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Asymmetric Reduction of Alkyl Aryl Ketones in the Binding Domain of Bovine Serum Albumin

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The asymmetric reduction of alkyl aryl ketones with sodium borohydride ($NaBH_4$) or sodium cyanoborohydride ($NaBH_3CN$) in the presence of bovine serum albumin (BSA) was studied. The asymmetric induction in the product alcohols was largely dependent on the BSA concentration and the pH value of the buffer solution. The maximum optical yields obtained ranged over 14–78%, varying with the alkyl and aryl substituents, using ca. $\frac{1}{3}$ molar eq of BSA to the ketone and at pH > 9. With two exceptions, the predominant enantiomer had the *R* configuration in 13 ketones investigated.

INTRODUCTION

During the last decade asymmetric synthesis has been of outstanding importance in organic chemistry as a result of the growing demand for optically pure materials in quantity by flavor, fragrance, and pharmaceutical industries (*1*). Therefore, organic chemists have paid much attention to the high stereoselectivity in enzyme-catalyzed reactions (*2*). It is most important for high stereoselectivity that strict chiral recognition is accomplished in a chiral environment of the enzyme active site (binding domain). In the field of chemistry there are some molecular aggregates and compounds with chiral binding sites capable of including guest compounds, e.g., cholesteric liquid crystals (*3*), chiral micelles (*4*), cholenic acid clathrate crystals (*5*), and inclusion compounds of cyclodextrins, etc. (*6*). However, the extent of observed stereoselectivity is generally very low when asymmetric reactions are conducted in these chiral binding sites. When compared with enzymes, obviously these systems are lacking structural rigidity to meet the stringent steric requirements for accommodating the substrate in an anacomeric transition state.

Although chemists dream of completely synthetic organic systems endowed with properties of high stereoselectivity and rate enhancement, they have to overcome a number of synthetic difficulties in order to reach the goal. On the other hand, naturally occurring globular proteins furnish a chiral environment in their binding domains, and so chemists might possibly construct a semiartificial enzyme with considerably less effort for modification. We chose a BSA protein as a candidate, because the structure and properties of this protein have been elaborately investigated by many researchers. Prior to any modification of

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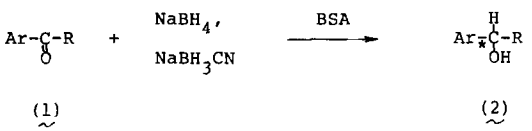
catalytic functions, one is obliged to understand the chiral characteristics of this binding domain in asymmetric reactions. This paper deals with asymmetric reduction of alkyl aryl ketones (**1**) by the use of achiral reducing agents, $NaBH_4$ and $NaBH_3CN$, in the binding domain (Scheme 1), which has proved to produce a strong asymmetric bias in the product alcohols (**2**) (*7*).



SCHEME 1

RESULTS

The asymmetric reduction of ketones was carried out at various BSA concentrations and in different pH's ranging over 3–11. First, at a given pH the effect of BSA concentration on the optical yield of alchol was examined. Figure 1 shows the results of asymmetric reduction of acetophenone (**1a**) and trifluoroacetophenone (**1b**) with $NaBH_4$ in a 0.01 *M* borate buffer solution (pH 9.2). The ketone **1a** (5.0 m*M*) was reduced with 2 molar eq of $NaBH_4$ in the presence of BSA to give predominantly the *R*-enantiomer of phenylmethylcarbinol (**2a**) in a quantitative chemical yield. The optical yield increased gradually with an increasing amount of BSA, reaching a constant value of 45% at 1.5–1.7 m*M* of BSA. The change of optical yield with respect to BSA concentration displayed a sigmoidal curve. This is contrary to the expectation that normal ketone binding of BSA should give a saturation change. This was also the case with **1b**. Particularly striking was the reduction of **1b** with $NaBH_3CN$ (Fig. 2). At a concentration below 0.8 m*M* the resulting phenyltrifluoromethylcarbinol (**2b**) had the *R* configuration.

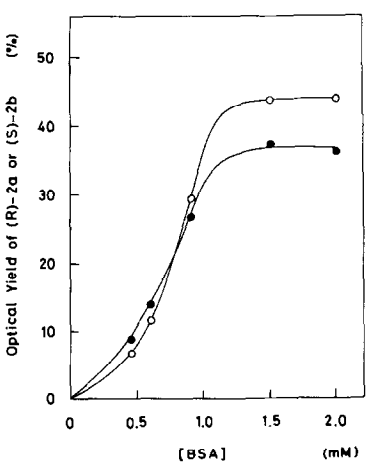


FIG. 1. The effect of BSA concentration on the optical yields of *R*-**2a** (○) and *S*-**2b** (●) in the reduction of **1a** and **1b** with $NaBH_4$ at pH 9.2.

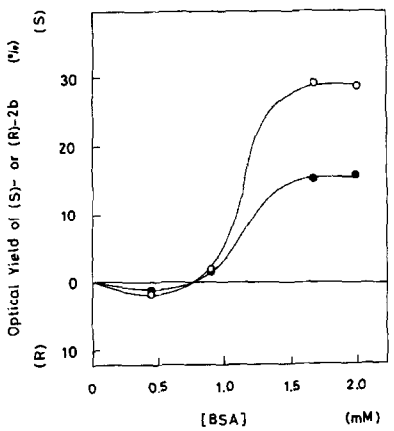


FIG. 2. The effect of BSA concentration on the optical yield of **2b** in the reduction of **1b** with $NaBH_3CN$ at pH 9.2 (○) and 5.6 (●).

On the other hand, the *S* counterpart was obtained in preponderance above this concentration, and then the optical yield increased with increasing BSA concentration. The maximum optical yield obtained was 29% at 1.5–1.7 m*M*. From the above experiments ca. $\frac{1}{3}$ molar eq of BSA was needed to produce maximum optical yields of alcohols. Also at pH's other than 9.2 a similar change of optical yield with respect to BSA concentration was observed. For example, the result in the reduction of **1b** with $NaBH_3CN$ at pH 5.6 is included in Fig. 2.

Next, we examined the pH effect on the optical yield of alcohol in the present system. Since the BSA exhibits a gross structural change at a different pH (*8*), a striking dependence of optical yield on pH was anticipated. The maximum optical yield of **2b** at each pH was plotted against pH of the buffer used in the reduction of **1b** with $NaBH_3CN$ (Fig. 3). This reduction was suitable for investigating the pH effect on asymmetric induction in the broad pH region from 3 to 11, because

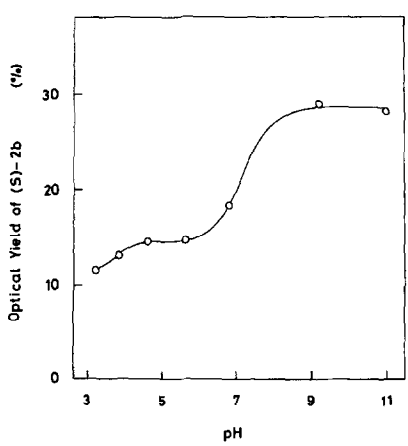


FIG. 3. The pH effect on the maximum optical yield of **2b** obtained in the reduction of **1b** with $NaBH_3CN$.

$NaBH_3CN$ is stable in this region and can smoothly react with **1b**, whose carbonyl is activated by the electron-withdrawing CF_3 group (*9*). Obviously the highest optical yield (29%) was obtained in the range of 9–11, but below pH 9 the yield decreased gradually with lowering of the buffer pH, declining to ca. 10% near pH 3. Within the above pH range the configuration of the predominant alcohol was *S*. In particular, striking changes in optical yield were observed near pH 6–9 and 3–4. This is not an exceptional case. The reduction of **1a** and **1b** with $NaBH_4$ also resulted in the best asymmetric induction in the pH range of 9–11, although the experiment was not realized in the region below pH 7 because of instability of this reducing agent.

In a similar manner alkyl aryl ketones other than **1a** and **1b** were reduced with $NaBH_4$ in the presence of BSA. In all cases the alcohols were obtained in quantitative yields, and the optical yields reached the maximum value when ca. $\frac{1}{3}$ molar eq of BSA was used in a buffer solution of pH > 9. The observed optical yields were in the range of 14–78%, varying with the alkyl and aryl substituents (Table 1). As can be seen from the table, in the reduction of a series of alkyl phenyl ketones, there is no definite trend of stereoselectivity by changing alkyl substituents. Nevertheless, higher stereoselectivities in general were observed when the substituents were the less branched and shorter alkyl groups. It should also be noted that the stereoselectivity was sensitive to changes in aryl substituent structures, e.g., **1a**: 45%, 2-acetonaphthone (**1j**): 65%, 2- and 3-acetylphenanthrenes (**1l**) and (**1m**): 21 and 20% in aryl methyl ketone reduction, respectively. The predominant enantiomeric alcohols possessed the *R* configuration, with two exceptions. The configuration of phenanthrenylmethylcarbinols [(**2l**) and (**2m**)] are not noted in the table since their configurations are unknown. However, a series of *R*-alkyl aryl carbinols was highly dextrorotatory, so that (+)-**2l** and (+)-**2m** may also reasonably be assigned the same *R* configuration. The (+)-**2b** and (+)-1- and 2-naphthyltrifluoromethylcarbinols [(**2i**) and (**2k**)] are configurationally correlated to the corresponding (+)-**2a** and (+)-1- and 2-naphthylmethylcarbinols [(**2h**) and (**2j**)], but the former is designated *S* and the latter *R* since CF_3 takes nomenclature preference to C_6H_5 in the Cahn–Ingold–Prelog system. It then follows that BH_4^- preferentially attacks the *re*-face of carbonyl group in all the ketones with exception for valerophenone (**1f**) and 1-trifluoroacetophenone (**1i**), where poor stereoselectivities are observed.

DISCUSSION

A number of studies have demonstrated that the BSA protein can bind a variety of hydrophobic compounds, including **1a** and the other aryl ketones (*10*). The structural study was done with a small-angle X-ray scattering method, which showed the presence of three analogous main binding domains (*11*). In agreement with the above observations, it is most likely that one protein molecule binds, at the most, three guest molecules into the binding domains, probably in the mode of one guest molecule per domain. The stringent stereochemical requirements of domains are conducive to an effective chiral recognition of the domain-included

TABLE 1

MAXIMUM OPTICAL YIELDS OF ENANTIOMERIC ALCOHOLS OBTAINED FROM THE REDUCTION OF 1[a] WITH $NaBH_4$[b] IN A 0.01 *M* BORATE BUFFER SOLUTION (pH 9.2) CONTAINING BSA[c] AT 25°C

	2[d]		
1	$[\alpha]_D^{25}$ (*c*, solvent)	Optical yield[e] (%)	Configuration
(a) Acetophenone	+20.3 (0.4, methanol)	45	*R*
(b) Trifluoroacetophenone	+5.4 (1.2, benzene)	36	*S*
(c) Propiophenone	+25.4 (0.8, ethanol)	78	*R*
(d) *n*-Butyrophenone	+9.3 (0.4, benzene)	27	*R*
(e) Isobutyrophenone	+31.8 (0.8, ether)	66	*R*
(f) Valerophenone	−4.4 (0.5, benzene)	14	*S*
(g) *tert.*-Butyl phenyl ketone	+7.6 (1.2, ether)	21	*R*
(h) 1-Acetonaphthone	+50.3 (0.5, ethanol)	67	*R*
(i) 1-Trifluoroacetonaphthone	−3.0 (1.7, chloroform)	16	*R*
(j) 2-Acetonaphthone	+27.0 (1.3, ethanol)	65	*R*
(k) 2-Trifluoroacetonaphthone	+12.5 (0.8, chloroform)	39	*S*
(l) 2-Acetylphenanthrene	+27.5 (0.3, chloroform)	21	—
(m) 3-Acetylphenanthrene	+15.9 (0.8, chloroform)	20	—

[a] 5.0 m*M*.

[b] 0.01 *M*.

[c] Maximum optical yields were obtained with the use of 1.5–1.7 m*M*.

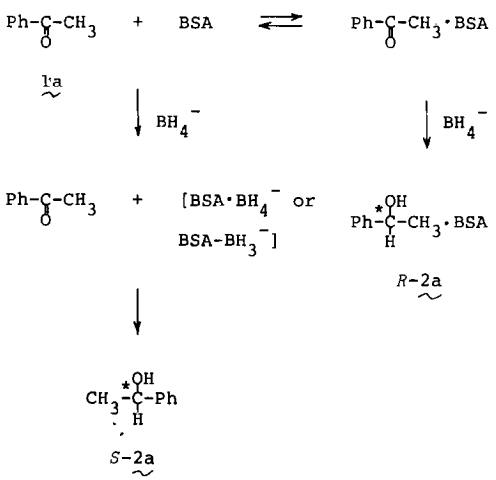
[d] Satisfactory spectral data were obtained for these alcohols.

[e] The optical yields were calculated from $[\alpha]_D$(obs)/$[\alpha]_D$(max) × 100 (%) and the values obtained in the table include an error of ±2%. The optical rotations and configurations of the optically pure alcohols were referred to the following reports: $[\alpha]_{Hg}^{25}$ +45.5° (methanol) for ***R*-2a** (R. Huisgen and Ch. Ruchardt, *Annalen* **601**, 31 (1956)); $[\alpha]_D$ +14.76° (benzene) for ***S*-2b** (H. M. Peters, D. M. Feigl, and H. S. Mosher, *J. Org. Chem.* **33**, 4245 (1968)); $[\alpha]_D^{r.t.}$ +32.48° (ethanol) for ***R*-2c** and $[\alpha]_D^{r.t.}$ +74.39° (ethanol) for ***R*-2h** (R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, 1115 (1914)); $[\alpha]_{Hg}^{24}$ +35.8° (benzene) for ***R*-2d** and $[\alpha]_{Hg}^{24}$ +31.3° (benzene) for ***R*-2f** (P. A. Levene and R. E. Marker, *J. Biol. Chem.* **97**, 379 (1932)); $[\alpha]_{Hg}^{25}$ +48.3° (ether for ***R*-2e** (D. J. Cram and J. E. McCarty, *J. Amer. Chem. Soc.* **79**, 2866 (1957)); $[\alpha]_{Hg}^{20}$ +36.2° (ether) for ***R*-2g** (R. MacLeod, F. J. Welch, and H. S. Mosher, *J. Amer. Chem. Soc.* **82**, 876 (1960)); $[\alpha]_D^{25}$ + 18.8° (chloroform) for ***S*-2i**, $[\alpha]_D^{25}$ + 32.2° (chloroform) for ***S*-2k**, $[\alpha]_D^{25}$ 130.8° (chloroform) for **2l**, and $[\alpha]_D^{25}$ 79.2° (chloroform) for **2m** (W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.* **89**, 5485 (1967)); $[\alpha]_D^{r.t.}$ +41.3° (ethanol) for ***R*-2j** (T. A. Collyer and J. Kenyon, *J. Chem. Soc.* 676 (1940)).

guest molecule with a reduced conformational mobility. Indeed, this has proved to be the case in the present asymmetric reduction, i.e., ca. $\frac{1}{3}$ molar eq of BSA to ketone is needed for the maximum optical yield of alcohols to be obtained, and the ketones included in the domains are asymmetrically reduced to the alcohols with high stereoselectivity. This asymmetric reduction however shows the great sensitivity of stereoselectivity to small changes in ketone structure; such a sensitivity would not be expected from experience with asymmetric reductions using chiral chemical reducing agents. This reflects the sensitive discrimination between enantiotopic faces of carbonyl group in the domains. In addition, the selectivity of BH_4^- attacking the enantiotopically discriminated carbonyl face must be considered. As expected, stereoselectivity markedly decreased when the

BSA was denatured to an unfolded random polymer by addition of 0.8 *M* urea (*12*). A similar observation was also made in the case of the protein whose binding domains were saturated beforehand with a good guest compound, naphthalene (*10c*) or sodium lauryl sulfate (*13*). For example, the reduction of **1a** at 0.6 m*M* of BSA gave the *R*-**2a** in 12% optical yield (see Fig. 1), but this diminished to 2% (*R*) in the protein denaturation and 1 (*S*) and 4% (*S*), respectively, in the presence of naphthalene and the surfactant.

Asymmetric induction does not always occur only in the binding domains when all the ketones are not completely included in the domains. As shown from a preliminary experiment, the binding of **1a** by BSA displayed a saturation curve, indicating that the three analogous domains are governed by a respective analogous binding constant.[1] Therefore, the observed optical yield of **2a** at a given BSA concentration is a sum of yields obtained at the respective domain, and should change in a saturation curve with respect to BSA concentration, if only the included ketones are asymmetrically reduced. This is not the case, however. In fact, a sigmoidal curve was obtained (Fig. 1). This discrepancy can be reasonably interpreted by assuming that the nonincluded ketones were asymmetrically reduced as well to give the enantiomeric alcohol of the opposite *S* configuration to the *R* counterpart obtained in the domains (Scheme 2). This is supported by the



SCHEME 2

fact that the *S*-**2a** is produced in preponderance from the reduction in the presence of a competitive inhibitor of naphthalene or the surfactant. The BSA concentration dependence of optical yield of **2b** can also be explained in terms of the

[1] The ketone **1a** (5.0 m*M*) was added to a buffer solution containing BSA at various concentrations in the range of 0–2.0 m*M*, and the mixture was gently stirred for several hours at 25°C. Then, the mixture was subjected to separation of the nonincluded ketone and the included ketone by equilibrium dialysis at 25°C. The nonincluded and included ketones were isolated by ether extraction, and the respective amount was determined by glc. The plot of the included ketone concentration against the BSA concentration seemingly showed a saturation curve. Nevertheless, this method is questionable as a definite determination of binding constant.

simultaneous production of enantiomeric alcohols with an opposite configuration inside and outside the domains. The BH_3CN^- reduction is remarkable in that the asymmetric induction on the outside is predominant over that on the inside at the low BSA concentration below 0.8 m*M*. However, as the BSA concentration increases, the nonincluded ketones on the outside sharply decrease and the asymmetric induction becomes less significant. The asymmetric reduction of the other ketones outside the domains was investigated, and this asymmetric induction was shown to take place in a highly stereocontrolled manner with preferential *si*-face attack of BH_4^-. In the most probable scheme for asymmetric induction BH_4^- reacts with amino acid residues of BSA to generate a chiral reducing agent such as a BSA · BH_4^- ion pair or a covalently bonded BSA–BH_3^-, which in turn reduces the nonincluded ketones into the chiral alcohols. A similar type of chiral reducing agent can be prepared from $NaBH_4$ with chiral aminocarbinols and monosaccharide derivatives, which have been used extensively for the production of chiral alcohols (*1, 14*).

The remarkable pH dependence of stereoselectivity is another characteristic of this asymmetric reduction. This can be visualized in the reduction of **1b** with $NaBH_3CN$ (Fig. 3). The stereoselectivity tends to decrease with lowering of the pH. In particular, dramatic changes occur near pH 3–4 and 7–9. These pH's are parallel with those of the conformational changes in gross protein structure. The structural changes are called N–F (*8a*) and N–B (*8b*) transitions, respectively. That is, this protein exists in an F-form structure below pH 3, in an N-form structure in the pH range of 4–7 and in a B-form structure above pH 9. The gross structural changes also have influence on the structure in the neighborhood of the binding domains, accompanying the chirality change. For the present asymmetric reductions, the B-form structure is most desirable in order to achieve the highest stereoselectivity.

The present study proves that the BSA binding domain can induce a fairly strong asymmetric bias of the product alcohols in a stereocontrolled manner. Nevertheless, the noncatalytic nature of reaction puts the present system at an unavoidable disadvantage in synthetic utility, even if the demerit can be offset by the recycling of protein. Recently, Whitesides *et al.* (*15*) have prepared a chiral catalyst from an avidin protein and a diphosphinerhodium(I) complex, and investigated asymmetric hydrogenation of α-acylaminoacrylic acids by the use of this catalyst. Kaiser *et al.* attached a flavine group to hydroxylases such as α-chymotrypsin and papain, so as to achieve an oxidoreductase-type enzyme model (*16*). In the future, studies aiming at success in artificial enzyme models comparable with native enzymes will receive growing attention. Study of BSA modification with catalytic functions as an effective chiral catalyst is currently in progress in our laboratory.

EXPERIMENTAL

The nmr spectra were recorded with a Varian EM-360 spectrometer with tetramethylsilane as an internal standard and carbon tetrachloride or chloroform-

d as a solvent. The mass spectra were obtained with a JASCO JMS-01SG mass spectrometer. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter using a quartz cell with a 100-mm path length at 25°C. A Varian Model 920 instrument was used for glc preparative separations with a PEG-6000 or EGSS-X, 1-m column. Separation by tlc was carried out on a silica gel 60 F_{254} tlc plate (20 × 20-cm layer; thickness, 0.25 mm) from Merck with ether as an eluent.

Materials. Distilled water was from Daiwa Chemical Ltd., and solvents for optical rotation measurement were spectroscopic grade from Nakarai Chemical Ltd. The BSA protein was Fraction V grade from Armour. The alkyl aryl ketones other than **1b, 1i, 1k, 1l,** and **1m,** were from Nakarai Chemical Ltd., and used without further purification. The commercially nonavailable ketones were prepared according to the known literatures, and their purities were checked by nmr and glc. Reducing agents of $NaBH_4$ and $NaBH_3CN$ were extrapure grade from Nakarai Chemical Ltd.

The effect of BSA concentration on the optical yield of alcohol obtained from the reduction of 1. An appropriate ketone **1** (0.25 mmol) was added to 50 ml of a buffer solution at a given pH containing a various amount of BSA in the range of 1.0–6.6 g (0.015–0.1 mmol) (the molecular weight was taken as 66,000). The mixture was gently stirred at 25°C for 2 hr, then $NaBH_4$ or $NaBH_3CN$ (0.5 mmol) was added, and the reaction was continued by stirring for another 2 hr at 25°C. The reaction mixture was extracted three times with 100 ml portions of ether or 10% benzene–ether, dried over magnesium sulfate, filtered, and concentrated to give the residue containing a small amount of solvent. The nmr and mass spectra indicated a mixture of the product alcohol and an unknown material contaminated with the used BSA. The pure alcohol was obtained in almost quantitative yield by separation on glc or tlc. To the measured amount of this isolated alcohol was added 2 ml of the solvent used in the literature, and the optical rotation was measured at 25°C. The optical yield was determined from $[\alpha]_D(\text{obs})/[\alpha]_D(\text{max}) \times 100$, (%), using the known optical rotation of the optically pure alcohol measured in the same solvent. The nmr spectrum of the crude alcohol in the presence of chiral europium shift reagent, tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III) (*17*), also provided an estimate of the optical yield. Values from optical rotation and nmr spectroscopy were in good agreement. The configuration was also determined from the sign of optical rotation reported in the literature. The effect of BSA concentration on the optical yield of alcohol can be shown well by plotting the optical yield against BSA concentration at a given pH. Representative examples are given in the reduction of **1a** with $NaBH_4$ at pH 9.2 (Fig. 1), and also in the reduction of **1b** with $NaBH_4$ at pH 9.2 (Fig. 1), and with $NaBH_3CN$ at pH 5.6 and 9.2 (Fig. 2). In all the ketones including **1a** and **1b,** the maximum optical yields were achieved with the use of ca. $\frac{1}{3}$ molar eq of BSA to the ketone in the range of pH 3–11.

The pH effect on the optical yield of alcohol in the reduction of 1b with $NaBH_3CN$. In the same procedure as that of the above experiment the asymmetric reduction of **1b** (0.044 g, 0.25 mmol) with $NaBH_3CN$ (0.031 g, 0.5 mmol) was carried out in 50 ml of a buffer solution containing BSA at the following pH levels in the range of 3–11: pH 3.1, 3.8, and 4.6 (AcOH-HCl), pH 5.6 and 6.8

(KH_2PO_4–Na_2HPO_4), pH 9.2 ($Na_2B_4O_7$), and pH 11.0 ($Na_2B_4O_7$–NaOH). At all the pH's the maximum optical yields were obtained with the use of 5.5–6.0 g (0.083–0.10 mmol) of BSA. Figure 3 represents the plot of the maximum optical yield against the buffer pH.

Asymmetric reduction during protein denaturation and in the presence of a competitive inhibitor. The BSA protein was denatured to an unfolded random polymer by addition of urea (2.4 g, 0.4 mol) to 50 ml of a borate buffer solution (pH 9.2) containing BSA (2.0 g, 0.03 mmol). To the solution was added **1a** (0.030 g, 0.25 mmol), and the reaction mixture was gently stirred for 2 hr at 25°C. The reduction with $NaBH_4$ was carried out by the same procedure as the above experiment. For the asymmetric reduction in the presence of a competitive inhibitor, naphthalene or sodium lauryl sulfate (0.25 mmol) was added to the native BSA solution above, and then the reaction mixture was thoroughly stirred at 25°C. After further addition of the ketone the same reduction was conducted.

Experiment on the repeated reuse of BSA. The reduction of propiophenone (**1c**) provides a most illustrative example. According to the above reduction procedure the alcohol (**2c**) was quantitatively obtained in 78% optical yield from the ether extract of the reaction mixture. On the other hand, the contaminated solvent was thoroughly removed *in vacuo* from the remaining aqueous protein solution. The ketone was newly added to the solution and the reduction also gave the chiral alcohol in 77% optical yield. If the loss of BSA in large amounts and/or the denaturation are avoidable during this repeated reuse experiment, this procedure can be repeated many times. However, in practice three repetitions are most desirable, because in further repetitions a considerable amount of protein is lost and/or a gradual denaturation begins to occur.

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REFERENCES

1. (a) J. D. MORRISON AND H. S. MOSHER, "Asymmetric Organic Reactions," Prentice-Hall, Englewood Cliffs, N.J., 1971; (b) J. W. Scott AND D. VALENTINE, JR., *Science* **184**, 943 (1974); (c) D. VALENTINE, JR. AND J. W. SCOTT, *Synthesis* 329 (1978).
2. For textbooks, see the following: (a) T. C. BRUICE AND S. J. BENKOVIC, "Bioorganic Mechanisms," Benjamin, New York, 1966; (b) W. P. JENCKS, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, 1969; (c) E. T. KAISER AND F. J. KEZDY, eds., "Progress in Bioorganic Chemistry," Wiley-Interscience, New York, 1974; (d) M. L. BENDER, "Mechanisms of Homogenous Catalysis from Protons to Proteins," Wiley-Interscience, New York, 1971; (e) K. G. SCRIMGEOUR, "Chemistry and Control of Enzyme Reactions," Academic Press, New York/London, 1977.
3. (a) F. D. SAEVA, P. E. SHARPE, AND G. R. OLIN, *J. Amer. Chem. Soc.* **97**, 204 (1975); (b) L. VERBIT, T. R. HALBERT, AND R. B. PATTERSON, *J. Org. Chem.* **40**, 1649 (1975); (c) W. H. PIRKLE AND P. L. RINALDI, *J. Amer. Chem. Soc.* **99**, 3510 (1977); (d) M. NAKAZAKI, K. YAMAMOTO, AND

K. FUJIWARA, *Chem. Lett.* 863 (1978); (e) C. ESKENAZI, J. F. NICOUD, AND H. B. KAGAN, *J. Org. Chem.* **44**, 995 (1979).

4. (a) T. DOIUCHI AND Y. MINOURA, *Isr. J. Chem.* **15**, 84 (1976/1977); (b) T. SUGIMOTO, Y. MATSUMURA, T. IMANISHI, S. TANIMOTO, AND M. OKANO, *Tetrahedron Lett.*, 3431 (1978); (c) S. I. GOLDBERG, N. BABA, R. L. GREEN, R. PANDIAN, J. STOWERS, AND R. B. DUNLAP, *J. Amer. Chem. Soc.* **100**, 6768 (1978).
5. For reviews, see the following. (a) B. S. GREEN AND M. LAHAV, *J. Mol. Evol.* **6**, 99 (1975); (b) D. D. MACNICHOL, J. J. MCKENRICK, AND D. R. WILSON, *Chem. Soc. Rev.* **7**, 65 (1978); (c) B. S. GREEN, M. LAHAV, AND D. RABINOVICH, *Acc. Chem. Res.* **12**, 191 (1979).
6. (a) F. CRAMER AND W. DIETSCHE, *Chem. Ber.* **92**, 1739 (1959); (b) N. BABA, Y. MATSUMURA, AND T. SUGIMOTO, *Tetrahedron Lett.*, 4281 (1978).
7. For a preliminary report see T. SUGIMOTO, Y. MATSUMURA, S. TANIMOTO, AND M. OKANO, *J. Chem. Soc. Chem. Commun.*, 926 (1978).
8. (a) K. AOKI AND J. F. FOSTER, *J. Amer. Chem. Soc.*, **78**, 3538 (1956); (b) G. MARKUS AND F. KARUSH, *J. Amer. Chem. Soc.* **79**, 3264 (1957).
9. (a) R. F. BORCH, M. D. BERNSTEIN, AND H. D. DURST, *J. Amer. Chem. Soc.* **93**, 2897 (1971); (b) E. R. H. WALKER, *Chem. Soc. Rev.* **5**, 23 (1976).
10. (a) A. WISHNIA AND T. PINDER, *Biochemistry* **3**, 1377 (1964); (b) D. B. WETLAUFER AND R. LOVRIEN, *J. Biol. Chem.* **239**, 596 (1964); (c) F. HELMER, K. KIEHS, AND C. HANSCH, *Biochemistry* **7**, 2858 (1968).
11. (a) J. R. BROWN, *Fed. Proc.* **34**, 591 (1975); (b) P. O. BEHRENS, A. M. SPICKERMAN, AND J. R. BROWN, *Fed. Proc.* **34**, 591 (1975); (c) J. R. BROWN, *Fed. Proc.* **35**, 2141 (1976); (d) M. GEISOW, *Nature (London)*, **270**, 476 (1977).
12. H. A. MCKENZIE, M. B. SMITH, AND R. G. WAKE, *Biochim. Biophys. Acta* **69**, 222 (1963).
13. (a) T. PETERS, *Adv. Clin. Chem.* **13**, 37 (1970); (b) E. A. NIKKILA, *Progr. Biochem. Pharmacol.* **6**, 102 (1971); (c) A. A. SPECTOR, *Progr. Biochem. Pharmacol.* **6**, 130 (1971); (d) A. A. SPECTOR, J. E. FLETCHER, AND J. D. ASHBROOK, *Biochemistry* **10**, 3229 (1971); (e) C. TANFORD, "The Hydrophobic Effect," Wiley–Interscience, New York, 1973; (f) R. P. TAYLOR, V. CHAU, C. BRYNER, AND S. BERGA, *J. Amer. Chem. Soc.* **97**, 1934 (1975).
14. A. HIRAO, H. MOCHIZUKI, S. NAKAHAMA, AND N. YAMAZAKI, *J. Org. Chem.* **44**, 1720 (1979).
15. M. E. WILSON AND G. M. WHITESIDES, *J. Amer. Chem. Soc.* **100**, 306 (1978).
16. (a) H. L. LEVINE, Y. NAKAGAWA, AND E. T. KAISER, *Biochem. Biophys. Res. Commun.* **76**, 64 (1977); (b) T. OTSUKI, Y. NAKAGAWA, AND E. T. KAISER, *J. Chem. Soc. Chem. Commun.*, 457 (1978); (c) H. L. LEVINE AND E. T. KAISER, *J. Amer. Chem. Soc.* **100**, 7670 (1978); **102**, 343 (1980).
17. H. L. GOERING, J. E. EIKENBERRY, AND G. S. KOERMER, *J. Amer. Chem. Soc.* **93**, 5913 (1971).