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Catalytic antibodies: prospects for the use in organic synthesis

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Abstract. Data on catalytic antibodies as a new generation of biocatalysts are reviewed. The principles used in the design, and the methods for the preparation of all presently known classes of catalytic antibodies, which catalyse particular conversions of organic compounds, are discussed. Data on the efficiency, regio- and stereospecificity and mechanisms of action of catalytic antibodies are presented and their possible applications in fine organic synthesis are considered. The bibliography includes 124 references.

I. Introduction

Elaboration of an immense variety of methods designed for the formation of the carbon skeleton and its directed functionalisation makes it possible currently to obtain organic compounds of virtually any degree of complexity. Usually, the synthesis of structurally complex compounds is accomplished in several steps. The efficiency and unambiguity of each of these steps are the two crucial factors that determine the success of the synthesis. The efficiency of the reaction is characterised by its rate and the product yield, while its unambiguity is characterised by its regio- and stereospecificity. This enables one to achieve the maximum degree of purity of the target compound and to avoid tedious isolation and purification procedures. The progress in fine organic synthesis and the access to more and more complex structures necessitates enhancement in reaction efficiency and specificity. However, the use of purely chemical approaches in multistep syntheses is often insufficient. Recent trends are towards the use of enzymic reactions for the formation of the carbon skeleton and its functionalisation at particular stages of the synthesis.

The use of enzymes often makes it possible to carry out the reaction at higher rates and to achieve higher degree of purity of the reaction products due to high regio- and stereoselectivity of enzymes. Therefore, the methods of enzymic and chemoenzymic synthesis are becoming more and more popular in the routine practice of fine organic synthesis. However, low availability of natural enzymes endowed with the desired activity and specificity is an obstacle for the wide application of biosynthetic procedures. This demands additional tedious work aimed at a search for suitable and accessible enzyme sources, elaboration of methods for isolation and purification of the enzymes and detailed analysis of their specificity. Therefore, the laboratory and industrial use of natural enzymes that catalyse definite chemical conversions is rather limited.

Preparation of artificial enzymes with high efficiency and, which is the most important, with high predetermined selectivity has long been the dream of organic chemists. Such enzymes have to be prepared in a laboratory to be used for accomplishment of a particular reaction. Such artificial enzymes, which are endowed with predetermined specificity (the so-called 'tailor-made enzymes'), would make it possible to enhance considerably the efficiency of organic synthesis and open up new ways to the synthesis of novel classes of organic compounds.

Natural enzymes are high-molecular-weight proteins possessing a unique structure. Their catalytic activity and the selectivity of their action are determined by the strictly specific amino acid sequence of the protein chain. This, in turn, predetermines the conformation of the protein molecule and, ultimately, the formation of the spatial structure of the active site of the enzyme.

Directed synthesis might be considered as the simplest way to obtain an artificial enzyme. However, despite the generally acknowledged progress in synthetic protein chemistry, this approach is fairly inefficient not only because it is labour-consuming but because it requires information about the primary structure of the target enzyme. However, determination of an amino acid sequence is by itself a complicated and labour-consuming task.

The solution of this problem came from the use of a different approach to the preparation of artificial enzymes with predetermined specificity. This is based on the biosynthesis and rests on tremendous potentialities of the cell immunological apparatus, which can produce a great variety of protein molecules.

It is well known that a specific feature of the immune system of animals is its ability to synthesise antibodies, *i.e.*, protective

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proteins that 'neutralise' alien molecules and more complex entities (the so-called antigens) getting into the organism. The immune system generates a protein molecule of an antibody the structure of which relates to the structure of the antigen in such a way that the active site of the antibody appears to be complementary to the specific part of the antigen called the hapten. The antibody forms a rather stable complex with the antigen and thus 'neutralises' its effect. In other words, the biosynthetic apparatus of the immune system provides the synthesis of the protein molecule in which the active site is formed, the structure and spatial configuration of which are predetermined by the structure of the antigen administered to the organism. It is this principle that is being employed in the synthesis of artificial enzymes.

According to the current views, the catalytic effect of a natural enzyme consists primarily in the facilitation of the transition of the reaction substrate from the ground state to the excited transition state. The latter is stabilised in the active site of the enzyme to be further converted to reaction product(s). Therefore, the formation of an active site promoting the generation and stabilisation of the transition state of the substrate is a prerequisite for manifestation of catalytic activity by the protein molecule. Thus, the biosynthesis of the antibody possessing catalytic activity by the immune apparatus requires the use of an antigen the structure of which mimics the transition state formed in the catalytic reaction. It is impossible, however, to obtain a stable molecule corresponding to the transition state, since the latter represents an unstable complex. For this reason, only compounds the molecules of which mimicked the spatial and electronic structure of the transition state were used as antigens generating catalytically active antibodies. Being incorporated into immune cells, such antigens ought to generate antibodies that stabilise the transition state and, hence, manifest catalytic properties like the natural enzymes.

Although this idea was first forwarded long ago,[1,2] its practical implementation faced serious difficulties because immune cells synthesise a great number of antibodies ('polyclonal antibodies') in response to antigens. Isolation of an antibody manifesting the highest catalytic activity and selectivity from a complex mixture of structurally similar proteins is very problematic, which made this method impracticable.

The situation has changed totally with the discovery and detailed development of the method of synthesis of the so-called 'monoclonal antibodies' that are fewer in number and less diverse.[3] The antibodies isolated with recourse to this method did manifest catalytic activity.

Such antibodies, which were named 'catalytic antibodies' (CA) or 'abzymes' (abbreviated from 'antibody enzymes'), were first synthesised about 10 years ago.[4,5] This area continues to develop rapidly since that time. By now, nearly a hundred CA that accelerate and specifically direct various reactions have been generated and the basic principles underlying the preparation of CA with predetermined specificity have been formulated. Their physicochemical characteristics appeared to be similar to those of the natural enzymes.

In some cases, the activity of CA was close to that of the natural enzymes. As a rule, these CA manifested high specificity and stereoselectivity which might be predetermined. Moreover, several CA were prepared that catalysed reactions (*e.g.*, the Diels–Alder reaction) for which natural enzymes had not been found. What is most remarkable and promising for synthetic chemistry is that CA could be obtained which made it possible to change the direction of a known reaction and to channel it through a route which is energetically forbidden under normal non-catalysed conditions. In other words, it was possible to change chemo-, regio- and stereospecificity of the reaction and to implement conversions unattainable with conventional chemical methods.

Hence, 'catalytic antibodies' represent a new generation of protein biocatalysts, and their preparation is a considerable step forward in theoretical and practical aspects of biocatalysis.

In this review, which encompasses the literature up to 1997, the main attention is given to the chemical aspects of the problem and the prospects of application of CA for the solution of current tasks of synthetic chemistry. As for other aspects, such as enzymic kinetics of CA and some other physicochemical problems, they are reviewed only briefly and where appropriate, *e.g.*, in relation to the specificity of CA, the structure of their active sites and the mechanisms of their catalytic action. The readers who are interested in this problem as well as in the problems of immunochemistry and molecular biology of the catalytic antibodies may be referred to the original literature.

II. The basic principles and techniques used in the synthesis of catalytic antibodies

The essence of action of catalytic antibodies, like that of ordinary enzymes, is facilitation of the formation of the transition state of the substrate molecule to favour its conversion into the reaction products. This is accompanied by a decrease in the energy barrier on the reaction coordinate during the formation of the transition state, so that the reaction rate increases. This is a result of several specific interactions occurring in the active site of the enzyme. The cavity of the latter matches the spatial structure of the substrate. The active site contains active groups in its surface favouring the corresponding conversions of the substrate molecule. The spatial and electronic complementarity of the active site and the substrate favours the 'recognition' of the substrate by the enzyme and the formation of the enzyme–substrate complex, while the active groups enable the conversion of substrate molecules into the reaction products which involves acid–base catalysis, nucleophilic or electrophilic interactions, *etc*.

As the enzyme–substrate complex is formed, the mobility of the substrate molecule is restricted, and the latter becomes 'fixed' in a conformation which is the most favourable for further conversions. This provides a significant gain in energy and creates the so-called 'entropic trap'. This catalytic effect is especially important for bimolecular reactions, where both interacting molecules are fixed in a reciprocal position favourable for the reaction. Other factors, such as the presence in the active site of hydrophobic zones favouring substrate conversions in non-polar aprotic ('non-aqueous') media, also play a substantial role in the manifestation of the catalytic effect.

In order to obtain an effective catalytic antibody (CA), it is necessary that its active site possesses the properties characteristic of the active site of the enzyme (see above), which ensures the manifestation of the catalytic effect.[6] This task is accomplished by an antigen, which induces the formation of the active site of a desired structure during the biosynthesis of the protein molecule by the immune apparatus of the cell in response to administration of the antigen.

The antigen used to obtain CA usually consists of two main fragments: a low-molecular-weight hapten and a high-molecular-weight carrier, usually, an available natural protein. It is the hapten that represents the fragment of the antigen molecule which is directly involved in the formation of the active site of CA. Its spatial and electronic structures must correspond to the transition state of this reaction and enable the formation of active groups, hydrophobic zones, *etc*., in the active site.

The protein fragment of the antigen (the so-called 'immunogen') is a high-molecular-weight carrier that ensures the generation of antibodies by the immune apparatus. Some available natural proteins, such as hemocyanin from the mollusc *Megatura crenulata* (keyhole limpet hemocyanin, KLH) or bovine serum albumin (BSA), are the most popular immunogens.

It is the proper choice of the hapten that is the key factor determining success in the preparation of CA. Naturally, the correct design of a hapten requires knowledge of the structure of the transition state, the pathways of its induction and subsequent decomposition, in short, a knowledge of the reaction mechanism. A number of CA catalysing many reactions which occur by

different mechanisms have been prepared. However, it is impossible to formulate general recommendations concerning the design of a hapten. The reasoning behind the choice of a hapten structure will be given below for each concrete case.

Sometimes, the choice of a hapten is semi-empirical because of the lack of exact knowledge of the reaction mechanism. In such cases, researcher's experience and even intuition come to the foreground. Thus in the early studies, a somewhat simplified concept of the correlation between the spatial and electronic structures of the hapten and the structure of the transition state have played a crucial role. This sufficed to prepare the first efficient CA. Mimicking of the transition state in the hydrolysis of the ester group by phosphoric or phosphonic acid groups provides an example (see Section III).

In addition to the main 'reactive' fragment, which is responsible for the formation of the active site of CA, the hapten must contain fragments providing the 'recognition' of the substrate by the antibody. This is achieved by incorporation into the hapten of groups analogous to those in the substrate. In addition, the hapten must contain a group which enables its immobilisation on the protein, so that the hapten fragment in the antigen is located at some distance from the protein chain. The role of such 'spacer' or 'linker' groups may be played by a short aliphatic chain having a terminal reactive functional group (*e.g.*, a carboxy group). In the immobilisation, the carboxy group of the spacer reacts with the amino group of the lysine residue of a protein and binds the hapten to the protein by a strong amide bond.

Normally, immobilisation of the hapten on the protein is performed under mild conditions that do not affect either the hapten or the immunogenic protein. The number of hapten molecules bound to the protein molecule depends on the immobilisation conditions. As a rule, the antigen synthesis is conducted so as to achieve binding of not less than 10–20 hapten residues per one protein molecule.

The antigen thus prepared is used for immunisation of animals (usually, inbred mice) using standard procedures[7] which differ from one another in some details for different CA. As a rule, two or three immunisations (as intramuscular injections) are performed in the presence of an adjuvant (most commonly, Freund's adjuvant) followed by one intravenous injection without the adjuvant in order to enhance the immune response. After the lapse of a definite time, spleen cells of the immunised animal are removed to prepare monoclonal antibodies by the classical hybridoma technology.[8] To this end, the spleen cells are 'fused' with myeloma cells in a polyethylene glycol solution. The hybridomas thus obtained are able to produce monoclonal antibodies against various fragments of the antigen molecule. From the total bulk of monoclonal antibodies, those generated against particular structural fragments of the hapten and able to form complexes with it due to complementarity of their structures are selected using the so-called enzyme-linked immunosorbent assay (ELISA).[9] The hapten immobilised on BSA is used as a specific reagent. The formation of a hapten–antibody complex points to the complementarity of the structure of the active site of the antibody to that of the hapten (and, correspondingly, to that of the transition state of the substrate which had been mimicked by the given hapten). Such antibodies are likely to manifest catalytic activity. The ability of the selected antibody to catalyse a specific reaction is assayed in direct experiments.

In order to obtain large quantities of CA for further analysis, the appropriate hybridoma selected by the ELISA technique is repeatedly injected into experimental mice after which the target CA is isolated from the ascites fluid where it is present in a high concentration. The CA thus obtained is further purified by conventional methods of protein chemistry (precipitation, chromatography and electrophoresis).

Being a protein, CA completely loses its activity upon thermal treatment (denaturation) or this is changed following treatment with typical protein modifiers (iodoacetamide, diethyl carbonate, tetranitromethane, *etc.*).

Sometimes, the preparation of active CA requires more sophisticated and specific procedures. These may include sequential immunisation of mice with two antigens differing in the hapten structure (the so-called 'heterologous immunisation').[10] This provides the formation of an active site which matches the features of both haptens and thus imparts a required specificity on CA. An alternative approach consists of the use of an antigen whose hapten not only is a stable model of the transition state generated in the course of the substrate conversion but can also interact with the active site of the CA formed in this process, thus reproducing the dynamics of conversion of the substrate molecule in the course of the catalytic reaction (the so-called 'reactive immunisation').[11]

In their properties and behaviour, CA are typical proteins pertaining to the class of immunoglobulins. Their physicochemical characteristics are close to those of natural enzymes. Thus the behaviour of CA in a catalytic process follows saturation kinetics and is described by the Michaelis–Menten equation. It thus follows that the catalytic effect of CA is based on the formation of the enzyme–substrate complex, which has been directly confirmed in mass spectrometric studies.[12]

The inhibition of the CA activity by the hapten or its analogues suggests that the cavity in the active site of CA is in fact complementary to the hapten used for its generation. Inhibitory analysis of CA is widely employed in the studies on the structure of the active site of CA and the mechanism of its catalytic effect. Quantitatively, the catalytic effect of CA is estimated by the ratio of the rate constants of the catalytic and non-catalytic (basic) reactions.

An important feature of CA is its specificity, which also includes stereoselectivity. Specificity of CA is established using a traditional approach, *viz.*, in reactions of structural analogues of the substrate, where the rates of their conversions and/or inhibitory effect in catalysis are determined.

In some cases, the structure of CA or its active site has been studied in more detail using X-ray structural analysis of CA itself or its complex with the substrate. These studies were mainly undertaken in order to elucidate the mechanism of action of CA at the molecular level, to compare the structure and mechanism of action of CA with those of the natural enzyme and to solve other enzymological problems with CA as the models.

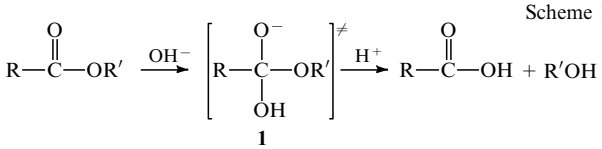
In the subsequent sections, we shall consider the procedures used to prepare CA catalysing reactions of various types which are classified according to the principle conventionally employed in organic chemistry and familiar to organic chemists. The chronological order may not be strictly followed.

III. Hydrolysis of the ester bond and other transacylation reactions

Hydrolysis of the ester bond is the first example of a chemical conversion for which catalytic antibodies have been specially raised and the principles of selection of haptens, which are the 'active' parts of the artificial antigens used in generation of specific CA by biosynthesis in the immune apparatus of an animal, have been considered.

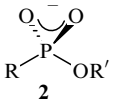
Success in the synthesis of these CA was achieved primarily due to the correct choice of a group mimicking the transition state **1** formed in the hapten in the cleavage of the ester bond (Scheme 1).

Scheme 1



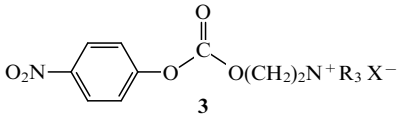
1

The RP(O)(OR′)O group (**2**) in phosphoric or phosphonic acid derivatives, which contains a tetrahedral phosphorus(V) atom and bears a negative charge, mimicked the transition state **1** of the reaction of cleavage of the ester bond containing a tetrahedral carbon atom and bearing a negative charge.[4,5]

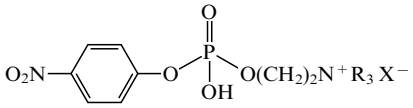


2 R = Ar, OAlk, OAr.

The idea to use phosphoric or phosphonic acid derivatives came about also from the consideration of the known data on the structure of synthetic inhibitors of natural esterases, particularly of choline esterases.[13] These inhibitors represent phosphate (or phosphonate) analogues of the corresponding substrates (*e.g.*, acetylcholine) with a phosphorus-containing group in place of the ester bond. They form complexes with choline esterase, which points to the complementarity of the phosphate group to the enzyme active site. It was found that some antibodies raised against such inhibitors manifested catalytic properties, *i.e.*, they enhanced the hydrolysis of the ester bond in the corresponding substrate. This confirmed the presence of the active site favouring hydrolysis. Thus the MOPS 167 antibody raised against the inhibitor of *O*-(4-nitrophenoxy)carbonylcholine (**3**) esterase formed a complex with this inhibitor (4-nitrophenylphosphorylcholine) (**4**) and possessed noticeable catalytic activity, *viz.*, it accelerated manyfold the hydrolysis of the choline derivative **3**.[5]



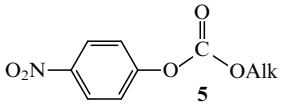
3



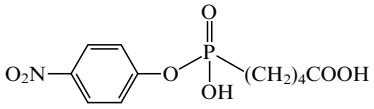
4

This antibody manifested the features of natural esterases (Michaelis–Menten kinetics, inhibition by compound **4**, characteristic dependence of its activity on pH, *etc.*). Its catalytic effect was regiospecific, *viz.*, the hydrolysis of substrate **3** analogues with an altered structure of the aliphatic chain or devoid of the quaternary nitrogen atom was accelerated in a much lesser degree or not at all. These data suggest that the phosphorus-containing group efficiently mimics the transition state which appears in the hydrolysis of the ester bond and can generate the active site in the CA possessing esterase activity. This circumstance has been widely employed in further preparation of this type of CA.

Thus a 4-nitrophenyl phosphonate derivative (**6**) containing a tetramethylene fragment with a carboxy group as a spacer was used as a hapten† to raise a CA catalysing the hydrolysis of 4-nitrophenyl carbonate (**5**).[14]



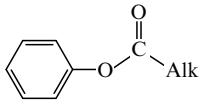
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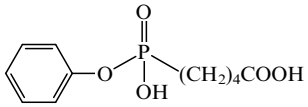
6

Immobilisation of phosphonate **6** through this carboxy group made it possible to obtain an artificial antigen containing, on the average, 15 hapten residues per one protein molecule. This antigen was used for immunisation, and a CA was isolated which accelerated the hydrolysis of carbonate **5** 800-fold in comparison with the non-catalysed reaction. This CA manifested the properties of an esterase, *viz.*, its hydrolysis followed Michaelis–Menten kinetics, suggesting the formation of an enzyme–substrate complex, and its activity showed a typical pH-dependence. This CA was inhibited by 4-nitrophenyl phosphate, the phosphate analogue of the substrate. Its effect was regiospecific, *viz.*, the rate of hydrolysis of 3-methyl-4-nitrophenyl carbonate was 10-fold smaller in comparison with the original compound, while the hydrolysis of isomeric 2-nitrophenyl carbonate was not catalysed at all.

Haptens with similar structures were used to obtain CA catalysing the hydrolysis of phenol esters. Thus the hapten **8** was used to raise CA catalysing the hydrolysis of the simplest *O*-acylphenols of the type **7**.

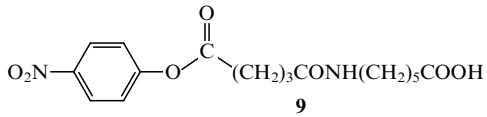


7

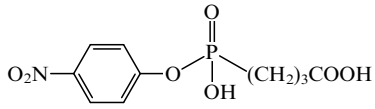


8

One of the CA prepared accelerated the hydrolysis of acylphenols more than 10^4-fold.[15] The hydrolysis of the ester bonds in nitrophenols **9** acylated with *N*-carboxyalkyl glutaramide, such as compound **9**, was accelerated 10^4-fold in the presence of CA raised against the hapten **10**.[16]

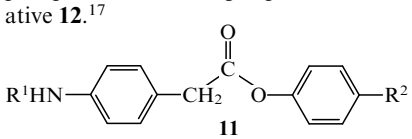


9



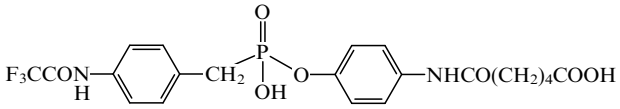
10

The hydrolysis of structurally more complex substrates **11** (aminophenol esters with substituted aminophenylacetic acids) would be accelerated in the presence of CA raised against phosphorus-containing haptens, *viz.*, a benzylphosphonate derivative **12**.[17]



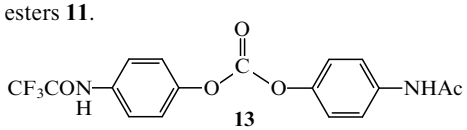
11

R^1 = Ac, $COCF_3$; R^2 = H, NHAc, $NHCOCF_3$, $NHCO(CH_2)_3COOH$



12

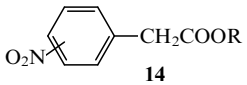
The structural similarity of the benzylphosphonic acid derivative to the acid moiety of the substrate allows more precise mimicking of the transition state of hydrolysis of the esters **11**. Several CA have been isolated from a mixture of antibodies raised against the hapten **12**. The most efficient of them accelerated the hydrolysis of the esters **11** 10^6-fold and manifested all the features of the enzyme. Thus its activity changed after treatment with specific protein modifiers (diethyl carbonate, tetranitromethane, *etc.*). The activity of this CA strongly depended on the nature of substituents in the aromatic nuclei of the substrate **11**. It was inactive in the hydrolysis of carbonate **13** structurally similar to the esters **11**.



13

The problem of specificity of the CA of the esterase type is of considerable importance for their practical applications in the solution of synthetic tasks. This problem and the factors determining the specificity have been studied with the hydrolysis of nitrophenylacetates **14** as an example.[18]

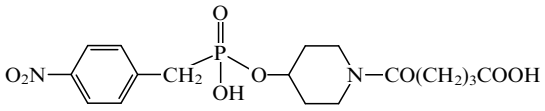
† For the synthesis of this hapten and other compounds mentioned in this review, see the original papers.



14

R = Alk, Bn, $O_2NC_6H_4CH_2$, $ClC_6H_4CH_2$ *etc.*

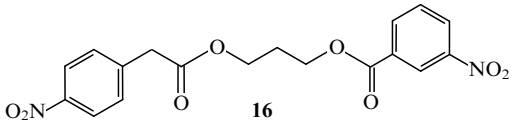
To this end, the hapten **15** containing a 4-hydroxypiperidine residue (enhancing the antigen immunogenicity) and a spacer was synthesised.



15

A catalytic antibody raised against the hapten **15** appeared to be highly efficient. It accelerated the hydrolysis of the esters **14** (3×10^4-fold, on average). The results of the kinetic analysis of hydrolysis of structurally different esters **14** revealed that the catalytic effect of CA was manifested only in the case of 4-nitro-substituted derivatives. The hydrolysis of the corresponding 3-nitro derivatives was not catalysed at all, which confirmed the previously established regularity.‡

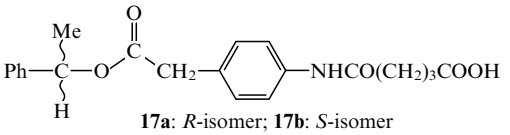
This specificity allowed the hydrolysis of a mixed ester of 1,3-propylene glycol **16** to occur in a strictly selective manner.



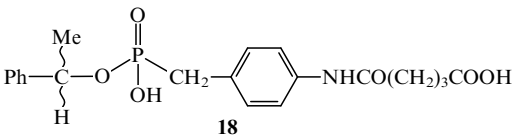
16

The effect of the nature of the alcoholic component is much less pronounced. Thus CA catalyse the hydrolysis of esters **14** containing residues of various primary alcohols, although the rates of this hydrolysis vary from 10^4 to 10^6. In contrast, the hydrolysis of the esters **14** containing residues of secondary alcohols is not catalysed at all, with the exception of 1,1,1,3,3,3-hexafluoropropan-2-ol derivatives.

High stereospecificity is one of the most distinguishing and important features of enzymic catalysis. This problem has received much attention in the studies of CA, and the generation of the first highly stereospecific CA has been a considerable achievement. For the first time, this has been studied in the preparation of a CA catalysing the hydrolysis of esters of substituted phenylacetic acid with α-methylbenzyl alcohol (**17**) containing a chiral carbon atom.[20] The hapten **18**, which is a phosphonate analogue of the substrate **17** and also contains an asymmetric carbon atom, was used to raise a specific CA.



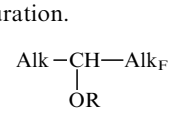
17a: *R*-isomer; **17b**: *S*-isomer



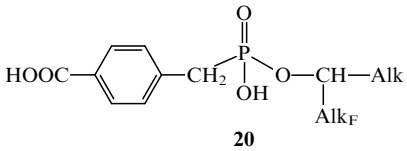
18

The ester **18** was used as a racemate. Eleven active CA catalysing the hydrolysis of the esters **17** were obtained. Study of the hydrolysis of pure enantiomers **17a** and **17b** in the presence of these CA showed that nine of them catalysed the hydrolysis of the *R*-isomer, while the other two catalysed the hydrolysis of the *S*-isomer. A detailed analysis revealed that all of these CA manifested the characteristic properties of esterases and high stereoselectivity. Thus the degree of hydrolysis of the enantiomer which corresponds to the specificity of the CA is 98%, while that of the other enantiomer is only 2%. The catalytic activity of stereospecific CA catalysing the hydrolysis of the *R*- and *S*-isomers is different. The catalytic antibody, which catalyses the cleavage of the *R*-isomer, accelerates the reaction 8×10^4-fold (*vs.* 1.6×10^3-fold increase in the case of the CA specific toward the *S*-isomer). Thus, the racemic hapten **18** generates CA manifesting two different stereospecificities and each of the enantiomers of **18** 'forges' the active site of CA with the complementary spatial structure. As has been mentioned above, the number of antibodies raised against each enantiomer of the hapten **18** is different due to the peculiarities of the protein chain biosynthesis in the immune apparatus.

Catalytic antibodies of different stereospecificities can be isolated in the individual state or be specifically generated using the corresponding optically pure hapten enantiomers. Such CA manifest high stereoselectivity and are used successfully in the synthetic practice, *e.g.*, for kinetic resolution of mixtures of stereoisomeric alcohols by selective hydrolysis of their esters. For example, racemates of the fluorine-containing alcohols **19a** were resolved by hydrolysis of the corresponding phenylacetates **19b**.[21] CA with predetermined stereoselectivity were generated using enantiomers of the hapten **20** having an *R*- or *S*-configuration.



19a,**b**

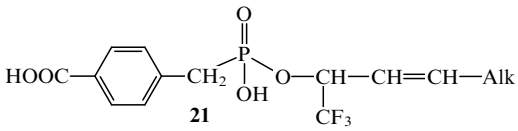


20

R = H (**a**), COBn (**b**);
Alk = C_6H_{13}, C_7H_{15}, C_8H_{17};
Alk_F = CHF_2, CF_3.

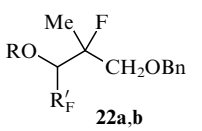
The hydrolysis of the racemic phenylacetates **19b** in the presence of these CA yielded the *R*- and *S*-isomers of the alcohols **19a** with optical purity 98%. It is noteworthy that the stereoselectivity of the resolution did not depend on the length of the aliphatic chain.

Kinetic resolution of the stereoisomeric alcohols **19a** containing the allylic group (Alk = CH=CHC$_6$H$_{13}$) was carried out in a similar way. With the use of CA against the enantiomers of the hapten **21** it was possible to isolate all the four isomers of the corresponding alcohols **19a** of high optical purity from a mixture of phenylacetates **19b** (Alk = CH=CHC$_6$H$_{13}$, Alk_F = CF_3).

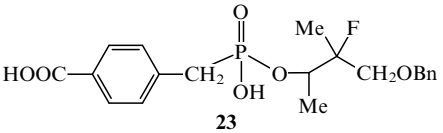


21

The diastereomeric alcohols **22a** were resolved by the hydrolysis of their phenylacetates **22b** catalysed by the catalytic antibodies raised against the haptens **23** having *syn*- and *anti*-configurations.[22]



22a,**b**



23

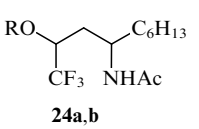
R = H (**a**), COBn (**b**).

The catalytic antibody against the *syn*-isomer of **23** hydrolysed the *syn*-isomer of **22b** to yield the alcohol with optical purity 99% (the degree of hydrolysis was 49%), while CA against the *anti*-isomer of **23** hydrolysed the *anti*-isomer of **22b** with the same high degree of purity. The resolution of the alcohols HOCH(R_F)C(F)(Alk)CH_2OBn, which differed from compounds **22a** in the length of the aliphatic chain (Et, Pr and Bu substituents instead of the methyl substituent), occurred in a similar way. After

‡ The specificity of antibodies against nitro-substituted substrates was established by Landsteiner [19] at the dawn of development of immunochemistry.

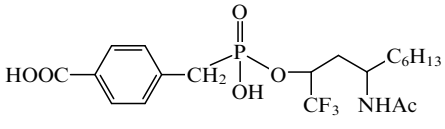
separation of the isomers subjected to hydrolysis, the diastereomers of phenylacetates **22b** resistant to hydrolysis were converted into the alcohols by conventional hydrolysis.

The resolution of all the four diastereomers of the amino alcohols **24a** from a mixture of their phenylacetates **24b** using a set of CA raised against the diastereomeric haptens **25** has been reported.[22]



24a,b

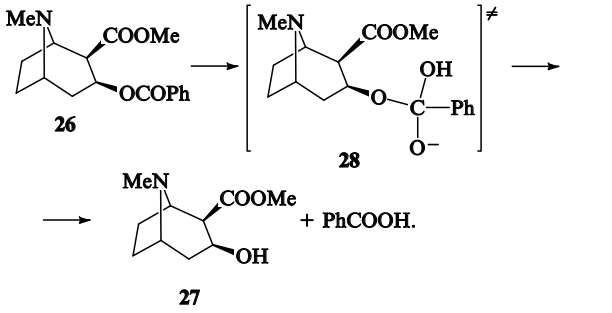
R = H (**a**), COBn (**b**).



25

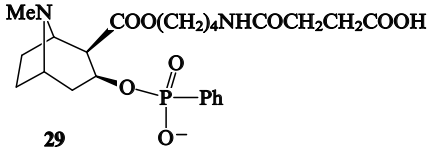
An attempt was undertaken to obtain a catalytic antibody of the esterase type that would efficiently cleave one of the most hazardous narcotic agents, *viz*., cocaine, and to use it in the design of a medicinal drug that would not merely bind the narcotic but also decompose it into nontoxic components. Hydrolysis of cocaine (**26**) leads to its degradation into the nontoxic ecgonine methyl ester (**27**) and benzoic acid (Scheme 2).

Scheme 2



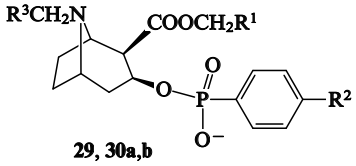
26 **28** **27**

This hydrolysis is catalysed by the natural butyrylcholine esterase, which is, however, insufficiently active. The needs of practical medicine are for a more efficient enzyme. Since hydrolysis of cocaine occurs *via* the transition state **28** and the mechanism of this reaction does not differ from that of analogous reactions, the choice of a hapten created on the same principle, *viz*., the cocaine analogue, compound **29** containing a phenylphosphonic acid residue instead of benzoic acid and a spacer, was used as a hapten to obtain CA.[23]



29

With this hapten, two CA were generated which accelerated cocaine hydrolysis 540- and 440-fold. However, this acceleration is insufficient for clinical application of these CA. Attempts were made to modify this hapten by changing the position of attachment of the spacer group. The haptens **29**, **30a,b** were synthesised specially for this purpose.[24]



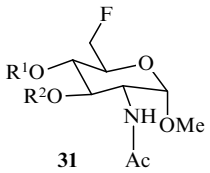
29, 30a,b

29: $R^1 = (CH_2)_3NHCOCH_2CH_2COOH$, $R^2 = R^3 = H$;
30a: $R^1 = R^3 = H$, $R^2 = (CH_2)_3NHCOCH_2CH_2COOH$;
30b: $R^1 = R^2 = H$, $R^3 = (CH_2)_3NHCOCH_2CH_2COOH$.

The respective CA manifested higher activity and the most efficient of them accelerated cocaine hydrolysis 2.3×10^4-fold. And even this enhancement did not meet the specific clinical requirements imposed on a medicinal drug. Analysis of enzymic kinetics and inhibitory analysis of the CA obtained revealed significant differences in their activities and a complex dependence of the activity on the structure of the protein chains of the antibody despite their high homogeneity. These studies open up realistic prospects for the synthesis of antinarcotic drugs, although the design of valuable drugs demands further investigation aimed at modification of CA by biotechnological methods.

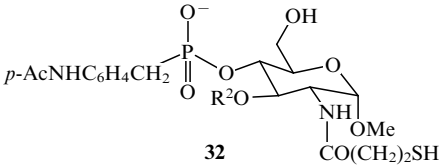
Catalytic antibodies of the esterase type endowed with high regiospecificity are also of interest, since they allow selective removal of structurally different acyl groups. Another no less important problem is selective removal of one of several structurally identical acyl groups which differ in their position in the molecule. Attempts have been made to obtain CA with predetermined regiospecificity of this type. An example can be found in the generation of CA for selective removal of an acyl group in the polyacyl derivative of a monosaccharide.[25] This approach could be a useful tool in the solution of many challenging problems of synthetic carbohydrate chemistry.

Selective removal of the 4-*O*-acyl group in the glucose 3,4-di-*O*-acyl derivative **31** ($R^1 = R^2 = COCH_2C_6H_4NHAc$) could be effected with the use of CA raised against the hapten **32**, the structural analogue of the substrate **31** in which the 4-*O*-acyl group is substituted by the benzylphosphonic acid residue.

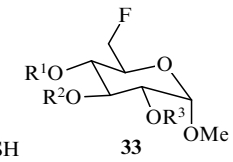


31

Compound **32** also contains a spacer at position 2 for immobilisation. One of the CA obtained regioselectively accelerated the hydrolysis of the 4-*O*-acyl group in the molecule of compound **31** 3000-fold. The ratio of 4-*O*- and 3-*O*-deacylation products was 20 : 1 (*cf*. 4 : 1 in conventional hydrolysis in the absence of CA). The ratio of 4-, 3- and 2-*O*-deacylation products in the hydrolysis of the triacyl derivative **33** ($R^1 = R^2 = R^3 = COCH_2C_6H_4$. .NHAc) catalysed by the same CA was 13 : 8 : 1.

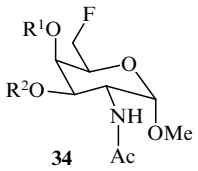


32



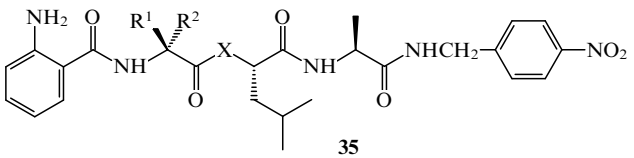
33

In addition to high regioselectivity, this CA manifested high stereoselectivity, *viz*., it did not catalyse the hydrolysis of the 4-*O*-acyl group in the galactose analogue (**34**) where this group occupied the axial position. This example shows a range of complicated and subtle problems which can be solved, in principle, with the use of CA, although the use of this particular CA is impracticable.



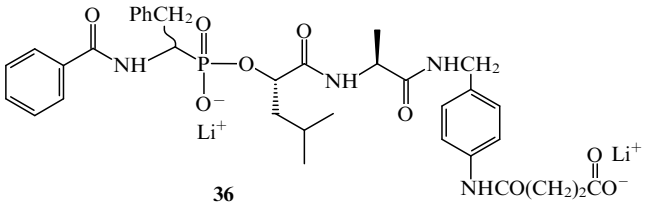
34

The catalytic antibody selectively catalysing the hydrolysis of the ester bond in one diastereomer of the depsipeptide **35** is an example of a catalytic antibody possessing chemo-, regio- and stereoselectivity.[26] It was raised against the hapten **36**, which represents the phosphonate analogue of the substrate and is a nearly equimolar mixture of diastereomers.



35

X = O, NH; R^1, R^2 = H, Bn.

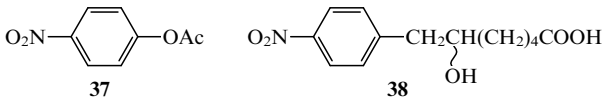


36

This CA hydrolysed only the ester bond (X = O) without affecting the peptide bond (X = NH) (chemoselectivity). It is noteworthy that this antibody exclusively catalysed the hydrolysis of the depsipeptide **35** containing the D-phenylalanine residue, whereas the depsipeptide containing the L-isomer was hydrolysed by no more than 0.5% (stereoselectivity). Evidence for the high regiospecificity of this CA followed from its inability to catalyse the hydrolysis of the depsipeptide **35** analogues in which leucine or tryptophan was substituted for the phenylalanine residue.§

All the above examples of preparation of CA are based on the use of substrate analogues as the haptens in which the ester bond was replaced by a residue of phosphoric or phosphonic acid and which mimic the transition state of ester hydrolysis. Catalytic antibodies against these haptens manifested chemo-, regio- and stereoselectivity. Haptens of this type are used in the synthetic practice most often and most successfully.

However, haptens of a different structure, among which haptens containing secondary alcoholic groups instead of the ester group in the substrate molecule are the most popular, can also be used for the preparation of CA possessing esterase activity.¶ Although the mechanism of action of such haptens is not clearly understood, several active CA were raised against them. For the first time, a hapten of this type was designed by analogy with pepstatin, a well-known inhibitor of pepsin.[28] Thus preparation of CA catalysing the hydrolysis of 4-nitrophenyl carbonate **5** or the corresponding acetate **37** was based on the use of 7-(4-nitrophenyl)-6-hydroxyheptanoic acid (**38**) as a hapten.

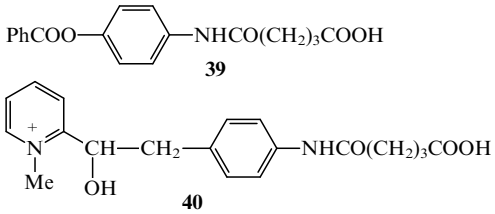


37 **38**

The acid **38** contains the structural elements of the substrates **5** and **37;** its structure resembles that of the phosphorus-containing hapten **6** used to obtain CA with the same character of action (see p. 1002).

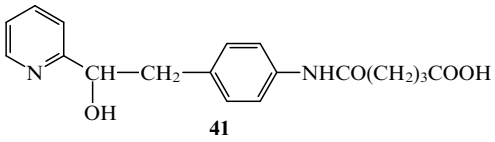
The catalytic antibody against the acid **38** accelerated the hydrolysis of the esters **5** and **37** 930- and 1225-fold, respectively. This CA displayed all the typical features of esterases, although its activity was much lower than that of CA generated against the phosphorus-containing hapten **6.** Nevertheless, this finding confirmed the possibility of using the secondary alcoholic group for mimicking the transition state of the hydrolytic reaction.

The use of haptens containing a positively charged group together with a secondary alcoholic group appeared to be more successful.[29] Thus a CA catalysing the hydrolysis of the ester bond in the benzoate **39** was raised against the hapten **40**.



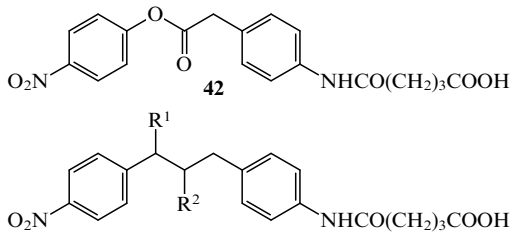
39 **40**

It was assumed that the positive charge of *N*-methylpyridinium generated an acidic group (presumably, a carboxy group) in the appropriate position of the active site of the antibody, while the tetrahedral carbon atom of the secondary alcoholic group provided the formation of its spatial structure. The catalytic antibody raised against the hapten **40** was found to be highly efficient; it manifested activity close to that of natural esterases and accelerated the hydrolysis of the benzoate **39** 10^6-fold. This was attributed to the fact that, in addition to the formation of the spatial structure of the active site, the hapten **40** induced the generation of a carboxy group which, in turn, facilitated the conversion of the substrate with the involvement of general acid – base catalysis. Indirect evidence for the presence of a carboxy group in CA and its role in the hydrolysis of the ester bond can be derived from the fact that the hapten **41**, the pyridine analogue of compound **40,** which is deprived of the positive charge, does not generate active CA.



41

Thus, the preparation of efficient CA requires not only the formation of an active site complementary to the transition state, but also the generation in the active site of groups favouring the very conversion of the transition state into the reaction products, *e.g.*, involving general acid – base catalysis. This views were confirmed [30] in studies of the hydrolysis of the ester bond in compound **42** catalysed by CA raised against the haptens **43.** These CA accelerated the ester hydrolysis 3×10^3-fold.



42 **43a – c**

43a: R^1 = NH_2, R^2 = OH;
43b: R^1 = N^+Alk_3, R^2 = OH;
43c: R^1 = N^+Alk_3, R^2 = .

A detailed study of CA generated against the haptens **43a – c** having different substituents using enzymic kinetics and, especially, inhibitory analysis has led to an important conclusion, *viz.*, that the lack of catalytic activity in CA depends not only on insufficient binding of the substrate because of insufficient matching of the active site but, rather, on the arrangement of groups responsible for the dynamics of conversion of the transition state into the reaction products in the active site. Thus the number of active CA raised against the hapten **43a** containing an amino

§ For the preparation of CA catalysing the hydrolysis of carbamates, see D van Vranken, D Panomitros, P G Schulz *Tetrahedron Lett.* **35** 3873 (1994); P Wentworth, A Datta, S Smith, A Marshall, L Partridge, G M Blackburn *J. Am. Chem. Soc.* **119** 2315 (1997).

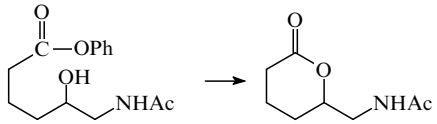
¶ For attempts to use haptens of a different structure (*e.g.*, containing the isosteric and isoelectronic CF_2 group) for preparing CA of the esterase type, see Ref. 27.

group (it virtually functions as the H_3N^+ group) exceeds that of CA raised against the hapten **43b** containing a bulkier Alk_3N^+ group, because the access to the positively charged centre of the hapten is hindered, which impedes the formation of the carboxy group in the appropriate position of the active site. The presence of an additional charged phosphorus group in the hapten **43c** also impedes the formation of the active centre, which results in less active CA. Thus the activity of CA against the haptens **43a**–**c** is believed [30] to depend primarily on the reciprocal position of the charged groups in the 1,2-amino-alcohol fragment of the molecule. This circumstance must be taken into consideration in the design of haptens for highly efficient CA.

Attempts have been made to introduce certain groups aimed at increasing the efficiency of CA by the methods common for natural enzymes, *viz*., by chemical modification and site-directed mutagenesis. The incorporation of a covalently bound imidazole residue into the active site of the antibody MOPS 315 possessing esterase activity [31] is an example of such a modification, which resulted in an increase in the activity of the CA 10^3-fold in comparison with that observed after mere addition of imidazole to the reaction medium in the same hydrolytic reaction.

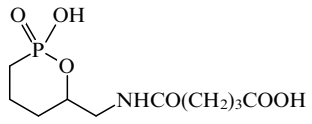
All the examples cited above are related to the preparation of CA catalysing the simplest transacylation reaction, *i.e*., the hydrolysis of esters where the acyl group is transferred to the water molecule present in huge excess. This reaction is of the pseudo-first order. In a wider sense, *O*-transacylation implies the transfer of an acyl residue to any hydroxy-containing compound and occurs according to a more complex mechanism.

The intramolecular transfer of the acyl group in the phenyl ester of the hydroxy acid **44** to the hydroxy group to yield the lactone **45** is the simplest example of the *O*-transacylation reaction.[32]



44 **45**

The hapten **46**, which is a phosphate analogue of the lactone **45**, was used to obtain CA catalysing this reaction. Like other haptens used to raise CA of the esterase type, the phosphonate **46** used (in the form of one enantiomer) mimicked the transition state which appears in the lactonisation of the ester **44**. In the presence of a CA obtained, the reaction was accelerated 167-fold in comparison with the non-catalysed process; this occurred with high stereoselectivity and followed the Michaelis–Menten kinetics.

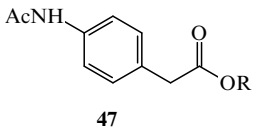


46

The cyclisation of the racemic ester **44** was terminated after conversion of 50% of the substrate to yield only one of stereoisomers of the lactone **45**, since only one enantiomer of compound **44** underwent conversion. The second enantiomer did not enter into the reaction. Thus, the enantiomer of the hapten **46** used has created the active site of CA complementary to only one of the enantiomers of the substrate. This CA can be regarded simultaneously as an enzyme promoting the formation of a new ester (lactone) bond and as a synthase.

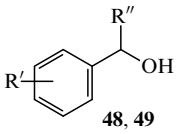
In addition to CA catalysing the intramolecular transfer of an acyl group within the same substrate, some CA of the transferase type have also been obtained. These CA catalysed the intermolecular transfer of acyl groups from one substrate to another. The reactions occurring in aqueous media, *i.e*., in the presence of large excess of water as the competing acceptor, present special interest. The preparation and examination of CA catalysing such reactions were performed mostly with the view of investigating the reaction mechanisms, the practical aspect of their use for the solution of problems of organic synthesis was studied relatively little. The complexity of catalysis of two-substrate reactions lies in that they can follow two fundamentally different mechanisms, *viz*., through the formation of a ternary complex (CA + substrate 1 + substrate 2) or in a more complex way which involves several steps. Realisation of such reactions in aqueous media implies the formation of an additional active site in CA responsible for 'ejection' of a competing water molecule from the reaction sphere, *e.g*., due to the presence of hydrophobic groups. This property must be 'preset' in the structure of the hapten mimicking the transition state.

A detailed study of one of such reactions, *viz*., the selective transfer of 4-acetylaminophenylacetic acid from the esters **47** to substituted benzyl (**48**) and α-methylbenzyl alcohols (**49**) in the water–DMSO system (10 : 1), has been carried out.[33]



47

R = Alk, Ar, Bn, CH_2=CH.



48, **49**

48: R″ = H, **49**: R″ = Me.

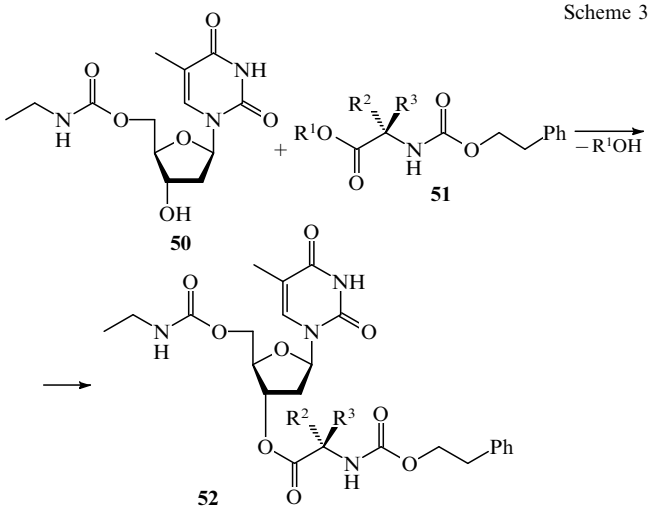
The corresponding CA was raised against the hapten **18**. This CA efficiently catalysed the transfer of the acyl residue not only to the water molecule (hydrolysis) (see p. 1003) but also to alcohols. The latter reaction is selective and unaccompanied by the hydrolysis of the ester **47** despite the presence of a large excess of water. Transacylation in a system of various acyl donors **47** (R = Alk, CH_2=CH, Ar, Bn) and acceptors (alcohols **48** and **49**) is highly efficient; the catalysed reaction occurs 10^4–10^6 times faster, even in the case of the secondary alcohols **49** the hydroxy group of which is much more sterically hindered than that of water. Interestingly, the vinyl ester **47** as an acyl donor proved to be especially efficient and convenient, since vinyl alcohol produced upon transacylation is isomerised into acetaldehyde and eliminated from the reaction.

Detailed studies of the catalytic effect of CA raised against the hapten **18** using the methods of enzymic kinetics and inhibitory analysis as well as X-ray analysis of several CA complexes with substrates have promped important conclusions about the mechanisms of two-substrate transacylation reactions which can be briefly summarised as follows.[34, 35] The transfer of the acyl residue from the ester **47** onto the alcohol occurs *via* the intermediate formation of an acyl derivative of the CA. Subsequent transfer of the acyl group onto the acceptor alcohol follows the so-called 'ping-pong' kinetics where the entering of the first substrate (the ester) into the active site of the CA, the removal of the leaving group with the concomitant formation of the acyl derivative of the CA, the entering of the second substrate (the acyl group acceptor) and the liberation of the transacylation product accompanied by the recovery of the catalyst (CA) regularly alternate. The whole sequence of reactions is then repeated. The active site of CA undergoes certain conformational changes in the course of the reaction depending on the structure of the second reaction substrate. For molecular mechanisms of the acyl transfer see Refs 36, 37

The reactions of the acyl transfer catalysed by natural transferases are widely represented in various biochemical processes occurring in living organisms. In this connection, the preparation of CA catalysing one of the most important reactions of this kind, *viz*., aminoacylation, which consists in the transfer of the amino acid residue onto a nucleotide residue is of considerable interest. In a cell, this reaction is catalysed by the natural enzyme (aminoacyl-tRNA synthase) and is the key stage in protein biosynthesis.

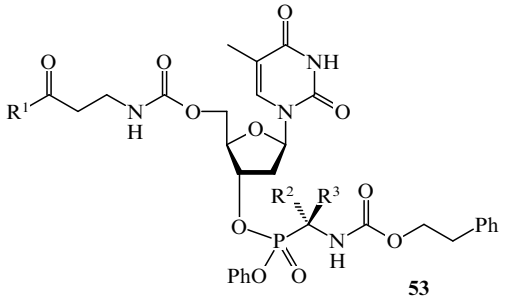
The transfer of the *N*-acylalanine residue onto the hydroxy group of the thymidine derivative **50** from the ester **51** resulting in the derivative **52** has been studied (Scheme 3).[38]

Scheme 3



50 **51** **52**

R^1 = Ph, 4-FC_6H_4, CH_2CN; R^2, R^3 = H, Me.



53

R^2, R^3 = H, Me.

The corresponding CA was raised against the hapten **53**, the phosphate analogue of the aminoacylation product **52**.

This hapten structure enables the formation of the active site of CA which ensures the mutually advantageous arrangement of the reaction substrates and gives a considerable gain in entropy in the formation of the transition state of this bimolecular reaction. The tetrahedral structure of the phosphonate group differs from the trigonal structure of the reaction product, which prevents the inhibition of the aminoacylation reaction by the reaction products.

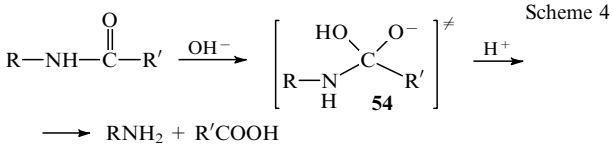
The aminoacylation of thymidine **50** took place in the case of phenyl ($R^1 = C_6H_5$), 4-fluorophenyl (R^1 = 4-FC_6H_4) and cyanomethyl ($R^1 = CH_2CN$) esters **51**, but not for the ethyl ($R^1 = C_2H_5$) esters **51**. Four of the CA raised against the hapten **53** catalysed the transesterification of L-alanine esters and three CA catalysed the transesterification of D-alanine esters. The most active of these CA accelerated the aminoacylation reaction more than 10^8-fold. A kinetic analysis showed that these CA only weakly recognised the alcoholic component but were more selective with respect to substrate stereochemistry, *i.e.*, the reaction was highly stereospecific.

IV. Hydrolysis and formation of the amide bond

The search for CA endowed with the ability to catalyse hydrolytic cleavage of the amide bond in a way similar to that of natural proteolytic enzymes is of considerable importance, because it opens up new ways to selective hydrolysis of a polypeptide chain of the protein molecule, which represents one of the basic challenges to bioorganic chemistry. This problem is much more difficult than hydrolysis of the ester bond, and achievements in this area are far more modest. Since the hydrolysis of the NH—CO bond is a more energy-consuming process and occurs according to a more complex mechanism, a more accurate choice of a hapten that would initiate the biosynthesis of highly specific CA able to lower the energy barrier of this reaction is required. Only CA efficiently catalysing the hydrolysis of only the simplest amides have been obtained so far.

The hydrolysis of the amide bond occurs through the transition state **54** which is further decomposed to give an amine and an acid (Scheme 4).

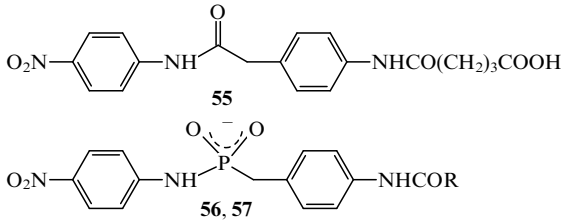
Scheme 4



54

The catalytic antibody must stabilise the ionic site in the transition state **54** and induce simultaneously protonation of the nitrogen atom as the amine is split off. Amide analogues in which the carboxamide bond was replaced by the sterically and electronically similar phosphamide bond were used as haptens mimicking the transition state **54** and forming the active site of CA.

For example, the hydrolysis of 4-nitroanilides of phenylacetic acids **55** was catalysed by CA raised against the hapten **56**.



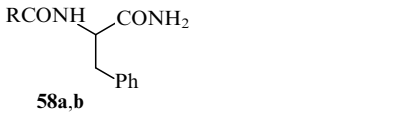
55 **56**, **57**

R = $(CH_2)_2COOH$ (**56**), Me (**57**).

The hapten **56** contained a phosphonamide group as well as functional groups analogous to the corresponding groups of the substrate **55** and essential for its 'recognition' and a spacer.[39] The most active of the CA obtained accelerated 2.5×10^5-fold the hydrolysis of the amide **55** at pH 9.0 compared to the non-catalysed reaction and manifested the features typical of natural amidases (Michaelis – Menten kinetics, inhibition with phosphonamide **57**, which is an analogue of the hapten **56**, *etc.*). The effect of this CA was highly specific, *viz.*, in contrast with 4-nitroanilide **55**, it did not catalyse the hydrolysis of 2- and 3-nitroanilides. Similar specificity was observed for CA of the esterase type (see Section III). The narrow specificity of CA against the acyl fragment of the substrate is the most essential.

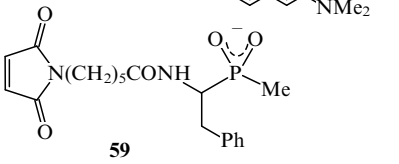
Unfortunately, the hydrolysis of 4-nitroanilides of various α-amino acids and some di- and tripeptides was not catalysed by these CA. Therefore, they are not suitable for the catalytic hydrolysis of the peptide bond.

Certain success has been achieved in the synthesis of CA catalysing hydrolysis of the simplest non-substituted amides of *N*-acylated phenylalanines **58**. The corresponding CA was generated using the hapten **59**, which is an analogue of the amides **58**; the carboxamide group in this hapten was replaced by the methylphosphinic acid residue, and the alkyl spacer had a terminal maleimide residue essential for immobilisation.[40]



58a,**b**

R = Me (**a**), $(CH_2)_5NHSO_2$ (**b**)



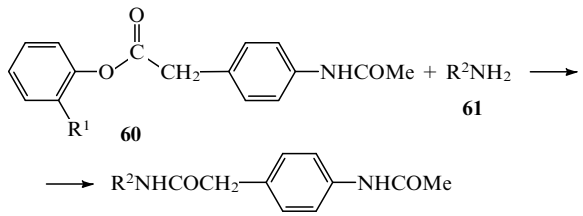
59

This CA accelerated the hydrolysis of the amide **58b** only 130-fold and catalysed the hydrolysis of D-phenylalanine derivatives only; it did not possess esterase activity. High regiospecificity of this CA was manifested in that it catalysed hydrolysis of only the non-substituted amide group, while the acylamide bond in the amides **58a**,**b** was unaffected. Although the practical application of this CA is impeded due to its narrow specificity, it turned out to be a useful tool in the study of the mechanism of the acyl transfer reaction (see p. 1006).

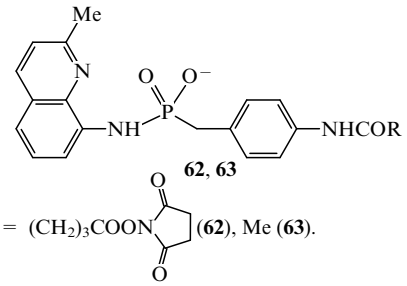
An alternative approach has also been used to obtain CA catalysing the cleavage of peptide bonds. Considering that many natural peptidases are metalloenzymes (*i.e.*, their active site contains a metal ion), it was suggested to use an amide complex containing a metal ion with the corresponding ligand as a hapten. It was assumed that this complex hapten will form a cavity in the active site which might accomodate the amide bond of the substrate coordinated with the metal ion. An attempt to obtain CA selectively hydrolysing the peptide bond of glycylphenylalanine is an example.[41] A hapten containing the trien (tri-ethylenetetraamine) complex of cobalt(III) in addition to the substrate residue was used to raise CA. One of them was found to be active and specific with respect to the peptide structure. Unfortunately, this study was not apparently continued and the CA obtained did not find any practical application. However, the fundamental importance of using metal complexes as haptens is indubitable.

The reverse reaction, *i.e.*, the CA-catalysed synthesis of the peptide bond, especially if this is efficient and selective under mild conditions, is no less interesting than the CA-catalysed cleavage of the amide bond. Although a great number of natural enzymes catalysing this reaction are known, interest in the preparation of CA with predetermined specificity is quite justified.

The chemical methods used in the formation of the amide bond are based on aminolysis of certain carboxylic acid derivatives, *i.e.*, the transfer of the acyl residue onto an amine. The specific function of CA consists in the facilitation of this transfer. Actually, the effect of this CA is close to that of CA of the esterase type catalysing the transfer of the acyl group to the water molecule. The essential difference is that in this case the concentration of the amine is commensurable with that of the acyl donor. This is a second-order reaction and occurs according to a more complex mechanism which may involve the formation of the intermediate acyl group carrier, *viz.*, the acylated derivative of CA (*cf.* Section III).[34–37] This must be taken into consideration in the design of the hapten mimicking the transition state arising in the synthesis of the peptide bond. The simplest aminolysis reaction, *viz.*, direct attack of the nucleophilic nitrogen atom on the ester bond (the transition state of this reaction is analogous to that existing in the hydrolysis of the ester or amide bonds) was used as a model. The transition state was mimicked with a hapten, a substrate analogue, in which the ester bond was replaced by the phosphamide bond. Thus in the preparation of a CA catalysing aminolysis of aryl esters of substituted phenylacetic acids (**60**) with the amines (**61**) (benzylamine, in particular), the 8-aminoquinaldine derivative (**62**) containing a phosponamide group was used as a hapten.[42]



R^1 = H, OMe; R^2 = $PhCH_2$, PhC(Me)H, Bu^n, .

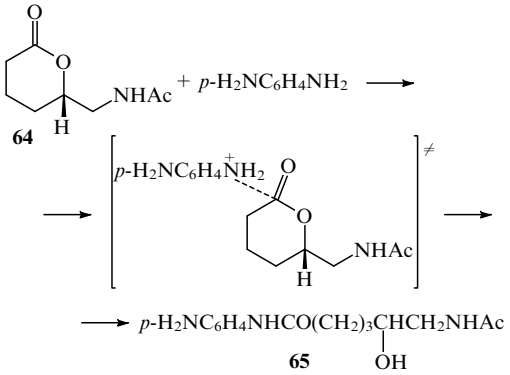


R = $(CH_2)_3COON$ (**62**), Me (**63**).

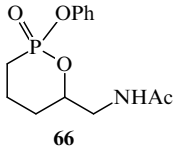
Like natural enzymes, the CA obtained noticeably accelerated the aminolysis which followed Michaelis–Menten kinetics with respect to both reaction components and was inhibited by the hapten analogue (**63**). The effect of CA was specific as regards the amine structure, which has been established in competitive reactions. The ratio of the rates of the CA-catalysed aminolysis with 8-aminoquinoline, benzylamine and butylamine was 30 : 3.12 : 1, whereas in the absence of CA the reaction rate was practically independent of the amine structure (the ratio was 0.9 : 0.7 : 1). In the presence of CA, the reaction was stereoselective: the rates of aminolysis of the ester **60** with epimers of α-methylbenzylamine were different ($R : S$ = 2.2 : 1).

A CA catalysing aminolysis of the lactone **64** with *p*-phenylenediamine was obtained in a similar way (Scheme 5). This reaction results in the ring opening and the formation of the amino alcohol **65**.[43]

Scheme 5



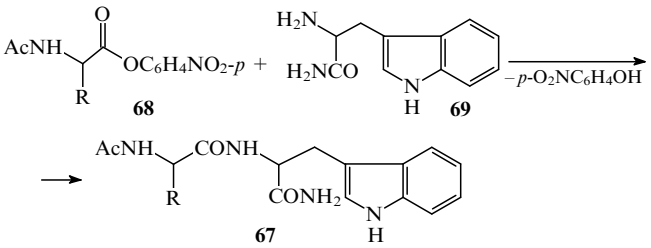
A catalytic antibody raised against the hapten **66**, the phosphonate analogue of lactone **64,** increased the rate of this reaction severalfold and produced a stereospecific effect by catalysing the aminolysis of only the (−)-epimer of the lactone **64**.



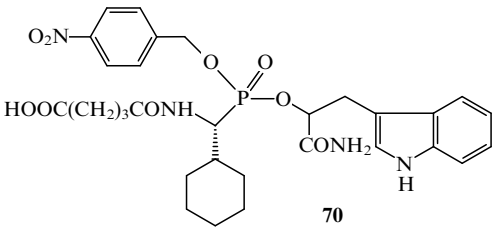
66

Initially, these studies were important for a comparison of the mechanisms of action of CA in similar reactions of hydrolysis and aminolysis (see Section III)[34–37] but did not present any interest for synthetic chemistry. However, recently these reactions have again attracted attention of investigators. The reason is that the design of efficient catalysts allowing selective formation of the peptide bond may facilitate the solution of a problem of synthesis of large proteins by directed coupling of peptide blocks that have now become available owing to solid-phase synthesis. The first steps in this direction have already been undertaken aimed at the preparation of CA the effect of which would be similar to that of such natural enzymes as ligases. Using phosphorus-containing compounds as haptens mimicking the transition state, the first representatives of CA catalysing the formation of the peptide bond between amino acids have been obtained. Thus CA efficiently catalysing synthesis of dipeptides **67**[44] from *p*-nitrophenyl

esters of the corresponding *N*-acetyl amino acids **68** with tryptophan amide (**69**) were raised against the hapten **70**.†

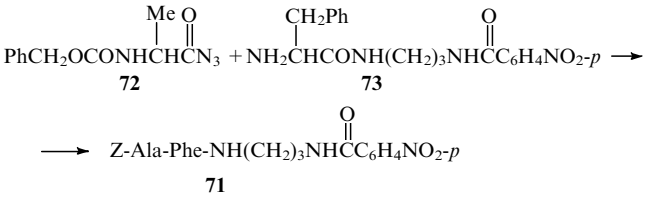


R = Pr^i, Bu^i, CH_2Ph.



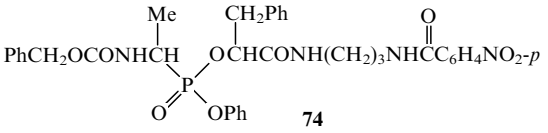
Compound **70** is a phosphonate analogue of the peptides **67** and contains all their characteristic groups. It is assumed [44] that the *p*-nitrobenzyl residue favours the removal, from the active site of the CA, of *p*-nitrophenol that is split off in the condensation, and the cyclohexyl residue mimics the aliphatic chain of α-amino acids. Two CA of approximately equal efficiency accelerated the synthesis of AcVal-Trp, AcIle-Trp and AcPhe-Trp peptides 220-, 380-, and more than 1000-fold, respectively, in comparison with the non-catalysed reactions. Thus, the CA manifested broad specificity essential for their synthetic application.‡ Very low racemisation of the original amino acids (0%–4%) (*vs.* 25% in the non-catalysed reaction) is an important advantage of conducting the condensation in the presence of CA. Interestingly, the configuration of the amino acid has little effect on the efficiency of CA.

A similar approach was used to obtain CA catalysing the formation of a dipeptide (alanylphenylalanine) derivative **71**.[46]



N-Benzyloxycarbonyl-L- or -D-alanine azide (**72**) prepared *in situ* from the corresponding phenyl ester and sodium azide was used as a peptidyl donor in the condensation reaction. The amino group of phenylalanine the C-terminus of which was protected as the amide with *N*-(4-nitrobenzoyl)-1,3-diaminopropane has served as an acceptor.

A phosphonate analogue of the dipeptide **71** to be synthesised containing protective groups of the original amino acids (compound **74**) was used as a hapten.



† The hapten **70** with the P–O bond has been employed instead of a less accessible phosphamide analogue.

‡ One of these CA has been used in the synthesis of tri- and tetrapeptides.[45]

The CA obtained accelerated the condensation of the amino acids **72** and **73** 10^4-fold in comparison with the non-catalysed reaction. The effect of the CA was stereoselective, *viz.*, the reaction with the L-alanine derivative occurred 7 times faster than that with the D-isomer.

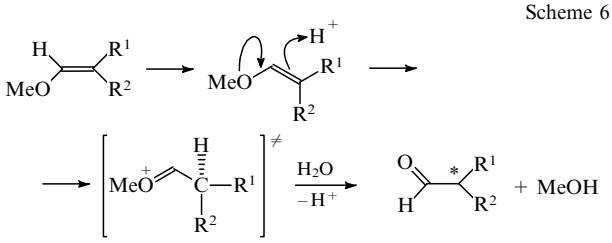
These studies [44–46] present substantial interest, since they can be regarded as the first steps en route to protein synthesis by coupling peptide fragments.§

V. Hydrolytic cleavage of the carbon–oxygen bond in enol ethers and acetals

A great number of papers have been devoted to the synthesis and investigation of CA catalysing the hydrolysis of the C–O bond in enol ethers and acetals to recover the parent carbonyl compounds. These reactions follow similar mechanisms and are involved in many synthetic schemes. Enhancement of the efficiency and selectivity (stereoselectivity, in particular) of these reactions would favour the solution of many synthetic problems.

Hydrolysis of alkyl vinyl ethers with recovery of the corresponding aldehyde or ketone is the simplest example of hydrolysis of enol ethers. This reaction occurs in the presence of an acid catalyst (Scheme 6).

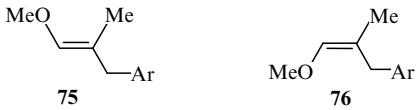
Scheme 6



Protonation of the β-carbon atom of the vinyl group of the ether is the rate-limiting stage. If the β-carbon atom of the vinyl ether is prochiral ($R^1 \neq R^2$), the reaction may yield a mixture of two stereoisomers. The stereochemical outcome of the reaction is important for its synthetic application, in particular, in asymmetric synthesis.

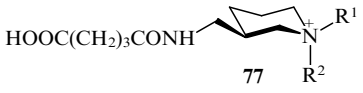
Hydrolysis of *O*-alkylenols normally occurs under relatively drastic conditions with moderate stereoselectivity. Therefore, preparation of CA catalysing this conversion under mild conditions and with satisfactory stereochemical control is of considerable interest. These CA will favour the formation of the oxonium centre in the substrate and the protonation of the β-carbon atom concomitantly with its sp^3-hybridisation ('pyramidalisation'), while it is the spatial direction of the proton attack that will determine the enantioselectivity of this reaction.

The preparation of CA catalysing the hydrolysis of the vinyl ethers **75** and **76** has been described.[48]



Ar = $C_6H_4CONH(CH_2)_3OH$

The *N*,*N*-dialkylpiperidinium derivatives **77** have been used as haptens.

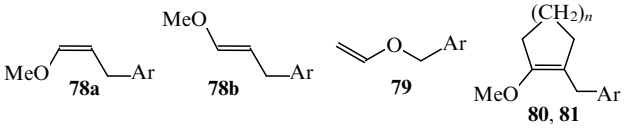


The positive charge on the nitrogen atom in the hapten **77** induces the formation of a negatively charged group in the active site of CA responsible for the formation of the oxonium site and

§ For the preparation of a CA catalysing rearrangement of the peptide bond in aspartylglycine, see Ref. 47.

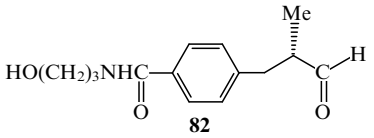
the protonation of the substrate. In turn, the tetrahedral structure of the nitrogen atom favours the formation of a cavity in the active site facilitating pyramidalisation of the β-carbon atom. The substituent at the C(3) atom of the piperidine ring stabilises the conformation of the ring in the molecule **77** and serves as a linker employed for coupling of the hapten to the protein in the immobilisation.

Several haptens differing in the nature and spatial arrangement of substituents at the nitrogen atom (R^1 and R^2 = H, Me, CH_2Ar) have been synthesised to study the relationship between the efficiency and stereoselectivity of the CA action. Active CA have been raised against all of these haptens. One of those, 14D9 raised against the hapten **77** (R^1 = Me, R^2 = CH_2Ar) has been selected for a more detailed examination. This CA manifested all the specific features of an enzyme and possessed relatively high efficiency, which strongly depended on the substrate structure. Thus, under comparable reaction conditions (pH 6.0), it accelerated the hydrolysis of the *trans*-isomer **75** 2500-fold and of the *cis*-isomer **76** only 290-fold. Further investigations into this CA revealed [49] that this antibody manifested broad substrate specificity, although its efficiency was not very high and varied within a very broad range. Thus the hydrolysis of the vinyl ethers **78**, which do not contain alkyl β-substituents, was accelerated 180-fold, while that of the vinyl ether **79** was accelerated only 110-fold. In contrast, this catalytic antibody manifested high activity in the hydrolysis of cyclic vinyl ethers and accelerated the hydrolysis of the methoxycyclopentene derivative (**80**) and methoxycyclohexene derivative (**81**) 12 500- and 370-fold, respectively.

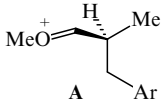


Ar = $C_6H_4CONH(CH_2)_2OH$-*p* **80** (*n* = 1), **81** (*n* = 2).

High enantioselectivity is the most essential property of this CA: it catalysed hydrolysis of the vinyl ethers **75** and **76** to yield the same *S*-enantiomer of the aldehyde **82** with very high enantioselectivity (93% – 96% *ee*).¶



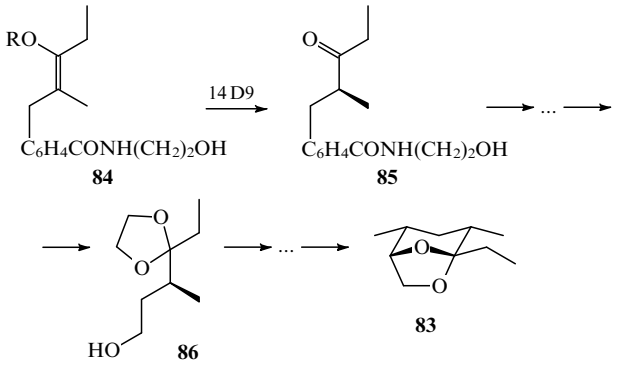
The hydrolysis of the cyclic vinyl ether **80** was also highly enantioselective and the cyclic ketone was obtained with 93% *ee* in the presence of this CA. Such a high enantioselectivity, which was comparable with that attainable with the most efficient chemical methods, was rather unexpected and was extensively investigated [49, 50] using numerous substrate analogues and structurally related inhibitors (including deuterated ones) by the enzymic kinetics method. The correlation between the enantioselectivity of the effect of the CA and the spatial structure of the hapten used for its generation as well as the origin of the enantioselective effect of the CA (which is the reason for the *S*- or *R*-configuration of the newly formed chiral centre) are of particular interest. The conformation of the hapten **77** (R^1 = Me, R^2 = CH_2Ar), which determines the peculiarities of the cavity formed in the active site of CA in the course of its biosynthesis by the immune apparatus of the cell, was established with the use of inhibitory analysis.[50] It was shown that the piperidine ring in the molecule **77** has a chair conformation with the *N*-benzyl group in the axial position and the *N*-methyl group in the equatorial position. The presence of a substituent at the C(3) atom of the piperidine ring, which also occupies the equatorial position, favours this conformation. The substrate molecule (the ether **75**) is arranged in the newly formed active site of CA to 'copy' the conformation of the hapten which had determined the steric configuration of the cavity in the active site of the CA. The position of the benzyl residue of the substrate responsible for its 'recognition' by the active site and fixation in the cavity of the active site of CA is similar to that of the benzyl residue of the hapten **77**. The presence of an anionoid (presumably, carboxy) group in the active site of the substrate induces the formation of a positively charged oxonium site, as a result of which the excited molecule of the substrate accepts the conformation **A**.



It is arranged in the active site as if it mimics the configuration of the hapten, making the β-carbon atom of the substrate accessible for enantioselective protonation on the *r,e*-site and providing the formation of the *S*-aldehyde **82**. Consequently, the enantioselectivity of protonation and the formation of the *S*-aldehyde are predetermined by the configuration of the hapten **77**.

It was emphasised [50] that the most characteristic feature of the enantioselective formation of the new chiral centre is that the hapten shapes the active site of CA which is complementary to the excited substrate molecule predisposed to the attack by the proton rather than to the neutral substrate molecule. This determines strict spatial directionality of this attack and high enantioselectivity of this process. Thus, the choice of the hapten structure mimicking the excited state of the substrate molecule is considered to be of crucial importance in the design of a CA endowed with high enantioselectivity.[49, 50]

Such catalytic antibodies may acquire great importance in the development of asymmetric synthesis, *i.e.*, the introduction of the first chiral centre with a predetermined absolute configuration into an achiral molecule.[51] The use of CA in the synthesis of the pheromone (−)-α-multistratin (**83**) is an example.



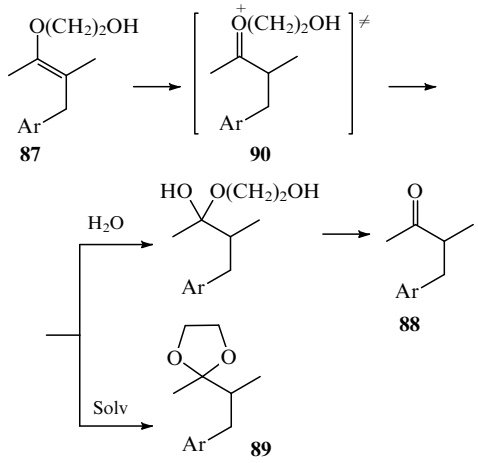
The key stage in this synthesis is the enantioselective introduction of a chiral centre into the molecule of a substrate (the vinyl ether **84**) using CA.[52] Hydrolytic cleavage of the ether **84** was accelerated 6.5×10^4-fold by the CA 14D9 and was highly enantioselective (95% *ee*). The ketone **85** obtained in 85% yield was further converted into the ketal **86** and then into (−)-α-multistratin (**83**).

A simple laboratory technique [53] specially developed for the synthesis of gram quantities of compounds catalysed by CA consists in the removal of the reaction product by dialysis. Since CA remaining in the dialysis sack are reusable, it is possible to use them in small quantities. A small semi-automatic laboratory unit has been designed for conducting the reaction in the presence of CA.[54]

¶ In order to avoid partial racemisation of the carbonyl compound, which may occur under reaction conditions and obscure the stereochemical result, the CA-catalysed conversion of enol ethers was carried out in the presence of cyanide. The latter converts the carbonyl compound into cyanohydrin and stabilises its configuration.

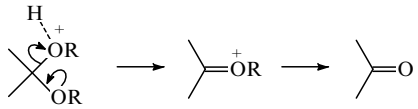
The use of CA in the hydrolysis of vinyl ethers permits one to interfere with the course of the reaction and to perform conversions that are unattainable under ordinary conditions. An amazing result was obtained in the hydrolysis of the vinyl ether **87** in the presence of the CA 14D9 (Scheme 7). This reaction can yield either the ketone **88** or the cyclic ketal **89**.[52]

Scheme 7



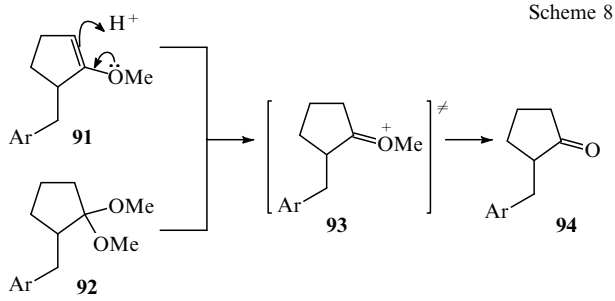
In an aqueous medium (in the absence of the CA 14D9), the acid-catalysed reaction gives only the ketone **88**, because the hydroxy group of the vinyl ether **87** cannot compete with excess water present in the reaction medium. In contrast, the cyclic ketal **89** is formed in organic solvents. However, when the reaction is carried out in an aqueous medium in the presence of CA, 12% of the cyclic ketal **89** is formed in addition to the ketone **88**. The formation of both products obeys the Michaelis–Menten kinetics, they have the *S*-configuration and are formed with very high enantioselectivity (99.5% *ee*), which testifies to the involvement of the active site of CA in their formation *via* the common transition state **90**. Thus, the ketal **89** is formed in a CA-catalysed reaction occurring in aqueous media. The involvement of water is excluded in this reaction because of the hydrophobic environment created in the cavity of the active site of CA where the reaction occurs.

The mechanism of hydrolysis of the acetal bond in the presence of an acidic reagent differs only slightly from that of vinyl ethers. In both cases, the reaction occurs through protonation. However, in contrast with the hydrolysis of vinyl ethers, it is the oxygen atom of the acetal group that undergoes protonation.

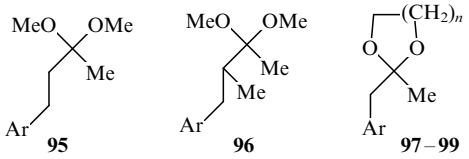


It was found that CA efficiently catalysing hydrolysis of vinyl ethers manifest catalytic activity in the hydrolysis of acetals.[55] This is apparent from comparative hydrolysis of the vinyl ether **91** and the ketal **92** in the presence of the same CA (Scheme 8).

Scheme 8



Both reactions occur *via* the same transition state **93** and result in the ketone **94**. In an aqueous medium and in the absence of CA, acid-catalysed hydrolysis of the acetal **92** usually proceeds at a substantially higher rate than that of the ether **91** (the ratio of hydrolysis rates is 0.09 : 1). In the presence of the CA 14D9, the ratio of the reaction rates changes drastically to the opposite (**91** : **92** = 1100 : 1). The dependence of the reactivities of acetals on their structure was studied under conditions of a hydrolytic reaction. It was found that the acyclic ketals **95** and **96**, which are hydrolysed more slowly in the absence of the CA than the cyclic ketal **92** (the rate ratio is **95** : **96** : **92** = 0.23 : 0.38 : 1), demonstrate the opposite reactivities in the presence of the CA (the rate ratio is **95** : **96** : **92** = 90 : 8 : 1).



n = 1 (**97**), 2 (**98**), 3 (**99**).

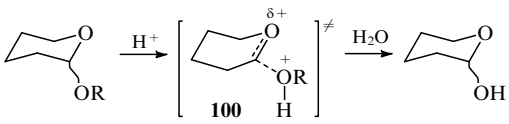
Presumably, the differences in reactivities are due to the fact that the role of steric tensions generated in the cavity of the active site of the CA in the protonation and further decomposition of the acetal is higher in the case of the conformationally more rigid cyclic ketal **92** than in the case of its conformationally flexible acyclic analogues **95** and **96**.

However, this is not the only reason. Recently, the relative effectiveness of the same CA (14D9) has been studied in the hydrolysis of other cyclic ketals (compounds **97**–**99**) with different ring sizes.[56] It was found that the effectiveness of the CA decreased with the increase in the ring size (the rate ratio was **97** : **98** : **99** = 1 : 0.37 : 0.04). These data and comparison of the binding constants of these acetals with those of the complementary azaalicyclic analogues in which the ammonium nitrogen atom corresponded to the acetal carbon atom led the authors to conclude that the effectiveness of the CA largely depended primarily on the electrostatic complementarity of its active site with the substrate, while the conformational tension generated during the reaction was of lesser importance.

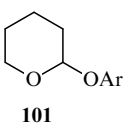
In conclusion, let us dwell briefly on attempts to prepare CA catalysing hydrolysis of mixed acetals (particularly, cleavage of the glycosidic bond).

The hydrolysis of the glycosidic bond under conditions of acid catalysis occurs through the transition state **100** with the oxocarbenium ion in the six-membered ring which has a half-chair conformation (Scheme 9).

Scheme 9

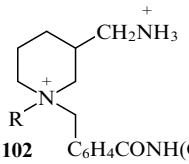


An attempt has been made [57, 58] to obtain CA catalysing the hydrolysis of the simplest model compound, *viz.*, 2-aryloxypyran **101**.

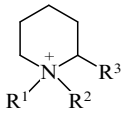


101

Ar = $C_6H_4NO_2$-*m*, $CH_2C_6H_4NHC(S)NHEt$-*m*



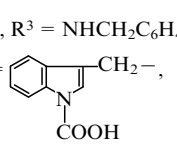
102 R = H, Me



103

$R^1 = R^2 = H$, $R^3 = NHCH_2C_6H_4NCS$-*m*,

$R^1 = H$, R^2 =



$R^3 = CH_2OCONH(CH_2)_4COOH$.

This was achieved using the haptens **102**, analogous to the hapten **77** and containing the $CH_2NH_3^+$ group at the C(3) atom, and the haptens **103** containing a substituted amino group at the C(2) atom. In both cases, the CA obtained moderately accelerated hydrolysis of the model substrate **101** but were ineffective in hydrolysis of more complex compounds.

These results show that it is possible, in principle, to obtain CA catalysing the hydrolysis of the glycosidic bond. However, the preparation of sufficiently active and selective artificial glycosidases poses the problem of choice of a hapten mimicking the half-chair transition state with a positive charge as well as a polysubstituted, polychiral molecule of a real monosaccharide.

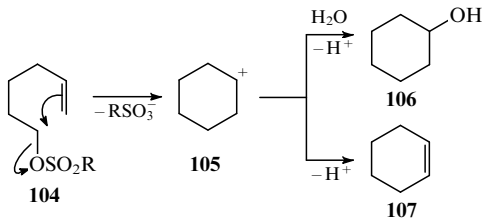
VI. The formation of carbon–carbon bonds

Apparently, preparation of CA that would catalyse the formation of new carbon–carbon bonds making the skeleton of organic molecules is of prime interest in preparative organic synthesis. These include CA that would increase the efficiency of the known reactions and enhances their regio- and stereospecificity and CA able to change the direction of certain reactions in comparison with the non-catalysed processes. This may eventually lead to the discovery of novel reactions that cannot be carried out by conventional chemical methods (the so-called 'unfavourable transformations').

Among a great multitude of reactions that result in the formation of the C–C-bond, only very few are known for which the corresponding CA have been obtained. In particular, several CA catalysing cationic cyclisations, sigmatropic rearrangements and pericyclic reactions as well as aldol condensation, which is one of the most common reactions of carbon–carbon bond formation, have been obtained thus far.

1. Cationic cyclisation

Cationic cyclisation is widely used in the synthesis of carbocyclic systems. Cyclisation of aryl sulfonates of unsaturated alcohols, which occurs in their solvolysis and gives cyclohexane derivatives (the Johnson reaction), has long been known.

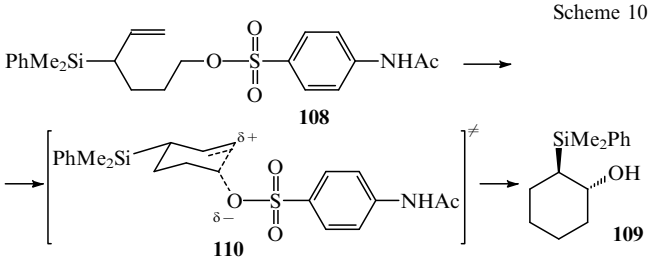


This reaction proceeds through intramolecular electrophilic attack, by the carbocation formed upon solvolysis of the C–O bond, at the double bond of the sulfonate **104**. The resulting cyclic carbocation **105** can be further stabilised either by addition of an external nucleophile to give, *e.g.*, the alcohol **106**, or by elimination of a proton to give the alkene **107**. Under ordinary conditions, this reaction is normally accompanied by the formation of a complex mixture of compounds, which strongly impedes its practical application. Therefore, a search for CA which would not only catalyse the reaction but also enhance its specificity is of considerable interest.

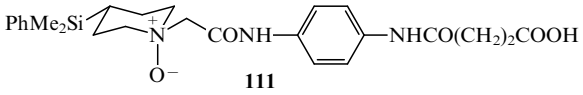
The cyclisation of the sulfonate **108** into the cyclohexanol derivative **109** was studied (Scheme 10).[59] It was assumed that this reaction occurs *via* the transition state **110** where the original substrate acquires a chair configuration, whereas the C–O bond of the sulfonate becomes polarised, which is accompanied by the appearance of an excess positive charge on the carbon atom. Thus the active site of CA would promote the formation and stabilisation of the carbocation and the formation of a chair conformation of the substrate and thus alleviate sterically the cyclisation.

The piperidine *N*-oxide derivative **111** was used as a hapten aimed at the formation of the active site of a CA which matches

Scheme 10



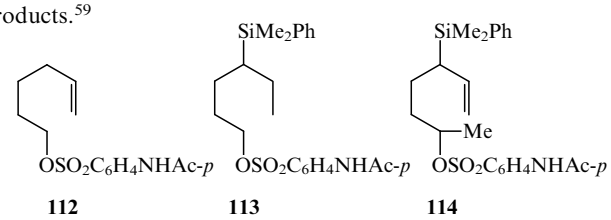
spatially and electronically the transition state. This derivative contains a spacer with a succinic acid residue needed for immobilisation.



In addition to the six-membered ring, which is spatially close to the transition state, compound **111** contains the $^+N-O^-$ fragment, which mimics sterically and electronically the formation and cleavage of bonds in the conversion of the sulfonate **108** into the cyclohexane derivative. The dimethylphenylsilyl group was introduced into the substrate **108** and, correspondingly, into the hapten **111** for convenience of monitoring the course of cyclisation. The authors assumed also that the silyl group occupying the quasi-equatorial position favoured the fixation of the chair conformation of the substrate ring in the transition state and the formation of a cyclic carbocation, the primary product of this cyclisation, owing to the β-effect of the silicon atom.

The hapten **111** was used to raise several CA which could catalyse the solvolysis of the sulfonate **108**. One of these, CA 4C6, catalysed the cyclisation of the substrate **108** to give the alcohol **109** and was studied in more detail.[59] The solvolysis of the sulfonate **108** in a biphasic system (pH 7.0) in the presence of the CA followed Michaelis–Menten kinetics and was accelerated several thousand-fold in comparison with the non-catalysed reaction. However, the most important feature is that this reaction was strictly stereospecific in the presence of the CA. The reaction mixture contained 98% of the *trans*-cyclohexanol **109** and only 2% of the elimination product, *viz.*, the cyclohexene derivative. Other side products were absent. It should be noted that in the absence of the CA the solvolysis of the sulfonate **108** yields a complex mixture of products with less than 30% content of the alcohol **109**.

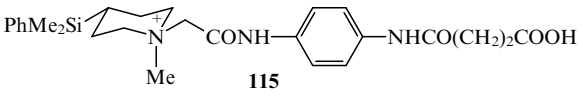
It is noteworthy that the catalytic antibody 4C6 manifests high substrate specificity. The conversions of compounds **112** and **113**, which are analogues of the sulfonate **108**, is not catalysed by this CA, whereas the sulfonate **114**, which has an additional methyl group and produces a secondary carbocation upon solvolysis instead of the primary one, yields a complex mixture of reaction products.[59]



These findings have led the authors to conclude that the CA-catalysed cyclisation reaction occurs by a concerted mechanism: the double bond in the sulfonate **108** anchimerically assists the formation of the carbocation, while the cyclic cation that formed (of the type **105**) undergoes immediately attack by the external nucleophile. Thus, the catalytic antibody 4C6 not only initiates the reaction by generating a carbocation but also promotes its further conversions (cyclisation) due to matching of the active site of the CA to the substrate. Additional evidence in favour of this

hypothesis can be derived from the fact that of all CA against the hapten **111** tested in this study only 4C6 induced the cyclisation, while other CA could merely accelerate the solvolysis of the sulfonate **108**, *i.e.*, they favour the formation of the carbocation without subsequent cyclisation (see below).

The nature and the ratio of the cyclisation products of unsaturated sulfonates of the type **108** depend also on the nature of CA, *i.e.*, on the structure of the hapten that had induced the CA. Thus it was found that the ratio of the cyclisation products of the sulfonate **108** could be fully reversed when CA 87D7 against the hapten **115**, very close to the analogue of the hapten **111**, was used instead of CA 4C6.

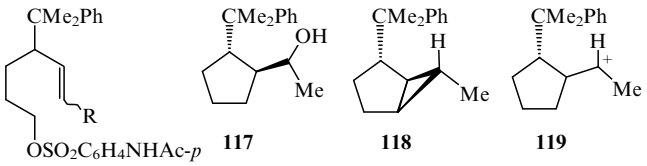


115

Compound **115** contains the N^+-Me group instead of the $N \rightarrow O$ group.[60] The cyclisation of the sulfonate **108** in the presence of this CA gives 90% of the cyclohexene derivative and only 10% of the cyclohexanol **109**. It was shown that the substrate **108** virtually adopts the same conformation in the active sites of both CA (4C6 and 87D7). Therefore, the difference in the ratios of the cyclisation products should be ascribed to the difference in the charge on the nitrogen atom rather than to the conformational effect, since the ammonium group induces a higher negative charge in the active site of CA than does the nitrogen atom of the N–O group.

Replacement of the substrate **108** by its analogue **116a** containing the CMe_2Ph group instead of the silyl group changes the direction of the cyclisation reaction catalysed by the CA 87D7. In this case, the reaction affords the corresponding cyclohexanol in 60% yield.

The direction of cyclisation of the sulfonate **116b** with the propenyl group instead of the vinyl group changes even more significantly.[61]

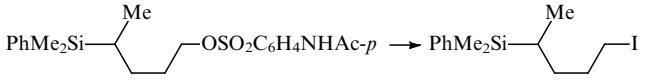


116a,b **117** **118** **119**

R = H (**116a**), Me (**116b**).

In the presence of CA 87D7, solvolysis of the *cis*-isomer of the sulfonate **116b** gives the alcohol **117** in 80% yield, whereas solvolysis of the *trans*-isomer yields the bicyclo[3.1.0]hexane derivative **118** as the main product. This unexpected result can be explained by the fact that the β-effect underlying the stabilisation of the six-membered carbocation is not manifested in the absence of the silyl group. This results in the carbocation **119**, which is further stabilised either by the attack of the external nucleophile to give the alcohol **117** or by the formation of the bicyclic compound **118** upon deprotonation.

As mentioned above, antibodies against the hapten **111** contain those which catalyse solvolysis and favour generation of the carbocation but not cyclisation. One of them, CA 16B5, was used as a convenient tool in elucidation of the mechanism of formation of carbocations in the solvolysis of arylsulfonates.[62] To this end, the conversion of the sulfonate **120** into the iodide **121** was studied under solvolysis conditions.



120 **121**

The catalytic antibody 16B5 was found to be an efficient catalyst of this reaction and accelerated it more than 500-fold. Analysis of reaction kinetics and performance of this reaction in the presence of other nucleophiles (NaSCN, NaN_3) revealed that this CA favoured the formation of the carbocationic centre in the transition state. This conclusion was also confirmed by measurements of the secondary isotope effects of α- and β-deuteriated analogues of the sulfonate **120**. Analysis of the CA-catalysed solvolysis of the deuteriated analogues revealed that substitution of the iodine for the sulfonyloxy group occurred with inversion of its configuration, which can be explained by the fact that the carbocation was generated as a tight ion pair in an equilibrium with the covalent sulfonate.

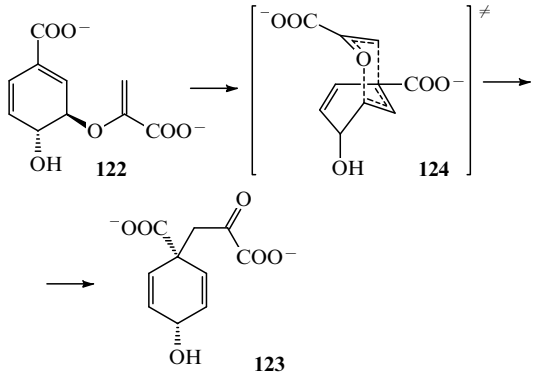
It may be concluded that the direction of the reaction of cationic cyclisation is determined by the peculiarities of both the active site of CA and the structural features of the substrate. These peculiarities affect all the stages of this reaction, such as generation of the carbocation, its stabilisation, the 'positive' charge value, possible ways of carbocation conversions and stabilisation of the secondary carbocation.

The examples of carbocationic cyclisation considered above † demonstrate high potentialities of the use of CA in directed synthesis of cyclic compounds and the possibility of their subtle modification by changing the type of the hapten and the structure of the substrate.

2. The Claisen rearrangement of enol ethers

The Claisen rearrangement of enol ethers, which consists in the cleavage of the O–C bond and the transfer of the enol residue and results in the formation of a new carbon–carbon bond, is widely used in the synthesis of complex organic structures, particularly, natural compounds. The possibility of preparing CA that would catalyse this reaction was examined [64–66] with the rearrangement of chorismic acid (**122**) into prephenic acid (**123**) as an example. This rearrangement is one key step in the biosynthesis of aromatic amino acids by bacterial or plant cells where this reaction is catalysed by the natural enzyme, *viz.*, chorismate mutase. The preparation of a CA catalysing this reaction was the first case of preparation of an artificial enzyme catalysing the formation of the C–C bond in a pericyclic reaction. The rearrangement of the acid **122** into the acid **123** is a [3.3]-sigmatropic concerted process and occurs *via* the transition state **124**, which is similar to the spatial structure of oxabicyclo[3.3.1]nonene with one six-membered ring in a boat conformation (Scheme 11).

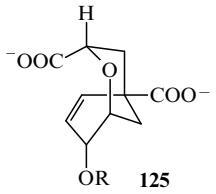
Scheme 11



The bonds corresponding to the structure of the substrate **122** in the transition state **124** are weakened, while the bonds of the future rearrangement product have just been outlined. The hapten derivative of the oxabicyclononene **125** containing a spacer (R) with the active succinimide group needed for its immobilisation on the protein was used to obtain CA the active site of which must promote the generation of a transition state with this spatial

† A recent report describes an attempt to obtain a CA catalysing carbanionic cyclisation, one of the versions of the Dieckman reaction.[63] Although the catalytic effect was observed in this case, data on the preparation of CA and its characteristics were absent.

structure. It is of note that the dicarboxylic acid **125** (R = H) is an inhibitor of the natural enzyme (chorismate mutase).



125

The resulting CA accelerated the conversion of chorismic acid (**122**) into prephenic acid (**123**) $2 \times 10^2 - 10^4$-fold in comparison with the non-catalysed rearrangement (data from two independent laboratories). This reaction proceeded with high regiospecificity at 10 °C and pH 7.0 and gave prephenic acid alone. (About 5% of hydroxybenzoic acid was formed in the absence of CA). The stereospecificity of this reaction is also very high. Experiments with (+)- and (−)-isomers of chorismic acid revealed that only the (−)-isomer was a substrate for the reaction. Only 50% of the substrate was consumed in the reaction with racemate. The kinetic measurements establish the enantioselectivity of this reaction in the presence of CA as 90 : 1.

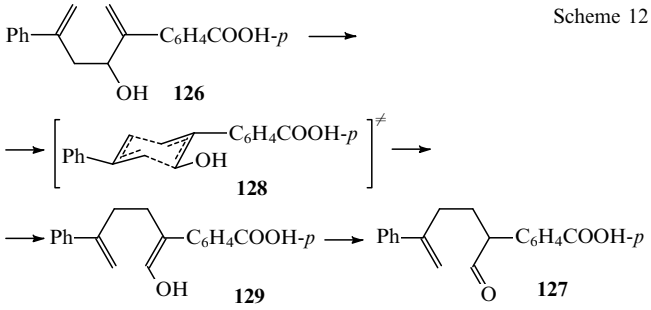
X-Ray structural analysis of a complex of the hapten **125** with the CA [67] confirmed the complementarity of the spatial structure of the active site of CA to the structure of the hapten and, correspondingly, to the structure of the transition state **124**. The lack of pH dependence of the Claisen rearrangement in the presence of CA and the absence of ionic groups in the active site testify to the fact that this rearrangement in the presence of the CA represents a pericyclic reaction. The role of the active site amino acid residues has been established in this reaction and the catalytic role of CA in this process has been suggested.[68] Although the effectiveness of the CA studied is inferior to that of the natural enzyme, the preparation and detailed analysis of this first CA catalysing a pericyclic reaction was a considerable breakthrough en route to artificial enzymes.

3. The Cope rearrangement

The Cope rearrangement is also related to pericyclic reactions and occurs as a [3.3]-sigmatropic rearrangement. No natural enzyme for this reaction has been found thus far.

The possibility to obtain CA catalysing this reaction was studied in the example of conversion of a diphenylhexadienol derivative **126** into the unsaturated aldehyde **127** (Scheme 12).[69]

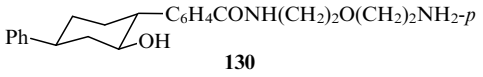
Scheme 12



126 **128** **129** **127**

It is suggested that this rearrangement occurs *via* the transition state **128** in which the central six-membered ring has a chair conformation. The enol **129**, which is formed first, is further converted into the aldehyde **127** fixed as an oxime.

With consideration of the accepted reaction mechanism, the diphenylcyclohexanol derivative **130** with a spacer group was used as a hapten. The specific role of this hapten consisted in the formation of the active site of CA and fixation there of the substrate in a conformation most closely matching the transition state. This active site would thus act as an 'entropic trap' (see Section II). The absence of a natural enzyme catalysing the Cope rearrangement made the preparation of this catalytic antibody especially attractive because it provided rationale for the general principles of design of haptens for the CA catalysing pericyclic reactions.



130

The most reactive catalytic antibody (AZ-28) raised against the hapten **130** accelerated this rearrangement 5300-fold. Of the two enantiomers of the original alcohol **130**, only the *S*-isomer and not the *R*-isomer entered into the reaction. The kinetic and inhibitory analyses of all the CA catalysing this reaction confirmed that they do generate the transition state **128** in the reaction.

The Fab fragment isolated from the CA AZ-28 was prepared in considerable quantity by genetic engineering methods.[70] This important experiment illustrates the possibility to use the catalytic antibodies generated in small quantities under laboratory conditions for large-scale productions using the most advanced biotechnological techniques.

An attempt has been made to establish the mechanism of the catalytic action of the CA AZ-28 and its spatial structure by molecular genetics methods and X-ray analysis.

4. The Diels – Alder reaction

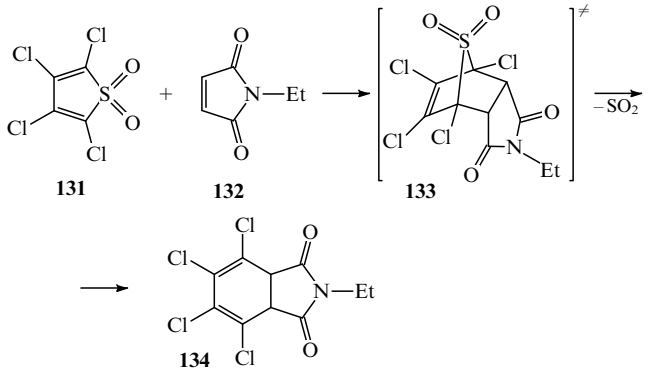
The Diels – Alder reaction is the basis for one of the main synthetic procedures used to obtain six-membered alicyclic systems by condensation of a diene with an alkene (dienophile). It represents a $4\pi + 2\pi$-cycloaddition reaction. The cleavage of particular bonds in the reaction components and the formation of other bonds occur as a single concerted process and conform to the regularities of pericyclic reactions. The success in the condensation depends on the mutual arrangement of the diene and the dienophile, which must be in the appropriate conformation in the transition state enabling the pericyclic process.

No natural enzyme has been found as a catalyst of the Diels – Alder reaction; therefore the preparation of an effective CA is of fundamental interest. Since the Diels – Alder condensation often occurs as a spontaneous exothermic process, the main function of the CA would be to alter its regio- and stereoselectivity rather than to increase the reaction rate.

The catalytic effect of the active site of the CA is reduced to the restriction of mobility of the diene and the dienophile and their fixation in the necessary conformation. Thus, the active site of the CA should primarily act as an 'entropic trap' (see Section II), while the presence of reactive groups in this site is less significant. In the design of a hapten for generation of the CA, one had to keep in mind that the CA prepared must not be inhibited by the reaction product. Otherwise, this would reduce the enzyme 'turnover number' and its effectiveness. Therefore, the spatial structure of the active site of the CA must be close to that of the transition state but differ, wherever possible, from the structure of the reaction product.

Several CA catalysing the Diels – Alder reaction have been obtained (Scheme 13).

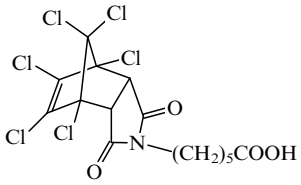
Scheme 13



131 **132** **133** **134**

The first of these CA catalysed the condensation of tetrachlorothiophene dioxide **131** with *N*-ethylmaleimide **132** which occurs *via* intermediate formation of the tricyclic sulfone **133**. Spontaneous elimination of SO_2 results in the dihydrophthalimide derivative **134** (Scheme 13).[71]

The tricyclic derivative **135** structurally analogous to the intermediate product **133** and containing a hexanoic acid residue essential for immobilisation was used as a hapten. At the same time, the structure of the hapten **135** differs from that of the final product, *viz*., the imide **134**.

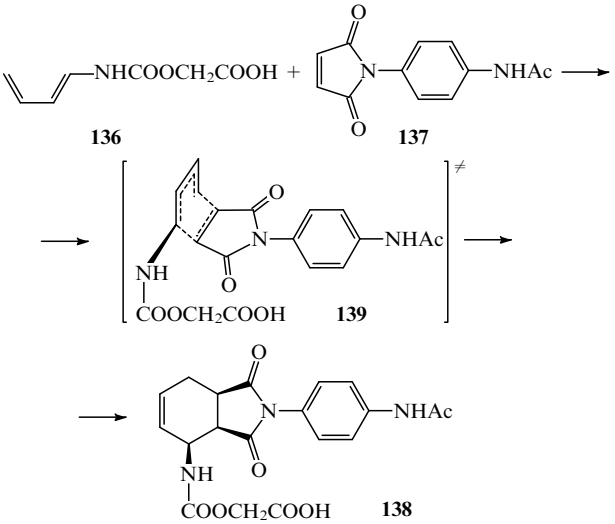


135

The reaction between the reactive sulfonyl group in the molecule **131** with the amino groups of lysine in CA might irreversibly change its function. To avoid this, the amino groups of lysine were methylated by reaction first with CH_2O followed by reduction with $NaBH_3CN$. The methylated CA thus obtained was found to be sufficiently active and accelerated the Diels–Alder condensation of the sulfone **131** with the imide **132** manyfold. Its effect was substrate-specific. No catalytic effect of CA was manifested when maleimide was used instead of *N*-ethylmaleimide.

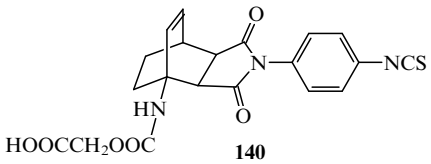
Preparation of a CA catalysing the condensation of the acyclic diene **136** with the maleimide derivative **137** is of certain interest. This reaction yields a derivative of *cis*-tetrahydrophthalimide **138**[72] and proceeds *via* the transition state **139** in which the diene acquires a cisoid conformation, while its complex with the dienophile **137** adopts a boat conformation. This favours the pericyclic reaction and ensures *cis*-fusion of the rings in the reaction product **138** (Scheme 14).

Scheme 14



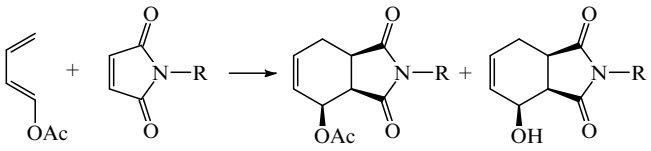
136 **137** **139** **138**

The hapten **140** containing a cyclohexane ring fixed in a boat conformation by an ethylene bridge and the isothiocyanate group necessary for its immobilisation was chosen for raising CA with the active site mimicking this transition state. The most active CA accelerated the condensation of the diene **136** with the imide **137** approximately 500-fold and followed Michaelis–Menten kinetics. The reaction product **138** was found to bind with the CA ~ 100 times weaker than the original compounds **136** and **137**. Thus, the CA is practically uninhibited by the reaction product.



140

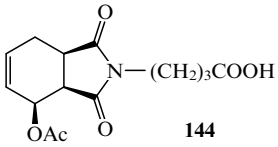
Preparation of a CA catalysing a similar reaction, *viz*., the condensation of 1-acetoxybutadiene **141** with *N*-methyl- and *N*-benzylmaleimides **142** to give the *N*-substituted tetrahydrophthalimides **143**, is documented.[73,74]



141 **142** **143** **143a**

R = Me, Bn.

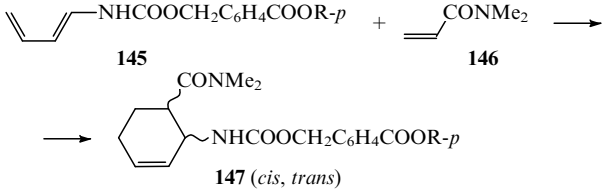
It was shown[73,74] that the CA raised against the hapten **144** accelerated the condensation of the diene **141** with the dienophile **142** more than 1000-fold. It manifested substrate specificity, since it was inactive when more reactive methoxybutadiene or piperylene were used instead of the diene **141**.



144

It was found also that the activity of this CA depended on pH, which was never observed with other CA catalysing the Diels–Alder reaction. This was rationalised as being due to the fact that this CA also manifested esterase activity and is a unique example of CA possessing dual activity. However, this explanation does not seem to be convincing enough, although the side product of this reaction, *viz*., alcohol **143a**, has been isolated.

The problem of stereospecificity of the Diels–Alder reaction is the key problem. It was specially studied in the condensation of two acyclic compounds, *viz*., *trans*-1-*N*-(alkoxycarbonyl)aminobutadiene **145** and *N,N*-dimethylacrylamide **146**, resulting in the cyclohexene derivative **147**.[75]

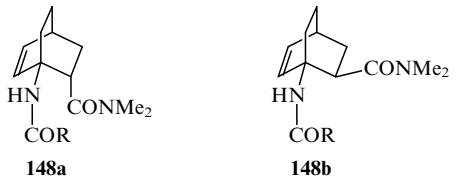


145 **146** **147** (*cis*, *trans*)

This reaction may, in principle, yield four compounds, *viz*., two 1,2-isomers with *cis*(*endo*)- and *trans*(*exo*)-configurations and two 1,3-isomers with *cis*-(*endo*) and *trans*-(*exo*) configurations. In the absence of CA, the condensation of compounds **145** and **146** yields only the 1,2-isomers in the *cis* : *trans* ratio 66 : 34 (thermal condensation) or 85 : 15 (condensation in a phosphate buffer). Thus, this reaction is not stereospecific. Analysis of molecular models and calculation of the transition states leading to the formation of each of these isomers ‡ revealed that the energy of the transition state for the 1,2 isomers was 2–4 kcal mol^{-1} lower than that for the 1,3-isomers. It is noteworthy that the energy barrier for the 1,2-isomer having the *cis*-configuration is lower than for the *trans*-isomer, *i.e*., the formation of the *trans*-isomer (an 'unfavourable' reaction) is energetically less favourable.

‡ The calculation was performed with the use of a simplified model of the diene component of the reaction.

Catalytic antibodies able to selectively direct the condensation of compounds **145** and **146** towards the formation of either *cis*- or *trans*-isomers have been obtained. The haptens **148a** and **148b** with a rigidly fixed ethane bridge where the $CONMe_2$ and NHCOR groups corresponding to the groups of the reactants ('recognition groups') are in the *cis*- or *trans*-positions relative to each other were used to mimic the transition states.



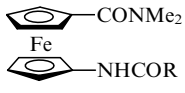
148a **148b**

R = $(CH_2)_3COONC(O)CH_2CH_2C(O)$.

Two groups of CA were raised against these haptens. One CA, each from both groups, were selected that catalysed the condensation of compounds **145** and **146** in an aqueous medium at pH 7.0 to give the *cis*- (CA 7D4, *endo*-'Diels-Alder'ase') and *trans*-isomers (CA 22C8, *exo*-'Diels-Alder'ase'). The effectiveness of these CA was not very high: they accelerated the reaction 960- (CA 22C8) and 740-fold (CA 7D4) in comparison with the non-catalysed reaction. It is noteworthy that the *exo*-CA catalysed the 'unfavourable' reaction of the *trans*-isomer formation more efficiently than the *endo*-CA. Both CA ensured complete stereoselectivity of the reaction: the isomer **147** was formed in the presence of the CA 7D4, whereas the *trans*-isomer **147** was formed in the presence of the CA 28C8 with the stereoselectivity exceeding 98%. Thus, artificial enzymes (*endo*- and *exo*-'Diels-Alder'ase') manifesting high stereoselectivity have been obtained.

A very interesting and a basically new approach to the preparation of stereospecific CA for the Diels–Alder reaction has been recently demonstrated.[76] In contrast to the above-described, traditional approach based on the use of conformationally rigid haptens, it was suggested to use conformationally flexible haptens. The authors proceeded from the opposite idea that the immune apparatus can produce antibodies against individual conformers of a conformationally flexible hapten including those the 'frozen' conformation of which would correspond to the transition state observed in the formation of certain stereoisomers. Thus, biosynthesis can yield a set of antibodies from which CA with the required specificity can be selected.

This idea was examined in the example of condensation of compounds **145** and **146**. The ferrocene derivatives **149a**,**b** were used as haptens to mimic the transition state of the reaction. They contained the substrate 'recognition groups' one of which served as a spacer. This is the first example of the use of organometallic compounds for such a purpose.



149a: R = $(CH_2)_3COOH$;
149b: R = C_6H_4COOH-*p*.

149a,**b**

It was taken into account that the π-delocalised system and two planar cyclopentadiene rings of ferrocene at a distance 3.3 Å rotating freely around the common axis can form a cavity in the active site of a CA the size and spatial configuration of which would favour the approach and the necessary mutual arrangement of the diene and the dienophile. The rotation of five-membered rings gives the desired steric combinations of the hapten which mimic various transition states that appear in the formation of the stereoisomeric reaction products.

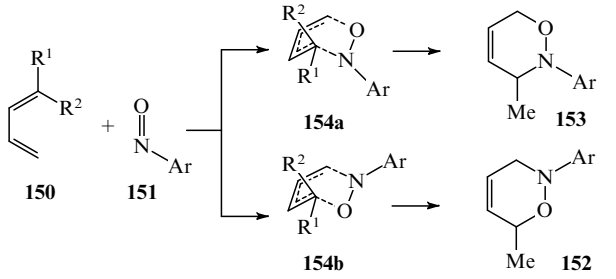
Two most active and stereospecific CA, *viz*., *endo*-CA (4D5) and *exo*-CA (13G5), were selected from a large group of antibodies raised against compounds **149a** and **149b**. Examination of these CA revealed that they accelerated the condensation only insignificantly and were inferior to the CA raised against 'rigid' haptens. However, the specificity of reactions was very high: the condensation of the substrates **145** and **146** catalysed by these CA yielded only the 1,2-isomers: *endo*-CA (4D5) gave the *cis*-isomer **147**, while *exo*-CA (13G5) gave the *trans*-isomer **147** with high diastereoselectivity (98% *de*) and enantioselectivity (95% *ee*). Thus, the concept on the possible use of conformationally flexible haptens that correspond in the geometric and electronic parameters to the transition state of the Diels–Alder reaction for the preparation of stereospecific CA has been fully confirmed.

Comparative characteristics of CA raised against 'rigid' haptens (*e.g*., bicyclo[2.2.2]octenes **148**) and conformationally flexible haptens **149** suggest that the 'antiferrocene' CA have a bulkier cavity in their active site, since the ferrocene molecule is larger than the bicyclo[2.2.2]octene molecule, and can react therefore with the 'rigid' haptens, whereas CA raised against 'rigid' haptens does not react with the conformationally flexible haptens.

Although the 'antiferrocene' CA are not yet practically valuable, this result is very essential for it offers a fundamentally new approach to the preparation of CA endowed with stereospecific activity through selection of CA from the group of antibodies raised against a conformationally flexible hapten.

In addition to the CA that catalyse the classical Diels–Alder reaction, the first CA catalysing the hetero-Diels–Alder condensation of *cis*-piperylene **150** with 4-nitrosobenzamide derivative **151** to give isomeric dihydro-1,2-oxazines **152** and **153** has been obtained (Scheme 15).[77]

Scheme 15



150 **151** **154a** **154b** **153** **152**

R^1 = H, R^2 = Me; Ar = $C_6H_4CONHPr$.

This CA was raised against the 'rigid' hapten **155** which mimics the hypothetical transition state **154** with a boat conformation.



155

This catalytic antibody accelerates the condensation of *cis*-piperylene **150** (R^1 = Me, R^2 = H) 2600-fold; however, its effectiveness in condensation of *trans*-piperylene (**150**) (R^1 = H, R^2 = Me) is much lower.[78] The regioselectivity of this reaction in the presence of the CA is also enhanced: the non-catalysed reaction of the diene **150** with an excess of the nitroso compound **151** gives the oxazines **152** and **153** in the ratio 48 : 52. In the presence of 10% CA, this ratio alters to 32 : 68, whereas in the presence of an equimolar amount of CA, 95% of the isomer **153** with high enantioselectivity (82% *ee*) is formed. Apparently, this results from inhibition of the sluggish parallel non-catalysed reaction. The action of the CA is fully inhibited by the hapten analogue **155** in which the n-propyl group is used instead of the $(CH_2)_5COOH$ group. This proves that the catalytic reaction occurs in the cavity of the active site of CA.

5. Aldol condensation

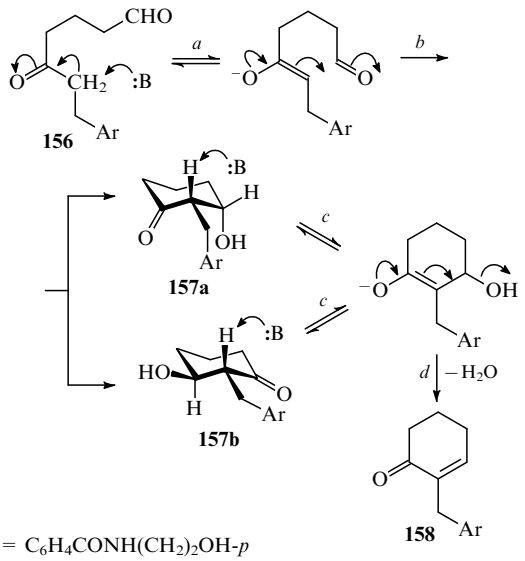
Aldol condensation is one of the most well-studied reactions. It occurs under conditions of acid–base catalysis and results in the formation of a new C–C-bond and a β-hydroxycarbonyl (aldol) system. This reaction occupies a prominent position among the

processes occurring in living cells. Despite numerous multiple modifications and versions allowing for this reaction to be carried out efficiently and in a highly regio- and stereoselective way by purely chemical methods, interest in specific CA with enabling enhanced selectivity is never lost. A great number of natural enzymes (aldolases) catalysing this reaction are presently known; however, all of them manifest relatively narrow specificity which prevents their practical use. The preparation of CA manifesting broader and/or 'predetermined' specificity might be very useful in organic synthesis.

Several approaches are currently used to obtain CA catalysing aldol condensation. One of them is based on general chemical concepts on the mechanism of formation of the β-hydroxycarbonyl system in the condensation of two carbonyl compounds under conditions of acid–base catalysis. Others reproduce, to a certain extent, the catalytic process catalysed by natural enzymes, *viz*., aldolases.

The first approach can be exemplified by the preparation of a CA catalysing intramolecular cyclisation of the oxo aldehyde **156**, which occurs *via* the formation of two stereoisomeric cyclic aldols **157a** and **157b**. The latter are at equilibrium, and elimination of the water molecule eventually results in the cyclohexenone derivative **158** (Scheme 16).[79]

Scheme 16



Ar = $C_6H_4CONH(CH_2)_2OH$-*p*

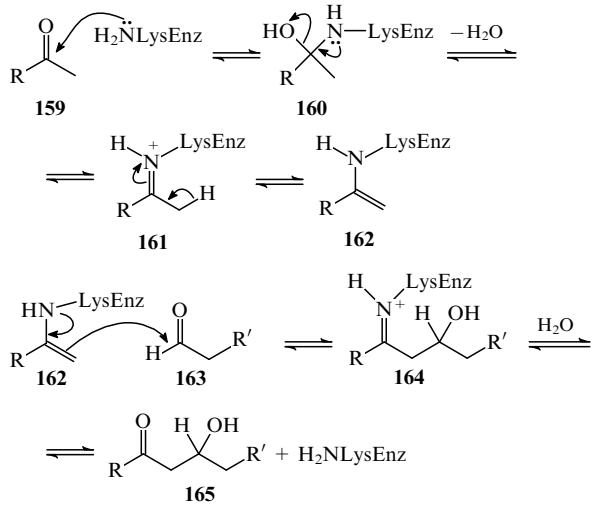
Preparation of a CA catalysing this reaction seems to be a more complicated task than those described above, because this concerns a multistep process. The overall acceleration of the cyclisation of the oxo aldehyde **156** into the enone **158** requires a catalyst which stabilises several transition states by lowering the energetic barrier on the common coordinate of this reaction.

This problem has been solved successfully owing to the fact that all the steps of this reaction occurred under the conditions of acid–base catalysis and the intermediate products (as well as the corresponding transition states) were similar as regards their spatial and electronic structures. This made it possible to raise CA using a single hapten **77** responsible for the formation of the active site of the CA, which exerted a catalytic effect at all the steps of this reaction. The hapten **77** is structurally similar to the cyclic aldols **157a** and **157b** and the final product **158**. The positive charge on the nitrogen atom in the hapten **77** generates a carboxy group in the complementary position of the active site of the CA and thus provides for acid–base catalysis in enolisation (Scheme 16, stage *a*) and elimination (Scheme 16, stages *c* and *d*).

Among other antibodies against the hapten **77**, two CA were catalytically active in the cyclisation of the oxo aldehyde **156**. This reaction followed Michaelis–Menten kinetics and was inhibited by one of the hapten analogues, which testified to its occurrence in the active site of the CA. One of these CA (78H6) was studied in more detail. It was found that general base catalysis takes place and the CA 78H6 is its very potent catalyst. It is of note that the dehydration stages (*c* and *d*) represent energetically less favourable ('adverse') *trans*-elimination reactions in the intermediate aldol **157a** and are regio- and stereospecific. Kinetic studies showed that this reaction was accelerated more than 10^5-fold in aldolisation (stages *a* and *b*) and more than 10^4-fold in elimination (stages *c* and *d*) as compared to the reaction carried out under ordinary conditions, *viz*., in the presence of acetate. This example illustrates the possibility to obtain a unified CA that catalyses a multistage intramolecular process in those cases where the catalytic mechanism and the intermediate products (and, correspondingly, transition states) formed at all stages are sufficiently close.

Another approach to the preparation of a CA the mechanism of action of which is similar to that of natural aldolases seems to be more interesting and versatile.[80] According to the present concepts of the mechanism of the catalytic action of aldolases, this may be represented by Scheme 17.

Scheme 17

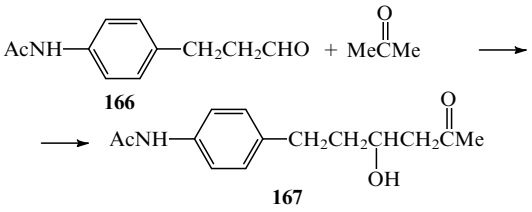


The ε-amino group of lysine in the enzyme molecule reacts with the carbonyl group of the aldol donor **159** to yield the hemiaminal **160**, which is further converted into the enamine **162** *via* the immonium ion **161**. It is the enamine **162** that is the genuine aldol donor (the nucleophile) and the key intermediate of the overall reaction. The enamine **162** attacks the aldol acceptor **163** to form a new C−C bond and the immonium ion **164**. Its subsequent hydrolysis gives the aldol **165** and results in the regeneration of the amino group of lysine in the aldolase. Since all the reactions in this chain are reversible, aldolase can also catalyse the reverse reaction, *i.e*., retroaldol degradation.

The active site of a CA catalysing this complex chain of reversible reactions must be able to lower the energy barriers in all the stages of this process. It is difficult to imagine the generation of such a CA in a traditional way where a stable and nonreactive hapten does not virtually react with the antibody raised against itself but, rather, merely mimics the transition state.

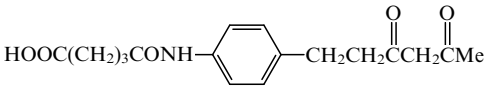
A fundamentally new approach, which is named 'reactive immunisation', has been proposed. In this case, the hapten reacts with the antibody to form a covalent bond. The active site of the CA that formed acquires the ability to 'perceive' and favour these conversions. Such a hapten not only favours the formation of the transition state of the CA but virtually mimics the reaction itself. Here, structural elements favouring the whole chain of conversions involved in this multistage process are generated in the active site of the CA.

Condensation of 3-(4-acetamidophenyl)propionaldehyde (**166**) with acetone resulting in 4-hydroxy-6-(4-acetamidophenyl)-hexanone (**167**) was used as a model reaction aimed at obtaining CA of the aldolase type.[80,81]



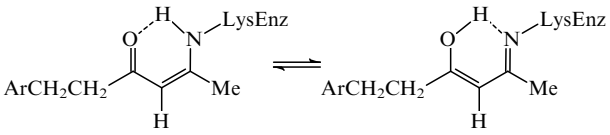
166 **167**

A β-diketone derivative **168**, which is structurally similar to compounds **166** and **167** and contains the substrate 'recognition groups' and a spacer, [$CO(CH_2)_3COOH$], was used as a hapten.



168

The hapten **168** induced the formation of structural elements that favoured the appearance of a lysine residue in the active site of the CA and the interaction of the NH_2 group of this lysine residue with the substrate to give the key intermediate **169** with an enamine structure.



169a **169b**

Ar = $C_6H_4NHCO(CH_2)_3COOH$-*p*

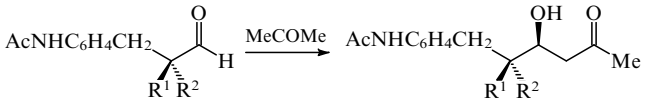
The stabilisation of the intermediate **169** was achieved owing to the equilibrium **169a** ⇌ **169b** and the formation of a conjugated system, *viz*., carboximide vinilogue.

In addition, this hapten favoured the necessary mutual arrangement of the aldehyde **166** and acetone and thus lowered the entropic barrier to the bimolecular reaction. Thus, owing to its conversions in the active site of the CA that is formed, the hapten **168** generated all the elements needed for this reaction to occur. The formation of the enamine, the main driving force of aldol condensation, is crucial for this reaction. It is the formation of the enamine structure of the type **169** that indicated that the reaction of the antigen with the antibody generated against it did occur. This circumstance was taken into consideration in primary monitoring of reactive antibodies by chemical methods [instead of commonly employed monitoring by immunoenzymic methods (see p. 1001)].

The antibodies against the hapten **168** were studied by spectral analysis as complexes with the hapten. It was assumed that all the complexes having absorption bands at 316 nm characteristic of the carboximide vinylogue **169b** and, correspondingly, capable of forming enamines possessed catalytic properties. The two CA (38C2 and 33F12) thus selected did manifest high activity and catalysed the aldol condensation of the aldehyde **166** with acetone. This catalytic reaction followed Michaelis–Menten kinetics and was inhibited by the hapten analogue or acetylacetone. These and some other data suggest that the CA in fact functions as a natural aldolase. Kinetic measurements showed that these CA accelerate the reaction 10^9-fold; their activities were close to that of the natural enzyme.

It is essential that both CA manifest broader substrate specificity than the natural aldolase, which catalyses an analogous conversion and thus demonstrates the benefits of the artificial enzyme. Thus the CA 38C2 catalyses the condensation of **166** not only with acetone but also with other ketones (fluoro- and chloroacetone, butan-2-one, pentan-3-one, pentan-2-one, dihydroxyacetone), although its efficiency is lower in this case. The condensation with asymmetrical butan-2-one and pentan-2-one was regioselective, *viz*., the reaction occurred predominantly at the CH_2 group of the ketone to give branched aldols. The catalytic effect of the CA in the reaction of the aldehyde **166** was manifested only towards ketones, the reaction with acetaldehyde was not accelerated. However, the CA 38C2 catalysed reactions of acetone with branched analogues of the aldehyde **166** (*e.g*., with 3-aryl-2-methylpropionaldehyde derivative **170**) with even higher efficiency.

The action of the CA was highly stereoselective. Thus the addition of acetone to the stereoisomeric aldehydes **170** occurred with high diastereoselectivity: the *S*-isomer yielded the 4*S*,5*S*-isomer of the aldol (**171a**) (*de* > 95%), while the *R*-isomer gave the 4*S*,5*R*-aldol **171b** (*de* > 83%).



170a,b **171a,b**

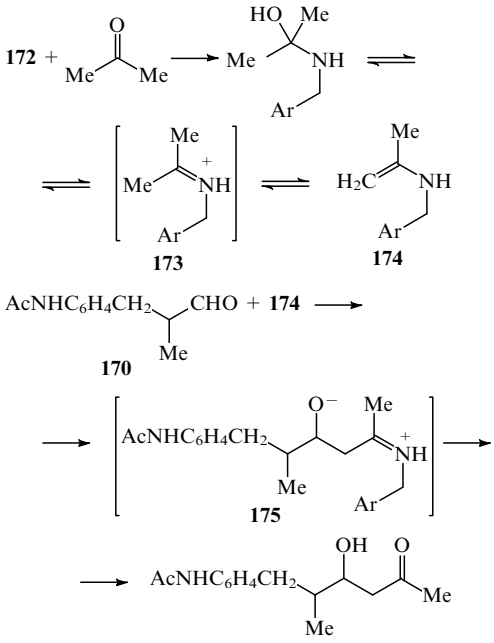
a: R^1 = Me, R^2 = H; **b**: R^1 = H, R^2 = Me.

It is noteworthy that in both cases the addition of the acetone molecule occurs from the *re*-side irrespective of the configuration of the chiral centre in the aldehyde **170**. The rate of this addition is the same for the both isomers **170**. Therefore it cannot be used for kinetic resolution of the racemate of the aldehyde **170**.

Yet another possibility of catalysis of aldol condensation with CA has been studied.[82] Since the catalytic effect of aldolases is based on the acceleration of the reaction of the carbonyl group of an aldol donor with the amino group of the lysine residue with subsequent formation of a nucleophilic enamine, it was decided to carry out aldol condensation in the presence of a CA against the hapten of the type **77** using an amine with the appropriate pK_a as a cofactor. This amine would react with the CA in its active site to yield a 'modified' CA the mechanism of action of which would be then typical of that of the natural aldolase.

This possibility has been examined in the condensation of 3-aryl-2-methylpropionaldehyde **170** with acetone in the presence of a CA against the hapten **77** and a benzylamine derivative *p*-$H_2NCH_2C_6H_4CONH(CH_2)_2OH$ **172** as a cofactor. This amine is structurally similar to the hapten **77** and has the corresponding value of pK_a (8.5). It may therefore be assumed that it will react with CA against this hapten and modify it in the appropriate way; the reaction of aldol condensation will occur *via* the formation of the immonium ion **173**, the enamine **174** and the product of its condensation with the aldol acceptor **175** (Scheme 18).

Scheme 18



173 **174** **170** **175**

Ar = $C_6H_4CONH(CH_2)_2OH$-*p*

Indeed, the condensation of the aldehyde **170** with acetone in the presence of the CA 72D4 and a cofactor (amine **172**) at

pH 7–9 occurs with high efficiency and follows Michaelis–Menten kinetics with respect to each component. Evidence was obtained that this reaction involves the formation of the intermediates **173** and **175**. The use of the tertiary amine as a cofactor does not result in a catalytic effect.

This reaction is stereoselective: the racemate of the aldehyde **170** gives *S*,*S*- and *R*,*S*-diastereomers of the aldol **171** with *de* 95% and 65%, respectively in 1 : 2.8 ratio.§

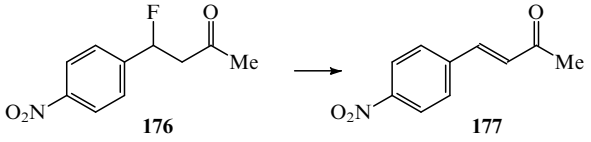
The aforementioned examples of the preparation of artificial aldolases possessing high stereoselectivity and a broader specificity than natural enzymes illustrate one of the most remarkable achievement in the design of a new generation of biocatalysts.

VII. 1,2-Elimination reactions (β-elimination)

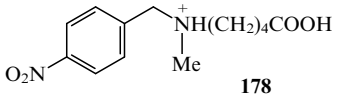
Reactions of 1,2-elimination (dehydrohalogenation, dehydration, *etc*.) underlie many synthetic procedures that are used for introduction of multiple bonds into the carbon skeleton of a molecule. Catalytic antibodies which catalyse these conversions are mostly of interest because they can alter the stereochemical result of some reactions resulting in alkene isomers that are inaccessible in energetically forbidden reactions of non-catalysed elimination. Most frequently, elimination of hydrogen halide, water and other small molecules from the vicinal carbon atoms with the formation of a multiple bond occurs as a concerted process.

Several examples of preparation of CA catalysing 1,2-elimination are presently known. The action of these CA is based on promotion of the reaction by introduction, into the active site of CA, of a group favouring removal of either a proton or a substituent (*e.g*., the OH group in the dehydration), thereby initiating a concerted elimination process.

The preparation of a CA catalysing elimination of HF from the fluoroketone **176** to give the benzylideneacetone derivative **177** is an example.[84, 85]



The design of a hapten aimed at generation of the active site of CA was based on an assumption that facilitation of α-deprotonation would accelerate the reaction. Therefore, compound **178** was selected as a hapten, which contained the groups similar to those in the ketone **176**. The CH_2 group in this ketone was replaced by the ammonium group. It was assumed that the latter would generate a group (presumably, a carboxy group) in the complementary position of the active site of CA which would facilitate deprotonation of the CH_2 group of the substrate **176**.

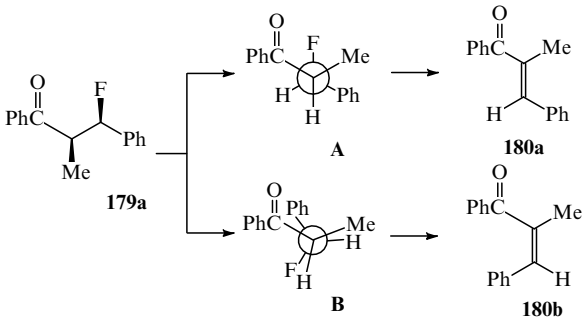


Indeed, compound **178** immobilised on the protein through a $(CH_2)_4COOH$ spacer group generated a CA that accelerated the elimination of HF from the fluoro ketone **176** 10^4-fold. This reaction followed Michaelis–Menten kinetics and was inhibited by the hapten and its analogues. Modification of the CA with chemical reagents and the character of the pH dependence of the CA activity confirmed the presence of a carboxy group (in the form of aspartate or glutamate) in the active site of the CA. This was confirmed by isolation of the corresponding peptide upon hydrolysis with trypsin of a CA modified with diazoacetamide. The effect of CA was substrate-specific, *viz*., the elimination from the *meta*-isomer of the ketone **176** was activated less strongly than from the *para*-isomer. It was found that the racemate of the substrate **176** yielded a mixture of *cis*- and *trans*-isomers of the unsaturated ketone **177**.

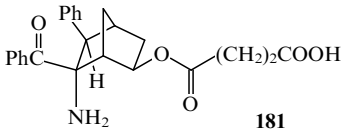
The stereochemical outcome of the elimination reaction is of particular interest. The reactions of all four diastereomers of the ketone **176** containing one deuterium atom in the CH_2 group were studied. Because of free rotation around the $CHF-CH_2$ bond in the molecule of the ketone **176**, the stereochemistry of β-elimination could be judged from the ratio and the structure of the *cis*- and *trans*-isomers of the unsaturated ketone **177** formed. The experimental results suggest that β-elimination takes place when the fluoro ketone molecule **176** is in the staggered conformation, *i.e*., when the F and H (or, correspondingly, D) atoms are in the *anti*-periplanar position (*anti*-elimination). It thus follows that the active site of the CA favours *anti*-elimination without discriminating considerably between the *R*- and *S*-isomers of the substrate. This suggests that the carboxy group in the active site of the CA is conformationally flexible and can approach that particular proton the splitting of which is most favourable for the elimination from the *anti*-periplanar position.

In this context, the preparation of CA catalysing elimination of two 'eclipsed' atoms (or groups), *i.e*., promoting '*syn*-elimination', is of special interest.[86] In the absence of a catalyst, this reaction is impeded, since the 'eclipsed' conformation is energetically most unfavourable because of the strong steric and/or electronic interaction of the neighbouring groups. Thus *syn*-elimination leading to a *cis*-alkene is an example of the so-called 'unfavourable' reaction and can hardly be realised by purely chemical methods. In order to perform this energetically unfavourable (or even forbidden) reaction, the active site of the CA must fix the substrate molecule in an unfavourable, 'eclipsed' conformation. Then the group facilitating deprotonation (*e.g*., the carboxy group) present in the active site of the CA must be fixed in a position close to the proton to be eliminated from the substrate.

This idea has been successfully materialised in the preparation of a CA catalysing *syn*-elimination of HF from the ketone **179a** to yield the *cis*-isomer. In the absence of CA, this reaction cannot be performed, since the molecule **179a** adopts the staggered conformation **A**, as a result of which the H and F atoms in the *anti*-periplanar position are eliminated to give the *trans*-alkene **180a**.



A CA raised against the hapten **181** with a rigid bicycloheptane skeleton was used to direct the reaction towards the formation of the *cis*-alkene **180b**.



The hapten **181** forms such a cavity in the active site of the CA, where the PhCO and Ph groups of **179a** are fixed in an 'eclipsed' position relative to the central C–C bond (the conformation **B**). This determines their *cis*-position in the elimination product.

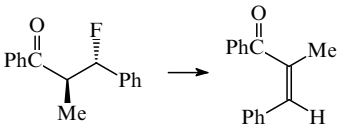
The position of the NH_2 group in the hapten **181** corresponds to the eclipsed position of the proton in the molecule **179a** relative to the fluorine atom that is eliminated. The NH_2 group, which is

§ This reaction is reversible; the aldol formed can undergo retroaldol degradation or be converted into an unsaturated ketone. For the effect of spatial structure of the aldol on its further conversions, see Ref. 83.

converted into the NH_3^+ group in the CA biosynthesis, generates a carboxy group ensuring the catalysis in a strictly specific position of the active site of the CA.

The catalytic antibody against the hapten **181** catalyses the elimination of HF from the ketone **179a** to give only the *cis*-alkene **180b**, *i.e.*, ensures '*syn*-elimination' with high stereospecificity and fully changes the stereochemical result of this reaction in comparison with the non-catalysed process. The catalytic reaction follows Michaelis – Menten kinetics and is inhibited by the hapten **181** and its structural analogues which points to its occurrence in the active site of the CA. Thus, conditions exist which compensate for the consumption of additional energy (~ 5 kcal mol^{-1}) needed for the 'unfavourable' *syn*-elimination reaction to take place.

It is of note that this CA catalyses the conversion of the ketone **179a** into the *cis*-alkene **180b** more effectively than the conversion of the diastereomeric ketone **179b**.



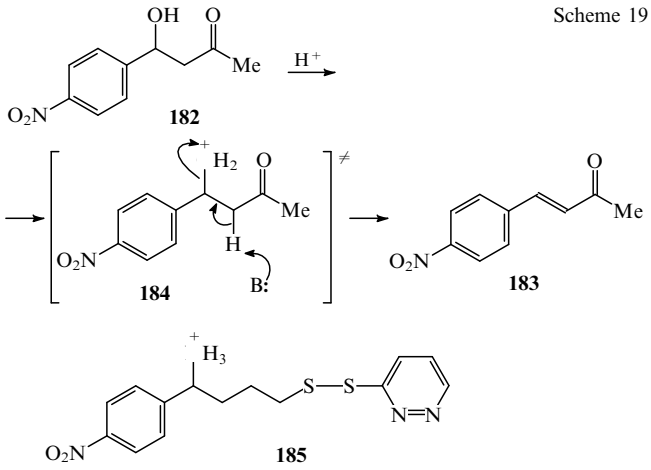
179b **180b**

The conversion **179b** → **180b** occurs as the *anti*-elimination, which suggests that the active site of the CA binds specifically with the conformer where the PhCO and Ph groups (and, correspondingly, the H and F atoms) are 'eclipsed' and is less suitable for the fixation of the conformers with the *anti*-periplanar position of these substituents.

The reaction of 1,2-dehydration is yet another type of β-elimination. One plausible mechanism of dehydration of hydroxy ketones involves protonation of the hydroxy group, the formation of an oxonium intermediate in the transition state and subsequent elimination of the substituent as the water molecule, which is energetically more favourable.

The possibility to obtain a CA catalysing this reaction was studied using the dehydration of the β-hydroxy ketone **182** into the unsaturated ketone **183** as an example (Scheme 19).[87]

Scheme 19

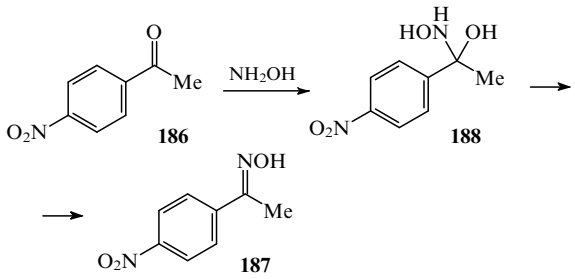


182 **184** **183** **185**

The hapten **185** used to obtain such a CA contained the ammonium group instead of the hydroxy group in the ketone **182**. The ammonium group would induce the formation, in the complementary position of the active site, of a group responsible for the protonation of the hydroxy group in the molecule **182** and stabilisation of the transition state **184**. The disulfide group was used in a spacer to provide binding of the hapten **185** with the protein.

This CA accelerated the elimination more than 1000-fold. It is specific to the substrate structure, since it does not catalyse the dehydration of the *meta*-isomer of the ketone **182**. The nature of the group in the active site of CA that stabilises the oxonium centre in the transition state remains obscure.

It is of note that the same CA catalyses stereoselective transformation of 4-nitroacetophenone **186** into the oxime **187**, which occurs as a consecutive addition and dehydration.[88]

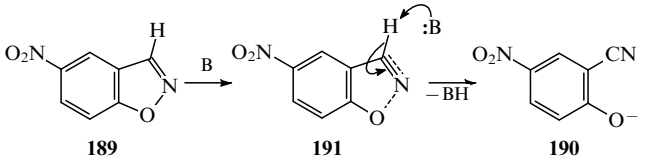


186 **188** **187**

The dehydration of the intermediate **188** in the absence of the CA is not stereochemically unambiguous and yields a mixture of *syn*- and *anti*-oximes **187** in a 3 : 1 ratio. The reaction conducted under identical conditions but in the presence of the CA is accelerated 10^4-fold and gives predominantly the *syn*-oxime (the *syn : anti* ratio is 9 : 1). It is remarkable that the same CA induces the elimination of small molecules not only from the C – C bond, but also from the C – N bond, if the substrate molecules are structurally close to each other.

Transformation of benzoisoxazole **189** into the *o*-cyanophenol derivative **190** is another example of dehydration for which a highly effective CA has been obtained.[89]

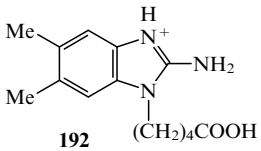
This reaction is initiated by deprotonation to give a partially delocalised transition state **191**. This reaction occurs as a concerted *E*2-elimination.



189 **191** **190**

In order to initiate this reaction, the active site of a CA must contain a group favouring deprotonation. Obviously, a carboxy group with appropriate acidity ($pK_a = 7.8$) matching the proton to be split off meets this demand.

The benzoimidazole derivative **192**, which is planar like benzoisoxazole and bears a spacer providing its immobilisation on the protein, was used as a hapten. The N(3) atom in the hapten **192** is protonated and is positively charged, which induces the formation of the carboxylate ion in the active site of a CA.



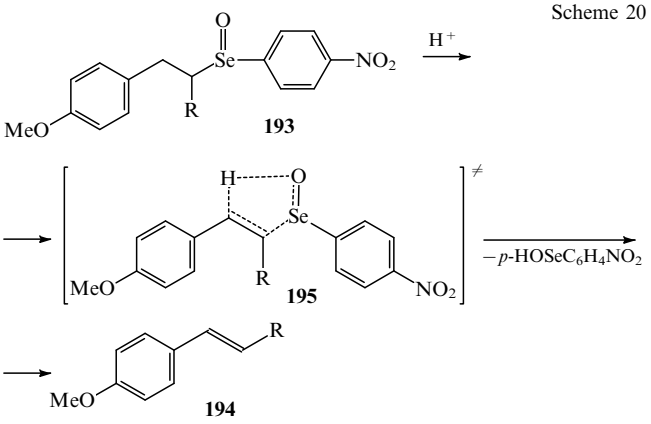
192

The most active CA raised against the hapten **192** accelerated the reaction $\sim 10^8$-fold compared to the reaction catalysed by the acetate ion. Such efficiency is one of the highest attained with CA so far.

The results of kinetic and inhibitory analyses and the character of pH dependence of the CA effectiveness suggest that initially the CA forms a complex with the substrate. It is followed by deprotonation under the action of the carboxylate ion in the cavity of the active site, resulting in the ring opening. Such a high catalytic effect is attributed[89] to the correct choice of the hapten stimulating the formation of a carboxy group of proper acidity and at the necessary place of the active site of the CA.

A recent study[90] describes the preparation of CA catalysing β-elimination of *p*-nitrobenzeneselenenic acid from the selenoxide **193** to yield the unsaturated derivative **194** (Scheme 20).

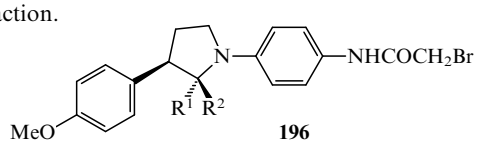
Scheme 20



R = H, Me, CH_2OH, COOH.

This reaction, which has recently attracted considerable attention of synthetic chemists, occurs as a [2.3]-sigmatropic process. It is believed that the planar five-membered pericyclic transition state **195**, which is less polar than the original molecule **193**, is formed in the first stage.

The proline derivative **196**, which was deemed to form a relatively low-polar medium in the active site of a CA,[90] was used as a hapten. Such a medium favours the pericyclic reaction; the carboxy group and the benzene rings form a cavity in which the substrate is fixed in a conformation favourable for the reaction.



R^1, R^2 = H, COOH.

Two CA against the *cis*-isomer of the hapten **196** (R^1 = H, R^2 = COOH) and one CA against its *trans*-isomer (R^1 = COOH, R^2 = H) have been obtained. All of them accelerated the elimination reaction several hundred times (2000-fold in case of the most active CA). The reaction rate increased with a decrease in the size of the substituent R in the molecule of the original selenoxide **193** (COOH < CH_2OH < Me < H).

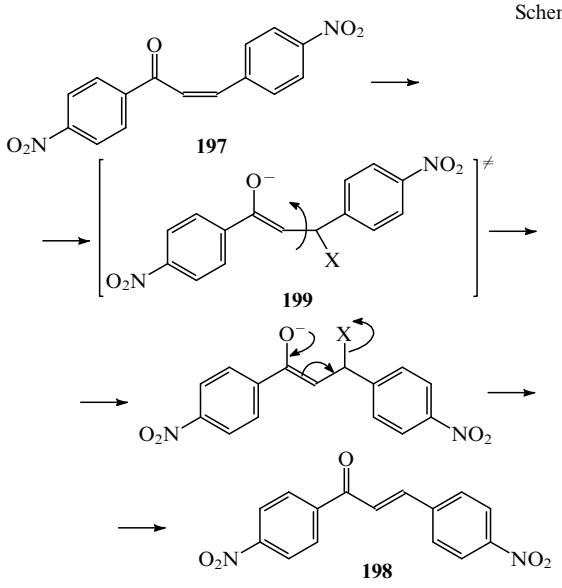
Of particular interest is the stereochemical result of this reaction, which opens up new opportunities for its application in organic synthesis. The non-catalysed elimination of selenenic acid from the selenoxide **193** results in *trans*-alkenes for steric reasons. If the reaction is performed in the presence of a CA raised against the *trans*-hapten **196** (R^1 = COOH, R^2 = H), the racemic selenoxide is converted into the *trans*-alkene **194** (90%), while the *cis*-isomer is formed in insignificant amounts (10%).

On the contrary, in the presence of catalytic antibodies against the *cis*-hapten (R^1 = H, R^2 = COOH) only a half of the racemate **193** (*i.e.*, one stereoisomer) undergoes conversion. It is noteworthy that whereas one CA gives exclusively *trans*-alkenes, while the other yields both *cis*- and *trans*-isomers **194** in the 45 : 55 ratio. This unusual result was rationalised as being due to the fact that the cavity of the active site of this CA was split into two parts, so that the CA could interact with both stereoisomers. Detailed analysis of the reaction kinetics revealed strong effects of the medium polarity and solvation on the catalytic activity of the CA.

There is a single study[91] devoted to the preparation of CA which catalyse *cis*–*trans*-isomerisation of a double bond. These CA effect the conversion of the *cis*-isomer of the α,β-unsaturated ketone **197** into the *trans*-isomer **198** (Scheme 21).[91]

It is believed that the isomerisation occurs by addition of a basic (or a nucleophilic) group to the double bond to give the transition state **199**. After rotation around the ordinary bond that

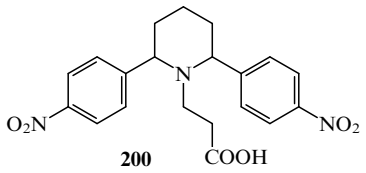
Scheme 21



formed, the substrate acquires a more favourable transoid conformation, while elimination of X^- gives the *trans*-isomer **198**.

Such an isomerisation could take place provided that the active site of the CA contained a group X that could add to the double bond and the spatial configuration of the cavity in the active site of the CA favoured subsequent conformational transition.

The hapten **200** was used to obtain a CA in which the nitrogen atom of the piperidine ring generated a carboxy group (a carboxylate ion) in the active site of the CA, while the benzene rings in the molecule **200** form a cavity favouring the conformational transition of the substrate by lowering the energy barrier to this reaction.



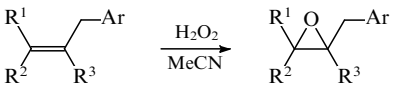
The catalytic antibody against the hapten **200** accelerated the isomerisation of the alkene **197** into the alkene **198** 1.5×10^4-fold. This reaction was inhibited by the *trans*-isomer of the hapten **200** but not by its *cis*-isomer. The presence of a carboxy group in the active site of the CA was confirmed by chemical modification of this CA. Thus, the CA plays the role of an ordinary acid which lowers the energy barrier to isomerisation.

VIII. Formation and opening of the oxirane ring

Several papers[92–97] devoted to the preparation of CA catalysing the formation and opening of the oxirane ring present special interest for synthetic chemists.

Thus a CA catalysing synthesis of oxiranes by epoxidation of a non-activated double bond of alkenes under the action of oxidants has been prepared.[92] This study was focussed mainly not on the acceleration of this well-known reaction but on the possibility of its stereochemical control, *i.e.*, an increase in its enantioselectivity. The catalytic effect of the CA was studied with the conversion of alkenes of the type **201** into the oxiranes **202** as an example.[92]

Hydrogen peroxide in acetonitrile was found to be the most suitable oxidant; in this case, the real oxidant is peroxyacetimidic acid $CH_3C(=NH)OOH$. It is essential that the effectiveness of this oxidant is combined with its mild action; it did not destroy the protein.

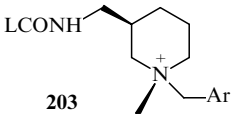


201a–e **202a–e**

Ar = $C_6H_4CONH(CH_2)_2OH$-*p*

Compound	R^1	R^2	R^3
a	Me	H	Me
b	H	$-(CH_2)_3-$	
c	H	Me	Me
d	H	H	Me
e	Me	Me	Me

The catalytic antibody must favour the pyramidalisation of carbon atoms, *i.e.*, their transition from sp^2- to sp^3-hybridisation. The active site of the CA was formed under the action of the hapten **203** with a positively charged tetrahedral nitrogen atom, which ensured the formation of a negatively charged group that facilitated acid catalysis.



203

L = spacer; Ar = $C_6H_4CONH(CH_2)_2OH$-*p*.

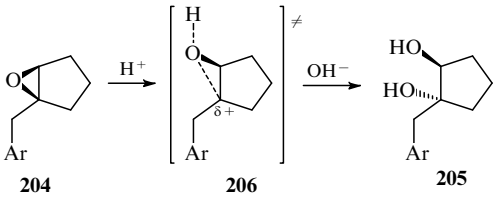
One of the catalytic antibodies raised against this hapten was relatively effective in the conversion of the alkenes **201** into the oxiranes **202**. It was found that epoxidation of trisubstituted alkenes (**201a–c**) was accelerated 40–125-fold, epoxidation of the disubstituted alkene **201d** and the tetrasubstituted alkene **201e** was accelerated 60- and 15-fold, respectively. High enantioselectivity of this oxidation presents substantial interest, although it depends on the alkene structure. The alkenes **201b** and **201c** yielded the corresponding oxiranes **202** with optical purity 98% (with allowance made for the reaction product formed in the parallel non-catalysed reaction). Enantioselectivity of the oxidation of the alkene **201a** was 67%, which can probably be ascribed to the ability of this alkene to react in two different conformations.

Catalytic epoxidation of styrene in the presence of a CA raised against a metalloporphyrin derivative with iodosobenzene as an oxygen source has been described.[93] This study was undertaken with the view of elucidating the mechanism of action of natural oxidases of the cytochrome type.

Studies aimed at the synthesis of CA that accelerate the opening of the oxirane ring are of considerable interest. Such CA enable a control over the regiospecificity and enantioselectivity of this reaction.

A catalytic antibody of this type was used[94] to catalyse the hydrolytic opening of the oxirane ring in the alicyclic oxirane **204** (Scheme 22).

Scheme 22



204 **206** **205**

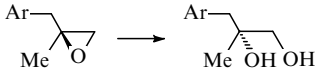
Ar = $C_6H_4CONH(CH_2)_2COOH$.

This acid-catalysed reaction occurs *via* the transition state **206** in which the tertiary carbon atom bears an excess positive charge and is subject to a nucleophilic attack, in this case, by the OH^- ion. The catalytic function of the active site of the CA is reduced to the promotion and stabilisation of this positively charged centre and facilitation of 'pyramidalisation' of the corresponding carbon atom.

The hapten **203** was used to generate the CA. This hapten is thought[94] to form a cavity in the CA complementary to the transition state and the positive charge on the nitrogen atom favours the formation of a negatively charged group stabilising the excess positive charge of the transition state.

This antibody accelerated the conversion of the oxirane **204** into the 1,2-diol **205** 440-fold in comparison with the reaction carried out under identical conditions but in the absence of the CA and, which is especially important, with high enantioselectivity: the racemate of the epoxide **204** yields only the *R*,*S*-diol **205** (*ee* 87%). This reaction obeys Michaelis–Menten kinetics and is inhibited by an analogue of the hapten **203**, which confirms its localisation in the active site of the CA.

The catalytic antibody raised against the hapten **203** manifests broad substrate specificity and accelerates 100-fold the ring opening in simple epoxides of the aliphatic series, for example, the conversion of the epoxide **207** into the diol **208**. However, in this case the reaction is devoid of enantioselectivity, *viz.*, the *R*- and *S*-isomers of **207** are hydrolysed at the same rate, while hydrolysis of the corresponding racemate does not yield an excess of the *R*- or the *S*-enantiomer.



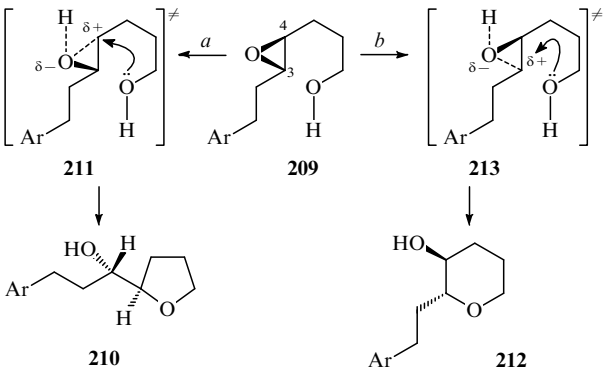
207 **208**

The difference in the behaviour of the oxiranes **204** and **207** is ascribed to the presence of an additional five-membered ring in the molecule **204**. In this case, the substrate and the active site of the CA created against the cyclic hapten are in a tighter contact.

Of particular interest is the use of CA in the catalysis of intramolecular opening of the oxirane ring in epoxy alcohols where a CA can change the regiospecificity of the reaction. The opening of the oxirane ring in the epoxy alcohol **209**, which culminates in the formation of a heterocyclic system, is an example.

This reaction may proceed by two different routes. In the absence of CA, it proceeds only by a route (*a*) as the nucleophilic attack at the C(4) atom of the epoxide ring and gives the tetrahydrofuran derivative **210** in accordance with the so-called 'Baldwin rule'.[96] Apparently, the reaction occurs *via* the transition state **211** with a partial positive charge on C(4) (Scheme 23).

Scheme 23

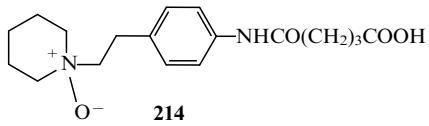


211 **209** **213**

210 **212**

Ar = C_6H_4NHAc-*p*, C_6H_4OMe-*p*.

It was found that this reaction can be fully reversed in the presence of the corresponding CA and channeled through the energetically less favourable route (*b*) to give the tetrahydropyran **212**, which requires special conditions, that would direct the reaction *via* the transition state **213**. This demand was met with the use of a CA the active site of which corresponded to the transition state **213** different from the transition state **211**. The piperidine *N*-oxide derivative **214** the geometry of which is close to that of the transition state **213** was used as a hapten.

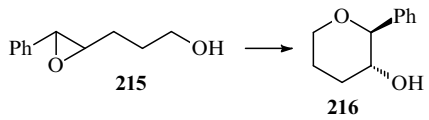


214

The hapten **214** has the N^+-O^- group which matches the carbon and oxygen atoms bearing excess charges in the transition state **213**. All this promoted the formation of groups stabilising this transition state in the active site of the CA.

Indeed, one of the CA obtained (26D9) favoured the strictly regiospecific opening of the oxirane ring to give the tetrahydropyran **212** alone. The action of this CA was also highly stereoselective: only the *S*,*S*-isomer of the epoxy alcohol **209** was converted into the *S*,*R*-isomer **212**. This alteration in the reaction course in the presence of the CA was due to the formation of a complex between the transition state **213** and the complementary active site of the CA, which decreased the free energy of the transition state **213** and lowered the energy barrier to this reaction. Route (*b*) proved to be energetically more favourable than route (*a*).

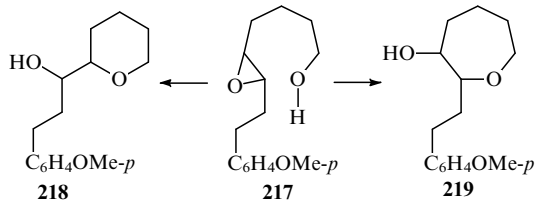
An analogous CA-induced change in the regiospecificity of the reaction of intramolecular opening of the epoxy alcohol **215** has been reported.[54] Under conditions of ordinary acid catalysis, only a tetrahydrofuran derivative is formed, whereas in the presence of the CA tetrahydropyran **216** is exclusively formed.[54]



215 **216**

This reaction is highly stereoselective: the racemate **215** gives the *S*,*R*-isomer **216**, while the *R*,*R*-isomer of the epoxy alcohol **215** does not enter into the reaction.

Further experiments showed that such changes in regiospecificity are common for the cyclisation of epoxy alcohols. Thus it was found unexpectedly that the cyclisation of the epoxy alcohol **217**, which under normal conditions is converted into the tetrahydropyran derivative **218** in accordance with the Baldwin rule, yields exclusively the oxepane **219** in the presence of the same CA (26D9).



218 **217** **219**

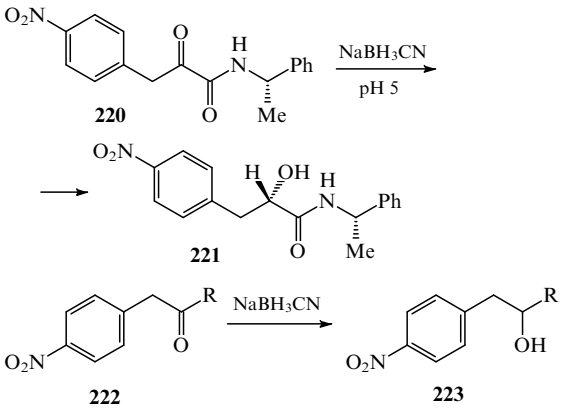
Thus, in this case, too, the CA channels the reaction through an energetically 'unfavourable' route.[97] Since the geometry of the hapten involved in the formation of the active site of the CA corresponded to the transition state with a six-membered ring, the formation of the oxepane **219** is unexpected at first glance. The authors interpret this phenomenon by the fact that the role of the CA consists primarily in the formation of a group in the appropriate position of the active site which facilitates the strictly specific opening of the oxirane ring. The size of the heterocyclic ring formed in the process depends on the length and conformation of the aliphatic chain (tether) in the epoxy alcohol **217**.

The above examples demonstrate various applications of CA in directed organic synthesis and elaboration of new synthetic procedures.

IX. Redox reactions

Studies aimed at the search for CA catalysing redox reactions have been neither extensive, nor systematic, apparently because of the existence of numerous chemical and biochemical methods for their efficient realisation. Nevertheless, several CA catalysing the reduction of carbonyl groups have been prepared. The use of artificial CA pursued the objective to make reduction more selective.

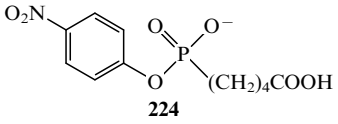
Some CA catalysed the reduction of the α-oxo amide **220** into the α-hydroxy amide **221**[98] and the reduction of the ketones **222** into the corresponding alcohols **223** with $NaBH_3CN$.[99]



220 **221** **222** **223**

R = Et, Pr^i, Bn.

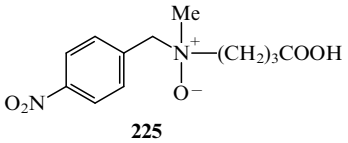
In the former case, the corresponding phosphonic acid derivative **224** was used as a hapten.



224

The phosphorus atom in the hapten **224** is in the same position as the oxo group in the substrate **220** to be reduced. The hapten **224** ensured the formation of a group, in the active site of CA, responsible for the polarisation of the oxo group and the tetrahedral structure of the phosphorus atom provided the stereoselectivity of the nucleophilic attack on the carbon carbonyl atom by the hydride ion in the transition state. The resulting CA accelerated the reduction of the oxo amide **220** 300-fold in comparison with the non-catalysed reaction. High stereoselectivity is the main merit of this reduction, because the alcohol **221** with the *S*-configuration formed in the presence of the CA was of 99% purity, whereas the *R*- and *S*-isomers were formed in approximately equal amounts in the absence of the CA.

Since the oxo group in the ketone **222** is less reactive than that in the oxo amide **220**, a more effective CA raised against the hapten **225** was used to catalyse this reaction.

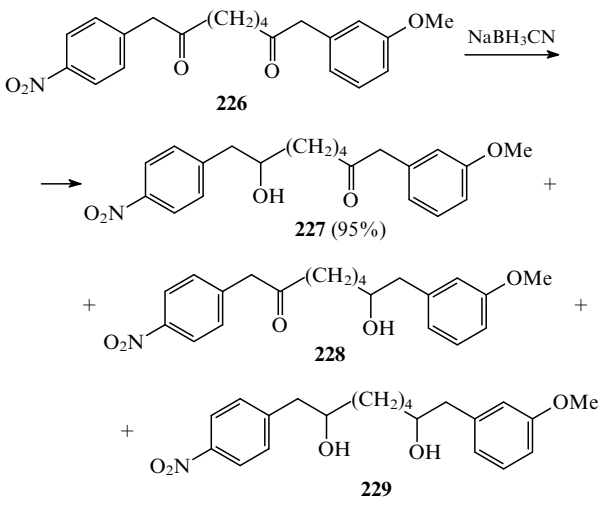


225

The $^+N-O^-$ group in the molecule **225** ensured the formation of the CA. Its active site ensured both the stabilisation of the tetrahedral transition state and enhancement of the substrate polarisation, which are essential for the nucleophilic attack of the reducing agent.

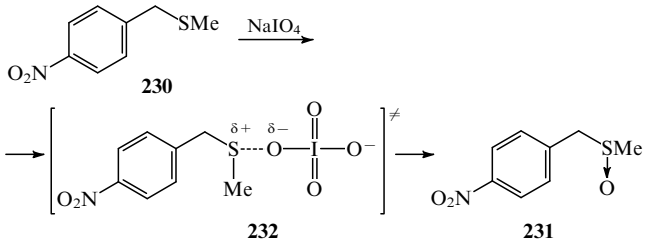
This CA accelerated the reduction of the oxo group in the ketones **222** approximately 100-fold; the alcohols **223** having the *S*-configuration were formed with high stereoselectivity (88%–95% *ee*) irrespective of the size of the alkyl substituent. The effect of CA was also highly regioselective. The reduction of the unsymmetrical diketone **226** in the presence of this CA afforded the *S*-enantiomer of the hydroxy ketone **227** in 95% yield, whereas other probable reaction products (compounds **228** and **229**) were formed in insignificant amounts. The regiospeci-

ficity of the CA is determined by the structure of the initiating hapten **225**, *viz*., by the presence of the NO_2 group.



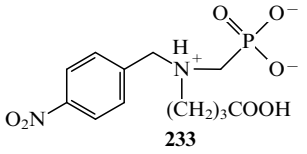
Among oxidation reactions for which effective CA have been obtained, special mention should be made of the oxidation of the sulfide **230** into the sulfoxide **231** (Scheme 24).

Scheme 24



It is believed that this reaction involves the attack at the sulfur atom of the sulfide **230** with the oxygen of the periodate ion which occurs *via* the transition state **232**.

Based on these concepts, the hapten **233**, a benzylaminophosphonic acid derivative, the amino group of which was transformed into the ammonium group under conditions of the CA biosynthesis, was used to generate the CA. The ammonium group favoured the formation of a negatively charged group stabilising the positive charge on the sulfur atom in the transition state, whereas the phosphonic acid residue formed a cavity binding the periodate ion in the active site of the CA.[100]



One of the CA raised against this hapten accelerated the oxidation of the sulfide **230** 2×10^5-fold; its effectiveness was close to that of natural oxidases, although it differed from the latter by low enantioselectivity ($\sim$ 15% *ee*). This CA was practically inert with respect to periodate. The mechanism of its catalytic action and the structure of its active site were established by X-ray analysis of a CA – hapten complex.[101]

It can also be noted that CA catalysing redox reactions have been used to solve some problems of biochemistry and enzymic catalysis, *e.g*., for determining the reduction potential of flavin [102] and for estimating the contribution of two fluorescein fragments to the free energy of binding in the active site of CA.[103]

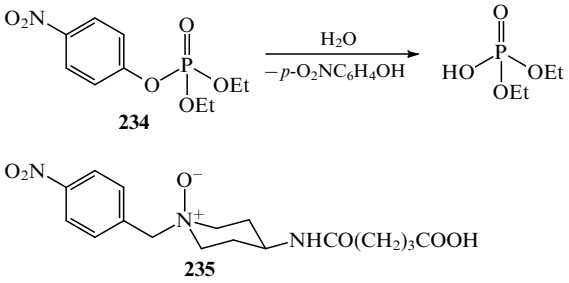
X. Miscellaneous reactions

The catalytic antibodies described below have not found general use; yet they present certain interest since they demonstrate not only the broad range of conversions for which active CA can be obtained but also the high potentialities of these artificial enzymes.

1. Hydrolysis of phosphotriesters

The interest in the synthesis of CA catalysing hydrolysis of phosphotriesters [104] arose in connection with medicinal and environmental problems, since phosphotriesters and their derivatives are the basis for many pesticides and war gases and intermediate products for large-tonnage chemical industry. Preparation of CA providing mild hydrolysis of these toxic compounds and their practical application might become useful in curing poisoning with pesticides, war gases, *etc*.

Thus the preparation of a CA catalysing hydrolysis of a well-known pesticide paraoxone (**234**), which occurs with the elimination of 4-nitrophenol, was undertaken with compound (**235**) as a hapten.



The charges on the N^+-O^- group of the molecule **235** generate specific groups in the cavity of the active site of the CA, which favour protonation and/or stabilisation of the negative charge on the oxygen atom of the phosphate group, whereas the spatial structure of the hapten forms a cavity which corresponds to the optimum structure of the transition state.

The CA obtained accelerated hydrolysis of the phosphate **234** $\sim$ 1000-fold. The linear dependence of the reaction rate on the concentration of the hydroxide ion suggests [104] that the catalytic effect of this CA is most probably associated with the stabilisation of the transition state. The CA possessed rather narrow substrate specificity. Hydrolysis of phosphate **234** analogues with a methoxy or an acetylamino group in the benzene ring and of cyclic phosphates was practically not accelerated by this CA.

2. Cleavage of triphenylmethyl ethers

Selective splitting of the triphenylmethyl (trityl) group presents certain practical interest, since this group is widely employed in synthetic practice for temporary protection of the hydroxy group, especially in the synthesis of nucleosides and carbohydrates. Detritylation is usually performed under rather drastic conditions of acid hydrolysis. Preparation of CA which would permit one to perform detritylation under neutral conditions and with required selectivity presents substantial interest.

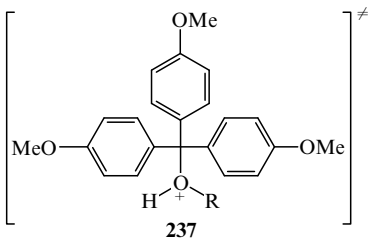
Detritylation of the ether **236** served as an example.[105]

$$(p\text{-}MeOC_6H_4)_3COR \longrightarrow (p\text{-}MeOC_6H_4)_3COH + ROH$$

236

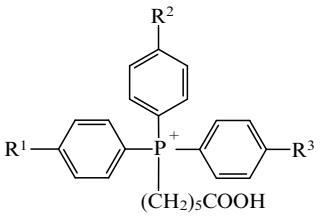
R = $(CH_2)_2O(CH_2)_2O(CH_2)_2OH$.

The protonation of the oxygen atom possessing enhanced nucleophilicity is the rate-determining step in the detritylation of tris(4-methoxyphenyl)methyl ether **236**. The resulting transition state **237** is further decomposed to give tris(4-methoxyphenyl)methanol.



237

The triphenylphosphonium derivative **238** containing a spacer was used to obtain the CA catalysing this reaction.



238

This hapten was structurally similar to the substrate **236**. The positive charge on the phosphorus atom of this hapten favoured the formation of a negatively charged group in the CA.

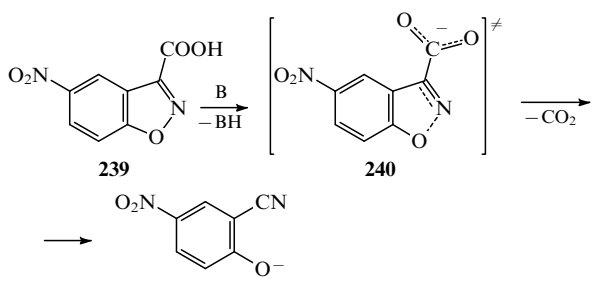
The CA obtained accelerated the hydrolysis of the ether **236** 270-fold in comparison with the non-catalysed reaction at pH 6.0 and manifested broad specificity as it was active in detritylation of analogues of the ether **236**, *e.g.*, such compounds as $(p\text{-MeOC}_6H_4)_2C(Ph)O(CH_2)_5COOH$ and $(p\text{-MeOC}_6H_4)\text{–}C(Ph)_2O(CH_2)_5COOH$, by accelerating their hydrolysis 200- and 230-fold, respectively. It is of note that hydrolysis of the thio analogue of the ether **236** was also accelerated 70-fold by this CA, whereas that of the amine Ar_3CNHR was not catalysed.

The effect of pH on the detritylation of compound **236** in the presence of the CA suggested [105] that its catalytic action was not owing to the direct effect of the carboxy group but rather due to electrostatic stabilisation of the oxonium centre of the transition state **237** in the cavity of the active site of the CA. This study on the preparation of a CA catalysing detritylation, was not explored further but it gave an impetus to the preparation of CA for other reactions associated with the cleavage of oxygen–carbon bonds (see Section V).

3. Decarboxylation reactions

Preparation of CA catalysing one specific reaction, *viz*., the decarboxylation of 5-nitrobenzo[*d*]isoxazole-3-carboxylic acid **239** resulting in 4-nitro-2-cyanophenol, has been described (Scheme 25).[106]

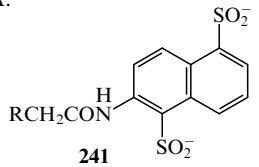
Scheme 25



239 **240**

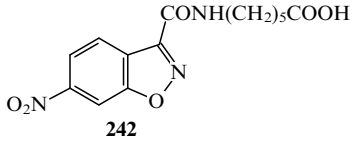
This first-order reaction¶ occurs as a concerted process. It is known that it is not associated with acid–base catalysis but its rate depends critically on the reaction medium. This reaction is accelerated by several orders of magnitude in aprotic solvents, in micelles and in the presence of organic polymers. Presumably, it proceeds *via* the transition state **240** in which the bonds are partially delocalised. A CA catalysing this reaction must contain a hydrophobic active site with polar groups favouring the splitting of CO_2.

The hapten **241** with a spacer group (R) was used to generate CA.



241

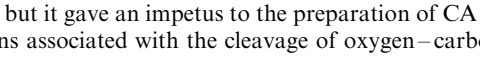
It was suggested [106] that the naphthalene nucleus of the hapten provides the formation of a cavity complementary to the substrate in the active site of the CA, while the sulfo groups induce the appearance of positively charged groups, which facilitate splitting of CO_2. The authors' arguments in favour of this hapten are not completely clear; nevertheless, a highly effective CA able to accelerate the reaction 2×10^4-fold in comparison with non-catalysed decarboxylation has been raised. It is of note that no active CA could be raised against the hapten **242**, which matches more closely the substrate but is devoid of polar sulfo groups.



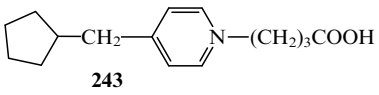
242

This reaction has been studied in detail using inhibitory analysis, fluorescence spectra and the kinetic isotope effect.[107] It was shown that the active site of the CA is very hydrophobic, and that the catalytic effect of the CA is achieved owing to the formation of a hydrophobic environment around the substrate. This conclusion was confirmed, in particular, by comparison of the rates of CA-catalysed reactions occurring in solvents of different polarity.[108]

Preparation of a CA catalysing decarboxylation of 4-pyridylacetic acid has been reported.[109]



Compound **243** with a cyclopentyl substituent, which was introduced to form a hydrophobic cavity in the active site of the CA, was used as a hapten.



243

The CA obtained accelerated the decarboxylation of 4-pyridylacetic acid and some of its methyl-substituted analogues $10^4\text{–}10^5$-fold. It was shown that the catalytic effect of such CA was largely due to the formation of a hydrophobic environment around the substrate in the complementary region of the active site of the CA. The main role in the formation of such a hydrophobic environment is played by the cyclopentyl substituent in the hapten **243**. Preparation of this kind of CA is of fundamental importance, since it demonstrates the possibility to attain a catalytic effect by the formation of hydrophobic zones in CA. This allows one to gain more insight into the mechanisms of action of natural decarboxylases.

4. Isomerisation of cyclic biphenyl derivatives

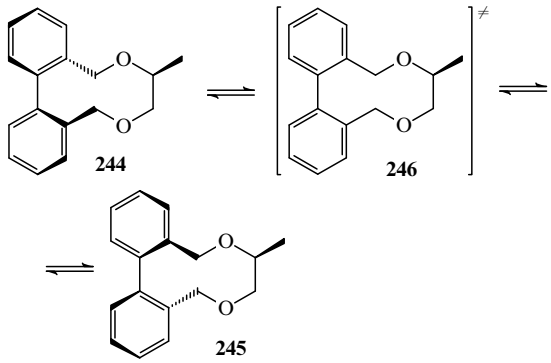
Isomerisation of the biphenyl derivative **244** incorporated in a cyclic system into its diastereomer **245** requires rather high ($\sim$ 20–26 kcal mol^{-1}) activation energy (Scheme 26).[110]

At room temperature, this reaction proceeds very slowly, because the rotation of phenyl nuclei around the common axis is

¶ For an analogous CA-catalysed conversion of nitrobenzoisoxazole into a cyano phenol derivative, see Section VII.

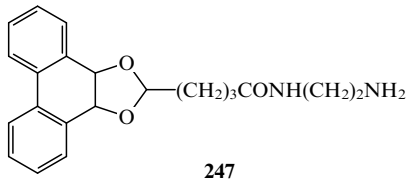
impeded due to the presence of methylene groups in *ortho*-positions.

Scheme 26



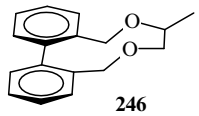
244 **246** **245**

Presumably, the effect of a CA is associated exclusively with the geometric factors favouring isomerisation rather than with stereoelectronic effects or acid–base catalysis. Assuming that this isomerisation occurs *via* the planar transition state **246**, a dihydrophenanthrene derivative **247** with fixed coplanar benzene rings was used in the preparation of CA as a hapten.



247

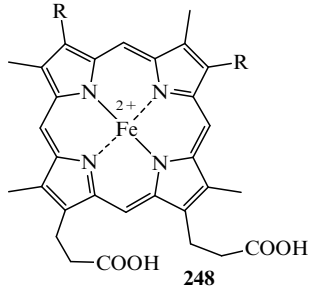
The most active CA obtained accelerated the isomerisation **244** → **245** approximately 3000-fold. This CA was subjected to a more detailed physicochemical analysis. It was demonstrated that the transition state **246** actually has a step-like conformation but its phenyl nuclei lie in the same plane.



246

5. The formation of metal–porphyrin complexes

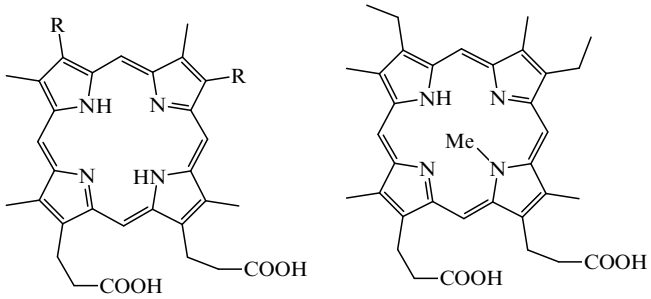
The formation of metal–porphyrin complexes is the key step in many vital processes. Thus, Fe^{2+} incorporates into protoporphyrin IX at the final step of heme (**248**, R = CH=CH$_2$) biosynthesis.



248

This reaction is catalysed by a natural enzyme, ferrochelatase, and inhibited by *N*-methylprotoporphyrin IX. It is assumed that the inhibiting effect of the latter is due to the non-planar structure, which resembles that of the transition state formed in the reaction **249** → **248**. Therefore, *N*-methylprotoporphyrin IX competes with the transition state for binding with the active site of the enzyme.

N-Methylmesoporphyrin **250** in which propionic acid residues served as a spacer for immobilisation was used to generate an active CA catalysing the formation of metal–porphyrin complexes.[111]



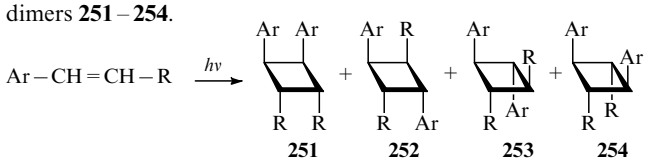
249 **250**

In this case, the choice of a hapten was based on the principle of mimicking the 'strained transition state'.

The most active CA obtained accelerated the formation of complexes of dihydroprotoporphyrin **249** (R = C_2H_5) with Zn^{2+} and Cu^{2+} 2600- and 1700-fold, respectively. This reaction followed Michaelis–Menten kinetics and was inhibited by the hapten **250**. The catalytic effect of this CA varied for different metals and depended specifically on the porphyrin structure. Thus complex formation of protoporphyrin **249** (R = CH=CH$_2$) and its analogue (R = H) was not catalysed by this CA. This makes the effect of CA strongly different from that of ferrochelatase, which also catalyses the reactions of these analogues.

6. Photochemical reactions

Several CA affecting UV-induced reactions (*e.g.*, dimerisation of methyl 4-nitrocinnamate in acetone) have been obtained.[112] Under ordinary conditions, this reaction gives a mixture of the dimers **251**–**254**.

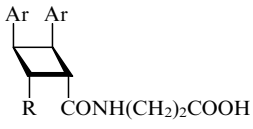


251 **252** **253** **254**

Ar = *p*-$O_2NC_6H_4$, R = COOMe.

With the use of a CA it was possible to change the ratio of the two main isomers **251** and **253** (0.45 : 1).

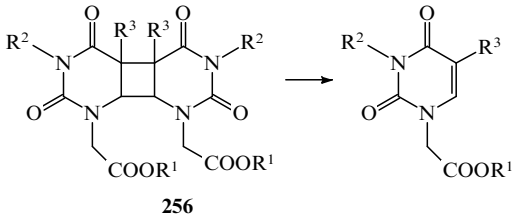
In order to increase the yield of the isomer **251**, it is necessary that the cavity of the active site of the CA matched the spatial structure of the dimer **251**. Therefore, the hapten **255** was used to generate the CA.



255

Irradiation of methyl 4-nitrocinnamate in the presence of the CA raised against the hapten **255** gave the dimers **251** and **253** in a 1.13 : 1 ratio. The reaction was accelerated by the CA. Thus, the formation of the active site favouring the appropriate configuration of the transition state directs the reaction towards the formation of the corresponding isomer. The structure of the group catalysing the dimerisation itself is still unknown.

Preparation of a CA catalysing UV-induced dissociation of the thymine dimer **256** (R^1 = R^2 = H, R^3 = Me) presents certain interest.[113]



256

UV-Induced dimerisation of the thymine residue of DNA is a cause of structural disturbances in this biopolymer, which has profound biological consequences. This reaction is reversible and DNA can be 'repaired' by irradiation in the presence of natural enzymes (DNA-photolyases).

The dissociation of the dimer **256** seems to occur through electron transfer with subsequent dissociation by a radical mechanism. Indole, quinone and flavin derivatives are sensitisers of the electron transfer. It was speculated that catalytic antibodies that catalyse effectively the dissociation of the dimer **256** ought to contain a group that sensitises the electron transfer in the cavity of the active site matching the substrate. The hapten **256** ($R^1 = NHCH_2COOH$, $R^2 = H$, $R^3 = Me$), which is the substrate analogue, was used to raise such CA. The efficiency of a CA raised against this hapten was comparable with that of the natural enzyme (DNA-photolyase from *E. coli*). The effect of the CA was specific: dissociation of the dimer **256** ($R^1 = H$, $R^2 = R^3 = Me$) was not catalysed. The changes in the fluorescence spectra during this reaction provide evidence that a tryptophan residue is responsible for sensitisation of this reaction in the active site of the CA.

The dissociation of the N_1-carboxymethyluracil dimer **256** ($R^1 = R^2 = R^3 = H$) was studied in order to elucidate the mechanism of photochemical dissociation of nucleic bases.[114] The corresponding CA was raised against the hapten **256** ($R^1 = NHCH_2COOH$, $R^2 = R^3 = H$), which is a structural analogue of the hapten used to obtain the CA catalysing the dissociation of the thymine dimer. The resulting CA accelerated the photodissociation of the uracil dimer 380-fold. A study of the effect of the wavelength of UV irradiation revealed the crucial role of a tryptophan residue on the electron transfer. Analysis of the isotope effect in the irradiation of C(5)- and C(6)-deuteriated uracil dimers has made it possible to suggest a general scheme for the photodissociation of nucleic base dimers.

XI. Conclusion

The creation of artificial catalysts having a protein structure and endowed with a predetermined specificity is one of the most impressive accomplishments in chemistry and biology. Considerable progress has been achieved over the decade elapsed since the preparation of the first catalytic antibody. A great number of such biocatalysts of a new generation have already been obtained. But what is most important is that the main principles of the design of catalytic antibodies of targeted action have been elucidated and the strategy of their preparation has been elaborated.

The design of a hapten determining the specificity of the antibody and the structure of its active site is the crucial step in the generation of a CA.

At present, the choice of a hapten is governed by different principles which reflect the diversity of mechanisms of conversion of organic compounds.[115] In the simplest case, a compound the structure of which is spatially and electronically similar to that of the transition state, is used as a hapten, which stabilises this transition state in the active site of the CA. Haptens generating active groups responsible for acid – base or nucleophilic catalysis in substrate conversions in the active site of CA are also used for this purpose. Catalytic antibodies that are effective in sigmatropic and pericyclic reactions are raised against haptens which form a cavity in the active site of a CA that enables optimum reciprocal arrangement of the reactants (in bimolecular reactions) or a required stereoelectronic state of the substrate. This lowers the entropy barrier to a chemical conversion (the 'entropic trap' principle). In some cases, the purpose of the hapten is to create local or more extended hydrophobic zones in the CA mimicking conversion of the substrate in a 'non-aqueous' medium. In practice, haptens meeting simultaneously two or several requirements have been employed, which made it possible to form an active site which acted on the substrate in several directions at once.

The most recent approaches make use of haptens which directly interact with the antibodies raised against them. In this way, CA acquire the ability not only to stabilise the transition state but also to mimic the very process of conversion of the substrate (the so-called 'reactive immunisation').[11] Yet another approach consists in catalysis in the presence of a cofactor which acts simultaneously with the antibody (and actually modifies it in the course of the reaction) and channels the catalytic reaction in the necessary direction.[82]

Further progress in this area will depend first of all on new efficient approaches to the design of haptens. It is due, primarily, to the development of theoretical chemistry and the use of computation and computer modelling for gaining a deeper insight into molecular mechanisms of biocatalysis and elucidation of mechanisms of catalytic reactions.

Great diversity of reactions for which catalytic antibodies have been already obtained testify to the high potentialities of this novel field. At first glance, it may seem that a catalytic antibody can be generated for virtually any conversion of an organic compound. However, it is still too premature to prognosticate and outline the real scope of the method.

The 'exploration' period in this area seems to be over. In addition to purely academic research, studies devoted to the preparation of artificial enzymes for practical purposes are currently under way. Yet, many general problems still await their solution.[116] Here it seems appropriate to mention two of such problems which present special importance from the point of view of a synthetic chemist.

In most cases, the activity of synthetic CA is inferior to that of natural enzymes. The inhibitory effect of reaction products on catalytic antibodies is one main reason for the low activity of CA which reduces their 'turnover' number and decreases their efficiency. The understanding of the mechanism of this phenomenon and the ability to overcome the problem through the correct choice of a hapten is the urgent task.

All in all, catalytic antibodies differ strongly from each other in their substrate specificity: some CA have narrow specificity and are effective with respect to only one substrate, while others are effective with respect to a vast variety of structurally close compounds. The former ensure high chemo-, regio-, and stereospecificity of a specific reaction and exclude parallel and side processes. The latter are more suitable for wide application and elaboration of a versatile synthetic procedure. As a matter of fact, both types of CA may coexist, and it would be important to learn how to control the range of their substrate specificity in the targeted preparation. This requires knowledge of mechanisms of the catalytic effect and the role of the fine structure of the active site in manifestation of specificity, and hence the correct choice of a hapten determining these properties.

Further improvement of the primary monitoring of CA raised and the procedure for the choice of the most efficient catalysts (see Ref. 117) are problems of substantial importance facing specialists in biological sciences. One may also hope that the efficiency of antibody biosynthesis in the immune apparatus will also be enhanced as a result of fundamental or methodological modifications of the existing immunisation protocols and further utilisation of the hybridomas obtained.

However, CA have already proved to be a very helpful tool in organic synthesis not only in enhancement of well-known reactions but, which is even more important, in increasing the specificity and stereoselectivity. It is therefore very tempting to obtain CA catalysing the reactions for which natural enzymes have not yet been found. Apparently, CA acquired the greatest importance in conversions of organic compounds which cannot be performed by purely chemical methods because of energetic and/or steric hindrances. In some cases, CA facilitate the formation of a required transition state and channel the reaction through a 'forbidden' route by changing its chemo-, regio- or stereospecificity, *i.e.*, opening up a way for a new synthetic method.

For chemists, the use of CA opens up new opportunities in the studies of the 'intimate' aspects of mechanisms of many reactions, the role of thermodynamic and kinetic factors, acid–base catalysis, effects of the medium, *etc*.

Catalytic antibodies may hold a particular position in bioorganic chemistry, specifically, in the chemistry of biopolymers where enzymes find especially wide uses.

Studies of CA are of considerable importance for the understanding of basic principles of biocatalysis.[115] The possibility to obtain CA with targeted action and their comparison with natural enzymes of analogous action patterns, studies of the molecular structure of the active site of CA by X-ray analysis and other physicochemical methods, the use of targeted chemical modification or selective mutagenesis and many other features make catalytic antibodies an indispensable tool in studies on the mechanisms of enzymic catalysis, the structure of enzyme–substrate complexes and their role in the catalytic process. The problem of specificity of natural enzymes can be followed by preparing a series of CA with analogous action, elucidating their molecular structures, structure–specificity relationships and so on. Studies in this area are currently under way and have been briefly mentioned in this review.

Investigations of CA will probably make a significant contribution to the elucidation of other problems related to immunochemistry, immunology, protein biosynthesis, *etc*. Thus it is known that CA have already been used to study such specific problems as the formation and alteration of the structures of natural enzymes in the course of chemical evolution.[118]

Attempts have been made to use CA in medicinal practice (see a review [119]), *e.g*., as preparations neutralising the effects of certain toxins, narcotics, *etc*. Their application in the creation of 'prodrugs' is another perspective trend.[120–122]

This creates a problem of a large-scale preparation of CA. The first steps in this direction aimed at obtaining large batches of CA and their utilisation in the synthesis of gram quantities of compounds as well as the design and construction of a laboratory unit operating in a semi-automatic regime have already been undertaken.[53, 54] In the state-of-the-art biotechnology and protein engineering, preparation of large amounts of highly purified catalytic antibodies does not face any serious problems. Of course, their practical application on a large laboratory or industrial scale will require the solution of many other problems including technological ones. One problem is related to the most rational form of their application, *viz*., in solution, in biphasic systems,[123] immobilised on carriers [124] or under conditions of micellar catalysis.[15] Many of these problems connected with the use of natural enzymes have been successfully solved, which facilitates their solution in the case of CA.

As far as the economical aspect is concerned, the benefits of CA applications (*e.g*., in asymmetric synthesis) may have fully paid off despite the labouriousness and expensiveness of the technological procedures involved in the preparation of these biocatalysts. However, their application in large-tonnage processes is still premature.

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