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# HOW LARGE ARE THE ACTIVE SITES OF THE LIPASES FROM CANDIDA RUGOSA AND FROM PSEUDOMONAS CEPACIA ?

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**Abstract:** Racemates of 2-Azido alcohols of large, polycyclic systems can be resolved by enzyme catalyzed hydrolysis of the respective butyrates using lipases from *Candida rugosa* (*cylindracea*) and from *Pseudomonas cepacia* (*fluorescens*) with excellent optical and chemical yields. An estimate of the size of the respective hydrophobic pockets of these lipases is given.

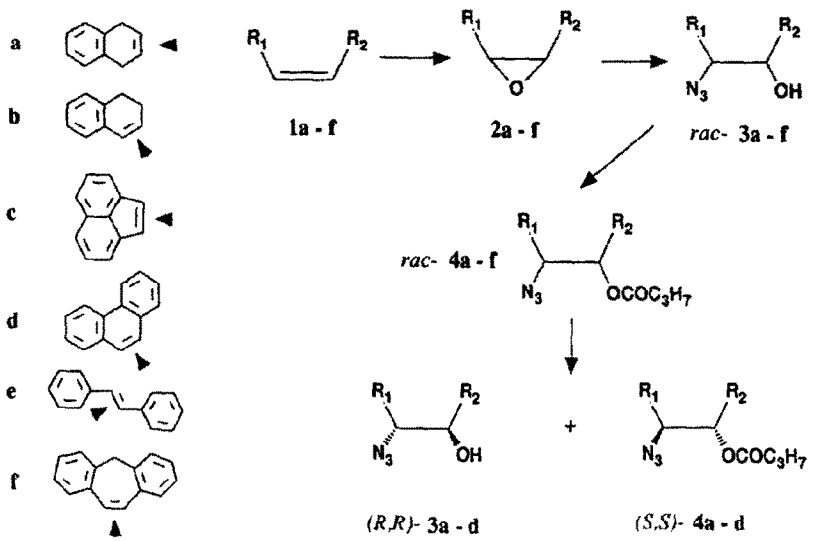
**Introduction:** Enzyme - catalyzed syntheses are well established methods for the preparation of enantiomerically pure compounds[1,2]. Especially hydrolases have proven to be extremely useful for synthetic organic chemists, because they are very efficient, do not require co-factors, the reactions can be carried out on large scale and they apply to a wide range of substrates. The appropriate enzymes are usually chosen by screening, because there is only very little information on the structures of lipases. Only a few high resolution x-ray crystal structures have been published recently[3,4,5]. Therefore, substrate models have been developed and are used to predict, which substrate under consideration could be transformed and whether there will be a stereoselection or not[6,7,8,9]. These models are usually accurate only for substrates similar to those used to generate the model. In some cases, more general models which define the sizes of the hydrophobic pockets around the active site have been developed[10]. They can be used to predict the reactivity for a wide range of substrates.

Lipases from *Candida rugosa* (*cylindracea*) and from *Pseudomonas cepacia* (*fluorescens*) are those most often used for enantioselective resolutions of chiral alcohols or acids in synthetic organic chemistry[11].

For these lipases the published models only predict the enantioselectivity of the enzymatic resolution, they are not concerned about the absolute size of the substrate but of the relative size or hydrophobicity of the substituents at the stereo center. In our ongoing work on the use of enzymes in the preparation of optically active amino alcohols we have published data for their respective precursors (2-azido cyclanols) with different ring sizes[12,13] and many acyclic structures[14,15,16]. We now report on an extension of this method to larger, conformationally fixed 2-azido alcohols in order to get some knowledge about the size of the hydrophobic pockets of the lipases used.

**Preparation of starting materials:** The epoxides **2a-f** with the exception of **2c** could easily be obtained by oxidations of the respective alkenes with peracetic[17] or m-chloroperbenzoic acid[18,19]. The oxidation of acenaphthene (**1c**), in contrary to the literature[20], did not yield the expected

epoxide but a mixture of different ketones. Thus **2c** was prepared by hydrobromination of **1c** with NBS in THF/water and subsequent treatment with KOH[21]. The azido alcohols **3a-f** were prepared by nucleophilic ring opening of the respective epoxides. The resulting azido alcohols showed the same physical data as described in the literature (**3a**[22], **3b**[23,24], **3c**[25], **3d**[26], **3e**[27]) and/or gave the expected NMR ($^{13}C$ and $^{1}H$) spectra. Esterifications were carried out according to standard procedures[28].




$^{13}C$-NMR- data of the hitherto unknown butyrates **4a-f** are summarized below:

| Compound[a] | C(OR) | $C(N_3)$ | arom. C (resolved resonances) |
|---|---|---|---|
| **4a** | 72.1 | 59 9 | 126.8 - 132.5 (6) [b] |
| **4b** | 72.2 | 62.4 | 126.4 - 136.0 (6)[c] |
| **4c** | 82.0 | 70.2 | 121.3 - 138.9 (9) |
| **4d** | 71.9 | 62.2 | 124.3 - 133.4 (11) |
| **4e** | 77.4 | 69.4 | 127.9 - 136.6 (7) |
| **4f** | 74.5 | 66.3 | 126.9 - 139.3 (11)[d] |

[a] $^{13}C$-NMR-shifts (in ppm) of the acyl residue varied from 173.0-173 5 (C-1'),36.4-36 5 (C-2'), 18 6-18.7 (C-3') and 13.7-13.8 (C-4'). [b] 33.5 and 33.8 ppm for the aliphatic ring atoms. [c] 25 3 and 25.8 ppm for the aliphatic ring atoms. [d]40.1 ppm for the aliphatic ring atom.

**Table 1.** $^{13}C$-NMR-data of the butyrates **4a-f** in $CDCl_3$, values in ppm.

**Enzymatic hydrolyses:** The results of the enzymatic hydrolyses of the butyrates **4a-f** are summarized below:

| substrate | enzyme[a] | time [h][b] | conv[%] | yield[%][c] | $[a]^{D}_{20}$[d] | ee[%][e] | E[f] |
|---|---|---|---|---|---|---|---|
| **4a** | CC | 3.5 | 50 | 42 | -75.9 | 54 | 6 |
| **4a** | P | 21 | 50 | 44 | -136.1 | >98 | >100 |
| **4b** | CC | 6.5 | 50 | 44 | +7.6 | 60 | 7 |
| **4b** | P | 15 | 50 | 37 | +12.6 | 98 | >100 |
| **4c** | CC | 9 | 40 | 37 | +16.2 | 31 | 2 |
| **4c** | P | 24 | 4 | 2 | +44.7 | 71 | 6 |
| **4d** | CC | 8 | 12 | 10 | -200.2 | >98 | >100 |
| **4d** | P | - | - | - | - | - | - |
| **4e** | CC | - | - | - | - | - | - |
| **4f** | CC | - | - | - | - | - | - |

[a] All reactions were performed in 0.1 M phosphate buffer (100 ml), ph 6.5 at 25°C, substrate 25 mmol, enzyme 0.5 g; [b] Time for the given conversion; [c] isolated yield; [d] c = 2, $CH_2Cl_2$; [e] determined by the value of the optical rotation and independently checked by $^1$H- and $^{19}$F-NMR-spectra of the respective MTPA esters; [f] Enantiomeric ratio[29].

**Table 2.** Enzyme catalyzed hydrolyses of butyrates **4a-f.**

**Results and discussion:** As can be seen by the results with **4d**, CC seems to be able to convert bigger substrates, while P always is more enantioselective. **4b** formally can be regarded as part of the structure of **4d** (the latter exhibiting one additional condensed phenyl-ring). **4b** is hydrolyzed by both lipases in reasonable time, with minor ee by CC and with excellent ee by P. **4d** however is only attacked by CC but with excellent ee. Thus it looks like the hydrophobic pocket of P being asymmetrically shaped in the vicinity of the binding site (the azido function). The limit of the more restricted half of the hydrophobic pocket of P seems to be the size of one half of an acenaphthene molecule. The striking difference between **4d** and **4e**, which easily can be rotated in a form of similar shape to **4d**, but apparently is not attacked by either lipase, to us at first sight was very surprising. Molecular mechanics calculations however revealed, that regardless of the orientation of the two benzene rings in **4e** (either like *cis*- or like *trans*-stilbene), the phenyl rings strongly tended to adopt a conformation almost perpendicular to the central C(O-acyl) - C ($N_3$) - bond. **4f** is also not attacked by either enzyme. The overall distance of the most distant phenyl hydrogens in **4d** (ca. 9.2 A) and in the more *cis*-stilbene like conformation of **4e** (9.3 A) in fact are identical. This seems to be the maximum width of the hydrophobic pocket of CC, which on the other side is very flat, at least its extension in the direction perpendicular to the plane of the aromatic rings is less than the size required for an upright phenyl ring. (ca. 5 A).

The limit for P in the direction of the binding site is about the same as for CC, whereas in the other direction (towards the active site), acenaphthene like molecules seem to represent the maximum size slowly attacked (one half of around 7.1 A).

**Conclusion**: Lipases CC and P can catalyze the hydrolysis of quite large but flat molecules, CC to a limit of around 9.2 A, P to a limit of around 7.1 A, although at the binding site of P, the substrate

can extend a little further. Thus P seems to exhibit a somewhat asymmetrically shaped hydrophobic pocket, the respective pocket of CC being almost symmetrical. If both enzymes are able to convert a substrate, P always exhibits the higher enantioselection.

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