A Cyclization Reaction Catalysed by Antibodies

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Monoclonal antibodies, elicited by transition-state analogues for either *exo*- or *endo*-lactonization of α-trifluoromethyl-γ,δ-unsaturated acids, catalyse the regiospecific formation of corresponding γ- or δ-lactones; the *exo*-lactonization proceeds with high diastereospecificity.

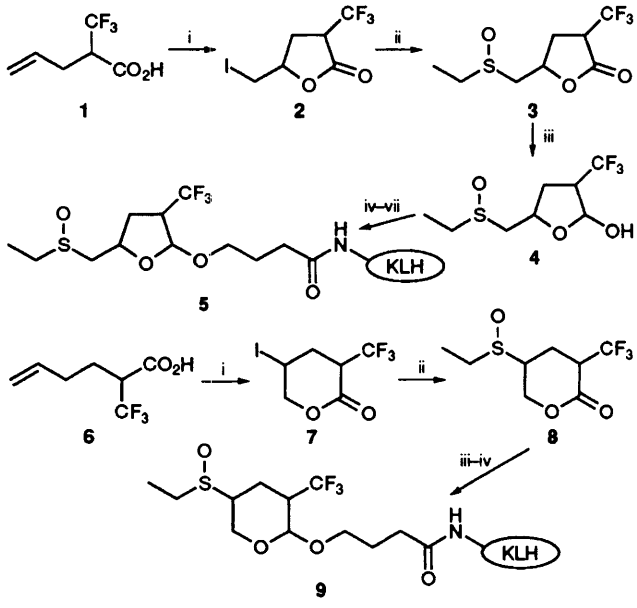
The use of antibodies as selective catalysts in various types of organic reactions has attracted the interest of organic chemists.[1–5] Recently, disfavoured chemical reactions catalysed by an antibody have been reported.[6] To date, acid-catalysed cyclization of unsaturated carboxylic acids is a reaction that has been used relatively infrequently in synthesis, particularly from the view point of stereocontrol.[7] Further, optically pure lactones possessing a fluoromethyl group have been receiving much attention in biological evaluation.[8] Accordingly, we have investigated the possibility of achieving stereocontrol in the cyclization of γ,δ-unsaturated acids by use of catalytic antibodies.

Enzyme-like catalyst design (antibody reagent design) requires the preparation of haptens with structures that mimic the transition state of the reaction. To form the desired antibody reagents, the immunogenic conjugate was prepared by reaction of the haptens with a carrier protein [bovine serum albumin (BSA) and keyhole limpet haemocyanin (KLH)].[9,10] Lymphocytes from the spleen of BALB/c mice immunized with each of the purified antigens (the KLH–hapten conjugate) were fused by standard protocols using mouse myeloma cells (P3/NS1/1-Ag4-1) as the fusion partner. Antibodies were screened by ELISA for cross-reactivity with the KLH–hapten conjugate, *i.e.* for inhibition of binding to the KLH–hapten conjugate by free hapten.

To prove the cyclization model, we employed as haptens cyclic ethers bearing a sulfoxide group (Scheme 1). When the antibody binds an electron-rich alkene, the produced acidic site[11] is correctly aligned to promote cyclization, thereby catalysing the lactonization of γ,δ-unsaturated acids. The antigen obtained from *trans*-iodo-γ-lactone **2** {$[\alpha]_D^{21}$ +9.25 (*c*. 1.14, MeOH), >94% ee, >98% de} produced five antibodies, that from *cis*-iodo-γ-lactone **2** {$[\alpha]_D^{21}$ −4.04 (*c*. 1.08, MeOH), >94% ee, >98% de}[12] produced six antibodies, and that from *trans*-iodo-δ-lactone **7** {$[\alpha]_D^{21}$ +6.17 (*c*. 1.09, MeOH), >97% de} produced eight antibodies. 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.

These antibody-catalysed lactonizations were inhibited by the addition of the transition state analogue, compound **3** or **8**. The inhibition constant K_i for the formation of the antibody–transition state analogue complex was determined by measuring the rate of lactonization of substrate (5 mmol) in the presence of antibody (15 μmol dm^{-3}) at varying inhibitor concentrations.

The antibody induced from antigen **5**, derived from the *trans*-iodo-γ-lactone **2**, acted to promote exclusively exocyclic ring closure of γ,δ-unsaturated acid **1** to the *trans*-γ-lactone {$[\alpha]_D^{21}$+14.8 (*c*. 0.71, $CHCl_3$)}, with a selectivity of >97% de (58% yield), and the antibody induced from antigen **5**, derived from the *cis*-iodo-γ-lactone **2**, acted to form the *cis*-γ-lactone {$[\alpha]_D^{21}$ −6.01 (*c*. 0.97, $CHCl_3$)} with a selectivity of >96% de (61% yield).† Further, the antibody induced from antigen **9**, derived from the *trans*-iodo-δ-lactone **7** {$[\alpha]_D^{21}$ +6.17 (*c*. 1.09, MeOH), >97% de}, catalysed the cyclization of γ,δ-unsaturated acid to form the δ-lactone {$[\alpha]_D^{21}$ +18.9 (*c*. 1.07, $CHCl_3$), >94% ee; δ-lactone:γ-lactone = 93:7}, and the antibody induced from antigen **9**, derived from the *cis*-iodo-δ-lactone **7** {$[\alpha]_D^{21}$ −11.5 (*c*. 1.14, MeOH), >96% de}, acted to form the δ-lactone {$[\alpha]_D^{21}$ +18.7 (*c*. 0.91, $CHCl_3$); δ-lactone:γ-lactone = 94:6}. In these cases, the endocyclic ring closure produces the δ-lactone (>94% ee; >50% yield) in more than 85% selectivity (ratio of δ-lactone:γ-lactone).



Scheme 1 *Reagents and conditions*: i, iodolactonization: I_2, MeCN; ii, $MeCH_2SH$, Et_3N, Et_2O; MCPBA, CH_2Cl_2; iii, diisobutylaluminium hydride, Et_2O, −78 °C; iv, $BrCH_2CH_2CO_2Et$, NaH, Et_2O; v, lipase P, H_2O; vi, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, KLH, phosphate buffer (pH 6.0); vii, dialysis, NaCl buffer, pH 7.4

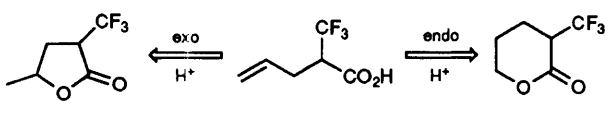


Table 1 Antibody-catalysed lactonizations

Hapten *cis* or *trans*	K_{cat}[a]/ min^{-1}	K_m[a]/ μmol dm^{-3}	K_i/ μmol dm^{-3}	Yield (%)	$[\alpha]_D^{21}$ (*c*, $CHCl_3$)	d.r.[b] (% de)
trans-γ-lactone	0.86 ± 0.2	190 ± 40	16 ± 3	58	+14.8 (0.71)	>97
cis-γ-lactone	0.64 ± 0.2	170 ± 30	15 ± 3	61	−6.01 (0.97)	>96
δ-lactone[c]	0.81 ± 0.2	180 ± 40	17 ± 3	58	+18.9 (1.07)	

[a] Kinetic constants were determined by the method of initial rate data. Kinetic parameters for the lactonization from the Lineweaver–Burk plots were determined. [b] d.r. = diastereoisomeric ratio, determined by ^{19}F NMR (470 MHz) intensities using an NMR shift reagent. [c] Ratio of *trans*-δ-lactone and γ-lactone 93:7.

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Footnote

† The antibody (15 μmol dm^{-3}, Lowry assay, with a molecular weight of 1.5×10^5 for immunoglobin G) was incubated at 25 °C in 50 ml of phosphate buffer at pH 7.3. (*S*)-(−)-2-(Trifluoromethyl)pent-4-enoic acid {$[\alpha]_D^{21}$ −9.77 (*c*. 1.13, MeOH), 0.84 g, 5 mmol} 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 oily materials were extracted with diethyl ether. On removal of the solvent, the lactone was separated by column chromatography on silica gel.

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