was maintained at 7.0 by addition of 1 M HCl using a pH stat (Stat Titrino, Metrohm). Silica was added and the reaction mixture was concentrated to dryness before dry loading onto a flash silica gel column. Following column purification (EtOAc/CH_3OH, 80:20), **2c** was isolated as an oil (40 mg, 30%). $[\alpha]_D^{20} = +2.0$ (*c* 2.0, $CHCl_3$), lit.[18] $[\alpha]_J^{25} = +3.0$ (*c* 0.017, MeOH); ν_{max} (KBr)/cm^{-1} 3415br s, 2923s, 1727s; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.36 (3H, s, OCH_3), 3.61 (1H, dd, *J* 9.9 and 4.4, C*H*HOMe), 3.70 (1H, dd, *J* 9.9 and 4.4, CH*H*OMe), 4.38 (1H, dd, *J* 4.4 and 4.4, C*H*OH), 4.43 (1H, d, *J* 19.7, C*H*HOH), 4.53 (1H, d, *J* 19.7, CH*H*OH); ^{13}C NMR (75 MHz; $CDCl_3$) δ 59.5 (OCH_3), 66.7 (*C*H_2), 73.4 (*C*H_2), 74.8 (*C*HOH) 210.8 (C=O); *m/z* (FTMS) [M+NH_4] calcd for $C_5H_{14}O_4N$, 152.0917; found 152.0919.

4.7. (2*R*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-hydroxy-4′-methoxy-2′-oxo-butyl ester 4c

The reaction was carried out under anhydrous conditions. To a stirred solution of **2c** (0.010 g, 0.07 mmol) in CH_2Cl_2 (3 mL) were added triethylamine (34 μL, 0.25 mmol) and (*S*)-MTPA chloride (20 μL, 0.11 mmol) in CH_2Cl_2 (2 mL) and the reaction mixture was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (EtOAc/hexane, 1:1) to afford **4c** as a colourless oil (0.015 g, 61%), (2*R*,3′*S*) 57% ee (from integrations of H_a and H_b ^{1}H NMR signals of **4c** and **5c**). R_f = 0.40 (EtOAc/hexane,1:1); $[\alpha]_D^{20} = +23.2$ (*c* 0.25, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3415br s, 2923s, 1727s; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.38 (3H, s, OCH_3), 3.61 (3H, s, OCH_3) 3.57–3.70 (2H, m, CH_2), 4.36 (1H, m, C*H*OH), 5.02 (0.16H, d, *J* 17.5, C*H*HO (2*R*,3′*R*)), 5.13 (0.84H, d, *J* 17.5, C*H*HO (2*R*,3′*S*)), 5.17 (0.81H, d, *J* 17.5, C*H*HO (2*R*,3′*S*)), 5.29 (0.16H, d, *J* 17.5, C*H*HO (2*R*,3′*R*)), 7.42 (3H, m, Ph), 7.62 (2H, m, Ph); *m/z* (FTMS) [M+NH_4] calcd for $C_{15}H_{21}F_3O_6N$, 368.1315; found 368.1318.

4.8. (2*S*,3′*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-hydroxy-4′-methoxy-2′-oxo-butyl ester 5c

The same procedure was used as that described above for **4c** using (*R*)-MTPA chloride to give **5c** as a colourless oil (0.010 g, 40%) (2*S*,3′*S*) 57% ee (from integrations of H_a and H_b ^{1}H NMR signals of **4c** and **5c**). R_f = 0.45 (EtOAc/hexane, 1:1); $[\alpha]_D^{20} = -10.6$ (*c* 0.25, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ 3.38 (3H, s, OCH_3), 3.61 (3H, s, OCH_3) 3.57–3.70 (2H, m, CH_2), 4.36 (1H, m, C*H*OH), 5.02 (0.74H, d, *J* 17.5, C*H*HO (2*S*,3′*S*)), 5.13 (0.81H, d, *J* 17.5, C*H*HO (2*S*,3′*R*)), 5.17 (0.38H, d, *J* 17.5, C*H*HO (2*S*,3′*R*)), 5.29 (0.81H, d, *J* 17.5, C*H*HO (2*S*,3′*S*)), 7.42 (3H, m, Ph), 7.62 (2H, m, Ph); ^{13}C NMR (150 MHz; $CDCl_3$) δ 55.8 (OCH_3), 59.5 (OCH_3), 68.1, 72.8, 75.0, 84.5 (q, J_{CF} 27, *C*CF_3), 123.1 (q, J_{CF} 285, *C*F_3), 127.5, 128.5, 129.8, 131.5, 166.1 (*C*=O ester), 202.9 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ −72.2.

4.9. (2*R*,3′*RS*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3′-hydroxy-2′-oxo-3′-phenylpropyl ester 4d

The same procedure was used as that described above for **4c** using (*S*)-MTPA chloride and **2d**.[13] The product was purified using flash silica chromatography (EtOAc/hexane, 1:4) to give **4d** as a colourless oil (0.014 g, 61%). ^{1}H NMR (500 MHz; $CDCl_3$) δ 3.62 (3H, s, OCH_3), 4.71 (0.25H, d, *J* 16.9, C*H*HO (2*R*,3′*R*)), 4.86 (0.25H, d, *J* 16.9, C*H*HO (2*R*,3′*S*)), 4.91 (0.25H, d, *J* 16.9, CH*H*O (2*R*,3′*S*)), 5.02 (0.25H, d, *J* 16.9, CH*H*O (2*R*,3′*R*)), 5.25 (1H, m, C*H*OH), 7.34–7.54 (6H, m, Ph), 7.70 (2H, m, Ph), 8.01 (2H, *J* 8.6, Ph).

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