202. Stereoselectivity in Reactions of Metal Complexes VII[1])

Asymmetric Synthesis of Amino Acids by Metal Ion-Promoted Transamination

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Summary

Enantioselective synthesis of phenylalanine was performed by reacting phenylpyruvic acid with pyridoxamine followed by ketimine-aldimine isomerization of the *Schiff* base formed catalyzed by an optically active copper(II)-complex. By UV and CD measurements it was shown that the enantiomeric excess strongly depends on the reaction conditions and on the reaction time. In favorable cases it reached values up to 80%. The selectivity of the reaction is discussed on the basis of possible structures of the intermediate mixed ligand complex.

Introduction. – Stereoselective interactions in metal complexes may be used *a*) for selective binding of one antipode to achieve separation of a racemic mixture, and *b*) to perform an asymmetric synthesis by means of a stereospecific reaction taking place in the coordination sphere of a mixed ligand complex. The crucial step in both types of reaction is the selective formation of a reactive diastereoisomeric complex obtained by combining a chiral or prochiral substrate (S) and a chiral and nonracemic auxiliary ligand (L*) with a metal ion, the substrate then being transformed into the chiral product (P*) *(Eqn. 1)*:

$$M + L^* \rightleftharpoons ML^* + S \rightleftharpoons ML^*S \rightarrow ML^* + P^* \qquad (1)$$

The optical yield of the reaction (% ee) depends on the way in which the stereochemical information is conducted through the reaction chain from the first inducing center, located in the auxiliary ligand, up to the final chiral center in the product P*. Several steps may be involved in such an information transfer: *i.* induction from the backside ligand center, which is usually an asymmetric C-atom, through asymmetric coordination atoms to result in a definite absolute configuration of the ML*-complex; *ii.* orientated and non-statistical binding of the substrate

[1]) Part VI, see [1].

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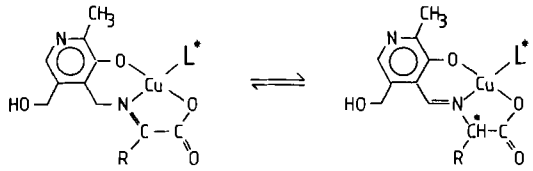
ligand forming the ML*S-complex; *iii.* evolution of the ML*S-complex towards the product P*, either by an intramolecular or by an intermolecular reaction. In the latter case the entering reactant may also need to be orientated in a specific way at the moment it reacts with the substrate ligand. As in each of these steps some factors favoring statistical arrangement may interfere, the structural information can be lost in different ways, and this loss could be important if several effects accumulate.

One of the most important conditions for successful planning of an asymmetric synthesis is therefore total elimination of such factors at any of the reaction steps where they may appear.

The problem of selective formation of labile ML*-type complexes was reviewed recently [2]. In the following study some results are presented on the asymmetric formation of amino acids by ketimine-aldimine isomerization in mixed ligand Cu(II)-*Schiff* base complexes formed from keto acids *(Eqn. 2)*. It is well-known that such isomerization reactions occur readily in weakly acidic solutions when pyridoxamine or analogous compounds are used in the *Schiff*-base formation [3], and indeed the reaction has also been used to obtain optically-active amino acids [4].



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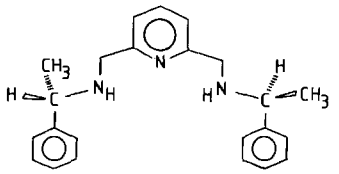
The choice of this system was based on the fact that the reaction proceeds by intramolecular rearrangement and enantiomeric excess is therefore only determined by diastereoselective differentiation of the various possible transition states. A special problem arose from the fact that the tridentate *Schiff* base is unsymmetrical. Its structure is such that in any arrangement where the N-atom of the *Schiff* base, the metal ion and the auxiliary ligand do not exhibit a twofold rotational axis, a mixture of *cis*- and *trans*-isomers may be formed in the reacting complex. Such geometrical isomerism is indeed one of the possible factors favoring random selection of the configuration of the amino-acid unit in the transition state. For this reason the optically-acive ligand L* must show C_2-symmetry and as a first example the tridentate compound 2,6-bis[(3*S*)-3-phenyl-2-azabutyl]pyridine (**1**) was chosen which has two identical molecular halves and which coordinates exclusively in a peripheric manner.

Results. – When an α-keto acid (KA) reacts with pyridoxamine (PM) in the presence of Cu^{2+}-ions and in a weakly acidic solution, two consecutive reactions



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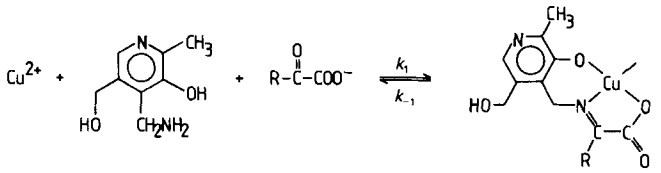
are observed: the formation of a Cu-ketimine complex *(Eqn. 3)* followed by the isomerization of the Cu-ketimine- into the corresponding Cu-aldimine complex *(Eqn. 4)*.



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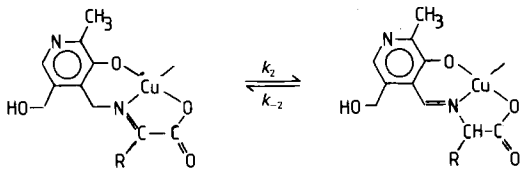


(4)

Both reactions can be followed by measurements of absorption spectra. Whereas the visible spectra of both the ketimine- and aldimine complexes are very similar, the aldimine complex shows a strong absorption band in the UV region with a maximum absorption around 390 nm [5].

The reaction rate of the Cu^{2+}-ketimine formation, measured at the wavelength corresponding to the isosbestic point ($\lambda = 692$ nm) of the spectra of Cu^{2+}-ketimine and Cu^{2+}-aldimine, follows first-order kinetics with respect to keto acid concentration. Ketimine-aldimine isomerization, on the other hand, depends only on the concentration of the ketimine complex. It therefore shows an increase in reaction rate with increasing keto-acid concentration when the latter is small, reaching, in general, a limiting rate at high keto acid concentrations. This limiting rate corresponds to the real rate of the isomerization. This behavior is shown in *Fig. 1* using pyruvic acid as an example

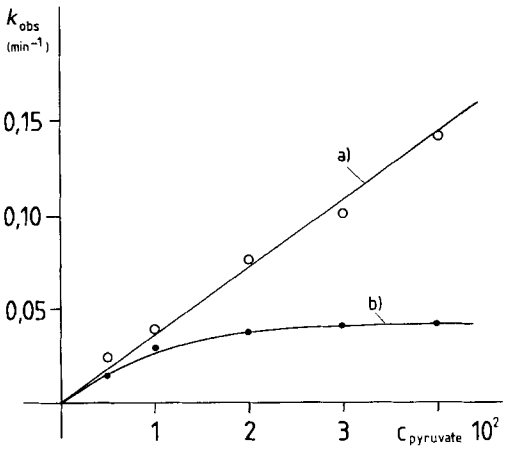


Fig. 1. *Observed rate for the reaction between Cu^{2+}, pyridoxamine and pyruvic acid: a) Cu^{2+}-ketimine formation* ($\lambda = 692$ nm); *b) Cu^{2+}-ketimine – Cu^{2+}-aldimine isomerization* ($\lambda = 395$ nm) ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$; pH = 5.0; acetate buffer; $\mu = 0.1$; $t = 25°$)

In *Table 1*, the observed rate constants for several keto acids are given. From these values – which must be considered as approximate – it is seen that 2-oxo-3-methylbutyric acid and phenylpyruvic acid show a particular behavior in that their rates increase proportionally with increasing keto-acid concentration for both *Schiff*-base formation and isomerization, even at the highest keto-acid concentration. In these two cases, the *Schiff*-base formation is therefore the rate limiting step of the overall transamination reaction.

Table 1. *Observed Pseudo-First-Order and First-Order Rate Constants for Cu^{2+}-Ketimine Formation* ($k_1 = k_{obs}$/[ketoacid]) *and Cu^{2+}-Ketimine – Cu^{2+}-Aldimine Isomerization* (k_2) ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$ M; acetate buffer ($\mu = 0.1$); t = 25°)

Keto acid RCOCOOH	k_1 (sec^{-1})	k_2 (sec^{-1})	k_1 (sec^{-1})	k_2 (sec^{-1})
R–	pH = 4.4		pH = 5.0	
–H	> 1.2	$1.8 \cdot 10^{-3}$		
$-CH_3$	$9.9 \cdot 10^{-2}$	$8.2 \cdot 10^{-4}$	$6.6 \cdot 10^{-2}$	$7.3 \cdot 10^{-4}$
$-CH_2CH_3$	$8.7 \cdot 10^{-2}$	$\sim 6.3 \cdot 10^{-4}$		
$-CH(CH_3)_2$	$3.3 \cdot 10^{-3}$	$> 1.4 \cdot 10^{-4}$	$4.2 \cdot 10^{-3}$	$> 1.5 \cdot 10^{-4}$
$-CH_2CH(CH_3)_2$			$2.1 \cdot 10^{-2}$	$5 \cdot 10^{-4}$
$-CH_2C_6H_5$[a])		$> 1.6 \cdot 10^{-3}$	$4.2 \cdot 10^{-2}$	$> 1.8 \cdot 10^{-3}$

[a]) 30% EtOH.

With the exception of phenylpyruvic acid, where special effects seem to occur, it may be concluded that the isomerization reaction is only slightly affected by the presence and the nature of the substituent in the keto acid, whereas the formation rate of the Cu^{2+}-ketimine species varies by several orders of magnitude for the different keto acids. When the reaction mixture contains an optically active ligand, the UV-absorption band is only very slightly modified, but the CD spectrum shows a band near to 390 nm, the intensity of which depends strongly on the nature of the substituent R of the keto acid used. During the isomerization reaction, the intensity of this CD band reaches a maximum, then decreases and finally completely disappears. *Fig. 2* shows the variation in UV and CD intensity during the formation of phenylalanine as a product of the reaction between pyridoxamine and phenylpyruvic acid.

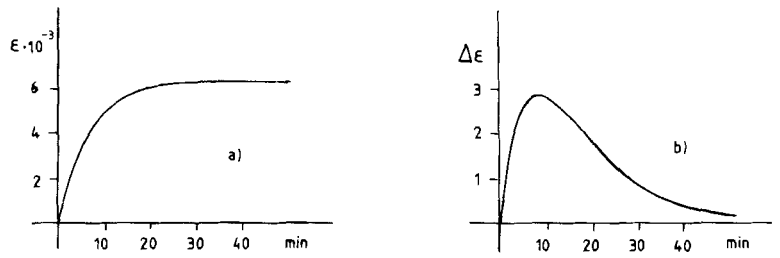


Fig. 2. *Variation at $\lambda = 395$ nm in UV-* (a) *and CD-absorption* (b) *during the reaction of the Cu^{2+}/pyridoxamine/phenylpyruvic acid/ligand system* ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$; $C_{(S,S)^{-1}} = 5.5 \cdot 10^{-3}$; $C_{phenylpyruvic\ acid} = 3 \cdot 10^{-2}$; pH = 5.0; acetate buffer; $\mu = 0.1$; 30% EtOH; t = 25°)

To use the intensity of the CD-signal at 395 nm as a measure of the diastereoselectivity of the ketimine-aldimine isomerization, it was important to show that the observed signal is due only to the presence of the asymmetric C-atom in the amino-acid moiety, and that the disappearance of the signal is the result of racemization. This could be achieved by direct formation of the Cu^{2+}-aldimine species through the reaction of the corresponding amino acid with pyridoxal, as is illustrated for the case of optically active (*S*)-phenylalanine in *Fig. 3.*

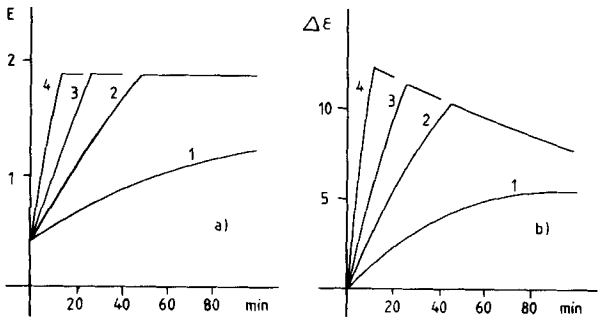


Fig. 3. *Formation and racemization of* Cu^{2+}*-(S)-phenylalanine-pyridoxylidene from UV* (a) *and CD* (b) *measurements* ($\lambda = 395$ nm; $C_{Cu^{2+}} = 2 \cdot 10^{-3}$; $C_{pyridoxal} = 2 \cdot 10^{-2}$; $C_{phenylalanine} = 2 \cdot 10^{-3}$ (1), $4 \cdot 10^{-3}$ (2); $6 \cdot 10^{-3}$ (3), $1 \cdot 10^{-2}$ (4); pH = 5.0; acetate buffer; $\mu = 0.1$; 30% EtOH; t = 25°)

On the other hand, the observed change in UV and CD absorption with time is in accordance with the proposed reaction mechanism [3], the complex formation between Cu^{2+} and the *Schiff* base being much faster than the *Schiff* base from the two components. The latter is therefore the rate-determining step of the reaction. From the UV absorption limit and by extrapolation of the CD absorption to zero-time, ε- and $\Delta\varepsilon$-values can be calculated. These values, which for phenylalanine are 7600 and 12.5 respectively, allow exact determination of the enantiomeric excess during the isomerization at any moment of the reaction.

Some results for different reaction conditions are presented in *Fig. 4.* The validity of the measurements was verified in several runs by gas chromatographic

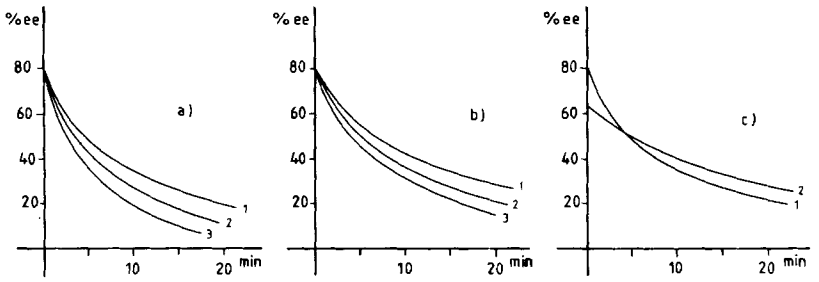


Fig. 4. *Enantiomeric excess in the (R)-phenylalanine formation by transamination between pyridoxamine and phenylpyruvic acid under different reaction conditions* ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$; 30% EtOH; acetate buffer; $\mu = 0.1$; t = 25°; a) pH: 5.00 (1), 5.25 (2), 5.50 (3); $C_{(S,S)^{-1}} = 5.5 \cdot 10^{-3}$; b) ligand concentration: $3.7 \cdot 10^{-3}$ (1), $5.5 \cdot 10^{-3}$ (2), $7.4 \cdot 10^{-3}$ (3); pH = 5.0; c) overall concentration: $C_{Cu^{2+}} = 2 \cdot 10^{-3}$ (1); $C_{Cu^{2+}} = 1.2 \cdot 10^{-3}$ (2), concentration of all other reacting species decreased proportionally)

determination of the enantiomeric excess of the amino acid formed. Details of these determination will be given in the following paper [6]. *Table 2* shows, for some examples, the good agreement between the two modes of determination. This agreement exists although the CD measurement shows only the optically active amino acid contained in the complex, whereas the gas chromatographic determination gives the total amount of the amino acid formed. All the amino acid must be formed by the isomerization reaction and an exchange between free and coordinated amino acid takes place. In this way random distribution of the optically-active amino acid between the free and the coordinated part must occur.

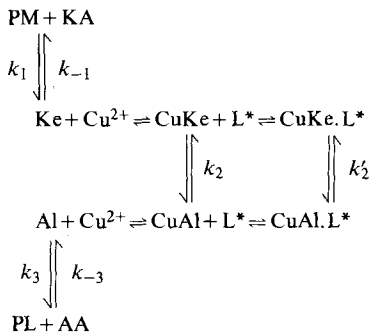
Table 2. *Enantiomeric Excess* (% ee) *of* (R)-*Phenylalanine*[a])

pH	% ee (CD)	% ee (GC)
5.00	40.0	40.9
5.25	42.5	45.5
5.50	41.0	41.2

[a]) Reaction conditions as indicated in *Figure 4a*. The reaction was stopped when the maximum CD intensity was reached.

Discussion. – The system used in the present study of enantioselective synthesis of amino acids is rather complicated; the main equilibria involved are given in the *Scheme*.

Scheme



The optically active amino acid can be formed exclusively through the isomerization reaction of the optically active mixed ligand Cu-ketimine complex. The enantiomeric excess of the reaction is therefore determined by the four following factors: *1.* the relative amount of the mixed species with respect to the free *Schiff*-base complex, *2.* the relative reaction rate characterized by the rate constants k_2 and k_2', *3.* the amount of racemization relative to isomerization, *4.* the stereospecificity of the isomerization in the mixed ligand *Schiff*-base complex. It is the last of these factors which is of special interest because of the way it reflects the real level of transfer of the stereochemical information from the auxiliary ligand to the substrate moiety of the mixed ligand complex.

As the Cu^{2+}-ketimine ligand complex is an intermediary species, formed in an equilibrium reaction, its actual concentration during the reaction is unknown. Approximate determination of this concentration is made even more difficult by the fact that the isomerization rate is approximately twice as fast for the mixed complex as for the unmixed species. On the other hand, as shown in *Fig. 5*, the isomerization rate of CuKe.L* is independent of the pH and excess ligand concentration, whereas the racemization of the Cu^{2+}-aldimine complex, which follows the isomerization, depends on both.

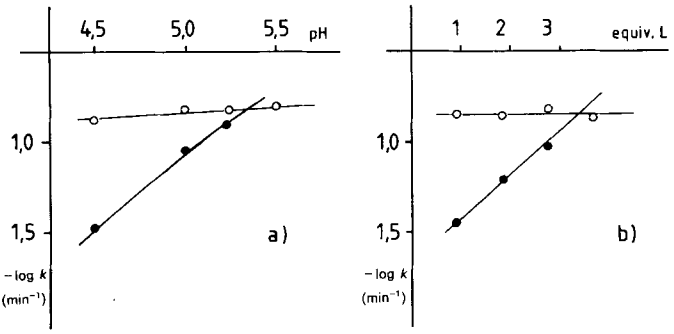


Fig. 5. *First-order rate constants of isomerization* (—○—○—) *and racemization* (—●—●—) *as a function of pH and ligand concentration* (Reaction conditions as indicated in *Fig. 4a* and *4b*)

The real stereoselectivity of the reaction can therefore only be estimated by extrapolation of the observed enantiomeric excess to zero reaction time and for an optimal concentration of the optically active ligand. This extrapolation gave a lower limit of about 80% *(cf. Fig. 4)*, representing a minimum difference of free activation energy $\Delta\Delta G^{\neq}$ for the two diastereoisomeric transition states of about 5.4 kJ/mol. With respect to this result it seemed interesting to investigate possible relationships which may exist between the structural orientation of the auxiliary ligand in the complex and the configuration of the product formed in excess.

It was first necessary to consider the possible configurations of the two asymmetric N-atoms with respect to the asymmetric C-atoms. A ligand having a given configuration for the latter, for example (*S, S*), allows three such arrangements: $C_{(S,S)}N_{(S,S)}$; $C_{(S,S)}N_{(R,S)}$ and $C_{(S,S)}N_{(R,R)}$. In all the transamination reactions performed so far, in the presence of the ligand exhibiting the $C_{(S,S)}$-configuration, the amino acid formed in excess has the (*R*)-configuration. The same (*R*)-amino acid is also enriched by the retroracemization reaction of the racemic mixture in a basic solution of the mixed ligand complex CuAl.L* [7]. These results indicate that the voluminous substituent lies preferentially in the same position in the transition state as in the product. For the (*R*)-amino acid such a favorable position is provided when the ligand shows the $C_{(S,S)}N_{(S,S)}$-configuration.

In the free ligand, the C—N-bond allows rotation of the asymmetric C-atom; this rotation seems to be strongly hindered in the mixed complex. Study of a model shows that the stable conformation is probably the one pointing the H-atoms in the direction of the *Schiff*-base ligand. In this arrangement the phenyl substituent

lies above the aromatic ring of the *Schiff*-base allowing a hydrophobic interaction between the two ligands, as shown in *Fig. 6*.

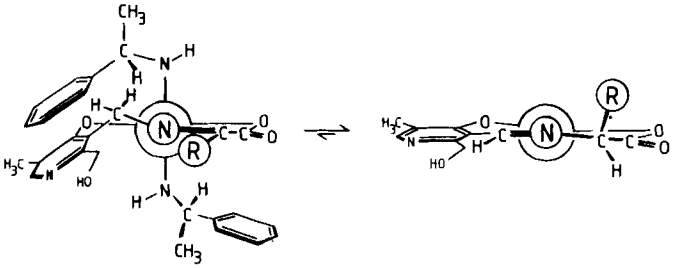
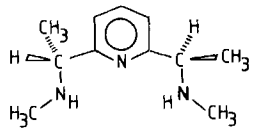


Fig. 6. *Proposed structure of intermediate mixed ligand Cu^{2+}-ketimine complex, and of its product after isomerization*

Some interesting observations seem to support the hypothesis of such a hydrophobic interaction. No stereoselectivity was observed when in identical conditions the optically active ligand 2,6-bis(3-aza-2-butyl)pyridine (**2**) [7] was used, which shows an identical basic structure with respect to **1** but contains no phenyl substituents. On the other hand, both ligands, **1** as well as **2** show retro-racemization in basic media with Cu(II)-salicylidene-aminoacid complexes [7]. The stability of the mixed ligand Cu(II)-ketimine complex with **1** may therefore be enhanced by a hydrophobic interaction between aromatic groups as was also observed with other systems [8].



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Another question worthy of discussion in this context is the relative stability of the mixed complex of Cu(II)-ketimine with respect to that of Cu(II)-aldimine. Zero-time extrapolation of the optical yield shows a value of about 80% which, in the concentration range used, is almost independent of the ligand concentration *(Fig. 4a)*. Taking into account the fact that isomerization in the mixed complex is only slightly faster than in the complex without an auxiliary ligand, this means that with Cu(II)-ketimine the mixed complex is almost quantitatively formed. However the mixed complex with Cu(II)-aldimine seems to be much less stable. This is shown firstly by the fact that racemization of the product is almost complete even in the presence of a large excess of the optically active ligand, and secondly as the racemization rate increases linearly with ligand concentration *(Fig. 5b)*. This may be due to the presence of a small amount of mixed complex which augments with increasing ligand concentration. The behavior of the system as a function of the overall concentration *(Fig. 4c)* can be explained in the same way. Whereas the dilution affects only slightly the enantiomeric excess of the isomerization, the initial racemization rate is strongly reduced.

The difference in the stability of the two mixed complexes is probably a consequence of the conformational change which occurs during the isomerization *(Fig. 6)*. Whereas in the ketimine compound, the pyridine ring of the *Schiff*-base is considerably drawn out of the coordination plane due to the presence of the CH_2-group in the six-membered chelate ring, the whole ligand is in an almost planar arrangement in its aldimine form. Model considerations suggested that ligand-ligand interactions between aromatic groups were most likely to occur in the ketimine structure with the pyridine group in the puckered six-membered chelate ring.

The observed expulsion of the tridentate auxiliary ligand by the only conformational change during the transformation of the substrate to product seems of some interest with respect to analogous mechanisms in enzyme-catalyzed reactions. Nevertheless, the given rationalization of stereoselectivity in terms of a given structure of the reactive intermediate needs further confirmation by the use of a more rigid auxiliary ligand. Synthesis of such ligands is currently in progress. In spite of this, the high stereoselectivity observed can certainly be considered as a consequence of the elimination of geometrical isomerism performed by using a C_2-symmetric metal-ligand system as a source of asymmetric induction.

We thank the *Swiss National Science Foundation* for financial support.

Experimental Part

General. Optical rotations were measured on a *Perkin-Elmer 241* polarimeter, UV and VIS spectra were measured on a *UVIKON 810* spectrophotometer and CD measurements were obtained from a *JASCO J-500C* spectropolarimeter. NMR spectra were recorded on a *Bruker WP-200* in D_2O with DSS as an internal standard.

Materials. (−)-(*S*)-1-Phenylethylamine was prepared according to [10] b.p. 80°/15 Torr, $[\alpha]_D^{22} = -40.03°$ (neat, optical purity: 99.3%). Keto acids, pyridoxamine and pyridoxal were of analytical grade *(Fluka)* and used without further purification.

2,6-Bis[(3S)-3-phenyl-2-azabutyl]pyridine. Pyridine-2,6-bis(carboxaldehyde) (11.7 g, 86.6 mmol) obtained by the method described in [9] is mixed with a solution of 21.0 g (173 mmol) (−)-(*S*)-1-phenylethylamine in 80 ml of dry EtOH. The mixture is hydrogenated in the presence of 10% Pd/C as a catalyst at r.t. and at a H_2 pressure of 3 atm. The mixture is filtered through *Celite*, and the filtrate diluted with 80 ml of H_2O. The pH is brought to 4.5 by dropwise addition of conc. H_2SO_4 and the solution is then evaporated to dryness. By crystallization of the crude salt from EtOH/acetone, 32.6 g (85%) of the sulfate are obtained, $[\alpha]_{436}^{25} = +36.1°$ (H_2O, $c = 0.2$). ^{1}H-NMR (200 MHz): 1.8 (*d*, $J = 7.5$, 6 H); 4.1–4.4 (*dd*, $J = 15$, 4 H); 4.5–4.7 (*q*, $J = 7.5$, 2 H); 7.2–7.4 (*d*, $J = 8.5$–9, 2 H); 7.4–7.6 (*s*, 10 H); 7.7–7.9 (*t*, $J = 8.5$–9, 1 H).

$C_{23}H_{29}N_3O_4S \cdot 2\,H_2O$ (479.23) Calc. C 57.62 H 6.89 N 8.94% Found C 57.63 H 6.30 N 8.66%

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