Molecular recognition in applied enzyme chemistry

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Abstract. Molecular recognition impinges upon many fields of biological chemistry, especially those involving catalytic processes. This review gives examples from studies at Strathclyde of both small and macromolecular systems. Mechanism-based enzyme inhibitors are described with reference to dihydrofolate reductase, dihydroorotate dehydrogenase, and cholesterol metabolism. Applications of molecular recognition related to synthetic transformations are discussed in terms of aromatic substitution, chemically modified papain, and catalytic antibodies for Diels-Alder reactions.

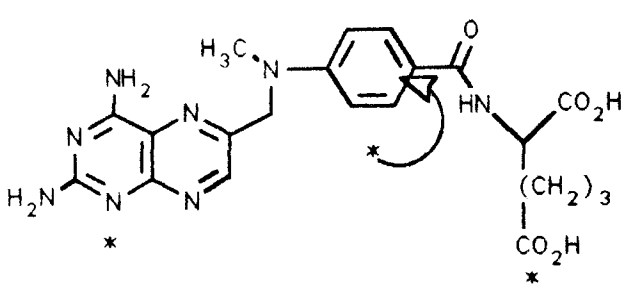
Key words. Enzyme inhibitors; biomimetic chemistry; chemically modified enzymes; catalytic antibodies.

Introduction

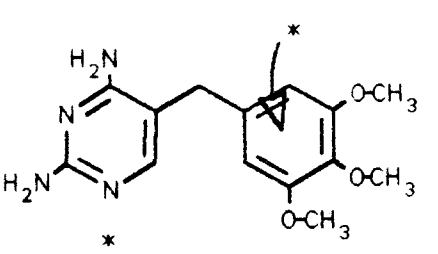
If one approaches molecular recognition from an investigative point of view, but with the possibility of application in mind, a natural question to ask is 'How similar to a natural ligand does a molecule have to be to bind at the same site as a natural ligand?'. This question has ramifications in many fields. For example, in medicinal chemistry, it relates to the design of enzyme inhibitors and to the binding of potential drugs to receptors typically for neurotransmitters or hormones. In synthetic chemistry, it relates to the ability of an enzyme to transform a close analogue to the natural substrate in a stereoselective manner. As mentioned in the introduction to this series of reviews, there are many potential strategies. At one extreme, one can ask what is the simplest biomimetic synthetic system that can support a preparatively useful selective reaction? At the other, and most recent, one can probe the ability of an antibody to catalyse a reaction involving a substrate that is related in structure to the hapten to which the antibody was raised. These fields have been studied at Strathclyde over the past 15 years. In this article, some of the most pertinent results and some with general significance will be discussed from the point of view of molecular recognition.

The inhibition of folate-transforming enzymes

A long-standing research interest at Strathclyde has been the inhibition of enzymes involved in the biosynthesis and metabolism of folic acid and its reduced derivatives [7]. Most recent work has centred on the enzyme dihydrofolate reductase (DHFR) and its inhibition by 7,7-spirocyclopropylpteridines [9]. This enzyme is one of the most well-characterised enzymes as a result of extensive crystallographic and NMR studies [3, 10, 20]. Consequently it has become one of the prime candidates for investigative studies of molecular recognition. Many important drugs act as inhibitors of DHFR and their interactions with the enzyme have been examined in detail. The principal components of recognition of diaminopyrimidine drugs to the active site have been identified as protonation of the diaminopyrimidine ring by an aspartic acid at the active site together with a hydrophobic interaction in antibacterial drugs such as trimethoprim or a specific binding of the glutamate side chain in anticancer drugs such as methotrexate (fig. 1). The toxicity of the latter together with the potential of achieving high selectivity using an enzyme inhibitor that became active only through binding and activation at the active site (a latent or suicide inhibitor) encouraged us to apply the chemistry of cyclo-

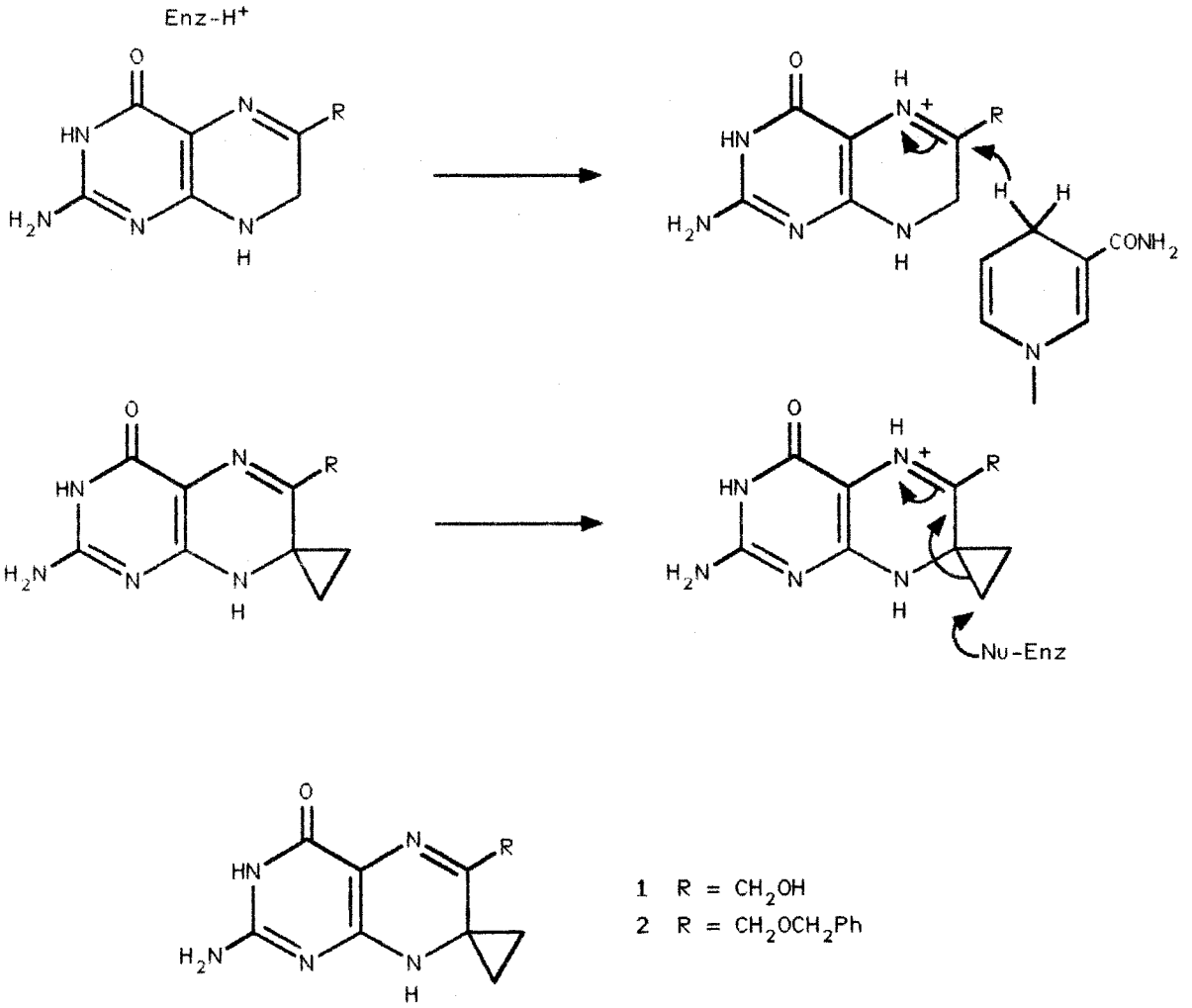


Methotrexate



Trimethoprim

Figure 1. Classical inhibitors of dihydrofolate reductase.* Principal structural features recognized by dihydrofolate reductase.



1 R = CH_2OH
2 R = CH_2OCH_2Ph

Figure 2. Protonation in catalysis by dihydrofolate reductase and its relationship to inhibitor design.

propanes to this enzyme[24]. The cyclopropane ring is small and its introduction to generate a substrate analogue should not make binding of the substrate analogue improbable.

The original mechanism proposed for the mechanism of action of this enzyme involved protonation of N-5 imine thereby activating it to reduction by hydride from NADPH. On the basis of this concept, we designed, prepared, and evaluated the spirocyclopropylpteridines **1** and **2** (fig. 2). The molecules were shown to be time-dependent irreversible inhibitors of DHFR, and were the first inhibitors to be discovered that were close substrate analogues, with the natural amino-oxo substitution pattern in the pyrimidine ring[9]. Whilst this work was in progress, however, results from crystallographic studies and stereochemical data forced a review of the mechanistic interpretation upon which the original design of inhibitors had been based (fig. 3)[3,10]. In the revised mechanism, protonation was viewed as taking place on the 4-oxo substituent of the pteridine ring; by rotation of a hydrogen bond, this proton could then be viewed as being transferred to the required destination on N-5. No independent reaction-based support for this view was, however, available. One possible avenue to explore was the possibility that a latent inhibitor might also be activated by protonation of a 4-oxo substituent. In order to investigate this possibility, it was necessary to block the possibility of direct protonation on N-5; this was achieved in compound **3**. This molecule was shown to be a time-dependent irreversible of DHFR also[16]. Clearly the enzyme recognises both types of pteridines **1**, **2** and **3** and is able to activate them presumably to nucleophilic ring opening.

This result is of interest in providing oblique support for a description of the mechanism of reduction by DHFR but in doing so, it poses significant problems of molecular recognition. Both inhibitors lack either a hydrophobic substituent or the glutamate side chain of the common drugs mentioned above. It is not therefore possible to describe directly by analogy with the drugs the way in

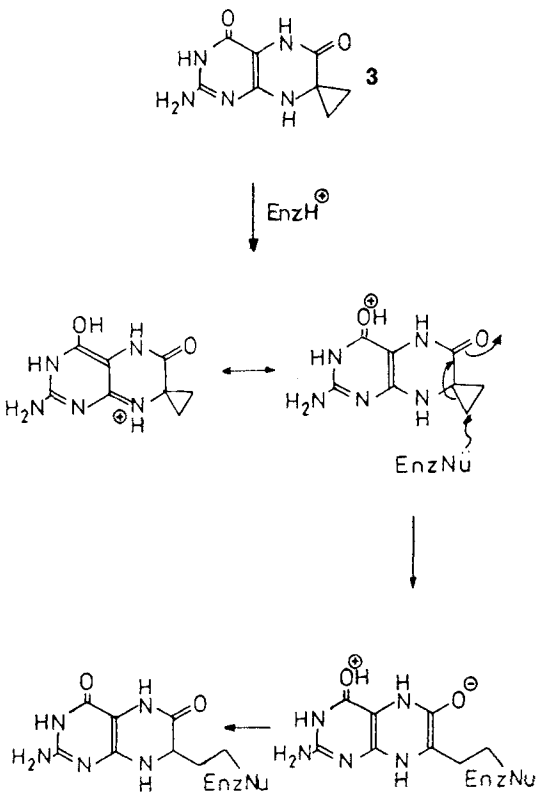


Figure 3. A second design for dihydrofolate reductase inhibitors.

which the three pteridines bind to DHFR; this question is currently under investigation. We can, however, suggest that in designing latent or suicide inhibitors, the extent of molecular similarity between the substrate and the inhibitor can be limited to a few essential features. Indeed this view has been mirrored in the discovery of some new substrates for DHFR [27]. The fullest expression of such a concept would be a molecule not belonging to the same structural series as the substrate, but nevertheless capable of being activated by the enzyme leading to irreversible inhibition. We accidentally discovered such a case in parallel studies on pyrimidine biosynthesis.

Inhibitors of dihydroorotate dehydrogenase

The inhibition of any pathway leading to the biosynthesis of nucleic acid components has potential for the discovery of anti-infective and anticancer drugs. DHFR inhibitors represent one such example in the biosynthesis of purines and of thymidine. Dihydroorotate dehydrogenase (DHODase) is an enzyme involved in the biosynthesis of pyrimidine bases and has been shown to be a target for the action of a new type of antimalarial drug [12]. During an investigation of the inhibition of the preceding enzyme in the pathway, dihydroorotase, we discovered that certain substituted hydantoins **4** acted as enzyme-activated irreversible inhibitors of DHODase from *Clostridium oroticum* (fig. 4)[6]. It is clear from figure 4 that the inhibitor **4** has no obvious structural relationship to the substrate **5**; the two molecules are amide-containing heterocycles, but their ring sizes differ; they both contain carboxylate functions, but there was no obvious way in which the inhibitor could be activated by the enzyme. We are clearly dealing here with a question of molecular recognition of a very dissimilar compound by an enzyme of potential chemotherapeutic significance,

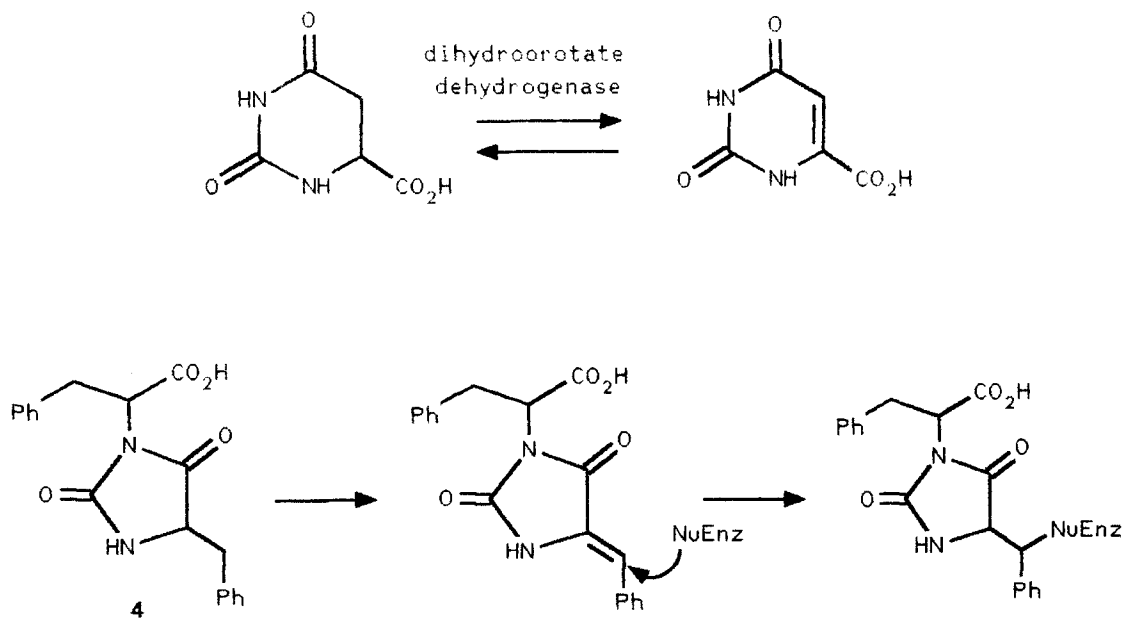


Figure 4. Dihydroorotate dehydrogenase: reaction and possible mechanism of action of hydantoin-based inhibitors.

that is exactly the situation outlined at the end of the 'Introduction' above.

Whilst no firm proof can be adduced because the enzyme is not presently available in sufficient quantities for study at a structural level, we derived a mechanistic rationale for the inhibition that is consistent with all results subsequently obtained [7, 11]. The key to this interpretation was the concept that an enzyme will accept a molecule if it recognises a small number of key structural features. In this case, it was necessary to incorporate a role for the carboxylate groups in both substrate and inhibitor; they would most probably interact with the same complementary residue on the enzyme, together with an oxidation site. The oxidation site in the substrate is clear and it seemed most likely that oxidation of the inhibitor would occur at the C-5 benzyl substituent on the basis of chemical reactions. The interpretation of the inhibition reaction was therefore derived by superimposing the two oxidation sites on the substrate and inhibitor and investigating the potential congruence of the structures by molecular modelling (fig. 5). After setting up the correct stereochemical relationships for dehydrogenation, it was apparent that the substrate and inhibitor rings could not be superimposed in such a way that the carboxylates and the dehydrogenation sites came into reasonable proximity. Thus although one might feel intuitively inclined to assign a similar role in recognition by DHODase to the heterocyclic rings of the substrate and inhibitor **4**, such a relationship cannot account for the results observed. The force of this argument is that there exists the potential for the design of enzyme inhibitors with or without chemical reactivity that differ greatly in molecular structure from the normal substrate. A thoughtless adherence to close structural molecular similarity as a guide to the design of enzyme inhibitors is therefore a danger. Many examples of potent enzyme inhibitors that are superficially unrelated to the substrate have, of course, been discovered and developed rather than designed [29].

Inhibitors of steroid metabolism

A second and quite different example in which a rational design of a biologically active compound has led to surprising results with respect to molecular recognition concerns inhibitors of steroid metabolism [26]. In order to treat diseases of cholesterol imbalance such as atherosclerosis, it is important to understand the effects that inhibiting a specific biochemical pathway in a cell would have on the overall handling of cholesterol by the cell. At the time our work began, there were no highly selective inhibitors of cholesterol 7α-hydroxlase, the first and rate-limiting enzyme in the biosynthesis of bile acids, and the effect of blocking this pathway on the overall metabolism of a cell was unknown. Cholesterol 7α-hydroxylase is a member of the cytochrome P-450 class. We reasoned that if it operated either by a radical mechanism or by insertion, the inclusion of fluoro substituents at the 6-position would generate an inhibitor of the enzyme. Accordingly, we prepared 6,6-difluorocholestanol **6**, and, for comparison, 7,7-difluorocholestanol **7** [5]. Both of these compounds were found to be acceptable substitutes for cholesterol in its physiochemical role as a rigidifier of bilayer membranes [19]. They were also investigated as inhibitors of several cholesterol-transforming enzymes including ACAT and cholesterol 7α-hydroxylase [26]. As planned, the 6,6-isomer **6** was found to be a specific inhibitor of the 7α-hydroxylase, and was also shown to be a substrate for ACAT. On the other hand, surprisingly, the 7,7-isomer **7** was not accepted as a substrate or inhibitor by either enzyme. There is thus a stark contrast in the recognition of the two isomers by the active sites of

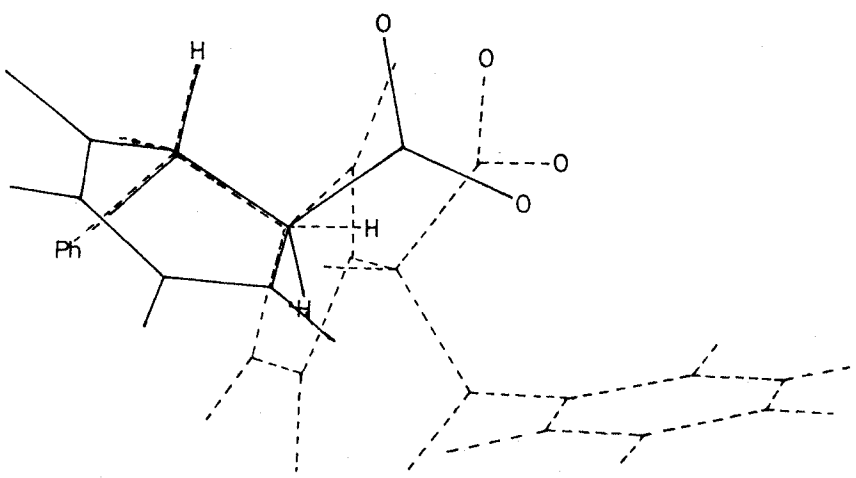


Figure 5. Rationalisation of the reactivity of substrate and inhibitors of dihydroorotate dehydrogenase by molecular graphics. The oxidation sites and carboxylate functions have been aligned as closely as possible. The inhibitor (**4**) is shown in broken lines and the substrate in continuous lines.

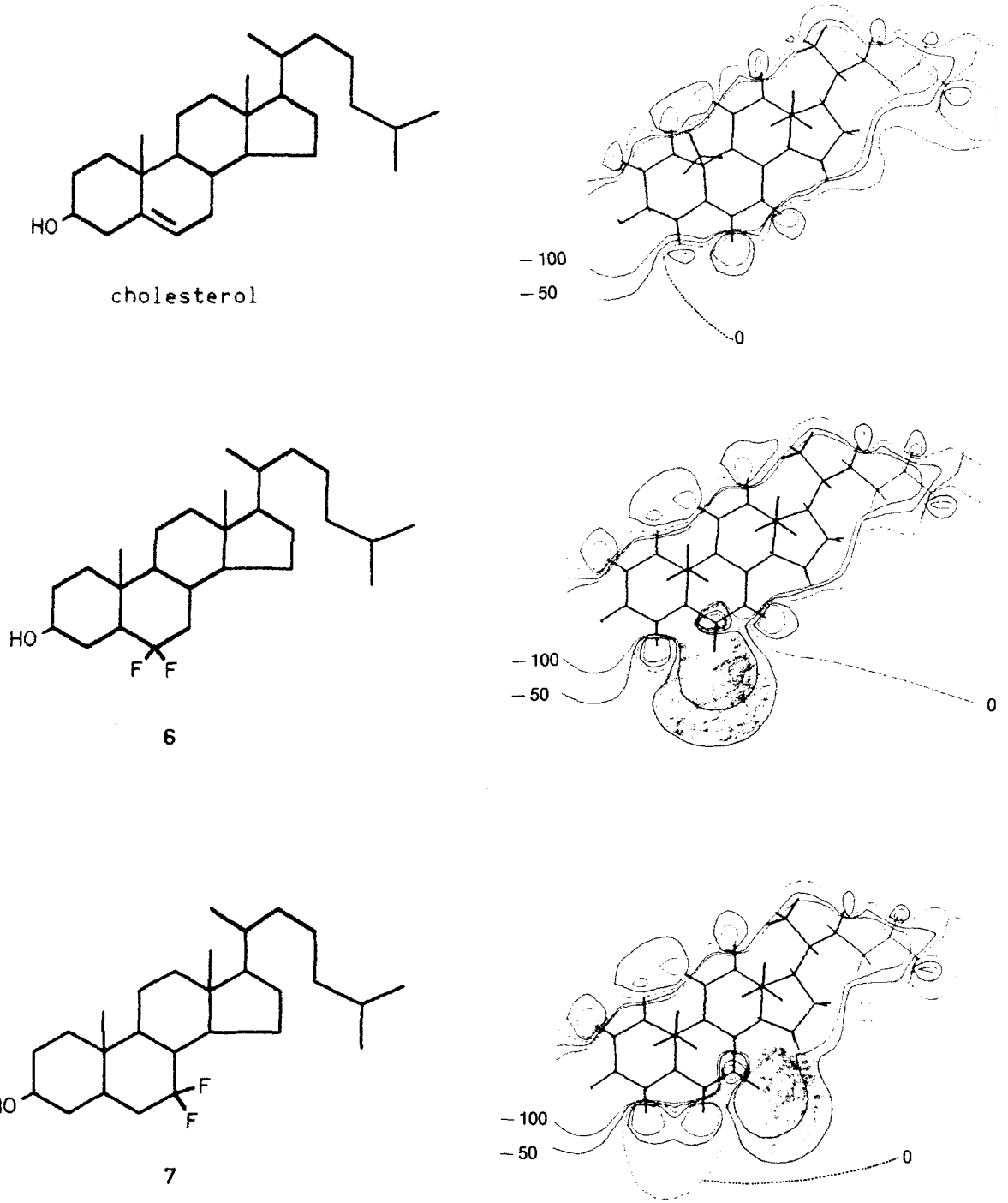
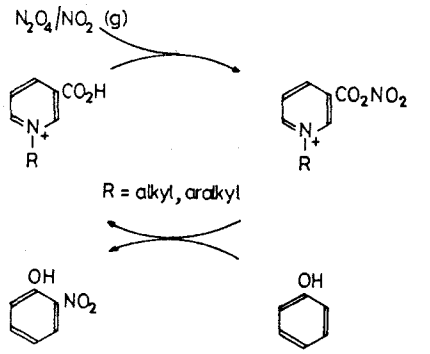


Figure 6. Cholesterol and fluoro-analogues: structures and potential maps. Calculations were carried out using semi-empirical methods including parametrisation for fluorine. The contours are shown at 100, 50, 0, – 50 and – 100 kJ mol^{-1} in the mean plane of the steroid ring system.

enzymes although the less discriminating bilayer membrane model did not distinguish between the two.

To obtain some insight into the basis of this molecular recognition phenomenon, we again consulted the electrostatic potential surfaces of the molecules (fig. 6; P. Bladon and C. J. Suckling, unpublished). No conclusions can be drawn from these results, but they do indicate how the two fluorosteroids may differ significantly from each other in the position of the negative potential associated with the fluorine atoms. As shown in figure 6, the 6,6-isomer shows negative potential in the region of the π-bond of cholesterol, although to a greater extent. On the other hand, the 7,7-isomer's negative potential bulge is substantially displaced around the 'bay' of ring

B of the steroid skeleton and quite different from that of cholesterol. It appears that this difference can be recognised by two different cholesterol-transforming enzymes which both reject the 7,7-isomer **7**. This result poses major questions about the way in which enzymes bind non-polar molecules such as cholesterol with the high specificity required for its selective transformation. It is also interesting to enquire which of the fluorines, 7α or 7β, or perhaps both, is responsible for the rejection of the 7,7-isomer.



Biomimetic synthesis

The replication of enzymic selectivity in small molecule chemistry was a goal of Fischer in the late 19th century. He lacked the technology to succeed despite having the correct concepts. In the late 20th century, however, the technology of organic synthesis is well up to the challenge of asymmetric reactions. Many such reactions have been described and most have been based upon the chirality of natural products or upon the discovery of chiral ligands such as binaphthyl derivatives. A second stream of work has mimicked the properties of enzymes by setting up binding sites in small molecules into which substrates can bind in a specific orientation leading to a selective reaction. Examples of such host-guest reactions have been indicated earlier in these articles; highly refined molecules were used to generate selective effects. In contrast, at Strathclyde we were interested in devising biomimetic systems sufficiently simple to be of potential in industrial applications. Surprisingly we found that, under suitable conditions, a molecule as small as 2-pyridone was capable of supporting a selective biomimetic reaction. As will be described, the mechanism of selectivity probably relies upon a donor-acceptor charge transfer type interaction together with a specific hydrogen bond.

Arising from earlier attempts to mimic the selective hydroxylation of aromatic and aliphatic hydrocarbons by cytochromes P-450, we discovered that micellar systems were capable of controlling the orientation of the chlorination of phenol[17,22]. This reaction could be understood in terms of the preferred orientation of phenolic substrates dissolved in micellar solution, a molecular arrangement that was consistent with NMR data[22]. The optimum selectivity was obtained when a functional group capable of forming a chlorinating agent, a tertiary hypochlorite, was included in the micelle-forming surfactant. Although this reaction was highly selective, there were many problems associated with its use preparatively, especially in work-up. During the NMR studies, we had observed an especially strong charge transfer interaction between pyridinium head groups of surfactants and phenols. This led to the idea that such an association could be the basis of a simple biomimetic reaction. Because of the environmental and operational problems

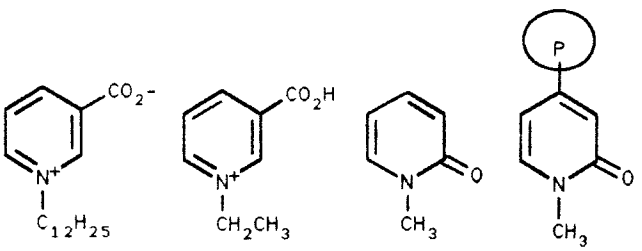


Figure 7. Biomimetic transfer nitration by pyridinium salts.

associated with aromatic nitration, we investigated the selective nitration of phenols[18].

The concept was to introduce a 'nitronium' carrier into the pyridinium salt so that in a reaction complex, the electron-rich phenol associated with the pyridinium salt would be drawn close to, and hopefully selectively, towards the reagent. This hope was realised initially using the surfactant pyridinium salt **8** (fig. 7) but it was quickly possible to show that small alkyl chains are sufficient, as in **9**. The reaction proceeded selectively to afford 2-nitrophenol as principal (> 98%) product under optimum conditions. At its simplest, 1-methyl-2-pyridone **10** was used as a selective nitrating agent carrier and this reagent was also established in polymer-supported form by derivatising a styrene-4-vinylpyridine copolymer **11**[18]. Using the polymer, selective nitration of phenol could be carried out simply by passing nitrogen dioxide into a suspension of the polymer in dichloroethane, filtering, washing, and resuspending the polymer in dichloroethane containing phenol. After filtration and evaporation, crystalline 2-nitrophenol was isolated in 95% yield and 98% purity by glc. No enzyme is known that catalyses aromatic nitration, let alone selectively. Unfortunately, however, the reaction was selective only for phenol itself; substituted phenols, whilst undergoing smooth nitration, afforded mixtures of products. Evidence was obtained from NMR and IR studies of N-ethyl-2-methoxypyridium methosulphate as an unreactive model for the pyridone-based nitrating system that a hydrogen bond between the phenolic hydroxyl group and the reactive group was a plausible mechanism for the

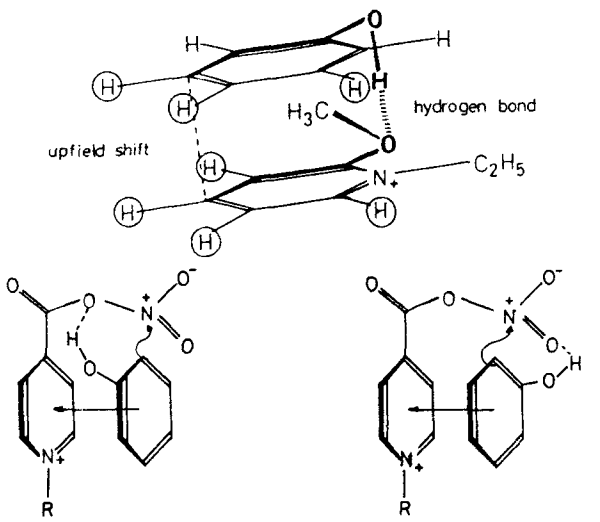


Figure 8. The probable mechanism of transfer nitration deduced from spectroscopic studies.

selectivity (fig. 8). It seems that the pyridinium-phenol interaction is disturbed with substrates other than phenol and several orientations are possible. In concluding this discussion, it is worth remarking that the concept of building binding sites into reagents is an effective way of stirring up new chemical surprises. In parallel with this work, we were investigating selective hydrogenation systems based upon micelle-forming structures; micellar hydrogenation catalysts were not discovered but a new type of palladium complex, apparently oligomeric, capable of selectively hydrogenating alkynes in the presence of alkenes and other reducible functional groups was discovered [14].

New catalytic proteins

The use of enzymes as catalysts in synthesis has now become widely accepted since it was first promoted strongly in 1974 [21]. Most of the reported experiments have used naturally occurring enzymes, either isolated from common organisms or tissues, or selected from microorganisms that have unusual biosynthetic pathways [23]. The inevitable urge of the chemist to create his own catalysts has nevertheless led to attempts to modify enzymes by all available technologies to obtain catalysts with specified properties. For example, site-directed mutagenesis has been used on lactate and dehydrogenase with some success [15]. However, to satisfy the chemist's creative urge fully, more must be done than simply extending by modification what was possible with nature's catalysts. Protein-based catalysts that promote reactions for which no naturally occurring enzymes are known must be invented. To attempt such a design, it is necessary to have a means to obtain a protein with specific binding properties and to include within it the required chemical reactivity. Since protein amino acids are rich in nucleophilic side chains but poor in electrophilic ones, catalysis of reactions involving carbanions, and oxidation-reduction reactions will require cofactors, as is the case with enzymes. The technologies that have been investigated for these purposes include imprinted proteins [4] and catalytic antibodies [2] for the protein component, and chemically modified enzymes and proteins for the cofactor [13]. Examples of the last two have been studied at Strathclyde in the past 3 years.

Our first successful experiments in this field were attempts to modify papain by introducing cofactors capable of mediating useful synthetic transformations by alkylation of the active site thiol following Kaiser [13]. The preparation of chiral acyloins was one target and accordingly, a thiazolium salt was introduced to the active site to give thiazolopapain (fig. 9; S. Onyiriuka and C. J. Suckling, unpublished). We were unable to establish any useful synthetic transformations of substrates such as aliphatic and aromatic aldehydes using this protein but the decarboxylation of pyruvate to acetaldehyde was

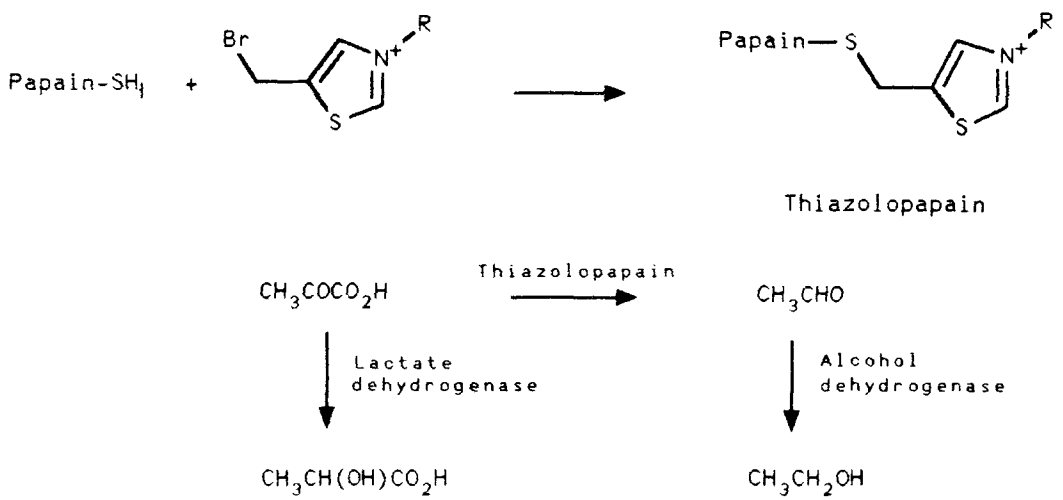


Figure 9. The preparation and reaction of thiazolopapain.

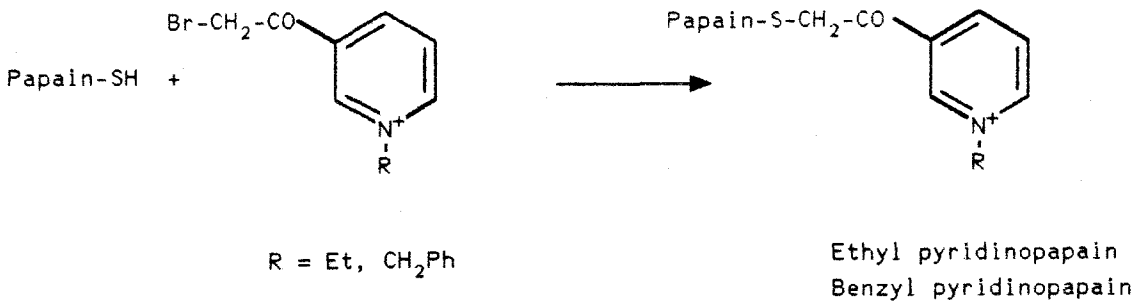


Figure 10. The preparation of pyridinopapains.

catalysed. It was not possible to obtain improved thiazolium-containing proteins using alternative modifying reagents bearing bromoethyl substituents; it seems that an activated halogen compound is necessary to modify papain under mild conditions. Following this argument, we have introduced pyridinium salts bearing bromomethyl ketones into papain and thereby generated proteins with a redox cofactor covalently bound. This protein, pyridinopapain, has been found to undergo reduction to the 1,4-dihydropyridine with sodium dithionite in aqueous solution; the reducing agent thus produced was also capable of reducing ethyl pyruvate to ethyl lactate in good yield. However, optimum conditions for recycling the covalently bound cofactor and the chirality, if any, of the product, have still to be established (Fig. 10; Z. Limin and C. J. Suckling, unpublished).

The potential for producing proteins with selective catalytic properties has been revolutionised by the establishment of catalytic antibody technology. In its original embodiment, the concept followed enzymic catalysis in which the importance of transition state stabilisation by the enzyme has been emphasised. If, therefore, an antibody to a transition state analogue can be raised, it should catalyse the reaction appropriate to the particular transition state. Interestingly, the concept of transition state analogues has been important in the design of selective inhibitors; in this sense, our work on catalytic antibodies provides a strong experimental and conceptual connection between the two branches of biological chemistry studied at Strathclyde. However, transition state stabilisation is not the only way to view catalysis by enzymes. Attention can also be focussed on contributing functional groups, such as acid-base catalysts, or upon the simple requirement to bring the reactants together in the right place at the same time. We have been interested in obtaining antibodies of importance in synthetic reactions. The aspect of selectivity selected for study was the preparation of homochiral synthons.

The Diels-Alder reaction is a mainstay of organic synthesis because of its predictable stereochemical course. Thus the relative stereochemistry of several chiral centres can be established in one reaction. However a mixture of diastereoisomers will usually result if the product is asymmetric, and the aim of our work was thus to prepare a diastereoisomerically selective catalytic antibody. Figure 11 shows the approach to this goal using 1-acetoxybutadiene and an N-alkyl maleimide to give a tetrafunctional compound with three chiral centres established. The hapten **12** was used to raise the antibodies by standard protocols[1]. Evaluation of the catalytic properties of the polyclonal serum showed that there were antibodies present that catalysed the reaction with selectivity for the terminal substituent on the diene, that is with selectivity for the acetoxy group which was present in the hapten. In contrast, the antibodies were indifferent whether N-ethyl or N-benzyl maleimide was used as reaction partner. When the monoclonal antibodies were isolated, some antibodies were found that showed no catalysis above the control rate shown by an IgG raised to a different antigen. Conventional immunisation followed by hybridoma technology inevitably leads to many antibodies that bind the hapten, but not necessarily catalyse the reaction of interest. However a catalytic antibody clone was established and it showed the same trends as the polyclonal serum had. The results were more clear cut, catalysis of the order of 1,000-fold being found only with acetoxybutadiene as substrate; the reaction of neither methoxybutadiene nor penta-1,3-diene as substrates was catalysed. More important than catalysis in this example, however, is the question of the chirality of the product, a question that was being addressed as this article went to press.

In a second series of experiments, we were able to characterise two antibodies, one of which was catalytic, and the other binding, but non-catalytic[1]. The hapten contained an amino group that would be partly protonated under the conditions of raising the antibodies. Presumably in some antibodies, a carboxylate or other general base was introduced to respond to the positive charge on the hapten. The catalytic antibody hydrolysed the 4-nitrophenyl ester **13** at rates exceeding 10^6 times the uncatalysed rates under the same conditions (fig. 12). Other esters, including ethyl, phenyl, and ethane thiol were not hydrolysed. It is interesting that this unexpected result emphasises the broadness of concept with which one can approach the design of catalytic antibodies. Originally, ester hydrolyses were approached through phosphonate-containing haptens, that is traditional transition state analogues. It

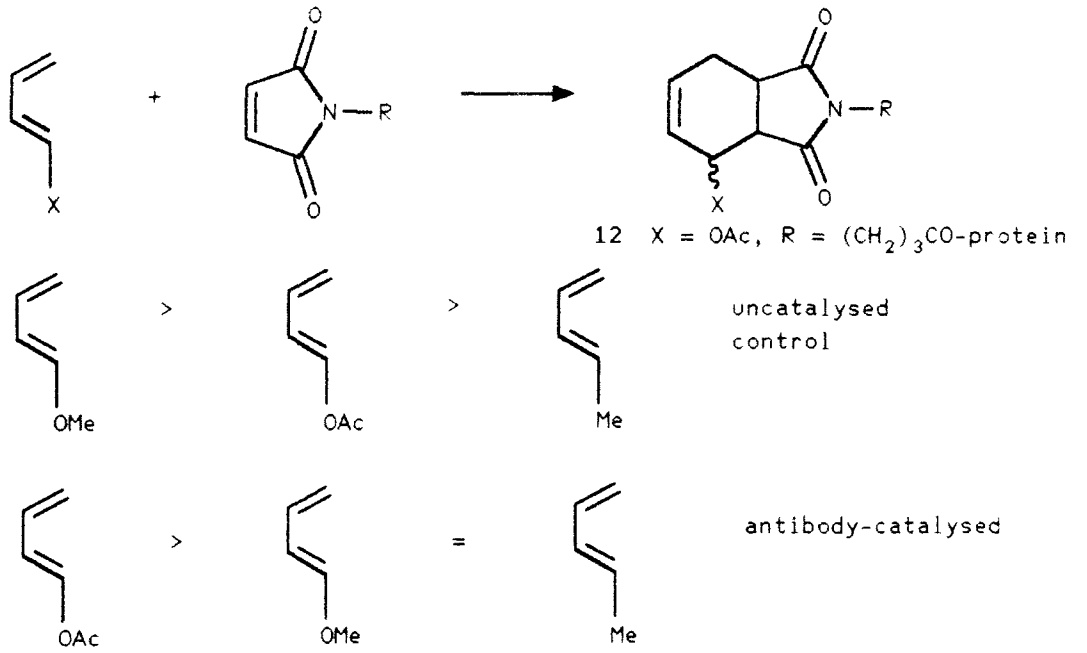


Figure 11. The Diels-Alder reaction catalysed by antibodies.

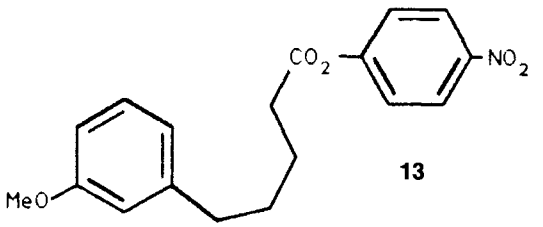


Figure 12.

is now clear that a similar effect can be obtained by specific introduction of a suitable functional group. Our antibodies raised to haptens that do not contain phosphonates are as efficient as many of those raised to phosphonates reported so far[2]. If two, or more, structural features relevant to catalysis can be incorporated into a hapten, it may be possible to achieve even higher rates of reaction. Nevertheless, our experience so far is that it is easier to inhibit an enzyme than to improve one or to generate a new one.

1 Bence, L., Khalaf, A., Proctor, G. R., Stimson, W. H., Suckling, C. J., and Tedford, M. C., in: Ciba Foundation Symposium 159, pp. 201–209. Wiley, Chichester 1991.
2 Blackburn, G. M., Kang, A. S., Kingsbury, G. A., and Burton, D. R., Catalytic antibodies (review). Biochem. J. *262* (1989) 381–390.
3 Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C., and Kraut, J., Crystal structures of *E. coli* and *L. casei* dihydrofolate reductase refined at 1.7 A resolution. J. biol. Chem. *257* (1982) 13650–13662.
4 Braco, L., Dabulis, K., and Kilbanov, A. M., Abiotic receptors by molecular imprinting of proteins. Proc. natl. Acad. Sci. USA *87* (1990) 274–277.
5 Brown, L., Lyall, W. J. S., Suckling, C. J., and Suckling, K. E., The synthesis of some cholesterol derivatives as probes for the mechanism of cholesterol metabolism. J. chem. Soc., Perkin Trans. *1* (1987) 595–599.
6 Buntain, I. G., Suckling, C. J., and Wood, H. C. S., Irreversible inhibition of dihydro-orotate dehydrogenase by hydantoins derived from amino acids. J. chem. Soc., Perkins Trans. *1* (1988) 3175–3182.
7 Cameron, R., Nicholson, S. H., Robinson, D. H., Suckling, C. J., and Wood, H. C. S., Syntheses of some functionalised 7,7-dialkyl-7,8-dihydropterins. J. chem. Soc., Perkins Trans. *1* (1985) 2133–2143.
8 Fraser, W., Suckling, C. J., and Wood, H. C. S., The inhibition of dihydro-orotate dehydrogenase by spirocyclopropanobarbiturates. J. chem. Soc., Perkins Trans. *1* (1991).
9 Haddow, J., Suckling, C. J., and Wood, H. C. S., Latent inhibition of dihydrofolate reductase by a pteridine cyclopropane. J. chem. Soc., Perkins Trans. *1* (1989) 1297–1304.
10 Howell, E. E., Villafranca, J. E., Warren, M. S., Oatley, S. J., and Kraut, J., The functional role of aspartic acid-27 in dihydrofolate reductase revealed by mutagenesis. Science *231* (1986) 1123–1128.
11 Howie, C., Suckling, C. J., and Wood, H. C. S., The inhibition of dihydro-orotate dehydrogenase by subsituted 5-benzylhydantoins. J. chem. Soc., Perkins Trans. *1* (1990) 3129–3135.
12 Hudson, A. T., Antimalarial hydroxynaphthoquinones, in: Topics in Medicinal Chemistry, pp. 266–283. Ed. P. R. Leeming. Royal Society of Chemistry, London 1988.
13 Kaiser, E. T., Catalytic activity of enzymes modified at their active sites. Angew. Chem. Int. Edn. Engl. *27* (1988) 913–922.
14 Kerr, J. M., Suckling, C. J., and Bamfield, P., Selective hydrogenation by a novel palladium(II) complex of salicylethylenediamine. J. chem. Soc., Perkins Trans. *1* (1990) 887–895.
15 Luyten, M. A., Bur, D., Wynn, H., Parris, W., Gold, M., Friesen, J. D., and Jones, J. B., An evaluation of the substrate specificity, and of its modification by site directed mutagenesis, of the cloned L-lactate dehydrogenase from *Bacillus stearothermophilus*. J. Am. chem. Soc. *111* (1989) 6800–6804.
16 McGill, J., Rees, L., Suckling, C. J., and Wood, H. C. S., Mechanism based inhibitors of dihydrofolate reductase, in: Pteridines and Folic Acid Derivatives, pp. 734–739. Eds C. H. Curtius and S. Ghisla. de Gruyter, Berlin 1990.
17 Onyiriuka, S. O., Suckling, C. J., and Wilson, A. A., The effect of functionalised and simple detergents upon the chlorination of phenol. J. chem. Soc., Perkins Trans. *2* (1983) 1103–1107.
18 Pervez, H., Onyiriuka, S. O., Rees, L., and Suckling, C. J., The nitration of phenols by pyridine derivatives carrying a transferable nitro group. Tetrahedron *44* (1988) 4555–4568.

19 C. J. Suckling, in: Biotechnology in Health Care and Medicine, NATO Advanced Research Workshop. Eds H. C. Alderweireldt and U. Pandit. Plenum Press, (1991) in press.
20 Roberts, G. C. K., NMR and mutagenesis studies of dihydrofolate reductase, in: Chemistry and Biology of Pteridines 1989, pp. 681–693. Eds H.-Ch. Curtius, S. Ghisla and N. Blau. de Gruyter, Berlin 1990.
21 Suckling, C. J., and Suckling, K. E., Enzymes in organic synthesis, Chem. Soc., Rev. *3* (1974) 387–406.
22 Suckling, C. J., and Wilson, A. A., ^{1}H studies of the orientation of aromatic molecules by surfactant micelles. J. chem. Soc., Perkins Trans. *2* (1981) 1616–1620.
23 Suckling, C. J., Selectivity in synthesis, chemicals or enzymes? in: Enzyme Chemistry Impact and Applications, pp. 95–170. Ed. C. J. Suckling. Chapman and Hall, London 1990.
24 Suckling, C. J., The cyclopropyl group in studies of enzyme inhibition and metabolism. Angew. Chem. Int. Edn. Engl. *27* (1988) 537–552.
25 Suckling, K. E., and Stange, E. F., The role of acyl-coenzyme A: cholesterol acyl transferase in cellular cholesterol metabolism. J. Lipid Res. *26* (1985) 647–671.
26 Suckling, K. E., Jackson, B., Suffolk, R. A., Houghton, J. D., and Suckling, C. J., Effects of 6,6-difluorocholestanol and 7,7-difluorocholestanol on hepatic enzymes of cholesterol metabolism. Biochim. biophys. Acta *1002* (1989) 401–404.
27 Thibault, V., Koen, M. J., and Gready, J. E., Enzymic properties of a new mechanism-based substrate for dihydrofolate reductase. Biochemistry *28* (1989) 6042–6049.
28 Umicharu, T., Tsuki, S., Tanabe, K., Benkovic, S. J., Furukawa, K., and Taira, K., Computational studies on pterins and speculation on the mechanism of dihydrofolate reductase. Biochem. biophys. Res. Commun. *161* (1988) 64–68.
29 Weisher, R. E., and Bristol, J. A., Selective inhibitors of phosphodiesterases, in: Comprehensive Medicinal Chemistry, vol. 2, pp. 505–509. Ed. P. G. Sammes. Pergamon Press, Oxford 1990.

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Molecular recognition: Models for drug design

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Abstract. The review takes examples, mostly from the recent literature, to illustrate how an understanding of physico-chemical properties and an appreciation of the molecular shape and electronic properties can lead to a better insight into molecular recognition processes. The techniques used to generate 3-dimensional structures of molecules and the influence this information has had on the drug design cycle, are briefly discussed.
Key words. Molecular recognition; modelling; NMR; structure; X-ray.

Introduction

The propagation of all life forms is dependent upon the recognition/response phenomenon. As described in other contributions to this multi-author review, molecular recognition plays a central role throughout the biological systems. It is the basis of the specificity seen during neurotransmitter response, cell cell, antigen antibody, substrate enzyme, and hormone receptor interactions. It is not a new concept, having occupied the minds of notable 19th century scientists like Pasteur and Ehrlich. Indeed, Ehrlich is widely credited with developing the concept of the pharmacophore. In a paper in 1900[25], he reasoned that toxins possessed two different combining groups, a hepatophore that bound the toxin to the cell and a toxophore that was responsible for the toxic action. Today, an acceptable definition of a pharmacophore would be that part of a molecule which contains a 3-dimensional pattern that exactly complements the shape and surface properties of the target protein.

The modern image of molecular recognition has developed as a direct result of the accessibility of 3-dimensional structural information and the means to visualise and interact with these structures. In 1965, Perutz and his colleagues published the first 3-dimensional picture of a protein structure[61], haemoglobin. This pioneering work on hemoglobin and subsequent work on related proteins led to an explanation, in molecular terms, of the way oxygen was transported in the blood stream. Even these early pictures were relevant to medicine because they provided a simple interpretation into the cause of the disease sickle cell anaemia. Haemoglobin is a tetramer consisting of two α chains, each of 141 amino acids, and two β chains, each of 146 amino acids. It is an allosteric protein in equilibrium between the oxy (R) structure and the deoxy (T) structure. The cause of the disease sickle cell anaemia can be traced to the 'natural' mutation of two glutamate residues to valine. The effect of these two mutations is to cause a disruption to the R/T equilibrium, in favour of the T-form, by creating a surface hydrophobic region which promotes the linear aggregation of haemoglobin and sickling of red blood cells. At least two approaches in drug design to alleviate this condition can be envisaged. First, compounds designed to alter the allosteric equilibrium in favour of the more soluble R-form, and second, compounds designed to alter the T-form in such a way as to destabilise the intermolecular interactions that lead to aggregation. Perutz[62] has pub-