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Structure versus enantioselectivity in *Pseudomonas cepacia* lipase catalysed transesterifications. Enantioselective acylations of primary 2-methylalcohols

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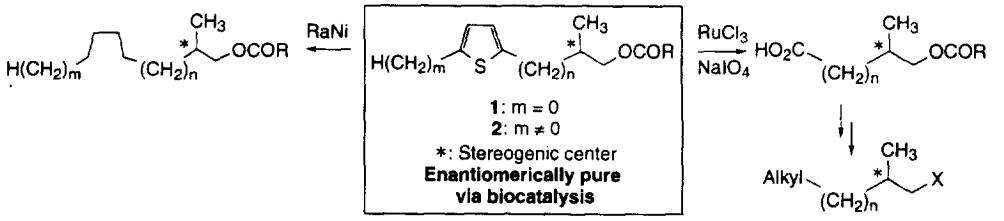
Abstract: The enantioselectivities of lipase from *Pseudomonas cepacia* (Amano PS) towards a series of primary 2-methyl-ω-(2-thienyl)alkanols using vinyl acetate as the acyl donor in transesterifications in organic solvents have been studied. It was found to be important to place the thiophene ring in the correct position in the chain. In terms of enantioselectivity, we found that the number of methylene groups between the stereogenic centre and the aromatic ring in chiral ω-aryl-2-methylalkanols ought to be one. © 1997 Elsevier Science Ltd

Enantiomerically pure primary 2-methylalkyl derivatives are useful building blocks in natural product synthesis.[1] For the preparation of these, the thiophene ring can be exploited either as a masked alkyl chain (via Raney-nickel reduction)[2a–c] or as a masked acid (via $RuCl_3$–$NaIO_4$–oxidation),[2a] the acid group of which can subsequently be transformed by elongation into an alkyl chain.[2d]



We have previously studied the preparation of primary 2-methylalkyl derivatives using enzyme-catalysed esterification of 2-methylalkanoic acids[3] and via baker's yeast reductions of *E*-2-methyl-3-(2-thienyl)propenals to (*S*)-2-methyl-3-(2-thienyl)propanols **S-1** and **S-2**, followed by RaNi-reduction.[2a,d]

In earlier work we and others have found that primary 2-methylalkanols such as 2-methyldecanol are not efficiently resolved using *Pseudomonas* lipase catalysed transesterifications with vinyl acetate as the acyl donor.[4] The E-values obtained are only around 10.[4] Previous results with *Pseudomonas* lipases show that not only are 2-methyl-3-arylpropanols much better resolved than 2-methylalkanols, they are also resolved much more efficiently than 2-methyl-2-arylethanols.[5]

Taking advantage of the results mentioned above the 2-thienyl group was used both as a phenyl mimic and as a masked chain by us[6a] and later by others.[6b] Thus, efficient resolution of *rac*-2-methyl-3-(2-thienyl)propanol **rac-1b** was achieved by *Pseudomonas sp.* lipase catalysed acylation with vinyl acetate as the acyl donor in organic solvents.[6] If one wants to exploit this type of resolution for the preparation of enantiomerically pure long-chain 2-methylalkyl building blocks, it essential to know in which position the masking thiophene should be placed in the racemic substrates. We have now studied the effect of moving the thienyl group away from the stereogenic centre.

We found that the position of the aromatic ring in compounds of this type is indeed crucial for obtaining a successful resolution. Thus, substrates having one methylene group between the stereogenic

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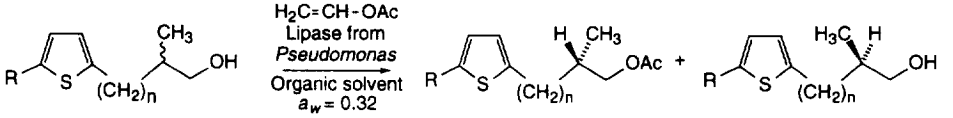
Table 1. Transesterifications of primary 2-methyl-ω-(2-thienyl)alkanols with vinyl acetate in different solvents, catalysed by lipases from *Pseudomonas sp.* (PFL or lipase Amano PS)

Entry[a]	Substrate	Solvent	c[b] %	ee_p[c] %	ee_s[d] %	E[e]	Enzyme	ref
1	**1a**	$CHCl_3$	39.9	31.0	20.5	**2.3** ± 0.1	PS	f
2	**1b**	$CHCl_3$	39.3	98.2	n.d.	**200** ± 40	PFL	4a
3	**1b**	$CHCl_3$	37.9	97.3	n.d.	**130** ± 20	PS	4a,f
4	**1b**	TBME[g]	42.2	97.5	n.d.	**170** ± 30	PS	f
5	**1c**	$CHCl_3$	40.1	76.6	45.6	**12** ± 1	PS	f
6	**1d**	TBME[g]	44.0	46.0	n.d.	**4.0** ± 0.3	PS	1c
7	**2a**	$CHCl_3$	39.9	68.8	42.2	**8.1** ± 0.5	PS	f
8	**2b**	TBME[g]	43.0	98.5	n.d.	**300** ± 60	PS	1c

[a]Reactions were carried out by dissolving the alcohol (1 mmol) in the solvent (1.8 ml) and equilibrating it to a water activity of 0.32 in a sealed container. Vinyl acetate (3.7 mmol) equilibrated to the same water activity was added.[6] [b]The conversion (*c*) was determined by GC by measuring the peak area of the produced acetate in relation to that of the alcohol substrate. [c,d]The enantiomeric excesses (p=product, s=substrate) were determined as described earlier.[6,7] n.d.: not determined. [e]The enantiomeric ratios E were calculated using the Sih formulae[8a] for entries 2–4, 6 and 7 or the Rakels one [8b] for entries 1, 2, 5 and 7. [f]This work. [g]TBME: tert-butyl methyl ether.

centre and the aromatic ring, as in compounds **1b**[6] and **2b**,[1c] were found to be the best substrates in terms of enantioselectivity (see Table 1 entries 2–4 and 8). If higher numbers of methylene groups were present [n=2 (**1c**) and 4 (**1d**),[1c] entries 5 and 6] or none [n=0, (**1a** and **2a**) entries 1 and 7], the E-values were much lower.



1a: R = H; n = 0. **2a:** R = n-C_4H_9; n =0.
1b: R = H; n = 1. **2b:** R = n-C_3H_7; n =1.
1c: R = H; n = 2.
1d: R = H; n = 4.

R-Ac-**1a** or *R*-Ac-**2a**
S-Ac-**1b-d** or *S*-Ac-**2b**

S-**1a** or *S*-**2a**
R-**1b-d** or *R*-**2b**

Thus, the resolution of substrates of type **1** or **2**, combined with subsequent RaNi-reduction, represents a clear improvement compared with direct resolutions of primary 2-methylalkanols. However, the replacement of four methylene groups in e.g. 2-methyldecanol by thiophene can lead to a highly enantiomerically pure terminal ω-position of the chain as in **1d**, the E-value (4, entry 6) is in fact significantly lower than that obtained with 2-methyldecanol (≈10)[4] itself.

Alkylation of the 2-thienyl group in the 5-position led to significantly increased E-values. When comparing the E-values obtained from the transesterifications of alcohols **1a** with that from the alkylated derivative **2a** and those of alcohols **1b** with that from the alkylated derivative **2b**, one finds increases of approximately 3.5 and 2 orders of magnitude, respectively (compare entries 1 with 7 and 4 with 8).

Since, to our knowledge, no X-ray structure of the enzyme from *Pseudomonas cepacia* is available, the spatial requirements for high substrate enantioselectivity of the enzyme have to be deduced by variation of the substrate structures. Models of enzyme pockets based on substrate structures are well established for secondary alcohols (Figure 1A), favouring *R*-substrates.[9] A similar model was recently suggested for 2-substituted primary alcohols (Figure 1B), in which the conformation of the preferred *S*-substrate is the one with the hydroxy group and the M- and L-substituents in approximately the same positions as in secondary alcohols.[9d,10,11] It has been suggested, that when a substrate of type **1** is docked inside the active site of the enzyme, there is a restricted empty volume available close to

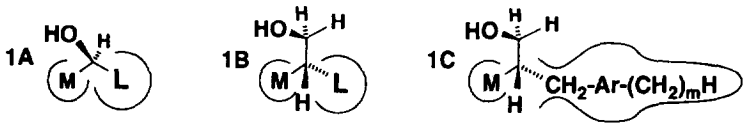


Figure 1. Empirical rules for the enantiopreference of *Pseudomonas* lipases. (1A) for secondary alcohols.[9] (1B) for 2-substituted primary alcohols[11] and (1C) for a 2-substituted primary alcohol based on a combination of our results, model 1B and a model suggested[5a] earlier for primary 2-methyl-ω-arylalkanols. L=large substituent; M=medium substituent.

the position where the inner methylene group is located in compounds **1b** (i.e. the methylene group between the aryl group and the stereogenic centre).[5a] The fact that the 2-methyl-3-(2-thienyl)propanols **1b** and **2b** gave higher E-values than the 2-methyl-2-(2-thienyl)ethanols **1a** and **2a** seems to support this hypothesis. A combination of the models from Figure 1B, that from ref.[5a] and our results is presented in Figure 1C. If the pocket in Figure 1C also represents the model of the enantiopreferences of *Pseudomonas* lipase for secondary alcohols, one would expect lower rates and enantioselectivities for secondary alcohols with no methylene group between the stereogenic centre and the aromatic group compared with those which have such a methylene group. However, this is not the case.[9d] Therefore, model 1C cannot represent the whole truth about the observed differences in reactivity of the compounds studied here.

The significant difference in enantioselectivities observed between compounds **1a** and **2a** and between compounds **1b** and **2b** can hypothetically be explained either by remote positive interaction with a hydrophobic pocket in the enzyme or as an electronic effect. In the latter case, the donor properties of the alkyl group would enhance the electron density in the aromatic ring leading to a more favourable interaction with complementary groups in the enzyme pocket. The latter hypothesis was tested by resolving 2-methyl-3-(5-propionyl-2-thienyl)propanol (propionyl replaces 1-propyl in **2b**), a compound with an electron withdrawing group on the thiophene ring. No significant difference between the E-values of the propionyl compound (E≈250) and the 1-propyl compound **2b** (E≈300, entry 8) was observed. Thus, by exclusion the first hypothesis is strengthened.

In summary, if one wants to prepare nonracemic primary 2-methylalkanols of high enantiomeric purities by enzymatic resolution using transesterifications, the use of a thiophene moiety as a masked tetramethylene unit is advantageous. However, as shown here, it is very important to place this thiophene residue in the correct position in the masked alkyl chain.

Acknowledgements

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