Bioorganic & Medicinal Chemistry Letters, Vol.3, No.8, pp. 1655-1658, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

The Effect of Aryl Fluorines in a Lipase Resolution.

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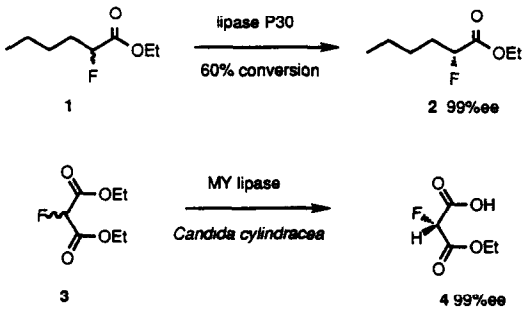
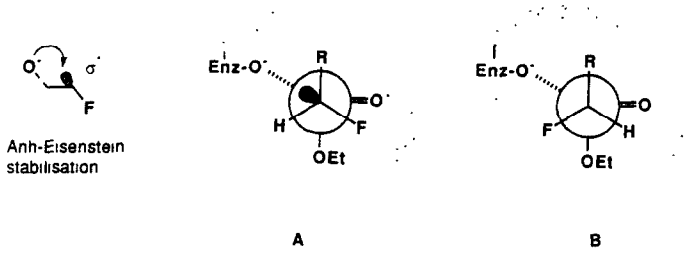
(*Received in USA* 29 *April* 1993)

Abstract; The steric influence of fluorine compared to hydrogen in enzymatic systems is probed by the resolution of a series of fluorinated benzhydrol acetates with lipases. Lipase AY-Amano-30 from *Candida cylindracea* can distinguish a single fluorine atom and the discrimination is most significant with fluorine in the ortho position close to the reaction centre. Increasing the number of fluorine atoms improves the discrimination and 2,3,4,5,6-pentafluoroaryl benzhydrol was resolved in 86%ee after 35% conversion.

It is an increasingly common strategy in the design of enzyme substrate analogues or enzyme inhibitors to replace a hydrogen for a fluorine atom [1]. The similar Van der Waal's radii, for hydrogen (1.2Å) and fluorine (1.35Å) suggest that enzymes in general will have difficulty in distinguishing between them on steric grounds[2]. On the other hand substitution of fluorine for hydrogen can be expected to induce an electronic perturbation of the system. Perhaps two of the most spectacular examples involving the direct discrimination of hydrogen and fluorine in enzymatic systems are illustrated in Scheme 1.

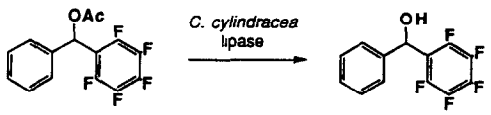


In the first case ethyl 2-fluorohexanoate **1** was resolved into its component enantiomers using the lipase from *Pseudomonas fluorescens* [3]. After 60% conversion the residual ester was judged to be soley the R-enantiomer **2** in 99.9%ee. In the second case Amano MY lipase (*Candida cylindracea*) exclusively hydrolysed the *pro*-S ester group of the *meso* compound, diethyl fluoromalonate **3**, to give **4** with an enantiomeric excess judged to be 99%[4]. These resolutions require the enzyme to make a direct distinction between hydrogen and fluorine atoms, and the exclusive nature of the discrimination in these cases suggest that the resolution is controlled by a combination of steric and electronic factors.



Recent theoretical analysis[5] of nucleophilic attack at sp^2 hybridised carbons with fluorine at an α chiral centre suggests that the preferred transition state geometry will have fluorine antiperiplanar to the incomming nucleophile. This is due to a n-σ* stabilising interaction of the Ahn-Eisenstein type[6,7] as shown for transition state **A**, which will be stabilised with respect to transition state **B**. This type of electronic effect may make a significant contribution to the enantioselectivity of the lipases shown for substrates **1** and **3**.

In order to probe the extent to which enzymes can distinguish hydrogen and fluorine on steric grounds alone, we have explored the steric influence of a series of aryl fluorines in various enzyme systems. For aryl fluorines the size of the fluorine atom is shielded to some extent by its conjugation into the aryl ring system, and therefore the steric difference between an aryl hydrogen and fluorine is expected to be smaller than that in sp^3 hybridised systems. This was therefore judged to be an appropriate investigative system to test the ability of an enzyme to make such a steric distinction. In the first instance pentafluorobenzhydrol acetate **5** was presented to a range of lipases.



5 **6**

35% conversion 89%ee

40% conversion 68%ee

There was no hydrolysis over extended periods of time (4-5days) using the *Mucor* (Lipase M Amano 10), *Rhizopus* (Lipase N Conc), *Pseudomonas* (Lipase PS Amano) and calftounge root lipase (PGE Amano), lipases from the Amano company. Lipase AP6 from an *Aspergillus* sp. hydrolysed the acetate to 40% conversion in 48hours, however the resultant alcohol **6** was racemic, and clearly no distinction was made by the enzyme between the aryl and pentafluoroaryl rings. A wheatgerm lipase (Sigma Type I) completely hydrolysed **4** in several hours and the reaction was judged too rapid to pursue further. However we found that Lipase AY-Amano-30 from *Candida cylindracea* was optimal and a 40% conversion was achieved in 24 hours affording alcohol **6** with a 68% enantiomeric excess. If the reaction was stopped after 35% conversion, then the alcohol **6** was isolated in higher optical purity (86% ee). The absolute configuration was not determined.

Having found an enzyme that was capable of distinguishing between the aryl and a pentafluoroarly ring system, a series of partially fluorinated benzhydrol acetates was presented to the lipase to determine at which sites the fluorine atoms were of steric significance. All of the reactions were terminated after 40% conversion and the %ee of the resultant alcohols determined. The results are outlined in Table 1. Mono-substitution of fluorine at the para position (4-fluoro, entry 2) resulted in racemic alcohol, and clearly no distinction was made by the enzyme between the aryl groups. However, the presence of fluorine in the 2-, and 3-positions induced varying degrees of enantioselectivity.

Entry	Fluorine substitution of benzhydrol acetate	%ee of alcohol at 40% conversion
1	2-fluoro	10
2	4-fluoro	0
3	2,4'-difluoro	22
4	2,5-difluoro	57
5	2,6-difluoro	52
6	3,5-difluoro	24

Table The %ee of recovered alcohol after hydrolytic resolutions of partially fluorinated benzhydrols with the lipase from *C. cylindracea*

Monosubstitution of the substrate with fluorine in the ortho position (2-fluoro, entry 1) resulted in a poor resolution (10%ee), however the enantioselectivity increased significantly when a second fluorine atom was placed in the other ortho site, (2,6-difluoro, entry 5) (52%ee). Two fluorine atoms in the meta positions (3,5-difluoro, entry 6), gave a significant but low enantioselectivity (24%ee), however with one fluorine in the ortho and the other in the meta position (2,5-difluoro, entry 4) 8, then the recovered alcohol had an enantiomeric excess of 57%.

From these observations it can be concluded that appropriate enzyme systems can distinguish aryl fluorines, most probably on steric grounds. In the two monosubstitited cases studied (2-fluoro and 4-fluoro) the fluorine in the ortho position closest to the reaction centre was sterically the more significant, although the effect is small (10%ee versus 0%ee). Interestingly when the fluorines were placed on the same molecule in different rings at the 2- and 4'- positions (entry 3) there was an improvement in the enantioselectivity (22%ee).

Two fluorines on the same aryl ring become sterically significant. For the 2,6- and 2,5-difluoro cases, enantioselectivities in the 50% region demonstrate this. However when the fluorines are in the meta positions (3,5-difluoro) the enantioselectivity is lower (24%). Again substitution on the ortho position improves the selectivity of the enzyme.

From this series of experiments we conclude that certain enzyme/substrate combinations will distinguish hydrogen and fluorine on a steric basis, however we remain aware that it is always difficult to delineate completely contributions due to electronic factors. It is noteworthy however

that the true size of the two atoms can be more representatively judged from their relative Van der Waal's volume (hemisphere) rather than their radii. This has been highlighted for CF_3 groups[8] and on this basis fluorine (5.15Å^3) commands a significantly greater volume than hydrogen (3.61Å^3).

EXPERIMENTAL

General Method for Lipase Resolutions. Typically 200mg of the fluorinated benzhydrol acetate was suspended in phosphate buffer (pH7)(20ml) and 300mg of Lipase AY-Amano-30 (9000 units) added and the reaction incubated with stirring at 40°C. The reactions were monitored by ^{1}H-NMR after removal of aliquotes until 40% conversion had been achieved. The product mixture was then extracted into diethyl ether, and washed with water. The organic extract was dried over $MgSO_4$ and the solvent removed under reduced pressure. The alcohol and acetates were purified by chromatography over silica gel eluting with hexane: dichloromethane (80:20).

All compounds had satisfactory spectroscopic data. For enantiomeric excess determinations the alcohols were reconverted to their acetates using acetic anhydride/pyridine, and purified by chromatography as above. %Ee's were assessed by ^{1}H-NMR analysis in CCL_4 after addition of the chiral shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato], europium (III). In the case of 2,3,4,5,6-pentafluorobenzhydrol **6**, $[\alpha]^{20}_D$ -21 (c=1.4, CH_2Cl_2)(89%ee) the %ee was confirmed by ^{1}H- and ^{19}F-NMR analysis after derivatisation as a (S)(+)methyl mandelate ester.

Acknowledgement

We thank the Amano Company UK, for a generous gift of lipase enzymes.

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