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Enzymatic Modification of Fullerene Derivatives

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Abstract: The activity of some representative enzymes has been tested on a series of fullerene derivatives. When the functional group that interacts with the enzyme active site is close to the fullerene spheroid, the reaction rates slow down, whereas relatively fast reactions are observed for reaction centres spatially distant from the carbon sphere. © 1998 Elsevier Science Ltd. All rights reserved.

A study of interactions between fullerenes and enzymes may potentially contain several points of interest. First of all, fullerene derivatives have shown a broad range of promising biological activities,[1,2] some of which are related to the inhibition of enzymes.[3,4] Secondly, enzymatic reactions involving bulky substrates are not common. Bulky substrates, in fact, are not easily accommodated inside the active site of most enzymes. Successful reactions may provide new insights on the mechanism of action of the enzyme. Finally, there may be some convenience in obtaining enantiomerically pure fullerene derivatives.[5] Many hydrolytic enzymes are commonly used to resolve racemic mixtures, but this practise has never been applied, so far, to fullerene derivatives.

We report herein the modification of some fullerene derivatives in toluene, successfully induced only by a restricted number of lipases among a wide range of enzymes tested.

The fullerene derivatives studied in the present work are reported in Scheme 1. They were prepared by cycloaddition of azomethine ylides to C_{60}.[6] Compound **1** was prepared by thermal ring-opening of the corresponding aziridine, whereas pyrrolidine **2** was obtained from DIBAL-H reduction of the parent ethyl ester. Compounds **3a-c** were synthesised from the reaction of N-functionalized glycine esters with formaldehyde. During the cycloaddition, the glycine ethyl ester derivative needed protection of the -OH group *via* the dimethyl-*t*-butylsilyl ether, which was then removed in the presence of stoichiometric boron trifluoride-ethyl etherate. Esters **4a-c** were obtained from the reaction of **3a-c** respectively with palmitoyl chloride.

Initial efforts in this work were directed to the preparation of optically pure fulleroproline derivatives.[7] So far, chiral proline derivatives for incorporation in fullerene-containing peptides have been obtained *via* enantioselective HPLC[7] or *via* diastereoselective additions.[7,8] However, the chiral discrimination with enzymes would offer the potential advantage of scaling up the reactions.[9] In this connection, proline **1** was a suitable target, since the trifluoroethyl group is a good leaving group in enzymatic reactions, and the N-(4-methoxybenzyl) protective group can be removed under acidic conditions.[7]

Attempts, to effort transesterification using **1** as a substrate, ethanol, and a number of enzymes failed.[10]

Compounds **3a-c** were then investigated for two main reasons. First of all, the variable length of the alkyl arm on nitrogen is a good probe for checking the influence of the distance of the reaction centre from the spheroid. Secondly, the steric hindrance of the ester group (ethyl *vs.* *t*-butyl) may also play a role.

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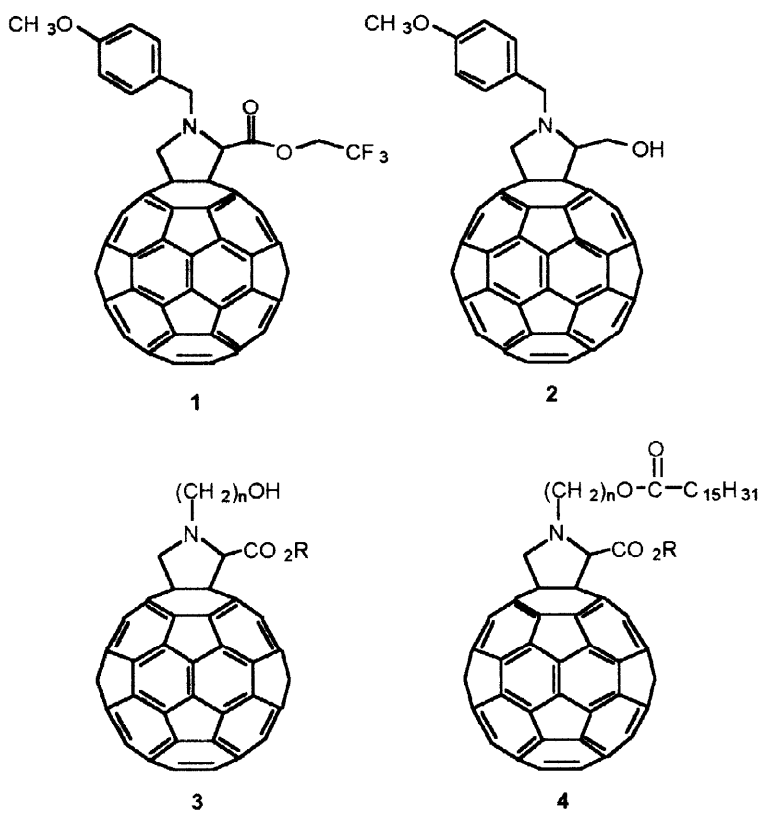

a, n = 3; R = Et
b, n = 2; R = Bu^t
c, n = 3; R = Bu^t

Scheme 1

Enzymatic transesterification of alcohols **3a-c** was successfully performed in toluene using 2,2,2-trifluoroethyl esters of palmitic and butyric acids as the acyl donors. Among the hydrolases tested, only lipase B from *Candida antarctica* (CALB) and lipoprotein lipase from *Pseudomonas specie* (LPL) were able to catalyse the acylation of fullerene derivatives, the former lipase being the more active. The results of the transesterification reactions, carried out with a series of enzymes using 2,2,2-trifluoroethyl palmitate as acylating agent, are collected in Table 1.

Enantioselectivity in the transesterification reactions, leading to resolution of the C_α centre of the proline ring, is expressed in Table 1 as ee% (enantiomeric excess). CALB shows no enantioselectivity towards substrates **3a**,**b**. Reaction of compound **3a** with LPL led to modest enantioselectivity (ee = 45% at 15% conversion), this may be significant if the distance between the chiral centre and the hydroxyl group reacting with the lipase active site is considered.

Steric requirements of the enzymatic reaction are clearly indicated by the initial rate data reported in Table 1. CALB and LPL are similarly affected by the steric features of substrates. The bulkiness of ester group leads to a heavy decrease of the reaction rates, whereas the length of alkyl arm on nitrogen exerts a negligible

influence. It is noteworthy that CALB proved unable to catalyse the transesterification of **1** with 1-pentanol (Table 1). Moreover, also the acylation of substrate **2** in the presence of 2,2,2-trifluoroethyl palmitate gave no result. As a consequence, it seems that the C_{60} moiety hinders the productive fitting of the substrate either in the acyl- or alcohol-binding sites when the reaction centre is close to the fullerene spheroid. If results are examined taking into account X-ray structural data of CALB,[11] it becomes evident that fullerenes, because of their size (≅10Å in Van der Waals diameter), cannot be accommodated inside the lipase active site. However, the C_{60} spheroid can establish hydrophobic interactions with more superficial regions of the enzyme, while the pyrrolidine moiety approaches the active site cavity of the lipase and the hydroxyl group interacts with the catalytic triad.

Table 1: Initial Rates and Enantioselectivity of Transesterification Reactions [a,b]

Substrate	Enzyme[c]	v_0 [d] *(mM/min)*	ee%[e] (at conv. %)	Final conversion % (time)
3a	CALB	$1.5 \cdot 10^{-1}$	<10 (50)	87 (3 days)
3a	LPL	$7.7 \cdot 10^{-5}$	45 (15) 27 (50)	61 (19 days)
3b	CALB	$4.6 \cdot 10^{-2}$	<10 (50)	93 (3 days)
3b	LPL	$8.6 \cdot 10^{-6}$	[f]	7.3 (19 days)
3c	CALB	$6.4 \cdot 10^{-2}$	[g]	82 (3 days)
3c	LPL	$4.1 \cdot 10^{-6}$	[g]	17 (19 days)
3a-c	CALBr	[f]	[f]	-
3a-c	CAL	[f]	[f]	-
1[h]	CALB	no reaction	-	-
2	CALB	no reaction	-	-

[a] Experimental conditions: [substrate] = 4.4mM; [2,2,2-trifluoroethyl palmitate] = 44mM; [CALB] = 2mg/mL; [CAL] = 10mg/mL; [CALBr] = 10mg/mL; T = 30°C; solvent = toluene dried over 4Å molecular sieves.

[b] Attempts of transesterification of **3a-c** working under the same conditions as described under note a and using lipase A12 (Amano), lipase from porcine pancreas (Sigma), Subtilisin Carlsberg (Sigma) and α-Chymotrypsin from bovine pancreas (Fluka) gave no appreciable reaction.

[c] CALB = lipase B from *Candida antarctica* immobilised (Novo Nordisk); LPL = lipoprotein lipase from *Pseudomonas specie* (Amano); CALBr = lipase B recombinant from *Candida antarctica* (Fluka); CAL = lipase from *Candida antarctica* (Fluka).

[d] Initial rates were determined by means of normal phase HPLC, using a Buckycluster 1 column from Regis. Eluents: A = 5% isopropanol in toluene, B = *n*-hexane (**3a,b**: 77%A, 23%B, flow = 1.1mL/min; **3c**: 74%A, 26%B, flow = 0.95mL/min). v_0 were calculated for mg of catalytic protein.

[e] *ee%* were determined by means of enantioselective HPLC, using a Welk-O column from Regis (**3a,c:** 50% *n*-hexane, 50% toluene, flow = 1.1mLmin; **3b:** 44% *n*-hexane, 56% toluene, flow = 1.15mL/min).

[f] Not determined because of low conversion.

[g] Under HPLC experimental conditions (see note e in this table) enantiomers were not separated.

[h] 1-pentanol was used as a nucleophile.

More insight into the comprehension of differences in reactivity and enantioselectivity will be gained by structural and computational studies of lipases and fullerene derivatives. It would be of particular interest to verify whether any correlation exists between the remarkable activity of CALB towards fullerene derivatives and the fact that the interfacial activation phenomenon is missing in the mechanism of action of this particular lipase.[11]

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