A Catalytic Antibody Elicited by a Hapten of Tetrahedral Carbon-type

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A monoclonal antibody, elicited by a α,α,α′,α′-tetrafluoroketone hapten, acted as an enzyme-like catalyst for the hydrolysis of an analogous carbonate, demonstrating that the difluoromethylene unit can been used as an isostere and isoelectronic mimic for the oxygen atom in an ether.

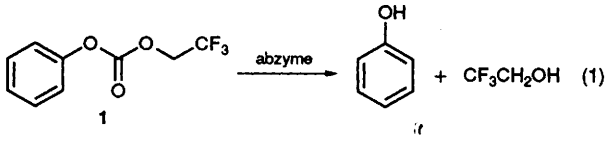
The transition state (TS)-analogue concept has been established as a valuable approach to the design of catalytic antibodies.[1–6] To date phosphonates and/or phosphates have been employed as TS-analogues for the hydrolysis reactions of esters and carbonates.[7–11] However, the rate enhancements exhibited by antibody reagents are generally lower than those of enzymes. Therefore, the challenge of preparing a new type of TS-analogue, for the development of antibody reagents with higher reaction rates remains.

In this paper we describe work aimed at designing and preparing a new type of hapten that mimics the TS for the hydrolysis of carbonates and esters more accurately. Furthermore, it is found that a monoclonal antibody, elicited by the above designed hapten for hydrolysis of carbonate and esters, acted as an enzyme-like catalyst. A fundamental objective of hapten design is to select the TS-analogue with an environment complementary in structure and electronic distribution to a TS of a given reaction. In fact, the tetrahedral TS for the hydrolysis of carbonates have been mimicked with tetrahedral phosphates.

To make clear more quantitative comparisons, important bond parameters have been evaluated by using the difluoromethylene functionality, which can be regarded as an isopolar and isosteric replacement for ether oxygen. The CF_2–C and C–CF_2 bonds in the PM3 optimized structure of a α,α,α′,α′-tetrafluorodiol are 15% longer than those of O–C or C–O bonds.† Furthermore, it is well known that α,α-difluoroketones, which have a highly electrophilic carbonyl group, exist in the stable hydrate form in aqueous media and as a result serve as ideal structural mimics of the putative tetrahedral intermediate [eqn. (1)]. The TS-analogue of sp^3 carbon-type in the hydrolysis of carbonate was designed upon the basis of these considerations.

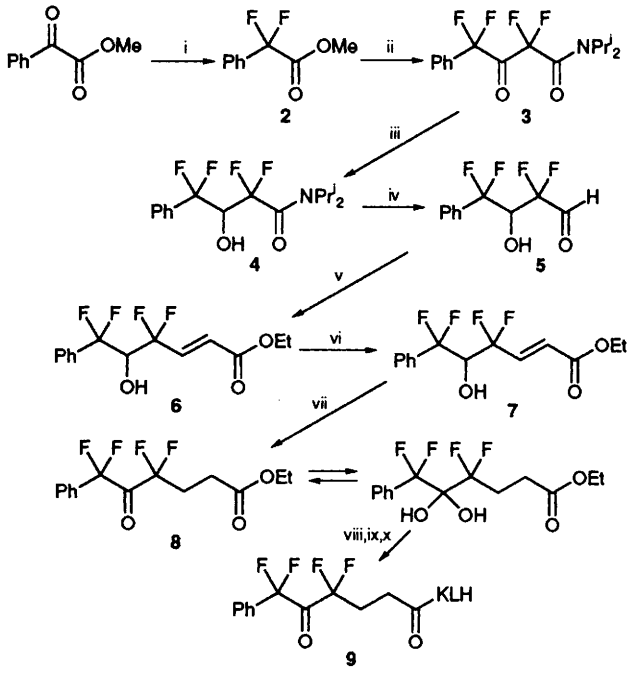
A convenient synthetic route to the designed hapten is shown in Scheme 1. The α,α-difluoro ester **2** was prepared from the direct fluorination of the ketoester. To attain the desired α,α,α′,α′-tetrafluoroketone unit, we required the precusor ethyl 4,4,6,6-tetrafluoro-3-hydroxy-6-phenylhexanoate **7**. Compound **3** was prepared by reaction of the α,α-difluoro ester **2** with *N*,*N*-diisopropyl α,α-difluoroacetamide. The reduction of the amide group with lithium alminium hydride in THF at −78 °C, was followed by coupling the resulting 2,2,4,4-tetrafluoro-3-hydroxy-4-phenylbutanal and triethyl phosphonoacetate in THF. Hydrogenation by Pd–C/H_2 in ethanol was required as the final step.

The next step in the synthetic strategy required the preparation of ethyl 4,4,6,6-tetrafluoro-5-oxo-6-phenylhexanoate **8** which proceeded to the formation of the hydrate form, ethyl 4,4,6,6-tetrafluoro-5,5-dihydroxy-6-phenylhexanoate in water. For the present purpose, compound **7** was oxidized with Dess–Martin reagent.[12] ^{19}F NMR analysis of the resulting compound **8** [δ from C_6F_6 as external standard; 55.9 (tt, J_{FH} 10.3, 17.2 Hz), 57.8 (t, J_{FH} 9.92 Hz)] in D_2O revealed the exclusive formation of the hydrate form [δ 50.7–50.8 (m), 54.1 (t, J_{FH} 20.6 Hz)]. To form the desired antibody reagents, an immunogenic conjugate[13,14] was prepared by reaction of the compound **8** with a carrier protein (bovine serum albumin or keyhole limpet haemocyanin). Lymphocytes from the spleen of BALC/c mice immunized with each type of the purified antigens (the BSA-hapten conjugate or the KLH-hapten conjugate)‡ were fused by standard protocols using mouse myeloma cells (P 3-X 63-Ag.8 U 1) as the fusion partner. Antibodies were screened by ELISA for cross-reactivity with the BSA-hapten conjugate or the KLH-hapten conjugate, *i.e.* for the inhibition of binding to the BSA-hapten conjugate by free hapten. Six or nine antibodies were obtained for each hapten. Antibodies were purified from ascites fluid by protein A Sepharose 4B affinity chromatography and were determined to be >95% homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

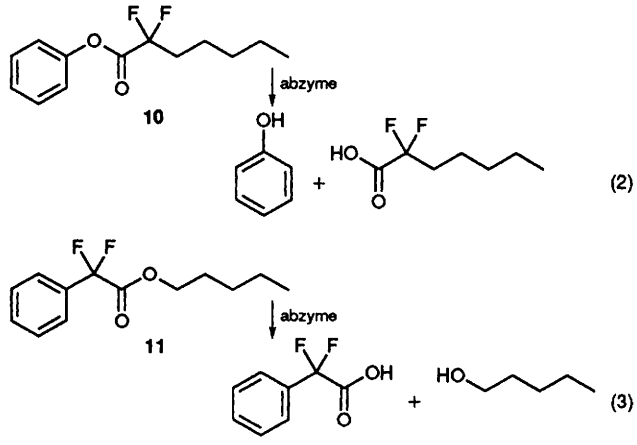


The rates of hydrolysis of carbonate **1** in the presence (k_{cat}) and absence (k_{uncat}) of 15 μmol dm^{-3} antibody were determined as a function of substrate concentration. Carbonate hydrolysis was monitored by ^{19}F NMR spectroscopy observing the increase of the signal intensity due to 2,2,2-trifluoroethanolate ion release.

Kinetic constants were determined by the method of initial-rates data. Kinetic parameters for the hydrolysis of carbonate **1** from the Lineweaver–Burk plots were determined. The value of k_{cat} and the Michaelis constant K_m, were found to be 0.94 ± 0.2 min^{-1} and 440 ± 90 μmol dm^{-3}, respectively. This antibody-catalysed hydrolysis of carbonate **1** was inhibited by



Scheme 1 i, DAST, 40 °C, 4 h, yield 87%; ii, $CHF_2CONPr^i_2$, LDA, THF, −78 °C; iii, $NaBH_4$, EtOH, 40 °C, 5 h; iv, $LiAlH_4$; THF, −78 °C → 20 °C; v, $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, room temp; vi, Pd–C, EtOH, room temp; vii, *o*-iodobenzoic acid, $KBrO_3$, H_2SO_4, Ac_2O, AcOH, 100 °C (Dess–Martin reagent, 1.6 mol dm^{-3} in CH_2Cl_2), 50 °C, 4 h; viii, lipase-MY; ix, keyhole limpet haemocyanin (KLH), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, dilute HCl, pH 5.0; x, dialysis, NaCl buffer, pH 7.4



eqn. (2)		eqn. (3)	
k_{cat}	0.51 ± 0.2 min^{-1}	k_{cat}	0.67 ± 0.2 min^{-1}
K_m	270 ± 40 μmol dm^{-3}	K_m	310 ± 40 μmol dm^{-3}
k_{uncat}	7.5 ± 10^{-5} min^{-1}	k_{uncat}	7.4 ± 10^{-5} min^{-1}
k_{cat}/k_{uncat}	6800	k_{cat}/k_{uncat}	9050

the addition of the transition-state analogue, compound **8**. The inhibition constant (K_i: 24 ± 4 μmol dm^{-3} at 25 °C) for the formation of the antibody-compound **8** complex was determined by measuring the rate of hydrolysis of carbonate (5 mmol dm^{-3}) in the presence of antibody (15 μmol dm^{-3}) at varying inhibitor concentrations. For the purpose of deriving a basic understanding of the relation between molecular structure and the antibody, we examined the affinity of the antibody induced by the designed hapten for molecules containing an ester group. A moderate effect on the rate of hydrolysis was observed on changing the OC(O)O group from $OC(O)CF_2$ (**10**) to $CF_2C(O)O$ (**11**). In these hydrolyses, the rates of the antibody-catalysed reaction relative to that of the uncatalysed reaction were 6800 and 9050 times faster in eqns. (2) and (3), respectively. Generally, it was found that the difluoromethylene group acted as an isopolar and isosteric replacement for ether-like oxygen.

We believe that these approaches (design and synthesis) open a new avenue for use of tailor-made catalysts in the field of synthetic and/or medicinal chemistry.

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Footnotes

† Calculations were performed by MOPAC v 6.10 (PM 3) included in CAChe Worksystem (SONY/Tektronix Corporation) for the conformers obtained from the rigid-search method with the key word PERCISE and the eigenvector following a minimization (EF) method. The final gradient norm was less than 0.01 kcal Å^{-1}.

‡ The antibody (15 μmol dm^{-3}, Lowry assay, with a molecular mass of 1.5×10^5 for immunoglobulin G) was incubated at 25 °C in 50 ml of phosphate buffer at pH 7.0. Carbonate (1.10 g, 5 mmol dm^{-3}) in acetonitrile (5 ml) was stirred at 25–27 °C in this solution. After 15 h of stirring, the antibody was removed by Centricon filtration; the hydrolysis ratio was determined by ^{19}F NMR signal intensities.

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