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Hapten Design for the Generation of Catalytic Antibodies

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ABSTRACT

This article brings together all of the kinetic data on catalytic antibodies available in the published literature at the time of writing (September, 1993). The data have been presented so that they can be analyzed for any significant trends that arise from relating the structure of the transition-state analog/hapten to the type and efficiency of the catalytic antibody activity elicited.

Index Entries: Catalytic antibody; abzyme; transition-state analog; synzme; pepzyme; phosphonate.

HAPTEN STRUCTURAL REQUIREMENTS

Sixty-two haptens were surveyed, the structures of which are given in Table 1. Of these, only eight do not contain aromatic residues, or have aromatic tautomers (*20,24,33,43,45,46,47,49*). Haptens containing aromatic groups appear to give, on average, a catalytic antibody for every three that display a high binding affinity (K_d in the range 10^{-8}–$10^{-10}M$) for the hapten. In contrast, those haptens lacking an aromatic moiety give, on average, one catalytic antibody for every 11 that bind the hapten ($K_i < 10^{-4}M$). The presence of aromatic groups in the hapten appears to make the hapten conjugate more immunogenic. This improved immunogenicity will lead to a larger antibody response and hence to an increased probability of finding catalytic clones, a notion exemplified by the work of Gibbs et al. (*38*), who isolated antibodies that catalyzed the aspartate/isoaspartate peptide isomerization. In this study, no immune response to the hapten

was observed unless the terminal aryl group was present (*66*). This suggests that aromatic groups should be included in the hapten design, where chemically feasible and where any desired specificity features in the substrate will not be compromised.

IS TRANSITION-STATE STABILIZATION THE MAIN FACTOR CONTROLLING REACTIVITY?

The hypothesis that catalytic antibodies operate purely by transition-state stabilization may be tested by comparing the values of K_m/K_i with those of k_{cat}/k_{uncat}, assuming the following approximation (*67*) holds:

$$K_m/K_i \approx K_s/K_{TS} \approx k_{cat}/k_{uncat} \quad (1)$$

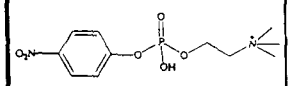
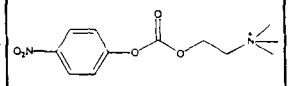
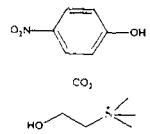
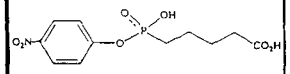
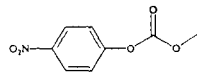
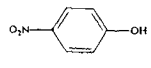
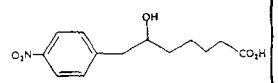
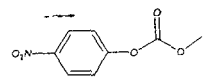
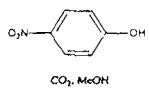
the assumption that $K_m \approx K_s$ is reasonable if k_{cat} is relatively small.

However, for the catalytic antibodies characterized, there appears to be little correlation between K_m/K_i and k_{cat}/k_{uncat}. One explanation for this could be that the K_m values for these substrates are not close to their true dissociation constants (the above approximation is only valid if $k_{-1} >> k_{cat}$, which may not be true in the case of catalytic antibodies where dissociation as well as catalysis are slow events). Alternatively, the k_{uncat} values, which in many cases are estimated or extrapolated from experimental data, are too low leading to a discrepancy between the two ratios. A third possibility is that a substantial element of the rate accelerations observed is attributable to some, or all of the following; the proximity/orienting effects of the binding pocket; participation of binding-site residues as acids/bases/nucleophiles; environment effects owing to desolvation of the substrate when entering a binding pocket with a lower dielectric than bulk water (*68*).

ARE ANTIBODIES WITH A HIGH AFFINITY FOR THE HAPTEN BETTER CATALYSTS?

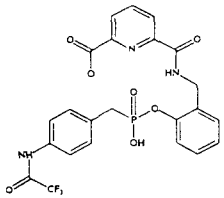
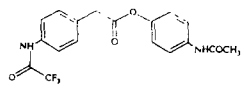
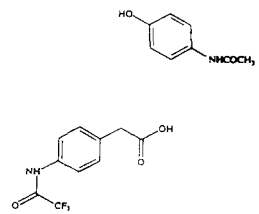
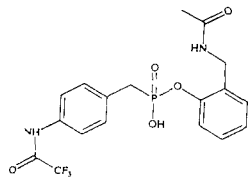
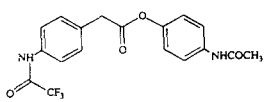
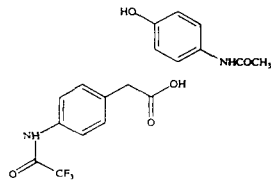
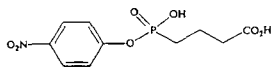
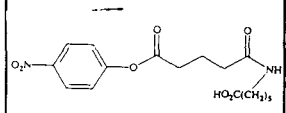
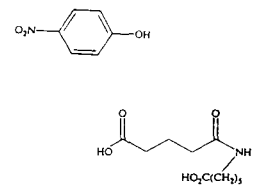
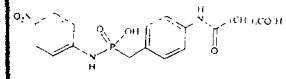
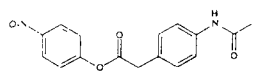
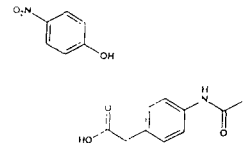
An examination of the inhibition/dissociation constant values for catalytic antibodies suggests that haptens that are bound with $K_i < 10^{-6}M$ give the largest accelerations, relative to the uncatalyzed reaction. However, several antibodies with very low K_i values have shown much lower rate enhancements (*10a,14,21,22,31,34,41,47*). This may be a reflection of the intrinsic difficulty of the reactions catalyzed in those examples, or that the haptens used were poor transition-state mimics, or the binding energy of the antibodies was diffused away from the transition-state region toward other chemical groups.

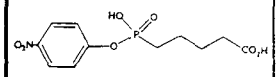
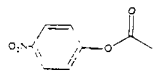
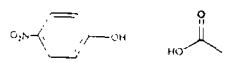
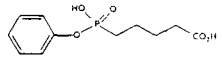
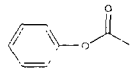
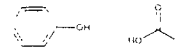
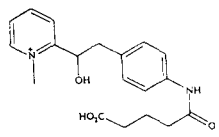
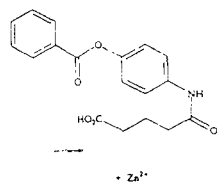
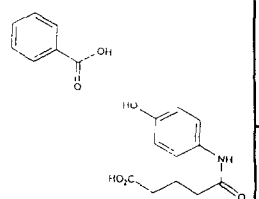
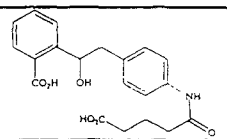
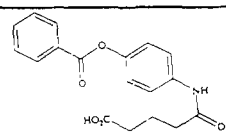
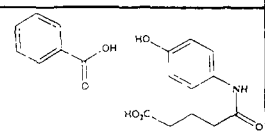
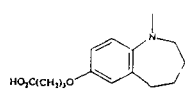
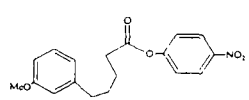
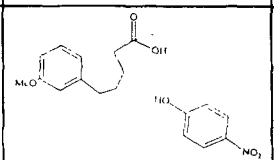
Table 1
Kinetic Data for Catalytic Monoclonal Antibodies Reported in Literature

Immunogen	Substrate	Product	Antibody/ Reaction Conditions	K_m (μM)	k_{cat} (s^{-1})	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	$\frac{k_{cat}}{k_{uncat}}$	Ref
Carbonate hydrolysis 			MOPC167 pH 7.0, 30°C k_i = 5 μM	208	0.007	3.36	770	1
			pH8.5, 30°C K_i = 3.3 μM	660	0.023	35	810	2a
			48G7-4A1 pH 8.1, 30°C Soluble Ab Immobilised Ab	 429 677	 0.67 0.38	 1560 560		2b
			7K16.2 pH 7.5, 30°C 1:5 K_i = 140μM	3330	5.2×10^{-3}	1.56	969	3

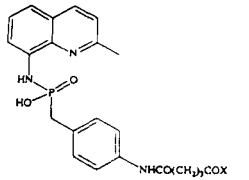
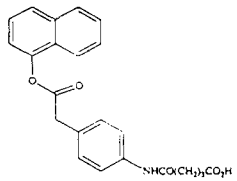
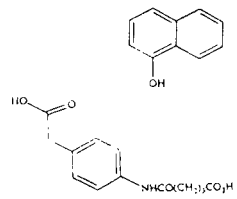
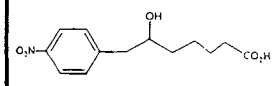
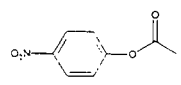
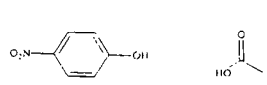
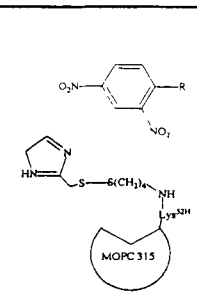
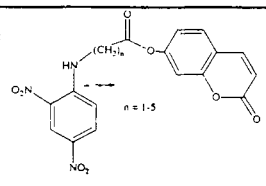
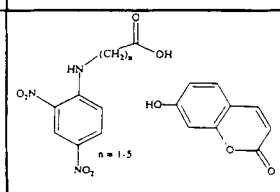
(Antibody/reaction conditions: Antibody name, reaction conditions, no. catalysts: no.of binders, and K_i of hapten (or similar).

(continued)

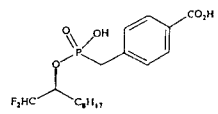
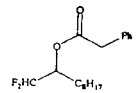
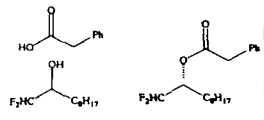
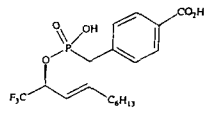
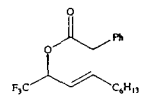
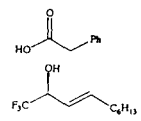
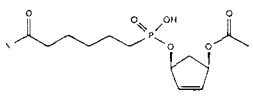
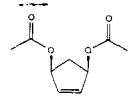
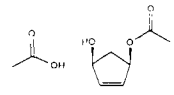
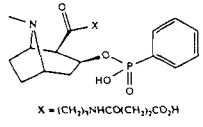
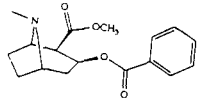
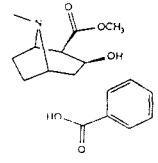
Immunogen	Substrate	Product	Antibody/ conditions	K_m (μM)	k_{cat} (s^{-1})	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	$\frac{k_{cat}}{k_{uncat}}$	Ref
Ester hydrolysis 	(O-aryl esters) 		6D4 pH8.0, 25°C K_i = 0.16 μM	1.9	0.027	1.42×10^4	960	4
			50D8 pH8.0, 5:20 K_i = 0.5μM	1500	20	1.33×10^4	6.3×10^6	5
			CNJ157 pH8.0 K_i= 3.4 mM	110	0.040	364	9700	6
			NPN43C9 pH 9.0, 37°C K_d = 0.8nM	30	40	1.33×10^6	1.5×10^5	7
			1D pH 7.2	115	4.4×10^{-3}	36	-	8

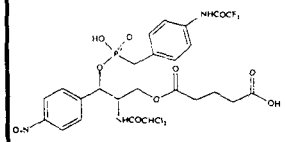
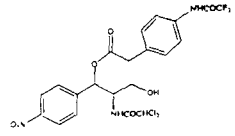
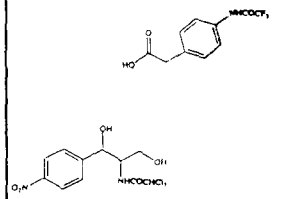
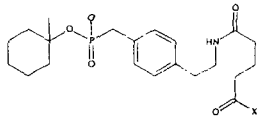
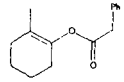
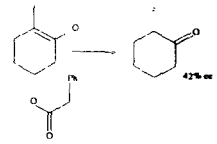
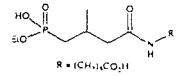
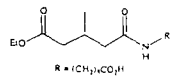
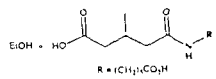
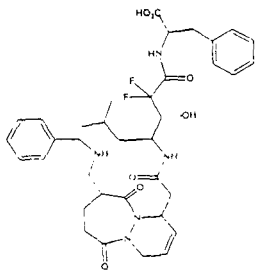
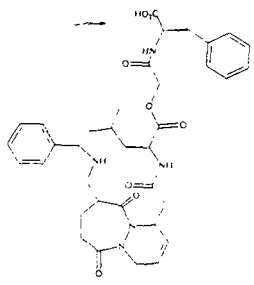
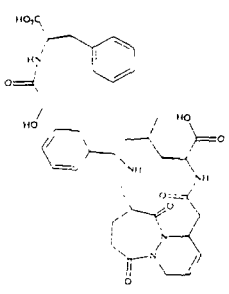
			KD2-260 pH 6.0, 20°C K_i = 0.12 μM	4.9	0.042	8600	3100	9
			20G9 pH8.8, 25°C K_i = 2.2 nM	300	0.152	500	8033	10a
			Reverse micelles pH 8.5, 35°C K_i = 39 nM	157	0.31	1970	1.7×10^4	10b
			30C6 pH 7.2, 37°C 7:23 K_i = 83 μM	1120	8.3×10^{-5}	0.07	1×10^6	11a
			84A3 pH 7.0, 25°C 1:25	3.5	0.045	12900	1230	11b
			27A6 pH 8.3, 37°C 3:18 K_i = 6 μM	243	1.6×10^{-4}	6.6×10^{-7}	-	12
			C3 pH 8.3 1:2 K_d = 23.5nM	400	2.4	6000	3.5×10^6	13

(continued)

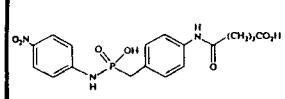
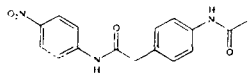
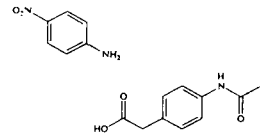
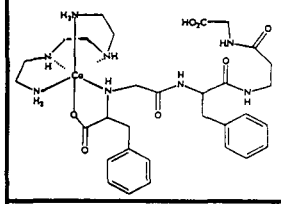
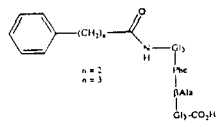
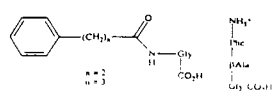
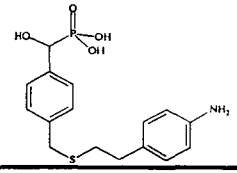
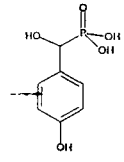
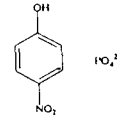
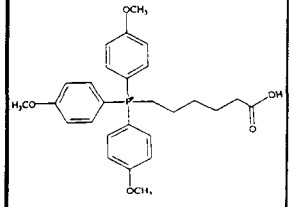
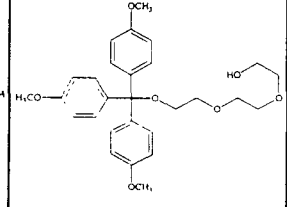
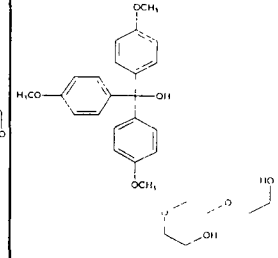
Immunogen	Substrate	Product	Antibody/ Reaction Conditions	K_m (μM)	k_{cat} (s^{-1})	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	$\frac{k_{cat}}{k_{uncat}}$	Ref
			37G2 pH8.0, 25°C 13:55 K_i - 0.5 μM	768	0.033	43	1976	14
			7K16.2 pH 7.5, 30°C 1:5	3650	0.012	3.3	2200	3
			MOPC315 pH 7.0, 10°C (semi-synthetic antibody)	1.2	0.0145	1.2×10^4	6×10^4	15

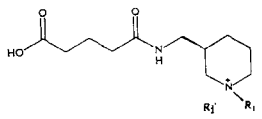
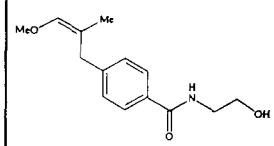
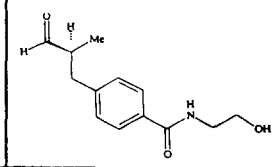
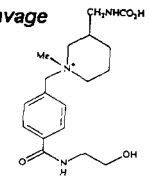
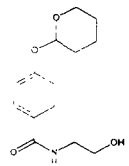
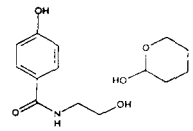
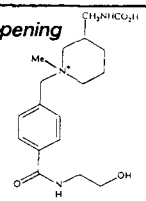
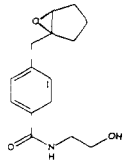
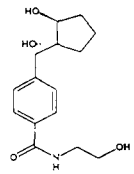
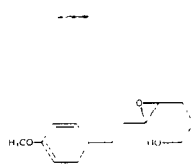
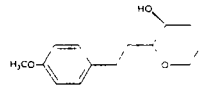
	(O-Alkyl Ester)		2H12E4 pH8.0, 24°C 18:31 K_i = 2.4 μM	14.8	3×10^{-5}	21	267	16
			2H6 pH 9.0, 21°C K_i = 2.0 μM	3994	7.7×10^{-2}	19.2	8×10^{4}	17
			D2.3 pH 8.25 9:970	280	0.1225	437.5	2.6×10^{5}	18

Immunogen	Substrate	Product	Antibody/ Reaction Conditions	K_m (μM)	k_{cat} (s^{-1})	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	$\frac{k_{cat}}{k_{uncat}}$	Ref
			A (R)-ester hydrolysis 49% ee	410	0.0157	38	914	19
			B (S)-ester 48% ee	380	0.0152	40	-	
			pH7.3, 25°C					
			Hapten config.					19
			(R)-(E)	390	0.0145	37	-	
			(R)-(Z)	370	0.0152	41	-	
			(S)-(E)	410	0.0157	38	-	
			(S)-(Z)	440	0.0148	34	-	
			pH7.3, 25°C					
			37E8 pH8.0, 37°C 1:33 K_i = 7μM	177	1.2×10^{-4}	0.66	1.5	20
 X = $(CH_2)_3NHCO(CH_2)_2CO_2H$			3B9 pH7.7 2:29 K_i < 2 μM	490	0.0018	3.74	540	21

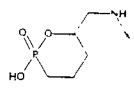
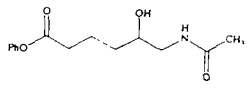
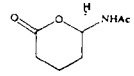
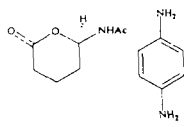
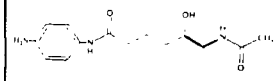
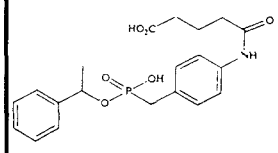
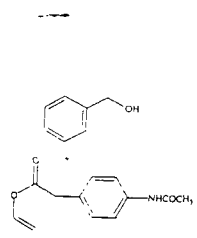
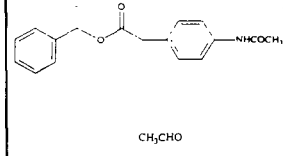
			6D9 pH8.0, 30°C 6:12 K_i = 0.06 μM	64	0.0022	34	1800	22
			27B5 pH9.0, 25°C 3:26 K_i = 4.3 μM	994	1.7×10^{-4}	0.170	300	23
			1C7 pH8.0, 30°C 3:21 K_i = 3.95 μM	285	0.033	115	-	24
			2E11.2E7 pH6.5, 37°C 3:9 K_i < 1 mM	4350	0.133	31	1.3×10^{7}	25

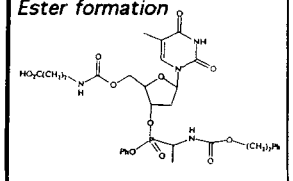
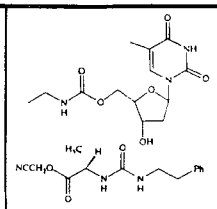
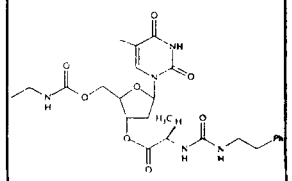
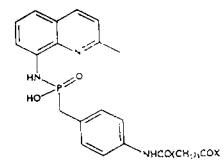
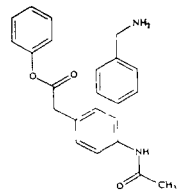
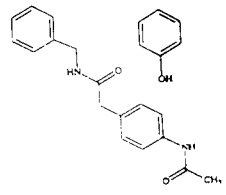
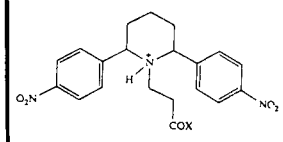
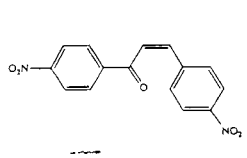
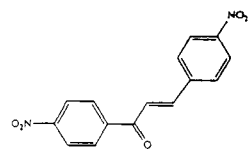
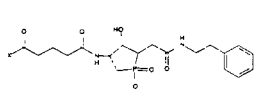
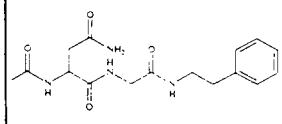
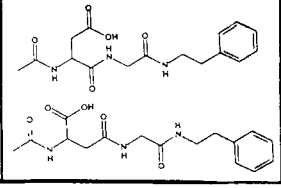
(continued)

Immunogen	Substrate	Product	Antibody/ Reaction Conditions	Km (μM)	kcat (s^{-1})	kcat / Km ($M^{-1}s^{-1}$)	kcat / kuncat	Ref
Amide Hydrolysis 			NPN43C9 pH9.0, 37°C K_d = 0.8 nM	370	8.3×10^{-4}	2.24	1.5×10^5	7
			28F11 pH6.5, 37°C 1:13	-	6×10^{-4}	-	2×10^5	26
Phosphate hydrolysis 			38E1 pH 9.0, 30°C 5:20 K_i = 34 μM	155	2×10^{-5}	0.13	8×10^3	27
Ether hydrolysis 			37C4 pH 6.0 8:11 K_d = 25 nM	31	1.6×10^{-3}	54	270	28

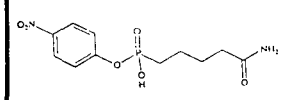
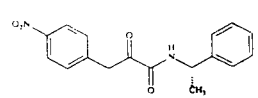
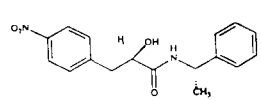
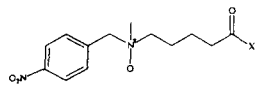
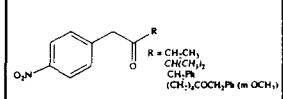
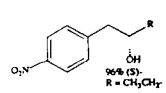
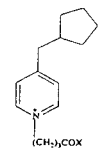
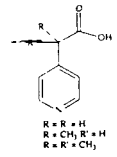
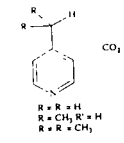
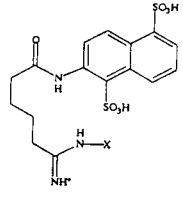
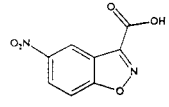
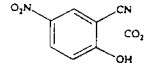
			14D9 pH 5.7, 37°C 13:23 K_i= 2 μM	340	9.5×10^{-5}	0.279	2500	29
Ketal cleavage 			14D9 pH5.7, 20°C 2:26 K_i= 2 μM	100	7.5×10^{-5}	0.78	70	30
Epoxide opening 			14D9 pH5.6, 24°C	25	2.5×10^{-5}	1.0	670	31
			26D9 pH 6.6, 2:26	356	1.5×10^{-3}	43	-	32

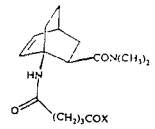
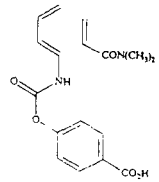
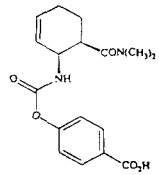
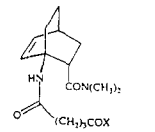
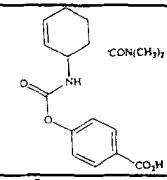
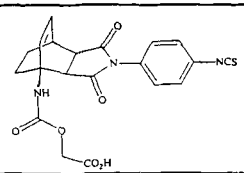
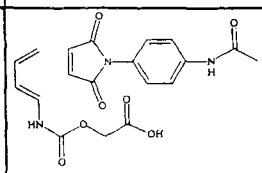
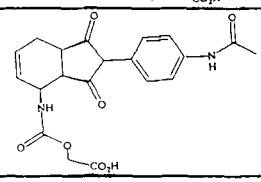
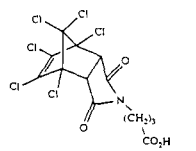
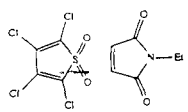
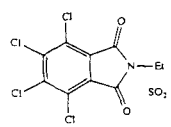
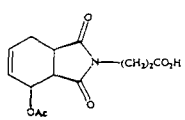
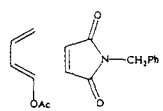
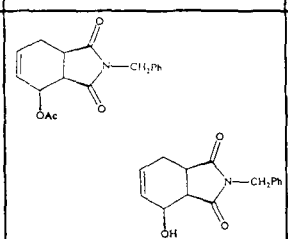
(continued)

Immunogen	Substrate	Product	Antibody/ Reaction Conditions	K_m (μM)	k_{cat} (s^{-1})	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	$\frac{k_{cat}}{k_{uncat}}$	Ref
Ester formation *Lactonisation/* *Amide formation* 			24B11 pH7.0, 25°C 1:24 K_i = 0.25 μM	76	8.3×10^{-3}	109	167	33
			24B11 (lactone) (amine) pH 7.0 K_i = 7.5 nM	4900 1200	1.1 x 10-3	0.24 1.0	16M	33
Trans-esterification 			21H3 11:18 K_i = 0.19 μM	394	1.5×10^{-3}	3.8		34a
			2H6-I K_i = 1.2 μM	2200	0.067	30	72×10^3	34b
			21H3-I K_i = 83 μM octane\ immobilised antibodies	200	1×10^{-3}	5	1100M	

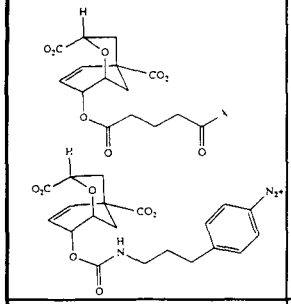
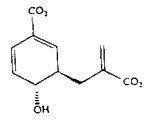
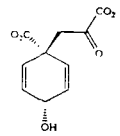
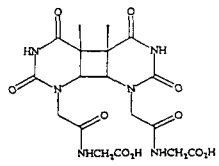
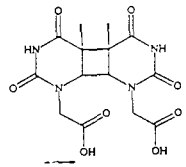
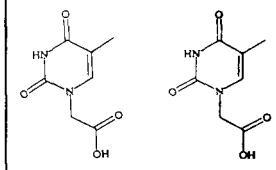
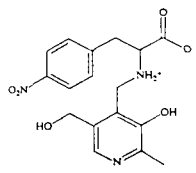
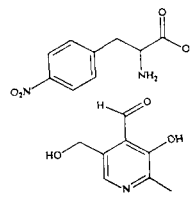
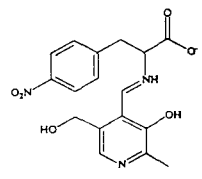
Ester formation 			18R.136.1 (thymidine) (ester) pH 6.5, 26°C 1:39 K_d= 240 pM	770 260	0.237	308 900	910M	35
Amide formation 			17G8 pH8.0, 23°C 13:55 K_i = 0.5 μM	2200	3.8 x 10^{-4}	0.173	10.5M	36
Isomerisation 			DYJ10-4 pH 7.5, 25°C 3:15 K_i= 6.7 μM	220	0.08	364	15000	37
			2E4 pH 9.0, 25°C K_i= 0.1 μM	193	1.2 x 10^{-4}	0.62	70	38

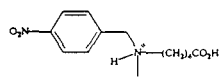
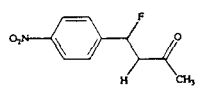
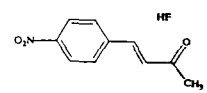
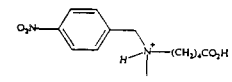
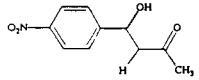
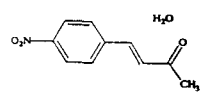
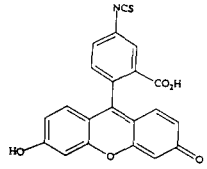
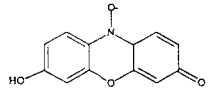
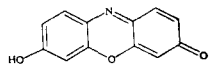
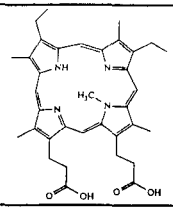
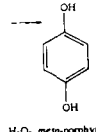
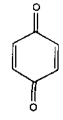
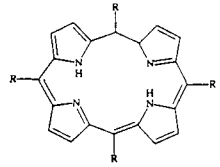
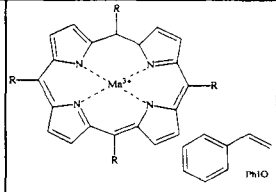
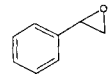
(continued)

Immunogen	Substrate	Product	Antibody/ Reaction Conditions	K_m (μM)	k_{cat} (s^{-1})	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	$\frac{k_{cat}}{k_{uncat}}$	Ref
Reduction 			A5 pH5.0, 22°C 2:8 K_d= 0.61M de > 99%	1240	0.0017	1.4	290	39
			37B39.3 pH 5.0, 22°C 12:25	52	0.0016	32	1.7×10^6	40
Decarboxylation 			CPD32A11 pH 5.5 K_i= 10 nM	1×10^5	4.7	3.2×10^{-5}	1.9×10^5	41
			21D8 pH 8.0, 20°C K_i= 6.4 nM	168	0.28	1667	19000	42

Diels-Alder 			7D4 (*Endo*) pH7.4, 37°C (diene) (dienophile) 2:22	 **960** **1700**	5.7×10^{-5}	 0.06 0.034	4.8M	43
			22C8 (*Exo*) (diene) (dienophile) 4:22	 **700** **7500**	5.3×10^{-5}	 0.076 7×10^{-3}	18M	43
			39A11 (diene) (dienophile) pH 7.5, 25°C K_d= 126 nM	 **1130** **740**	0.67	 583 900	0.35M	44
			1E9 (dienophile) pH 6.0, 25°C 1:5	 21000	0.070	3.3	>110M	45
			H11 (dienophile)	8300	0.055	6.6	1700M	46
			Hydrolysis pH8.0, 18°C	1100	9.2×10^{-4}	0.84		

(continued)

Immunogen	Substrate	Product	Antibody/ Reaction Conditions	K_m (μM)	k_{cat} (s^{-1})	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	$\frac{k_{cat}}{k_{uncat}}$	Ref
Claisen rearrangement 			1F7 pH 7.5, 14°C 1:15 K_i = 0.6 μM	51	1.2×10^{-3}	24	250	47
			11F1-2E11 pH 7.0, 10°C 1:8 K_i = 9.0 μM	260	0.0045	173	1×10^4	48
Retro 2+2 cycloaddition 			15F1-B1 pH7.5, 20°C 5:6 K_d < 1 μM	6.5	0.02	3080	218	49
Transimination 			17C5-11C2 (acid) (pyr.) pH7.0, 25°C 1:7 K_d = 6 nM (L) K_d =17nM (D)	 120 710	0.3 -	 2500 423	0.21M	50

Elimination 			43D4-3D12 pH 6.0, 37°C K_i= 0.29 μM	182	3.1×10^{-3}	17	8.8×10^4	51
Dehydration 			20A2F6 pH 7.0, 37°C K_i= 16 μM	1100	5.8×10^{-6}	5.3×10^{-3}	1200	52
Redox 			66D2 (sulphite) (resuzin) pH 5.8, 25°C 9:13	3000 0.6	0.02	6.7 3.3×10^4	60M	53
			7G12-A10- G1-A12 pH8, 10°C 2:3	24000	6.6	274	2.4	54
								55

(*continued*)

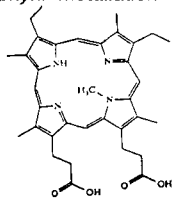
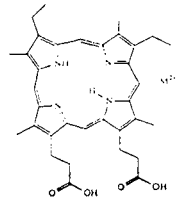
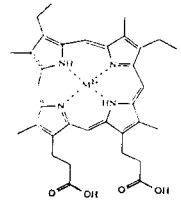
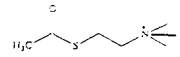
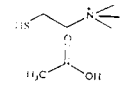
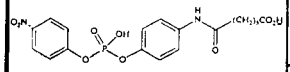
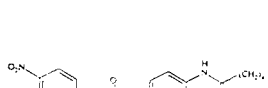
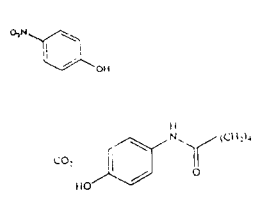
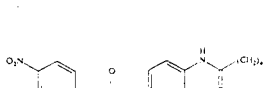
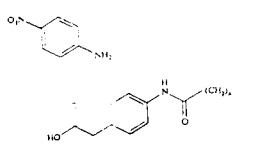
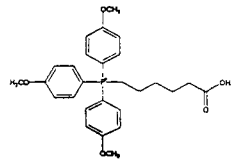
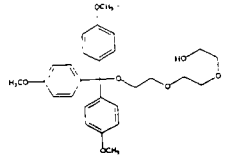
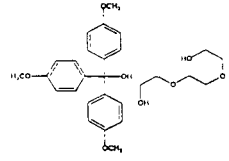
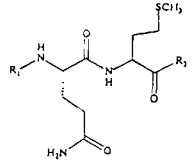
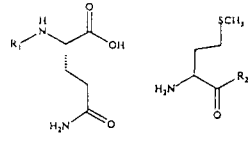
Immunogen	Substrate	Product	Antibody/ Reaction Conditions	Km (μ M)	Kcat (S^{-1})	Kcat Km ($M^{-1}S^{-1}$)	Kcat Kuncat	Ref
Porphyrin metallation 			7G12-A10-G1-A12					56
			Zn^{2+}	49	8.6×10^{-6}	0.17	2600	
			Cu^{2+}	50	1.4×10^{-6}	0.028	1700	
ANTI-IDIOTYPIC ANTIBODIES *Ester hydrolysis* mAb AE-2 (antibody against acetylcholinesterase)			9A8 (IgM) pH 7.4, (per binding site)	600	81	1.35×10^{5}	4.2×10^{8}	62
				1050	37	35×10^{3}		

Table 2
Kinetic Data for Catalytic Polyclonal Antibodies Reported in the Literature

POLYCLONAL ANTIBODIES *Carbonate Hydrolysis* 			PCA 270-29 pH8.0, 25°C	3.4	0.029 (100%)	8.8×10^{4}	1.5×10^{2}	57
					2.9 (1%)	8.8×10^{6}	1.5×10^{4}	
Amide Hydrolysis			PCA 270-29 pH9.0, 25°C	5.4	6×10^{-5} (100%)	11.0	3×10^{2}	58
					6×10^{-3} (1%)	1.1×10^{3}	3×10^{4}	

Ether hydrolysis 			pH7.2, RT	31	3.3×10^{-4} (12%)	11	125	59
AUTO-ANTIBODIES *Amide hydrolysis* unknown			pH 8.0, 38°C	0.0379	0.26	6.86×10^{6}		60
Phosphate (DNA)hydrolysis unknown								61

WHICH ARE THE BEST HAPTENS FOR CARBONATE/ESTER/AMIDE HYDROLYSIS?

Of the catalytic antibodies reported, half are for the hydrolysis of carboxylate derivatives. It is therefore among these antibodies that the widest range of hapten structures have been explored. Most of the haptens have been designed to mimic the high-energy tetrahedral intermediate/transition state that is produced during the cleavage of the ester/amide bond (Fig. 1).

A comparison (Table 1) of the reaction specificities of antibodies produced against haptens containing phosphonate or phosphoramidate haptens (Fig. 2) reveals that catalysts typically exhibit k_{cat}/K_M values in the range 1–40M^{-1} s^{-1}. A few exceptional catalysts with k_{cat}/K_M values of 10^3–10^4M^{-1} s^{-1} have been reported, although in these instances, the recruitment of an amino acid side chain (His,Arg [*7*], Arg [*9*], Tyr [*10a*]) that participates either as a general acid/base or as a nucleophile has been implicated.

Curiously, although a very potent polyclonal antibody has been reported using a phosphate-based hapten (*57,58*), no examples of monoclonal esterase or amidase antibodies using this structure have been reported (compare, however, the MOPC167 antibody [*1*], which binds phosporylcholine and catalyzes [poorly] the hydrolysis of a *p*-nitrophenyl methylcarbonate derivative). For the generation of other esterase antibodies, the 2-hydroxymethyl pyridinium hapten (*11*) has been particularly effective (k_{cat}/K_M = 1.29 × 10^4M^{-1} s^{-1}), but only when the reaction medium was supplemented with zinc ions (as a Lewis acid). However, when antibodies elicited to this hapten or the 2-hydroxymethylbenzoic acid (*12*) version are used in the absence of metal cofactors, the reaction specificities are considerably poorer (k_{cat}/K_M = 0.07 and 6.6 × $10^{-7}M^{-1}$ s^{-1}, respectively) than those of the antibodies generated using phosphorus-based reagents.

The α-difluoroketoamide-based hapten (Table 1; *25*), in which a difluoromethylene group is used as an isosteric replacement for oxygen, has been shown to be of similar potency to the phosphonate-based reagents (k_{cat}/K_M = 31M^{-1} s^{-1}). However, only one example of a hapten incorporating this group has been reported, making comparisons of its effectiveness with the phosphorus-based haptens difficult.

Suckling et al. (*13*) made the unexpected discovery that a tertiary amine-containing hapten produced an antibody capable of efficient (k_{cat}/K_M = 6 × 10^3M^{-1} s^{-1}) ester hydrolysis. Additionally, an antibody generated against hapten (*46*) not only displayed Diels-Alderase activity, but also some hydrolytic activity (although rather inefficient—k_{cat}/K_m = 0.84M^{-1} s^{-1}).

Sulfones (*58*) and sulfonamides/thiophosphates (*69*) have been investigated as potential transition-state analogs of the ester/amide hydrolysis reaction. No successful catalysts have been reported in either case.

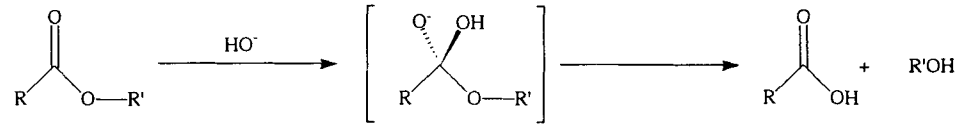


Fig. 1. Mechanism and Transition-state structure for ester/amide bond cleavage.

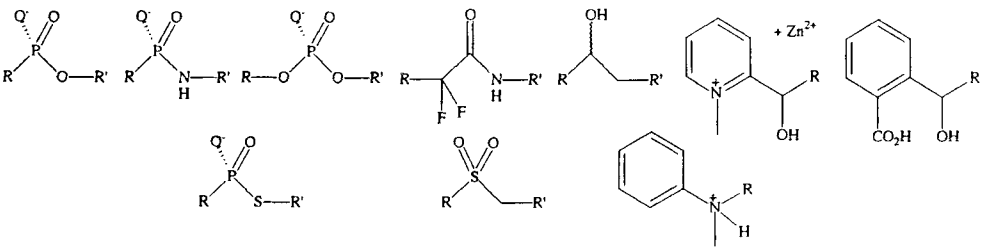


Fig. 2. Transition State analogs used as haptens for carbonate/ester/amide hydrolysis.

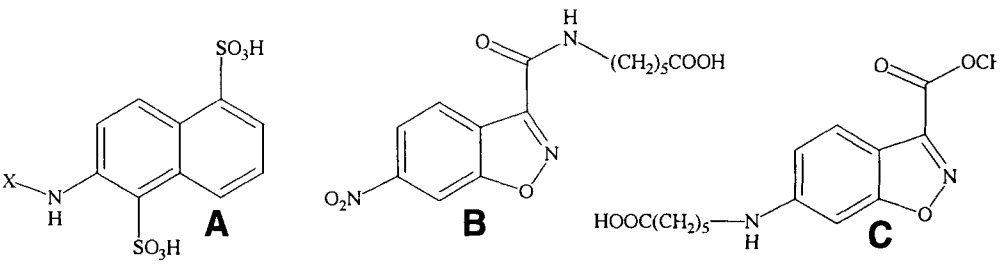


Fig. 3. Successful (A), and unsuccessful (B,C) haptens for generating a decarboxylase antibody.

STRUCTURAL REQUIREMENTS FOR HAPTENS TO ELICIT ANTIBODIES WITH DECARBOXYLASE ACTIVITY

Two different haptens have been used to generate antibodies with decarboxylase activity (Fig. 3). Interestingly, the contrasting chemical nature of the two haptens offers apparently contradictory explanations of the likely catalytic mechanism of this type of reaction. In the first example (*41*), a cyclopentyl ring was used to create a hydrophobic pocket for the carboxylate within the antibody-binding site. This type of environment should favor the production of the electroneutral carbon dioxide. The antibody produced had a k_{cat}/K_M of 3.2×10^{-5}. In the second example, preliminary attempts using hydrophobic haptens were unsuccessful. However, when the carboxylate to be cleaved was mimicked by a sulfoxide group in an otherwise hydrophobic naphthalene molecule, an effective catalyst was produced (*42*); $k_{cat}/K_m = 1.7 \times 10^3 M^{-1}\ s^{-1}$. It would be anticipated that the sulfoxide would induce a complementary postively charged residue (Lys, Arg) in the carboxylate-binding pocket. This could act either as a

base in the deprotonation of the carboxylic acid, so aiding the carboxylate cleavage, or by stabilization of the carboxylate through the formation of a salt bridge. This second action, however, should disfavor the decarboxylation.

The efficiency of the catalytic antibody suggests that the former is more likely, a conclusion supported by inspection of the X-ray structure of ornithine decarboxylase (*70*), which shows that the carboxylate-binding pocket is formed by a ring of hydrophobic residues (mainly aromatic) with an aspartic acid side chain at the base to act as a proton acceptor.

MONOCLONAL VS POLYCLONAL ANTIBODIES

Early (unsuccessful) attempts at the production of catalytic antibodies, including those of Raso and Stollar (*71*), were directed at the polyclonal antibody response. Recently, several groups have reported the isolation of polyclonal sera containing catalytic fractions (Table 2). At present, it has proved impossible to separate the chemically reactive fraction within the polyclonal mixture to give accurate kinetic data on individual antibodies, and so allow direct comparison with their monoclonal counterparts. However, a recently described polyclonal amidase appears to have an efficiency comparable with monoclonal catalysts. On the assumption that 1% of the mixture was catalytically active, Gallagher (*58*) estimated a $k_{cat}/K_m = 1.1 \times 10^3 M^{-1}\ s^{-1}$ for the amidase. Similarly, Stephens and Iversen (*58*) have estimated that, if 12% of the polyclonal population is responsible for an observed tritylase activity, the antibody will have a k_{cat}/K_m of 11 $M^{-1}\ s^{-1}$. This is less efficient than the monoclonal antibody ($k_{cat}/K_m = 54 M^{-1}\ s^{-1}$) raised against the same hapten (*28*). One issue, as yet unresolved, is whether the polyclonal mixture contains catalysts with a diverse range of catalytic activities or if the catalysis is essentially monoclonal. Although very high catalytic rates have also been observed for cleavage of vaso-intestinal peptide (VIP) by polyclonal autoantibodies (*60*) and for DNA hydrolysis (*61*), a similar quantification problem is encountered because of the difficulty of assigning the activity to a known homogeneous antibody fraction.

ANTI-IDIOTYPIC ANTIBODIES

This new form of catalytic antibodies (*62*) involves first generating an antibody bearing an "internal image" of an enzyme-active site. This antibody is then used as an immunogen to generate an anti-idiotypic antibody, thus regenerating the original active site. This approach has given rise to one of the most potent antibody catalysts seen ($k_{cat}/K_m = 1.35 \times 10^5 M^{-1}\ s^{-1}$), an activity that seems to originate from the presence of two of the

three residues (Ser and His) in the serine protease catalytic triad in the binding site of the anti-idiotypic antibody. This approach offers a wide range of opportunities for new catalysts, especially if the apoenymes of cofactor-dependent enzymes can be chemically modified so that the substrate specificity of the anti-idiotypic antibodies differs from that of the apoenzymes initially used as antigens.

ANTIBODY CATALYSTS VS SYNTHETIC CATALYSTS

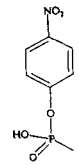
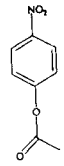
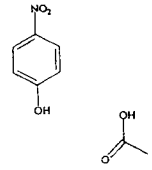
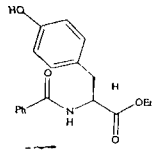
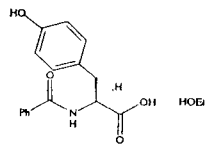
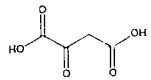
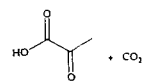
Table 3 compares three recent examples of synthetic approaches to the generation of catalysts displaying substrate and reaction specificities similar to those of enzymes. In the first of these examples, Robinson and Mosbach (*63*) have used the same transition-state principle that has been applied to the generation of catalytic antibodies. Binding pockets against a putative transition-state analog were created using molecular imprinting of polyvinyl-imidazole, which is rich in potential acid/base or nucleophilic imidazole groups, and ester hydrolysis was observed. In many ways, the generation of molecularly imprinted synthetic catalytic polymers (MIPS/synzymes) complements the research on catalytic antibodies, but it also allows for the possibility of studying catalysis at extremes of temperature and in organic solvents. The major disadvantage with the current synthetic polymers is that the catalysts produced have nonidentical binding pockets and so can be treated as synthetic equivalents of a polyclonal antibody mixture. Hence, it is difficult to quantify the catalytic activity of any single class of binding site.

Two examples of the use of small peptides to mimic the active site of enzymes have been reported (*64,65*). These so-called pepzymes have been shown to display both reaction and substrate specificity. Atassi and Manshouri (*64*) have synthesized two peptides that re-create the active sites of trypsin and chymotrypsin, using just 29 amino acids in each case. These catalysts were as efficient as the most efficient antibody-based systems and suggest that synthetic small peptide catalysts offer a viable alternative to antibody catalysts.

ANTIBODY CATALYSTS VS ENZYMES

Although none of the catalytic antibodies produced have come close to the reaction specificity of the "perfect enzyme" (k_{cat}/K_m = $10^9 M^{-1}$ s^{-1}) (*72*), specificites in the 10^3–$10^6 M^{-1}$ s^{-1} range have been reported for a number of hydrolytic catalysts.

Table 3
Examples of Other Catalytic Systems

	Substrate	Product	Material/ Reaction Conditions	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)	k_{cat}/k_{uncat}	Ref
Molecularly Imprinted Polymers (synzmes) *Ester Hydrolysis*  (imprint molecule)			poly[4(5)-vinyl-imidazole] pH 8.0, 25°C				60% faster than non-print polymer	63
Artifical Catalytic Peptides (Pepzymes) *Ester Hydrolysis*			ChPepz 29 Residue cyclic peptide TrPepz 29 Residue cyclic peptide pH 7.8, 25°C	1110 2420	147 85	1.32×10^5 3.5×10^4		64
Decarboxylation			Oxaldie 1 14 residue peptide pH 7.0, 25°C	8700	5.5×10^3	0.63	1×10^3 -fold higher than $BuNH_2$	65

The regio- and stereoselective controls exhibited by several of the catalytic antibodies rival those of comparable enzymes (giving products in enantio- or diastereomeric excesses of > 95%). The ability of individual clones from a single immunization to catalyze the same reaction on different stereoisomers or to produce selectively only one of several possible isomeric forms (for example, endo and exo-Diels-Alder adducts), is a property that should be exploited by chemists in the near future.

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