Computer-aided Substrate Design for Biocatalysis: An Enzymatic Access to Optically Active Propranolol

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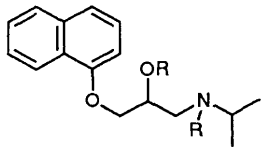
Computer-aided molecular modelling was used to examine the conformations of various propranolol derivatives, which has led to a facile enzymatic preparation of optically active propranolol.

A major impediment to the development of asymmetric biocatalysis has been the difficulty in perceiving the structure–selectivity relationships of enzymatic reactions.[1] Consequently, current practice of conducting biochemical resolutions requires a preliminary search for biocatalysts with useful enantioselectivity. Generally speaking, the probability of obtaining a discriminative process often depends upon the size of the enzyme–microorganism pool accessible to researchers. In particular, tedious evaluation of a large number of microbial agents is often necessary for sterically hindered molecules. Thus, in the course of exploring an efficient screening method, we turned to computer-assisted molecular modelling to examine structural requirements for enzymatic turnover and enantiomeric differentiation. In this communication, we report a successful case of applying computer analysis to the enzymatic resolution of propranolol **1**.

Although optically active **1** has been the focus of many asymmetric[2] and chemo-enzymatic[3] syntheses, reports on the direct enzymatic resolution of (±)-**1** are still lacking,[4] which prompted us to use this molecule as the target compound. In our preliminary experiment, *N,O*-diacetylpropranolol (±)-**2** was subjected to enzymatic hydrolysis. Among various proteases, lipases and esterases† tested, none of the enzymes was capable of effecting the deacylation of **2**, which is in

† Proteases included chymotrypsin, thermolysin and proteases from *Aspergillus oryzae, Aspergillus sojae, Aspergillus satoi, Rhizopus* sp. and *Streptomyces caeapitosus*. Lipases included crude lipase preparations from porcine pancrease, *Candida cylindracea, Aspergillus niger, Geotrichum candidum, Humicola lanuginosa, Mucor meihei, Pseudomonas* sp., *Rhizopus niveus* and *Rhizopus oryzae*. Esterases included pig liver esterase and cholesterol esterase.



1 R = H
2 R = C(O)Me
3 R = C(O)OMe
5 R = $C(O)CH_2Cl$
6 R = $C(O)CCl_3$

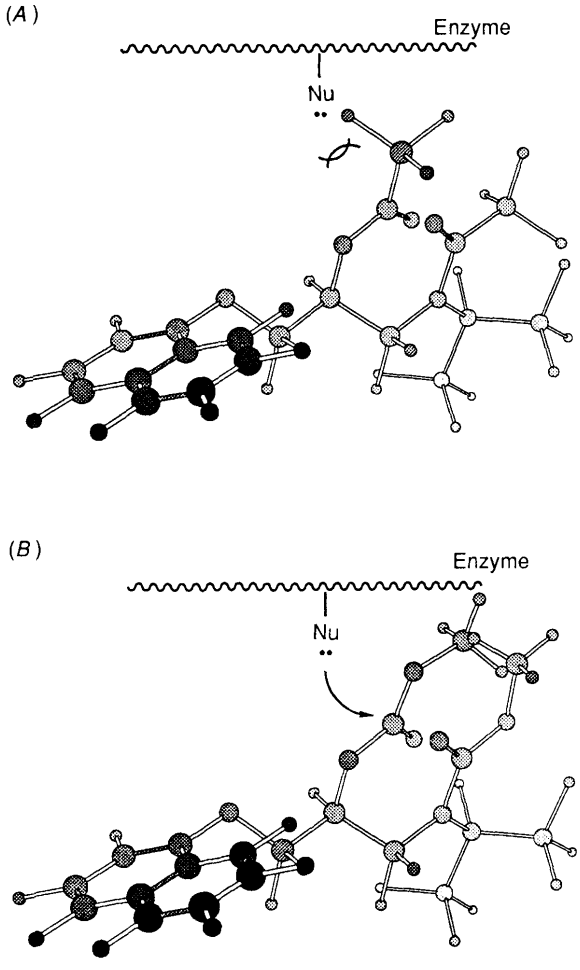
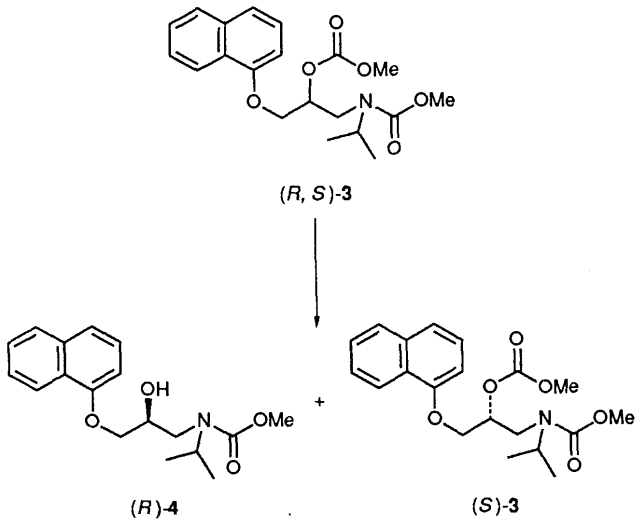


Fig. 1 Computer-generated diagrams of (*A*) *N,O*-diacetylpropranolol **3**, and (*B*) *N,O*-bis(methoxycarbonyl)propranolol **4**, Nu represents the nucleophilic residue (*e.g.* serine or cysteine) in the catalytic site

agreement with the results obtained by other groups.[5] Conceivably, the resistance to enzymatic cleavage stems from the intrinsic steric hindrance by the functional site. In principle, this steric congestion may be alleviated by modifying the acyl side chain to facilitate enzymatic hydrolysis. Here, we describe a computer-assisted strategy to achieve this goal by analysing the conformations of the parent molecule and its derivatives even though the tertiary structure of the biocatalytic domains remains unclear. Essentially, this approach allows one to envisage the substituent–conformation relationships and consequently provides a viable approach to identify potential substrates.

In our initial study, a computer graphic display of the possible conformation of **2** was drawn.[6] Subsequent refinement through molecular mechanics calculations (Allinger's



Scheme 1

Table 1 Enantioselective hydrolysis of (±)-**3** by esterases (Scheme 1)[a]

Esterase	Stereochemical preference	Conversion (%)	Enantiomeric excess (ee)		
			4	**3**	*E*[c]
Pig liver	*R*	35	0.11	0.06	1.3
Cholesterol[b]	*R*	38	0.56	0.35	5.0
Porcine pancreatic	*R*	40	0.83	0.55	19

[a] In a typical experiment, racemic **3** (375 mg), dissolved in dimethylformamide (0.7 ml), was introduced to 0.1 mol dm^{-3} potassium phosphate buffer (37.5 ml) containing 0.5% Tween 80. To the mixture was added 375, 37.5, and 500 units of PLE, CE, and PPE, respectively. One unit of PPE is that amount of enzyme catalysing the hydrolysis of 1 μmol of *p*-nitrophenyl acetate per minute at 25 °C. PLE and CE were obtained from Sigma, and the enzyme units were defined accordingly. The incubation times ranged from 48 to 96 h. [b] Both porcine and bovine cholesterol esterases exhibited the same selectivity. [c] The enantiomeric ratio (*E*) is calculated from $E = \ln\{1 - C[1 + ee(P)]\}/\ln\{1 - C[1 - ee(P)]\}$ where C is the conversion and ee(P) is the ee value of product. See ref. 7.

MM2 version) gave rise to the energy-minimized structure of **2** (Fig. 1*A*) from which factors contributing to the resistance to enzymatic hydrolysis might be delineated. In view of Fig. 1*A*, it appeared that the methyl group of the *O*-acetate might prevent the access of the nucleophilic residue in the enzyme pocket to the carbonyl group. Presumably, this problem may be circumvented either by reducing the size of the acyl moiety or by removing the methyl group away from the nucleophilic residue. Based on this notion, a number of structural variants of **2** were designed and subjected to computer graphic analysis. Especially noteworthy is the *N,O*-bis(methoxycarbonyl) derivative **3**‡ whose energy-minimized conformation (Fig. 1*B*) shows reduced steric hindrance by the carbonyl function as compared with **2**. Thus, (±)-**3** was prepared and exposed to various enzymes. In line with our supposition, the racemic compound was readily hydrolysed by several esterases to yield (*R*)-*N*-methoxycarbonylpropranolol, (*R*)-**4**, with different levels of enantiomeric discrimination (Table 1).

‡ Compound **3** was prepared by treating **1** with 10 equiv. of dimethyl pyrocarbonate in the presence of *N,N*-dimethylaminopyridine.

As shown, the esterase preparation from porcine pancreas§ gave the highest selectivity among several enzymes which have been examined. It should be noted that this enzyme is distinct from porcine pancreatic cholesterol esterase which exhibited a lower degree of stereoselectivity ($E = 5$).

Nonetheless, one valid argument may arise regarding the rate enhancement, *i.e.*, it may be caused by the electron-withdrawing nature of the methoxy moiety rather than the steric relaxation effect. To clarify this speculation, activated esters such as the *N,O*-bis(chloroacetyl) and *N,O*-bis(trichloroacetyl) derivatives **5** and **6**, respectively were also examined. Both compounds were similar to **2** with regard to energy-minimized conformation and degree of steric congestion (not shown). However, after being exposed to the enzymes, **5** and **6** did not show any sign of hydrolysis. This result thus confirmed the conclusion derived from our molecular-modelling study. In addition to **3**, several other analogues have also been suggested as potential substrates, and are currently under investigation.

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§ Porcine pancreas acetone powder (Sigma, 10 g) was suspended in 30 ml of 50 mmol dm^{-3} potassium phosphate buffer (pH 7.0), and subjected to homogenization. The tissue debris was removed by centrifugation at 12000 × g for 20 min, and the supernatant was applied to a DEAE-Sepharose CL-6B column (5 × 10 cm) equilibrated with 10 mmol dm^{-3} potassium phosphate buffer (pH 7.0). The column was first washed with 360 ml of the equilibrating buffer, and then eluted with a linear gradient (600 ml) consisting of 0 to 0.5 mol dm^{-3} NaCl in the same buffer. Fractions of 8 ml were collected. The esterase activity was assayed using *p*-nitrophenyl acetate as the substrate. Fractions 94 to 124, which contained the esterase, were collected, and the pooled solution was used directly for the enzymatic reaction. The synthetic utility of this enzyme is currently under investigation.

References

1 C. J. Sih, Q. M. Gu and D. R. Reddy, in *Trends in Medicinal Chemistry*, ed. E. Mutschler and E. Winterfieldt, VCH, New York, 1987, p. 181.
2 J. E. Backvall, E. E. Bjorkman and S. E. Bystrom, *Tetrahedron Lett.*, 1982, **23**, 943; T. Katsuki, *Tetrahedron Lett.*, 1984, **25**, 2821; S. Miyano, L. D.-L. Lu, S. M. Viti and K. B. Sharpless, *J. Org. Chem.*, 1985, **50**, 4350; J. M. Klunder, Y. K. Soo and K. B. Sharpless, *J. Org. Chem.*, 1986, **51**, 3710.
3 Numerous preparations of (2*S*)-propranolol which relied upon the elaboration of enzymatically prepared precursors have been reported. For examples, see: N. Matsuo and N. Ohno, *Tetrahedron Lett.*, 1985, **26**, 5533; C. Fuganti, P. Grasselli, P. F. Seneci and S. Servi, *Tetrahedron Lett.*, 1986, **27**, 2061; Y. Terao, M. Murata and K. Achiwa, *Tetrahedron Lett*, 1988, **29**, 5173; Y. F. Wang, S. T. Chen, K. Liu and C. H. Wong, *Tetrahedron Lett.*, 1989, **30**, 1917.
4 Our literature search indicates that there are only two microbial processes covered by Japanese patents. Jpn. Kokai Tokyo Koho JP 62 151, 196; Jpn. Kokai Tokyo Koho JP 63 94,992. (*Chem. Abstr.*, 1988, **108**, 110832d; 1989, **110**, 93558r).
5 G. Kirchner, M. P. Scollar and A. M. Klibanov, *J. Am. Chem. Soc.*, 1985, **107**, 7072; C. J. Sih, personal communication.
6 Chem3D plus program was used throughout the study.
7 C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294.