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Enzyme Catalysed Irreversible Transesterifications with Vinyl Acetate. Are they really Irreversible?

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Abstract: When racemic β–methyl-(2-thiophene)propanol (**1**) was resolved *via* transesterification catalysed by lipase from *Pseudomonas fluorescens* (*PFL*) using an excess vinyl acetate in chloroform the enantioselectivity was high $E \approx 200$ (conversion, $c = 39\%$) when calculated from the *ee* of the (*S*)-ester **S-1Ac**. However, based on the *ee* of the remaining (*R*)-alcohol **R-1**, assuming irreversibility and when measured at $c = 57$ %, $E = 37$ was obtained. Similarly, when the 5-(1-ethoxyethoxy)-3-pentyn-2-ol (**2**) was resolved using immobilised lipase B from *Candida antarctica* (Novozym SP435) under similar conditions, the (*R*)-ester product **R-2Ac** at $c = 22$ % gave $E \approx 140$ compared with that of the remaining alcohol **S-2** at $c = 65$ % which gave $E = 15$. These results are interpreted as a consequence of a reversible process occurring in transesterifications of this type, which are commonly referred to as irreversible.

In an irreversible enantioselective reaction performed on a racemic starting material it is possible to obtain the remaining substrate practically enantiomerically pure, as exemplified by the Sharpless oxidation of racemic allyl alcohols.[1] The conversion (*c*) must be higher than 50%. How much higher it should be depends on the rate ratio between the fast and slow reacting enantiomers, *i.e.* the enantiomeric ratio, *E*.[1] The enantiomeric purity of the remaining substrate increases continually with increasing *c*. On the other hand, in reversible reactions, low enantiomeric purity of the remaining substrate is often registered, even in such with high *E*-values. The enantiomeric excess of the remaining substrate reaches a maximum at a conversion higher than 50% and then drops to become zero when the equilibrium conversion is reached. Resolution of racemic alcohols using enzyme catalysed transesterification reactions is a widely used procedure.[2] In order to successfully perform such a resolution, irreversibility is highly desirable.[2] To achieve this, acyl donors such as enol esters have been used. These yield enols as the protonated leaving group, which rapidly tautomerises to an aldehyde or ketone. Since these are non-nucleophilic, they cannot react with the acyl-enzyme intermediate.[2,3] Alternatively, the released leaving group - nucleophile can be removed by evaporation, thus displacing the equilibrium.[4] Transesterifications of these types are generally referred to as irreversible.[2,3] Therefore, it is commonly assumed that the remaining substrates easily can be obtained enantiomerically pure in such reactions.[3] This is also a common assumption for enzyme catalysed ester hydrolysis reactions, but there is an example among, where the ee of the remaining substrate is much lower than expected.[5]

While studying some examples of such vinyl acetate transesterifications using different enzymes, we noted that these reactions, based on the produced esters, were highly enantioselective. However, based on the enantiomeric purities of the remaining substrates and assuming irreversibility, we found them to be of much lower enantioselectivities. We wish to propose that these observations are the effect of a reversible step in the reaction *i.e.* so called irreversible transesterifications are not irreversible.

The transesterifications first studied (Fig. 1) were performed at an initial water activity of $a_W = 0.32$ using vinyl acetate as the acyl donor and were the reactions of either racemic β–methyl-(2-thiophene)propanol[6a,7-9] (**1**) in chloroform as the solvent using a *Pseudomonas* lipase (*PFL*, Fluka) as the catalyst, or a diastereomeric mixture of the four stereoisomers of 5-(1-ethoxyethoxy)-3-pentyn-2-ol[10] (**2**) in *n*-heptane as solvent using immobilised lipase B from *Candida antarctica* (Novozym SP435) as the catalyst. In the case of substrate **2** which has an extra stereogenic center the terms enantiomeric excess, *ee* and enantiomeric ratios[11], *E*-values, are incorrect. However, since this additional stereocenter had no influence on the reaction, we subsequently use the terms with citation marks in case of compounds of type **2**. The *E*-values were calculated[12] from the *ee*:s of the produced esters at low conversions and were found to be very high (see Fig. 1: Experiments 1 and 3). However, much lower *E*-values were obtained when these were calculated from the *ee*:s of the remaining substrates assuming irreversibility[12] (see Fig. 1: Experiments 2 and 4).[8]

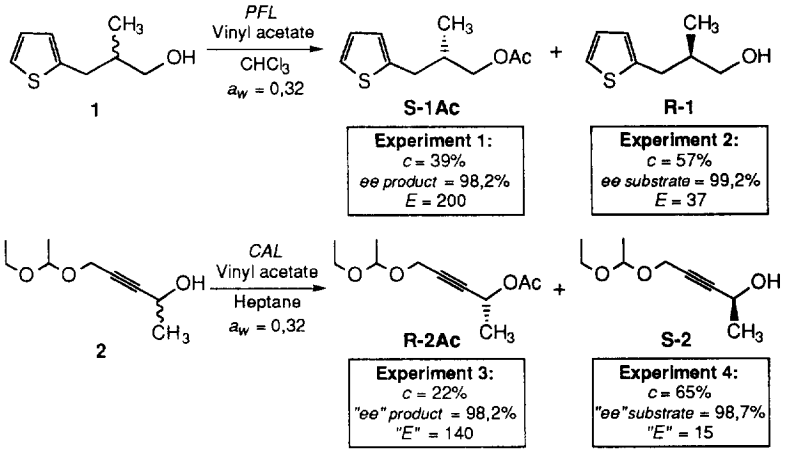

Fig. 1. Transesterifications of β–methyl-(2-thiophene)propanol (**1**) and 5-(1-ethoxyethoxy)-3-pentyn-2-ol (**2**).

Clearly, if these reactions were truly irreversible the *ee*:s of the remaining substrates should increase continuously after 50% conversion.[1-3]

We followed the esterification of the pentynol **2** both under initially dry conditions as well as at initial $a_W = 0.32$ (see Fig. 2a and b) and found that the *"ee"* of the remaining substrate actually decreased after reaching a maximum of 99.9% (dry) and 99.7% ($a_W = 0.32$). The apparent *E*-value calculated using the equation for an irreversible reaction diminishes with increasing conversions (see Fig. 2c, *cf.* ref[5]). This is clearly not possible and the only way to explain this is that all the reactions in the system are indeed not irreversible. The reaction of the primary acyl donor, vinyl acetate, is evidently irreversible. However, the produced ester **R-2Ac** can of course also function as an acyl donor liberating the secondary alcohol **R-2** thus contaminating the remaining substrate **S-2**.

In order to study if reversibility was responsible for the results of the transesterifications of *rac*-β–methyl-(2-thiophene)propanol (**1**) described (see Fig. 1: Experiments 1 and 2), a different set of experiments was used resembling quasi-racemate methods. This set was designed so that it should enable us to detect even very minute amounts of **S-1** formed from the acetate **S-1Ac** *via* enzymatic catalysis under conditions mimicking the reaction after 50% conversion (see Fig. 3). We knew from earlier experiments that the transesterification rates of (*R*)-β–methyl-(2-thiophene)propanol (**R-1**) and (*R*)-2-methyl-1-octanol (**R-3**) were of the same order of magnitude.[8] Therefore **R-3** should be a good mimic of **R-1**.

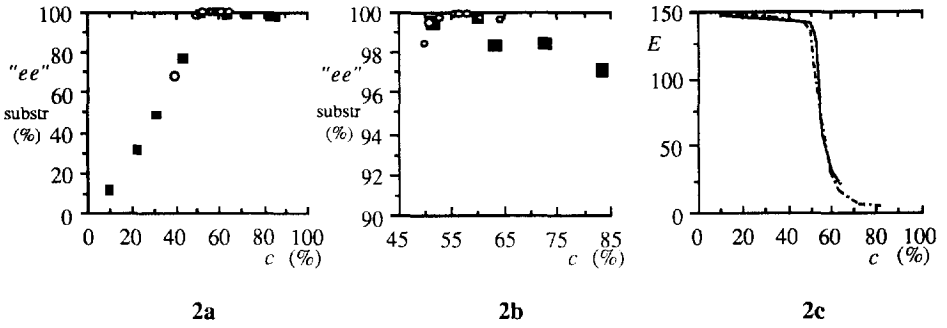

Fig. 2. %"*ee*" of remaining substrate **S-2** as a function of conversion in the transesterification of 5-(1-ethoxyethoxy)-3-pentyn-2-ol (**2**) with vinyl acetate in heptane catalysed by *CAL*. ○ : All reagents and the solvent predried ($a_w \approx 0$) ■ : All reagents and the solvent prequilibrated to $a_w \approx 0.32$. **a)** Left diagram shows the reaction from 0% - 85% conversion whereas the middle one **b)** shows the reaction between 45 - 85% conversion. The diagram **c)** on the right shows the variation of the apparent *E* with *c* (····· $a_w \approx 0.32$; —— dry)

First, we mixed 1 molar equivalent of β–methyl-(2-thiophene)propyl acetate (**1Ac**, 94% *ee*[6b,7]) with 1 *eq* of (*R*)-2-methyl-1-octanol (**R-3**, >99% *ee*) and added vinyl acetate without the addition of enzyme. No formation of the alcohol **S-1** was observed (see experimental part, under *Study of the formation of...*: Exp. I).

In order to establish that the acetate **S-1Ac** could serve as an acyl donor for the enzyme, the reaction was run at $a_W = 0.32$ in the absence of vinyl acetate (see *Study of ...*: Exp. II). After 20 h, ≈ 7% of the alcohol **S-1** was obtained, whereas only ≈ 5% of the acetate **R-3Ac** had been formed. Due to the water content in the reaction system partial hydrolysis of the ester must have occurred.

When equimolar amounts of the acetate **S-1Ac** and the alcohol **R-3** and 4 eq of vinyl acetate in the presence of enzyme were mixed under dry conditions, a small but significant amount of alcohol **S-1** formed rapidly and remained constant (≈ 0.05%, Exp. III) for 24 h. The same **S-1**-level was reached when the reaction was run in neat dry vinyl acetate without solvent (≈ 0.05%, see *Study of ...*: Exp. IV). When using dry conditions, except for the vinyl acetate which was used as received, slightly more of the alcohol **S-1** was formed (0.5%, Exp. V) after 2 h. When all the components in the reaction had been equilibrated to $a_W = 0.32$, the alcohol concentration reached 1.5% (see *Study of ..*: Exp. VI) after 2 h.

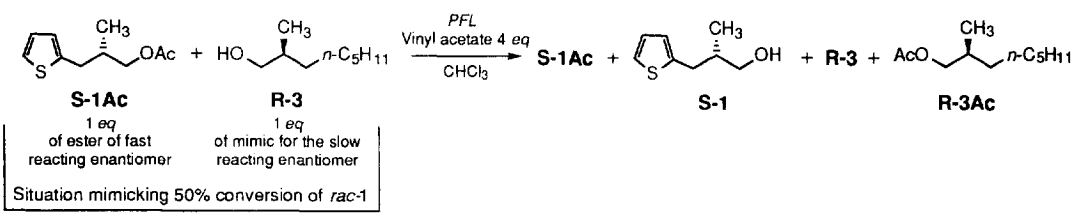

Fig. 3. Reaction designed to mimic conditions above 50% conversion in the transesterification of *rac*-β–methyl-(2-thiophene)propanol (**1**).

The results presented above provide strong evidence for the reversibility of enzyme catalysed acyl transfer reactions even though irreversible acyl donors like vinyl acetate are used. From our experiments it is evident that the produced esters in these reactions should be able to function as acyl donors also in these types of reactions.

The major reactions possible are described (see Fig. 4). Reaction ***1*** (***Rxn 1***) represents the irreversible acylation of the enzyme by vinyl acetate. Assuming that ***S***-OH reacts much faster than ***R***-OH, reaction ***2*** (***Rxn 2***) in the acylation (upwards) direction dominates before 50% conversion in an organic solvent. Soon after 50% conversion the concentration of ***S***-OH becomes very low and that of ***S***-OAc is high. Therefore, at this point the capacity of ***S***-OAc to act as an acyl donor becomes significant leading to a constant ratio of ***S***-OH / ***S***-OAc, since reaction ***2*** (***Rxn 2***) should reach an enzyme catalysed equilibrium. Reaction ***3*** (***Rxn 3***), the acylation of the slow reacting enantiomer ***R***-OH proceeds continuously but very slowly in the acylation direction during the whole reaction. The enzymatic hydrolysis of the product ***R***-OAc should be even slower. A second acylation reaction, namely reaction ***4*** (***Rxn 4***), where water present in the system acts as the nucleophile to give acetic acid is also possible in the system. When using *CAL*, a fatty acid has been shown to be an excellent acyl donor.[13] When acetic acid acylates the enzyme, water is liberated and a second equilibrium is established.

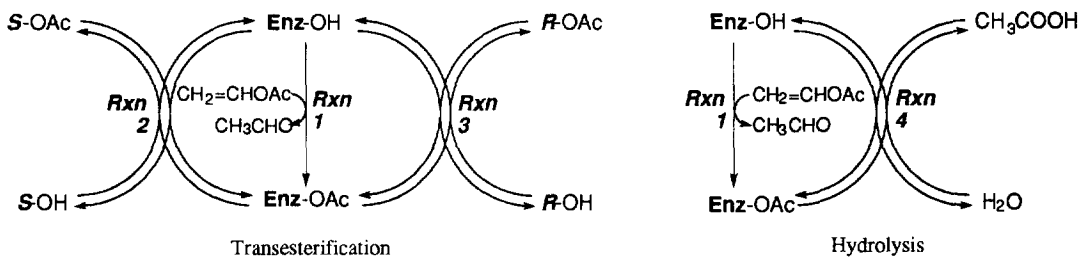

Fig. 4. Reaction schemes for an "irreversible" transesterification.

Thus, the decreased *"ee"* of the remaining substrate at high conversions ($c >> 50\%$) in the transesterification of the pentynol **2** should be the consequence of the reversibility of the reaction of the fast reacting enantiomer. Fitting the data of Fig. 2 to a recently developed[14] computer program for the calculation of *E*-values and the equilibrium conversion constant[12] $K = (1\text{-}c_{eq}) / c_{eq}$ gave for $E = 140$, $K = 0.001$ (dry) and $K = 0.004$ ($a_w = 0.32$). This means that racemic compounds are obtained at 99.9% and 99.6% conversion respectively! Before equilibrium conversion is reached the *ee* of the remaining substrate begins to deteriorate. Thus at 90% conversion the remaining substrate should be of < 99% *ee* (dry) or < 97% *ee* ($a_w = 0.32$).

The experiments with the alcohol **R-3** and the acetate **S-1Ac** clearly demonstrates that the acetate can function as an acyl donor leading to production of the alcohol **S-1** even in the presence of a large excess of vinyl acetate.

It is clear from the experiments described above that the water activity influences the molar ratio between the fast reacting alcohol enantiomer and the ester product from this at equilibrium. How this proceeds mechanistically is unclear. However, the results above suggest that if the highest possible *ee* of the remaining substrate is desired, the water activity should be kept as low as possible.

We have shown that some supposedly irreversible transesterifications are indeed reversible. This problem, which should be general, has not been emphasised enough in the major reviews on enzymatic reactions.[2,3] It might be minimised by using dry conditions, possibly a large excess of vinyl acetate and by interrupting the reaction at a conversion where maximum *ee* of the remaining substrate is obtained or better by interrupting the reaction just before $c = 50\%$, separate the ester and alcohol and then subject the latter to a second esterification.

In conclusion, one should not be surprised if remaining substrates in lower than expected *ee*:s are obtained from enzyme catalysed transesterifications using irreversible acyl donors.

Experimental

Commercially available chemicals were used without further purification unless otherwise stated. Dry chloroform ($CHCl_3$) free from stabilising agent (EtOH) was purified according to ref.[15] Vinyl acetate, *n*-heptane and pyridine was dried by addition of molecular sieves (4Å). The above dried materials were stored under argon in a refrigerator. Dry diethyl ether (Et_2O) was distilled from $LiAlH_4$. *n*-BuLi was titrated prior to use.[16] *PFL* (E.C.3.1.1.3) lipase from *Pseudomonas fluorescens* was obtained from Fluka. The specific activity was 31.5 U/mg. *CAL* (Novozym 435, LCC 0013-1), immobilised lipase B from *Candida antarctica* was obtained from Novo Nordisk A/S. The specific activity was 7400 PLU/g. *PSL* (E.C.3.1.1.3) lipase from *Pseudomonas sp.* was obtained from Amano Pharmaceutical Co, Ltd. The specific activity was 30.0 U/mg. The enzymes were stored at 4 °C in a desiccator over dried silica gel. Preparative liquid chromatography (MPLC) was performed on straight phase silica gel (Merck 60, 230-400 mesh, 0.040-0.063 mm) employing the gradient technique described[17] and using an increasing concentration of distilled diethyl ether in distilled *n*-pentane (0->100 %), as eluent. ^1H NMR spectra were recorded on a Jeol EX 270 spectrometer using $CDCl_3$ as solvent and TMS as an internal reference. FT-IR spectra were recorded neat between NaCl-plates using a Nicolet 5SXC spectrometer. Optical rotation was measured on a Perkin Elmer 241 polarimeter. Mass spectra were recorded using GC-MS (Varian 3300 and an ion trap detector, Finnigan ITD 800). Elemental analyses were carried out by Mikrokemi, Uppsala, Sweden.

Preequilibrated water activity, general method.[18] The substrates were dissolved in the appropriate solvent, and storage (24 h) in a vessel containing a saturated solution of $MgCl_2$ gave the mixtures a preequilibrated $a_W = 0.32$. Vinyl acetate was equilibrated similarly in another vessel. To be able to compensate for the loss of solvent from evaporation, pure solvent was equilibrated together with the substrate solutions. The enzymes were mixed with a small amount of solvent and treated similarly.

rac-*5-(1-Ethoxyethoxy)-3-pentyn-2-ol* (**2**). rac-1-(1-Ethoxyethoxy)-2-propyn (3.99 g, 31.2 mmol) was dissolved in dry Et_2O. The mixture was cooled to – 34 °C and *n*-BuLi (13.0 ml, 2.51 M in hexane, 32.8 mmol) was added dropwise to the mixture (0.5 h) The mixture was cooled to – 68 °C and acetaldehyde (1.85 ml, 32.8 mmol) in dry Et_2O (10 ml) was added dropwise keeping the temperature below – 60 °C. The mixture was kept at – 68 °C over night and was then slowly allowed to reach room temperature. The conversion was monitored by GC (OV101 packed column, carrier gas N_2, 5 ml/min, temperature programming: 80-140 °C, 8 °C /min.) The mixture was poured onto NH_4Cl (100 ml, sat. aq. soln.) followed by extractive workup with Et_2O (3 x 40 ml). The combined organic phases were washed with water (50 ml) and dried ($MgSO_4$) to give an oil which was distilled bulb to bulb (bath temp. 30-35 °C, 0.20-0.25 mbar) to give the alcohol (3.91 g, 22.7 mmol), in 73 % yield. Due to instability of the compound on storage at room temperature unsatisfactory combustion analysis was obtained: Calc. for $C_8H_{16}O_3$: C 62.77, H 9.36. Found C 61.8, H 8.8. n_D^{20} 1.4505. FT-IR: 3422, 2982, 2933, 2897, 2885, 2876, 1386, 1372, 1336, 1085 cm^{-1}. ^1H NMR (270 MHz): δ 1.19 (3H, t, $J = 6.9$ Hz), 1.32 (3H, d, $J = 5.3$ Hz), 1.43 (3H, d, $J = 6.6$ Hz), 2.33 (1 H, s), 3.50 (1 H, q of d, $J = 6.9$ and 2.3 Hz), 3.65 (1H, q of δ, $J = 7.3$ and 2.3 Hz), 4.21 (2H, t of d, $J = 13.2$ and 1.65 Hz), 4.55 (1H, q, $J = 6.3$ Hz), 4.82 (1H, q, $J = 5.3$ Hz) ppm. ^{13}C NMR (67,8 MHz): δ 15.2, 19.6, 24.1, 52.7, 58.2, 60.6, 80.0, 87.7, 98.5 ppm. Mass spectrum, *m/z* (*relative intensity*): 171 (M^+, 0.2 %), 157 (4), 83 (17), 73 (22), 55 (12), 53 (7), 45 (54), 44 (21), 43 (100), 40 (7).

rac-*5-(1-Ethoxyethoxy)-3-pentyn-2-yl acetate* (**2Ac**). *rac*-5-(1-Ethoxyethoxy)-3-pentyn-2-ol (**2**, 200 mg, 1.2 mmol) was dissolved in dry pyridine (5 ml). Acetic anhydride (2 ml) was added and the mixture was heated

to reflux for 2 min and then cooled to room temp. The solution was poured onto ice-water, stirred for 1.5 h and then the mixture was extracted with *n*-heptane. After washing with brine the organic phase was dried ($MgSO_4$) and the solvent evaporated off to give an oil which was distilled bulb to bulb (bath temp. 65-73 °C, 0.08-0.10 mbar) to give the acetate (154 mg, 0.7 mmol), in 62 % yield. This compound was unstable when stored neat and therefore gave unsatisfactory combustion analysis. n_D^{20} 1.4419. FT-IR: 2988, 1744, 1373, 1236, 1163, 1132, 1112, 1086, 1059, 1033 cm^{-1}. ^1H NMR (270 MHz): δ 1.15 (3H, d, *J* = 6.6 Hz), 1.27 (3H, d, *J* = 5.3 Hz), 1.43 (3H, d, *J* = 6.9 Hz), 2.02 (3H, s), 3.46 (1H, q of d, *J* = 6.9 and 2.3 *Hz*), 3.55 (1H, q of d, *J* = 7.3 and 1.65 *Hz*), 4.24 (2H, t of d, , *J* = 15.5 and 1.65 *Hz*), 4.77 (1H, q, *J* = 5.3 *Hz*), 5.44 (1H, q, *J* = 6.6 Hz) ppm. ^{13}C NMR (67.8 MHz) δ 15.1, 19.6, 20.9, 21.2, 52.6, 60.2, 60.7, 80.9, 84.0, 98.5, 169.7 ppm. Mass spectrum, *m/z* (*relative intensity*): 85 (41), 72 (19.5), 71 (61), 58 (38), 57 (100), 56 (19), 55 (18), 44 (30), 43 (92), 42 (30).

*Transesterification of racemic 5-(1-Ethoxyethoxy)-3-pentyn-2-ol (rac-***2***).* 5-(1-Ethoxyethoxy)-3-pentyn-2-ol (rac-**2**, 153.6 mg, 0.9 mmol), *n*-heptane (2.5 ml), tetradecane (51.27 mg, 0.3 mmol) and *CAL* (15.0 mg) were equilibrated to a_W = 0.32 (24 h). Vinyl acetate (280.5 mg, 3.3 mmol) was equilibrated similarly in another vessel and then added to the reaction mixture. Samples were taken from the stirred mixture for determination of conversion and optical purity. The conversion was determined with an internal standard method (tetradecane), and the optical purity of the remaining substrate after evaporation of the solvent and treatment with the chloride from (*R*)-(+)-MTPA in pyridine as described below (results see ■ in Fig 2a and b). In the dry experiment the experimental conditions were the same except that dry *n*-heptane and vinyl acetate was used. The substrate was dried in a dessicator over phosphorous pentoxide and the enzyme was stored over dried silica gel (results see ○ in Fig 2a and b).

*(*R*)-5-(1-Ethoxyethoxy)-3-pentyn-2-yl acetate (***R-2Ac***)* was obtained from a transesterification ("E"= 200) of rac-**2** using lipase from *Pseudomonas sp* (Amano PS). The reaction was interrupted at 44.3 % conversion, the product and remaining substrate were separated by MPLC and the acetate **R-2Ac** was purified by distillation. $[\alpha]_D^{25}$ + 9.5 (neat). *D.e.* (*"ee"*) ≈ 97.5 % (by GC-analysis, see below, after chemical hydrolysis and derivatisation with the chloride derived from (*S*)-(–)-MTPA).

*(S)-5-(1-Ethoxyethoxy)-3-pentyn-2-ol (***S-2***).* Further transesterification of the remaining substrate from above to a total *c* = 66.3 % (based on rac-**2**) gave the remaining substrate, **S-2** , after MPLC and distillation. $[\alpha]_D^{25}$ – 2.3 (neat). Spectral data was identical with those of rac-**2**. *D.e.* (*"ee"*) ≈ 99.8 % (GC-analysis after derivatisation with the chloride from (*R*)-(+)-MTPA).

Method[19] *for determination of optical purity in the remaining alcohol* **S-2** *in transesterification of 5-(1-ethoxyethoxy)-3-pentyn-2-ol.* A mixture of (2*S*)-5-(1-ethoxyethoxy)-3-pentyn-2-ol (**S-2**) with high optical purity (97.0 %) and racemic 5-(1-ethoxyethoxy)-3-pentyn-2-yl acetate was treated with the chloride derived from (*R*)-(+)-MTPA in dry pyridine at 50 °C. The optical purity of the MTPA-ester formed was analysed by GC (30 m x 0.32 mm I.D. capillary column coated with DB-WAX *df* = 0.25 μm; carrier gas He, 15 psi, split ratio 1/50, temperature programming: 130 °C, 1 min, 5 °C / min to 200 °C, 10 min) and after 24 h at 50 °C no statistically verified degradation of the optical purity of the formed MTPA-ester had occurred. Therefore this method could be applied on direct samples from the reaction vessel without any purification. Ret. time RR-ester: 20.3 min; RS-ester: 21.5 min. (The enantiomeric purity of the sample of chloride derived from (*R*)-(+)-MTPA used in all the analyses was checked by reacting it with enantiomerically pure (*R*)-1-phenylethylamine. The product was analysed by GC on the same column isothermal 200 °C. Ret. time RR-amide: 22.7 min; > 99.98% and SR- (or RS-)amide: 24.9 min < 0.02%. Thus the *ee* of this MTPA-chloride sample must be >99.95%.)

(S)–β–Methyl-(2-thiophene)propyl acetate (**1Ac**) was prepared as previously described[6], (*ee* = 93.6 %, determined by the method from ref.[8]).

*(*R*)-2-Methyloctanol* (**R-3**) came from the same batch as described[20] (*ee* > 99% determined by the method from ref.[8]).

Study of the formation of (S)–β–methyl-(2-thiophene)propanol (**S-1**) *from the corresponding acetate* (**S-1Ac**) *at a simulated conversion > 50 %. General method.*[8] (*S*)–β–Methyl-(2-thiophene)propyl acetate (**S-1Ac**, typically 30 mg) and (*R*)-2-methyloctanol (**R-3**, 22 mg) were dissolved in $CHCl_3$ (0.27 ml) in a reaction flask containing a magnetic stirring bar. *PFL* (4 mg) was mixed with $CHCl_3$ (0.34 ml) during the equilibration if the experiment were performed with controlled water activity. Otherwise with dry conditions, it was taken from the

dessicator with no further treatment. GC Analysis: The same DB-WAX column and conditions above but isothermal 150 °C, ret. times: **1Ac**, 8.3 min; **1**, 11.6 min. [Figures in percent is the amount of (*S*)–β–methyl-(2-thiophene)propanol (**S-1**) relative to that of the acetate **S-1Ac** and acetate of **R-3** relative to alcohol **R-3**].
Exp. I: No enzyme was added. After 24 h no significant formation of alcohol was noted.
Exp. II: Substrates and enzyme were equilibrated to a_W = 0.32 and then mixed: Alcohol **S-1**: (Reaction time/%**S-1**) 5 min/0.9; 10 min/1.5; 30 min/2.1; 1.3 h/2.8; 23 h/3.6; 20 h/6.7. At 20 h 4.7% of the acetate of **R-3** had formed.
Exp. III: Dry conditions, 3.7 equivalents of vinyl acetate and enzyme: Alcohol **S-1**: 10 min: 0.05 ±0.02%, 24 h: same.
Exp. IV: Dry conditions, vinyl acetate (neat, no solvent), enzyme: Alcohol **S-1**: 10 min: not detected, 24 h: 0.05 ±0.02%.
Exp. V: Substrates, solvent and enzyme were equilibrated to a_W = 0.32 whereafter equilibrated vinyl acetate was added: Alcohol **S-1**: 10, 30 and 130 min: constant level 1.5 ±0.1%.
Exp. VI: Solvent, substrate and enzyme were dry but the vinyl acetate (3.7 equivalents) was used as recieved without preequilibration. Alcohol **S-1**: 10, 30, 60 and 120 min: 0.5 ±0.1% alcohol **S-1** was present.

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6. **a)** As originally described by us[7] (*S*)-β–methyl-(2-thiophene)propanol (**S-1**) can be prepared by baker's yeast (*by*) reduction of (*E*)-2-methyl-3-(2-thienyl)-2-propenal. We now perform this reaction on a large scale using vigorous oxygenation and an improved work up by steam distillation. An alternative description of a large scale *by*-reduction of the same compound can be found in ref.[9] The enzyme catalysed resolution of β–methyl-(2-thiophene)propanol (**S-1**) to give **S-1Ac** and **R-1** was recently developed by us[8] and a similar procedure has later been reported also by others.[9] **b)** The presence of 3% of **R-1Ac** in the sample of **S-1Ac** should not have any consequences for the reasoning in this paper, since the former should be a much poorer acyl donor than the latter.
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