JOURNAL OF PHYSICAL ORGANIC CHEMISTRY. VOL. 2, 110–116 (1989)

MULTIFUNCTIONAL CATALYSIS FOR TRANSAMINATION REACTION OF α-AMINO ACIDS WITH GLYOXYLIC ACID IN SYNTHETIC BILAYER MEMBRANE

YUKITO MURAKAMI*, JUN-ICHI KIKUCHI† AND NOBUYUKI SHIRATORI
Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan

ABSTRACT

The transamination reaction of α-amino acids with glyoxylic acid as catalyzed by copper(II) ions was investigated kinetically in an aqueous medium at pH 5·0 and 30·0 °C. L-Phenylalanine transferred its amino group to glyoxylic acid most readily among seven different amino acids used here in the single-walled bilayer vesicle formed with *N,N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{16}$). Such rate enhancement was found to originate from the cooperative trifunctional catalysis: a coordination effect exercised by copper(II) ions, a general acid–base catalysis by the imidazolyl group of the lipid, and a hydrophobic field effect provided by the bilayer vesicle. Lack of any of the three functions failed to give out significant rate enhancement. As regards correlation between the reactivity and the nature of α-amino acids, the copper(II)-catalyzed transamination was progressively enhanced as hydrophobicity of the α-amino acid was increased in the $N^+C_5His2C_{16}$ vesicle.

INTRODUCTION

Bilayer aggregates formed with synthetic lipids have been extensively employed as novel and highly organized reaction media for various chemical reactions.[1] We have recently shown that synthetic peptide lipids, having an α-amino acid residue interposed between a polar head moiety and a hydrophobic double-chain segment as a hydrogen bonding component, constitute morphologically stable bilayer membranes in aqueous media,[2,3] and that single-walled vesicles formed with the peptide lipids provide effective reaction sites for simulation of several enzymatic reactions.[4,5]

In order to explore further novel features of the synthetic bilayer membranes as functionalized reaction media, we studied the copper(II)-catalyzed transamination reaction of various α-amino acids with glyoxylic acid in the single-walled vesicle formed with each individual cationic peptide lipid, $N^+C_5His2C_{16}$ or $N^+C_5Ala2C_{16}$. The results indicate that the single-walled vesicle formed with $N^+C_5His2C_{16}$ provides a very favorable reaction site for the

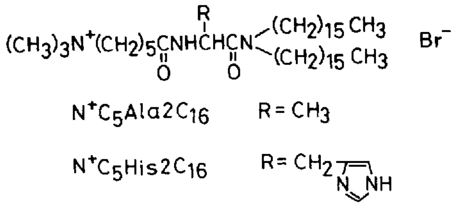
---

*Author for correspondence.
†Present address: Department of Applied Chemistry, Faculty of Science and Engineering, Saga University, Honjyo-machi, Saga 840, Japan.

0894-3230/89/020110–07$05.00

Received 28 April 1988

copper(II)-catalyzed transamination of hydrophobic α-amino acids with glyoxylic acid, exhibiting high substrate selectivity.



$$(CH_3)_3N^+(CH_2)_5\overset{\displaystyle O}{C}NH\overset{\displaystyle R}{C}H\overset{\displaystyle O}{C}N\begin{matrix}(CH_2)_{15}CH_3\\(CH_2)_{15}CH_3\end{matrix}\ Br^-$$

$N^+C_5Ala2C_{16}$ $R = CH_3$

$N^+C_5His2C_{16}$ $R = CH_2$–imidazolyl

EXPERIMENTAL

Materials

The following α-amino acids were obtained from Nakarai Chemicals, Kyoto, Japan as guaranteed reagents: L-2-aminobutyric acid, L-norvaline, L-norleucine, DL-2-aminooctanoic acid, L-leucine, and L-phenylalanine. Glyoxylic acid of 98% purity and disodium ethylenediaminetetraacetate (EDTA) of guaranteed reagent grade were purchased from Wako pure Chemical Industries, Osaka, Japan and Dojin Chemical Laboratories, Kumamoto, Japan, respectively. Dansyl chloride [5-(dimethylamino)-1-naphthylsulfonyl chloride] for fluorescent labeling of amino acids was obtained from Nakarai Chemicals as a guaranteed reagent. All the materials cited above were used without futher purification. Preparation and characterization of *N,N*-dihexadecyl-N^α-[6-(trimethylammonio)hexanoyl]-L-histidinamide bromide ($N^+C_5His2C_{16}$)[6] and *N,N*-dihexadecyl-N^α-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$)[3] have been reported elsewhere. Imidazole from Nakarai Chemicals was purified by sublimation at 60 °C/0·01 mmHg. Copper(II) perchlorate from Kishida Chemical Co., Osaka, Japan was dissolved in deionized water and standardized by conventional chelatometric titration.

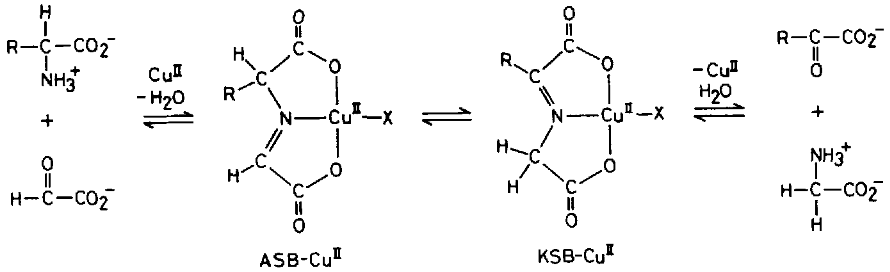
Kinetic measurements

A solution involving single-walled vesicles was prepared by sonication of a dispersion sample of each individual lipid in an aqueous acetate buffer (25 mmol dm^{-3}, pH 5·0, μ 0·10 with KCl) for 1 min with a probe-type sonicator (W-220F, Heat Systems-Ultrasonics) at 30 W and room temperature.[6] Each run was initiated by adding an aqueous solution of $Cu(ClO_4)_2$ (12 μl) to the vesicular solution (1·2 ml) containing an α-amino acid and glyoxylic acid, which was pre-equilibrated at 30·0 ± 0·1 °C in a thermostatted cell. The initial concentrations of chemical species were set as follows for most of the runs: α-amino acids, 5·0; glyoxylic acid, 5·0; $Cu(ClO_4)_2$, 0·05; peptide lipids, 1·0 mmol dm^{-3}. The reaction was followed by measuring amounts of glycine produced at appropriate time intervals in the course of the transamination. Glycine was dansylated and quantitatively analyzed on a reversed-phase HPLC column of TSK gel ODS-120T by employing a Hitachi 655A-11 liquid chromatograph, equipped with a Hitachi F-1000 fluorescence spectrophotometer, in a manner similar to that reported elsewhere:[5] mobile phase, a mixture of Tris aqueous buffer (10 mmol dm^{-3}, pH 8·0) and methanol at 7:3 v/v. For the control experiments carried out without $Cu(ClO_4)_2$, EDTA (0·05 mmol dm^{-3}) was added to the solutions to avoid any disturbance caused by a trace amount of metal ion species.

RESULTS AND DISCUSSION

Catalytic functions for transamination of L-phenylalanine with glyoxylic acid

The transamination of α-amino acids with α-keto acids is catalyzed by vitamin B_6-dependent transaminases in biological systems, as one of the most pivotal processes in amino acid metabolism. The vitamin B_6 coenzyme acts as a mediator for the amino-group transfer in the enzymatic transamination through the reversible transformation between pyridoxal and pyridoxamine species; the former reacts with an α-amino acid while the latter with an α-keto acid.[7] It is well known in general that the corresponding transamination reaction in nonenzymatic systems essentially requires the participation of vitamin B_6 or its derivative. However, the amino-group transfer from various α-amino acids to glyoxylic acid requires no coenzyme function though the presence of metal ions, such as Al(III) and Cu(II), is required.[8-10] In the light of the previous studies on this transamination reaction without participation of the vitaminB_6 function, the copper(II)-catalyzed reaction proceeds through the following sequential steps (see Scheme 1): (i) formation of a copper(II) chelate of aldimine Schiff-base (ASB-Cu^{II}) derived from an α-amino acid and glyoxylic acid; (ii) isomerization of ASB-Cu^{II} to the corresponding ketimine Schiff-base chelate (KSB-Cu^{II}); (iii) hydrolysis of KSB-Cu^{II} to give an α-keto acid, glycine, and the copper(II) ion.



Scheme 1

We examined the catalytic behavior of bilayer vesicles in this unique transamination reaction by employing L-phenylalanine as a hydrophobic α-amino acid. When the reaction was attempted in an aqueous acetate buffer (25 mmol dm^{-3}, pH 5·0, μ0·10 with KCl) at 30·0 °C with and without the $N^+C_5His2C_{16}$ vesicle (A and B in Figure 1), the formation of glycine was not detected to any extent in the absence of metal ions. On the other hand, the transamination reaction proceeded upon addition of copper(II) ions in an aqueous acetate buffer (C in Figure 1). The copper(II)-catalyzed reaction was somewhat enhanced when imidazole was added (D in Figure 1), presumably due to its general acid–base catalysis. Mix has observed a similar catalysis exercised by pyridine in the transamination of various α-amino acids with glyoxylic acid in the presence of copper(II) ions.[9] The reactivity was also increased to a certain extent in the presence of the $N^+C_5Ala2C_{16}$ vesicle (E in Figure 1). In this case, enhanced formation of ASB-Cu^{II} in the hydrophobic vesicular domain is responsible for acceleration of the overall reation (see below). Thus, three types of catalytic functions are involved in the present transamination reaction under the conditions applied to the case of F in Figure 1: a coordination effect caused by copper(II) ions, a general acid–base catalysis by the imidazolyl moiety of $N^+C_5His2C_{16}$ and a hydrophobic field effect provided by the bilayer vesicle.

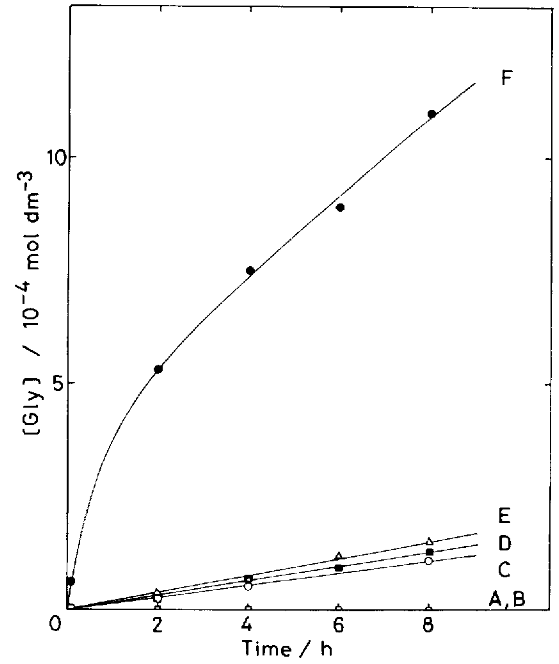


Figure 1. Time courses for transamination of L-phenylalanine (5·0 mmol dm^{-3}) with glyoxylic acid (5·0 mmol dm^{-3}) in an aqueous acetate buffer (25 mmol dm^{-3}, μ 0·10 with KCl) at pH 5·0 and 30·0 °C in the presence of the following additives: A, EDTA (1·0 mmol dm^{-3}); B, EDTA (1·0 mmol dm^{-3}) and $N^+C_5His2C_{16}$ (1·0 mmol dm^{-3}); C, $Cu(ClO_4)_2$ (0·05 mmol dm^{-3}); D, $Cu(ClO_4)_2$ (0·05 mmol dm^{-3}) and imidazole (1·0 mmol dm^{-3}); E, $Cu(ClO_4)_2$ (0·05 mmol dm^{-3}) and $N^+C_5Ala2C_{16}$ (1·0 mmol dm^{-3}); F, $Cu(ClO_4)_2$ (0.05 mmol dm^{-3}) and $N^+C_5His2C_{16}$ (1·0 mmol dm^{-3})

It is noteworthy that the $N^+C_5His2C_{16}$ vesicular system gave out marked rate enhancement in the presence of copper(II) ions (F in Figure 1). The initial rate of reaction was accelerated in this vesicular system by *ca.* 20-fold, relative to that for the copper(II)-catalyzed reaction in the identical aqueous buffer without other additives. The corresponding acceleration rates for the initial stage were observed to be much smaller when any one of the above three catalytic functions was eliminated. The initial reaction rates were accelerated for the Cu-(II)-$N^+C_5Ala2C_{16}$ and Cu(II)-imidazole systems only by 1·4- and 1·2-fold, respectively. Consequently, it is clear that the $N^+C_5His2C_{16}$ vesicle provides a extremely favorable reaction site capable of exerting multifunctional assistance in the copper(II)-catalyzed transamination reaction.

Substrate selectivity in the $N^+C_5His2C_{16}$ vesicular system

Figure 2 shows time courses for the copper(II)-catalyzed transamination of various α-amino acids with glyoxylic acid in the presence of the $N^+C_5His2C_{16}$ vesicle. The transamination reactivity varies sensitively by the nature of substituents placed in the α-amino acids. All the kinetic solutions were nearly clear and any detectable increase of turbidity was not observed for them during the reaction in a time range of 8 h, except for the case of DL-2-aminooctanoic acid. For the latter case, turbidity increased gradually after 4 h of the reaction period due to

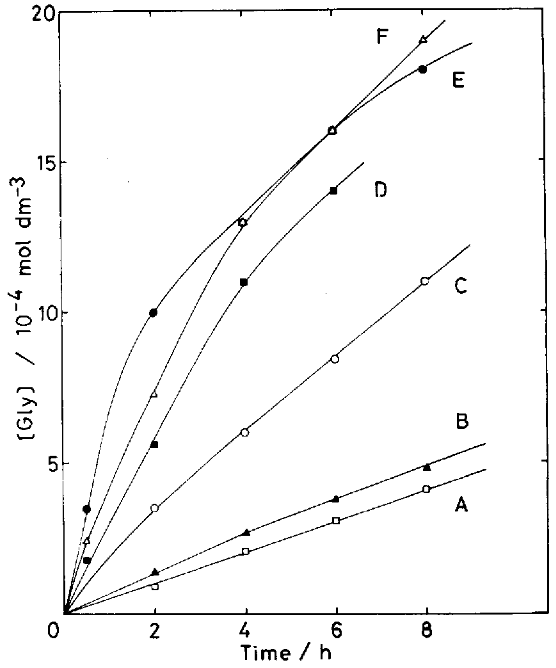


Figure 2. Time courses for transamination of various α-amino acids (5·0 mmol dm^{-3}) with glyoxylic acid (5·0 mmol dm^{-3}) as catalyzed by copper(II) ions (0·05 mmol dm^{-3}) and $N^+C_5His2C_{16}$ (1·0 mmol dm^{-3}) vesicle in an aqueous acetate buffer (25 mmol dm^{-3}, μ0·10 with KCl) at pH 5·0 and 30·0°C: A, L-2-aminobutyric acid; B, L-alanine; C, L-norvaline; D, L-norleucine; E, DL-2-aminooctanoic acid; F, L-leucine

low solubility of the produced α-keto acid in the bulk aqueous phase. Such an increase in turbidity led to lower the transamination reactivity to a certain extent (E in Figure 2).

When the reaction rate, as evaluated for the initial stage of each reaction in the $N^+C_5His2C_{16}$ vesicle (refer to Figures 1 and 2), is plotted against the hydrophobic parameter $(\pi)^{11}$ for the side-chain segment of each α-amino acid (R in Scheme 1), a good linear correlation was observed for the following five α-amino acids (Figure 3): L-2-aminobutyric acid, L-norvaline, L-phenylalanine, L-norleucine, and DL-2-aminooctanoic acid. Thus, the transamination reactivity of the α-amino acid with glyoxylic acid is primarily controlled by an extent of entrapment of the α-amino acid into the vesicle, and the reaction proceeds very favorably in the hydrophobic vesicular domain.

At this stage, some comments are required for the reactivities of L-alanine and L-leucine with glyoxylic acid. It has been reported that the copper(II) chelate of the aldimine Schiff-base of L-alanine with glyoxylic acid is quite unstable, so that isolation of the complex is not possible from aqueous media, and readily isomerizes to the corresponding ketimine Schiff-base chelate.[10] Cram *et al.* have claimed that the collapse ratio of an azaallylic carbanion formed by deprotonation of an imine is controlled by electronic and steric effects of a substituent attached to the allyl carbanion.[12] Thus, a high reactivity observed for the amino-group transfer of L-alanine, relative to the case of L-2-aminobutyric acid in the $N^+C_5His2C_{16}$ vesicular system, seems to originate from the difference in electronic nature between the side-chain segments (R in Scheme1) of the α-amino acids. On the other hand, a steric factor presumably prevails over an electronic effect as regards the reactivity of the L-leucine—glyoxylic acid system.

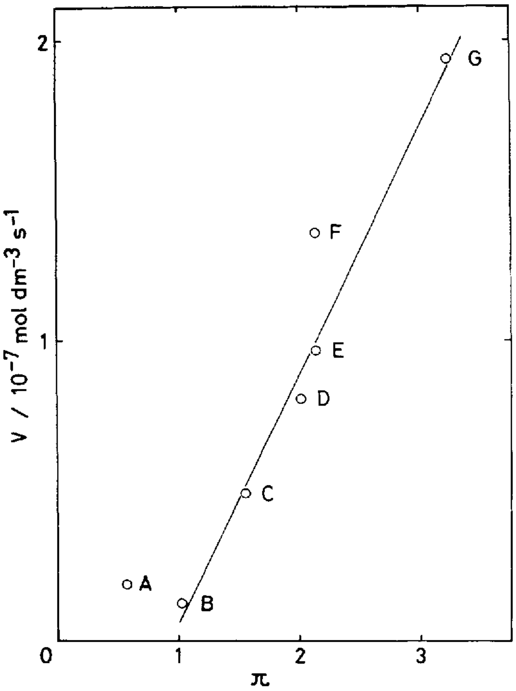


Figure 3. Correlation of the initial rate (V) of transamination with the hydrophobic paramer (π) for substituent R of an α-amino acid (refer to Scheme 1). The V values were evaluated from the data given in Figures 1 and 2: A, CH_3; B, CH_2CH_3; C, $(CH_2)_2CH_3$; D, CH_2Ph; E, $(CH_2)_3CH_3$; F, $CH_2CH(CH_3)_2$; G, $(CH_2)_5CH_3$

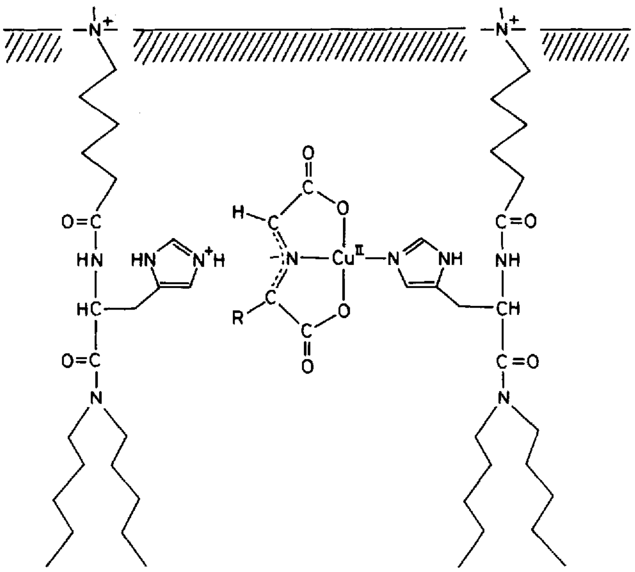


Figure 4. Schematic representation of multifunctional catalysis for the transamination of an α-amino acid with glyoxylic acid in the $N^+C_5His2C_{16}$ vesicle

Previously, we have investigated the copper (II)-catalyzed transamination of a hydrophobic pyridoxamine with pyruvic acid in synthetic bilayer membranes formed with the peptide lipids.[6,13] The general acid–base catalysis by the imidazolyl group of $N^+C_5His2C_{16}$ was observed in the isomerization process of the ketimine Schiff-base chelate formed with the pyridoxamine derivative, pyruvic acid, and the copper(II) ion to afford the corresponding aldimine Schiff-base chelate. In addition, such catalytic activity of the imidazolyl moiety is masked when the base is coordinated with the copper(II) ion.

On these grounds, an extremely high reactivity observed in the amino-group transfer from a hydrophobic α-amino acid to glyoxylic acid comes from cooperative assistance of the following functions (refer to Figure 4). (i) Formation of the copper(II) chelate of the aldimine Schiff-base is enhanced in the hydrophobic vesicular domain. (ii) Since the aldimne Schiff-base acts as a terdentate ligand toward copper(II) ions,[10] the fourth coordination site of the copper(ii) ion is occupied with the imidazoyl group of $N^+C_5His2C_{16}$. Such coordination behavior of copper(ii) ions with the imidazolyl group of the peptide lipid has been confirmed by electronic absorption spectroscopy.[13] Thus, the Schiff-base chelate is fixed in the hydrogen-belt domain. (iii) Under such conditions, the Schiff-base chelate is placed at the site close to the histidyl residue of $N^+C_5His2C_{16}$ and subjected to the effective general acid–base catalysis by the coordination-free imidazolyl group. In conclusion, the present work demonstrates that the bilayer membrane formed with $N^+C_5His2C_{16}$ provides an effective reaction site capable of generating multifunctional catalysis. The present concept of multifunctional catalysis may be extended further and serve as a basic guidepost for designing novel supramolecular assemblies as functionalized reaction media.

REFERENCES

1. T. Kunitake, H. Ihara and Y. Okahata, *J. Am. Chem. Soc.* **105**, 6070–6078 (1983); J. K. Hurst, L. Y. C. Lee and M. Grätzel, *J. Am. Chem. Soc.* **105**, 7048–7056 (1983); I. Tabushi *et al.*, *J. Am. Chem. Soc.* **106**, 219–226 (1984); P. Herve, F. Nome and J. H. Fendler, *J. Am. Chem. Soc.* **106**, 8291–8292 (1984); T. Mizutani and D. G. Whitten, *J. Am. Chem. Soc.* **107**, 3621–3625 (1985); J. van Esch, M. F. M. Roks and R. J. M. Nolte, *J. Am. Chem. Soc.* **108**, 6093–6094 (1986); R. A. Moss, S. Bhattacharya, P. Scrimin and S. Swarup, *J. Am. Chem. Soc.* **109**, 5740–5744 (1987).
2. Y. Murakami, A. Nakano and K. Fukuya, *J. Am. Chem. Soc.* **102**, 4253–4254 (1980); Y. Murakami, A. Nakano and H. Ikeda, *J. Org. Chem.* **47**, 2137–2144 (1982).
3. Y. Murakami, A. Nakano, A. Yoshimatsu, K. Uchitoni and Y. Matsuda, *J. Am. Chem. Soc.* **106**, 3613–3623 (1984).
4. Y. Murakami, A. Nakano, A. Yoshimatsu and K. Fukuya, *J. Am. Chem. Soc.* **103**, 728–730 (1981); Y. Murakami, J. Kikuchi, K. Nishida and A. Nakano, *Bull. Chem. Soc. Jpn.* **57**, 1371–1376 (1984); Y. Murakami, Y. Hisaeda and T. Ohno, *Chem. Lett.* 1357–1360 (1987).
5. Y. Murakami, J. Kikuchi, K. Akiyoshi and T. Imori, *J. Chem. Soc., Perkin Trans. 2* 1919–1924 (1985).
6. Y. Murakami, J. Kikuchi and K. Akiyoshi, *Bull. Chem. Soc. Jpn.* **58**, 1200–1204 (1985).
7. E. E. Snell and S. J. DiMari, *The Enzymes*, ed. P. D. Boyer, Academic Press, New York (1970), 3rd edn., vol. 2, pp. 335–370.
8. D. E. Metzler, J. Olivard and E. E. Snell, *J. Am. Chem. Soc.* **76**, 644–648 (1954).
9. H. Mix, *Hopper-Seyler's Z. Physiol. Chem.* **325**, 106–115 (1961).
10. Y. Nakano, K. Sakurai and A. Nakahara, *Bull. Chem. Soc. Jpn*, **39**, 1471–1476 (1966).
11. C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York (1979), pp. 65–167.
12. D. A. Jaeger and D. J. Cram, *J. Am. Chem. Soc.* **93**, 5153–5161 (1971); D. A. Jaeger, M. D. Broadhurst and D. J. Cram, *J. Am. Chem. Soc.* **101**, 717–732 (1979).
13. Y. Murakami, J. Kikuchi, A. Nakano, K. Akiyoshi and T. Imori, *Bull. Chem. Soc. Jpn* **57**, 1116–1122 (1984).