Dimolybdenum Tetraacetate as an Auxiliary Chromophore in Absolute Configuration Determination of Amino Acids from their Circular Dichroism Spectra – Foundations and Developments

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Abstract: A straightforward and versatile method for the determination of the absolute configuration of amino acids is presented. The proposed method involves the *in situ* formation of chiral complexes of optically active amino acids with the achiral dimolybdenum tetraacetate [$Mo_2(OAc)_4$] acting as an auxiliary chromophore. Optically active amino acids in reaction with [$Mo_2(OAc)_4$] in DMSO exchange *in situ* its acetate ligand to form chiral Mo-complexes. These complexes display several CD bands in the spectral range from 600 to 250 nm. The resulting CD spectra are suitable for the assignment of absolute configuration, since the observed sign of Cotton effects arising within the $d-d$ absorption bands of the metal core depends solely upon the chirality of the amino acid ligands. It is shown that two Cotton effects at around 300 nm and 400 nm are especially useful for the correlation between their sign and the stereostructure of amino acids by means of the hexadecant rule. The present study demonstrates that the hexadecant rule can be extended to differently *N*-protected amino acids. The dimolybdenum method can also be applied for determination of the absolute configuration of cyclic β-amino acids representing precursors of peptidomimetics.

Keywords: Circular dichroism, dimolybdenum tetraacetate, α-amino acids, β-amino acids, chiral Mo-complexes.

I. INTRODUCTION

Determination of the absolute configuration remains a major challenge in several fields of organic chemistry as well as in pharmaceutical research. The Bijvoet method [1], widely used in X-ray diffraction analysis, is applicable in the solid state only. Chemical correlation also belongs to the methods largely used in this task. However, this approach involves transformation of the compound in question into a product of known absolute configuration often by a long synthetic route. In this context, chiroptical methods, the circular dichroism (CD) spectroscopy, in particular, appears to be sensitive, fast and convenient method for the stereochemical assignment provided that the compounds studied are non-racemic. Application of circular dichroism additionally requires presence of a suitable chromophoric system situated close to a stereogenic center in the molecule.

Proteins play a crucial role almost in all fundamental processes in the living cell. All proteins are composed of the same basic building blocks: the 21 DNA-encoded amino acids (α-amino acids). With the exception of glycine, all are chiral, due to the presence of at least one stereogenic carbon atom and belong to the L-stereochemical series – consequently, they have mostly *S*-configuration [2]. During the last few decades peptides containing β-amino acid residue have become a growing interest [3].

Since α- and β-amino acids represent an important group of organic compounds due to their significant biological activity [4-5], interesting structural properties [3, 6], significant pharmacological activities [7], location within natural products [8-10], and utility as chiral auxiliaries and building blocks [11], the unequivocal and reliable determination of the absolute configuration of this class of compounds is a crucial part of their asymmetric synthesis. Therefore, the assignment of absolute configuration to amino acids has been the topic of interest for a number of research groups.

Recently, the field of optical rotations has achieved an important role for determining the 3D molecular structure with confidence. Quick calculations using Hartree-Fock theory, dynamic method, GIAOs, and smaller basis sets (such as 6-31G*) can provide absolute configuration with very little effort, but the predicted specific rotation magnitudes may deviate significantly from the experimental magnitudes [12]. For α-amino acids, however, it was found that the optical rotation is very sensitive to the molecular geometry, to the extent that it changes sign for the different conformers. This sensitivity makes the establishing correlation between the optical rotation and the geometrical structure a difficult task [13-14].

Ten years ago, new derivatives have been proposed for assignment of absolute configuration of α-amino acids by circular dichroism spectroscopy based on mononuclear palladium complexes [Pd(dmba)(acac)] [15]. In this method, the sign of the Cotton effects (CEs) at around 270 nm and 310 nm can be correlated with the stereochemistry of the investigated α-amino acid.

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Moreover, a CD exciton coupling method has been reported for assigning the absolute configuration of α-amino acids. This method consists of a one-step derivatization of α-amino acids with 2-(bromomethyl)quinoline following the formation of a Cu(II)-complexes affording CD spectra and allowing a correlation between signs of particular Cotton effects and stereostructure of α-amino acid ligands [16]. In the case of α-amino acids *N*-phthaloyl derivatives are also employed for determination of their absolute configuration. This method is based on the exciton coupling of the allowed transition of the phthalimide and the carboxylic acid derivative chromophores [17].

It was recently found that an achiral gadolinium(III) porphyrin complex is able to extract chiral amino acids from aqueous solution, forming a 1:1 chiral complex, through bidentate chelation with the carboxylate and amino groups. Intense bisignate ICD (Induced circular dichroism) spectra are observed in the Soret region of the porphyrin with the sign reflecting the absolute configuration of the amino acids. The strong CD of the porphyrin /amino acid complex suggests that the gadolinium(III) porphyrin may serve as a new sensitive chirality probe for unprotected amino acids [18]. The utility of porphyrin tweezer for determining the absolute configuration of α-amino acids after simple derivatizations by ECCD (excition couplet circular dichroism) was also reported earlier [19].

Due to the progress in instrumentation, the carboxylic acids and amino acids nowadays may be investigated by the circular dichroism without the need of preparation of derivatives possessing an appropriate chromophoric system. However, several difficulties may be encountered:

- in solutions, acids are present as a mixture of monomeric and dimeric forms, each one contributing with its own CD;

- the polar carboxylic group is heavily solvated in solution and such a "chiral solvation" will contribute to the CD in a non-predictable way;

- $n \rightarrow \pi^*$- and $\pi \rightarrow \pi^*$-bands lie very close to each other (appr. 220 and 200 nm, respectively), so the respective CEs may not unequivocally be disentangled;

- several conformers are generally present in solution;

- if additional chromophores are present in the molecule, the $n \rightarrow \pi^*$ Cotton effect of the acid chromophore might be overlapped by other Cotton effects.

All these drawbacks tend to vanish in the case where acids are studied in complexes with transition metals. One available procedure for such a derivatization is the *in situ* generation of so-called "cottonogenic" [20] derivatives by mixing the solutions of the chiral (but non-absorbing) ligand with an achiral transition metal complex acting as an auxiliary chromophore. Among various transition metal complexes employed for this purpose [21-26], [Mo₂(OAc)₄] has demonstrated considerable ability to form chiral complexes especially convenient for the chiroptical studies. In such chiral Mo-complexes of the acids their conformational mobility is very much reduced due to the restricted rotations of the acetate ligands coordinated to the metal atoms. Furthermore, transition metal ions, when complexed to an optically active ligand, become involved in the symmetry of the ligand. Thus, the Cotton effects related to electronic transitions of the metal atom are obtained and they are characteristic for the absolute configuration of the compound acting as ligand.
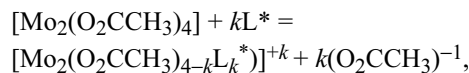
The aim of this work is to report a convenient and versatile method for determining the absolute configuration of optically active amino acids by *in situ* generation of chiral dinuclear complexes of amino acids with [Mo₂(OAc)₄]. Usefulness of these complexes for configurational assignment of amino carboxylic acids, based on the signs of CEs obtained in the CD spectra will be described. This paper will be divided into two general parts. In the first part, after general considerations, the foundations of the *in situ* dimolybdenum CD method will be discussed. Then, the development of the sector rule for correlation between structure of the amino acid and its CD will be shown. In the second part of the paper, some of the newest applications of the method to a variety of diversely substituted and *N*-protected amino acids will be discussed.

II. FOUNDATIONS OF THE *IN SITU* DIMOLYB-DENUM CD METHOD

General Considerations

Dimolybdenum tetraacetate is a commercially available and non air-sensitive in the solid state complex of D_{4h}-symmetry. It is also stable enough in solution to give with chiral ligands reproducible CD and UV-vis spectra over a relatively long period of time. As [Mo₂(OAc)₄] is practically soluble only in DMSO, the CD measurements are performed in this solvent. This stock complex is not dissolved sufficiently in other solvents most applied for CD-spectroscopy and alcohols decompose it quickly.

It is well-known that [Mo₂(OAc)₄] is kinetically labile in solution and therefore its acetate ligands can easily be exchanged by other carboxylates [27]. The chiral metal complexes, mostly of a bridging structure, can be formed *in situ* from dimolybdenum tetraacetate in DMSO by the replacement of acetate ligands with carboxylate groupings of the amino acid. However, an equilibrium mixture of several complexes is formed depending on the precise nature and relative concentration of the chiral ligand, according to the equation:

$$[Mo_2(O_2CCH_3)_4] + kL^* =$$
$$[Mo_2(O_2CCH_3)_{4-k}L_k^*)]^{+k} + k(O_2CCH_3)^{-1},$$

where L* means a chiral amino acid ligand and $k = 1 - 4$.

Altogether up to five optically active complexes may be formed, since the exchange of two of the original acetate ligands can lead either to a *cis*- or to a *trans*-product, as shown in Chart **1**.

The nature of the equilibrium formed in solution and the complexes involved still remain unknown. Nevertheless, provided the appropriate complexation takes place, chiral Mo-complexes provide a CD spectrum to match both pattern and sign with absolute configuration of amino acid ligand. On account of this, it is important to measure the CD spectrum always at similar concentration ratios.
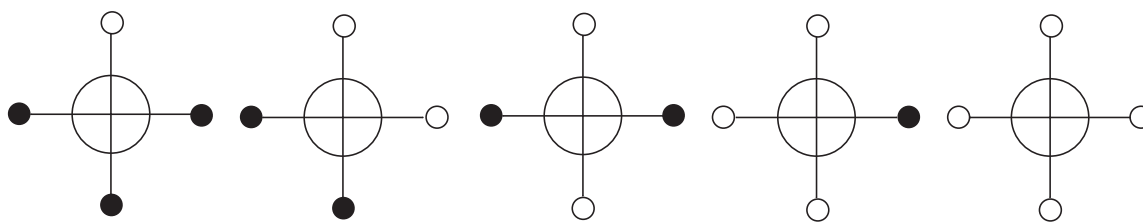
Chart 1. ○ – chiral ligand, ● – acetate ligand.

As has been proven by X-ray diffraction data [28], $[Mo_2(OAc)_4]$ has practically ideal D_{4h}-symmetry. The replacement of acetate ligand(s) of the Mo_2-complex with the chiral amino acid ligand does not result in any significant changes in the absorption spectrum (Fig. **1**). This suggests that ligand exchange within the Mo_2O_8 chromophoric system is not accompanied by any other considerable changes and may also indicate that only one acetate group of the Mo-cluster is replaced by an amino acid ligand. This means that as a result of ligand(s) exchange the symmetry of the chromophore remains D_{4h} and the skeleton of the complexes formed stays almost achiral, i.e., there is no twisting introduced into the chromophoric system due to the presence of chiral carboxylate ligand(s). Therefore, the signs of the individual Cotton effects should be determined by the sector rules. The simplest sector rule for the complexes discussed here, namely hexadecant rule, has been described by Snatzke *et al.* [20].

The proposed method consists of *in situ* formation of chiral Mo-complexes by mixing a solution of chiral but non-absorbing ligand – in this case amino acid – with $[Mo_2(OAc)_4]$ acting as an auxiliary chromophore. To obtain a solution for CD measurements the solid chiral amino acid (1-5 mg) has to be dissolved in a stock solution of the

$[Mo_2(OAc)_4]$ (5-7 mg) in DMSO (10 ml), so that the molar ratio of the stock complex to ligand equals 1:1, in general.

In order to establish whether the shape of CD curves depends on the concentration ratio, the CD spectra of D-phenylglycine (**1**) with the Mo_2-core in 0.5:1, 1:1, 3:1, and 5:1 ligand-to-metal ratios were recorded. The increase of the ligand concentration resulted only in a proportional increase of the band intensity (Fig. **2**). Both CD bands at 380 nm and 300 nm are well-developed and clearly visible even in the ligand-to-metal ratio amounting 0.5:1. Based on these results, we decided to measure the CD spectra of other amino acids with the Mo_2-core in DMSO solution with a ligand-to-metal ratio of 1:1.

The CD measurements can be performed immediately after the mixing of constituents because both the shape and sign of particular CEs do not depend on time substantially. In general, the signs of CEs and the relative intensities of the bands are not time dependent. However, as is evident from Fig. (**3**), the intensity of CD bands does change somewhat with time. In the case of D-phenylglycine (**1**), the intensity of CD bands at around 380 and 300 nm increased two-fold within the first hour. In the next timeframe the intensity of bands generally remains unchanged. Therefore, we decided to record the CD spectra of the Mo-complexes
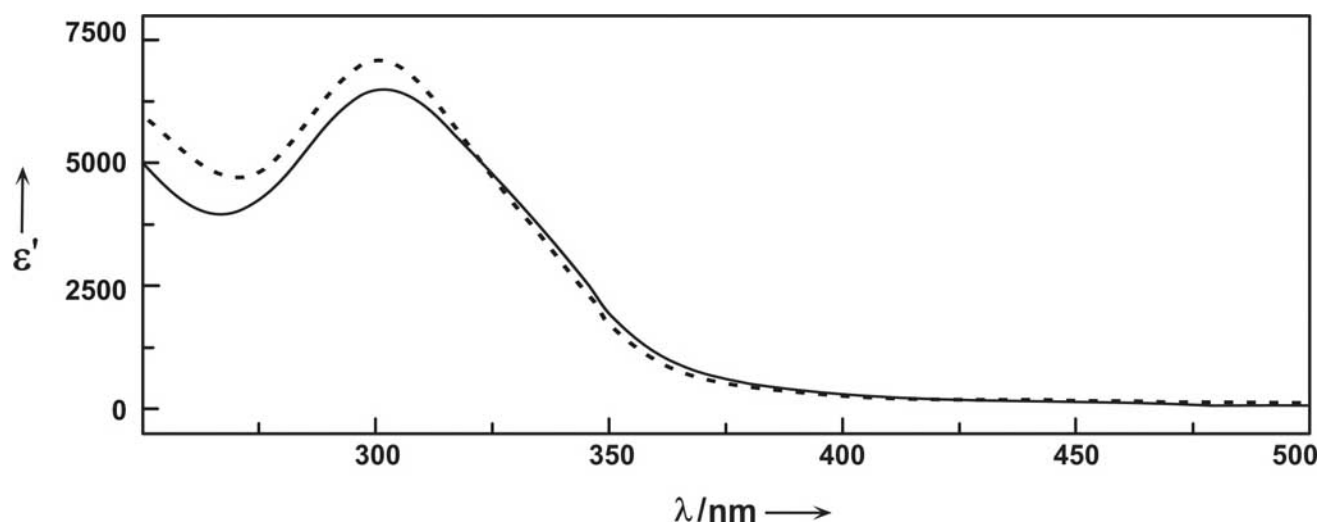
Fig. (1). UV-vis spectra of dimolybdenum tetraacetate (————) and its *in situ* formed chiral complex with L-phenylglycine (– – – –) recorded in DMSO immediately after dissolving of components.
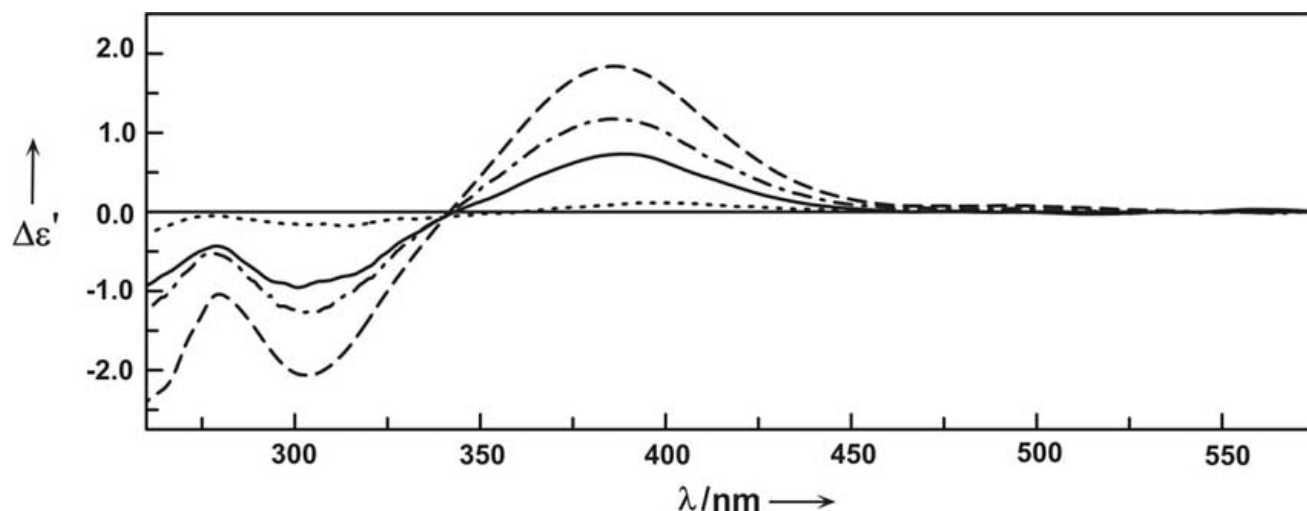
Fig. (2). CD spectra of *in situ* formed Mo-complexes of D-phenylglycine (**1**) at ligand-to metal ratios: 0.5:1 (·······); 1:1 (————); 3:1 (−·−··−··); and 5:1 (− − − −).
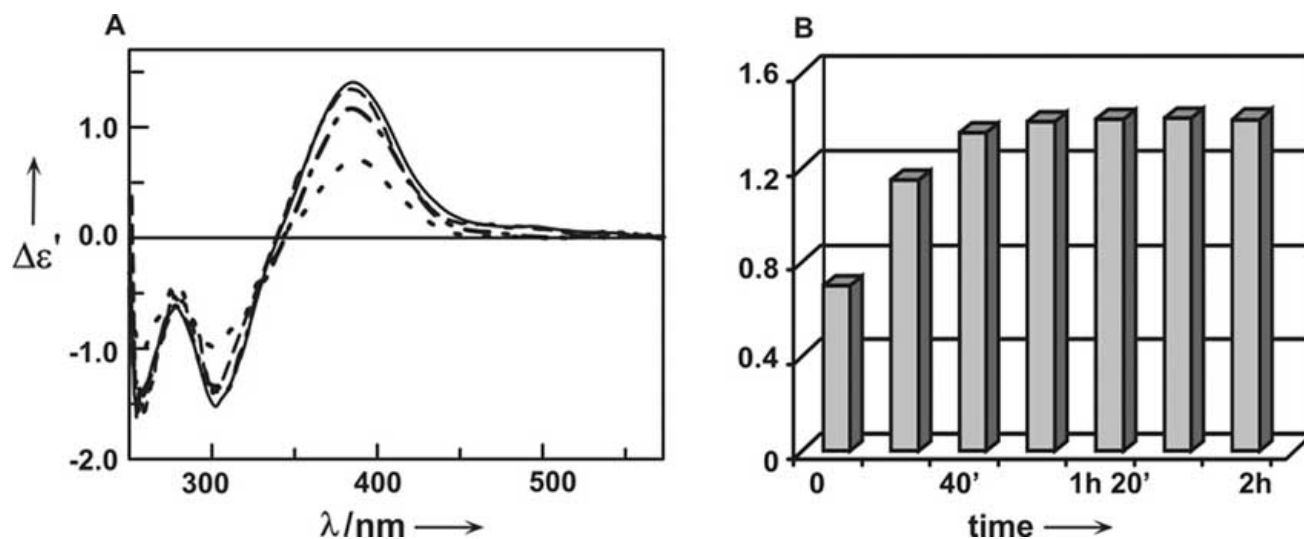

Fig. (3). CD spectra of *in situ* formed Mo-complexes of D-phenylglycine (**1**) recorded in DMSO A: immediately after dissolution (······), after 20' (−·−··−··), after 1 h (− − − −), and after 2 h (————). B: results of time-dependent measurements at 388 nm recorded every 20'.

with amino acids after 1 h from the moment of complex formation.

On the basis of the results presented above it can be concluded that the 1:1 molar ratio of the constituents is very convenient for the chiroptical study. Moreover, the CD spectra should be measured after approximately 1 h from the moment of chiral complex formation.

Using the *in situ* dimolybdenum CD method one does not obtain quantitative values since the real complex structure as well as the concentration of the chiral complex formed in solution is not known. Therefore, the CD data are presented as the $\Delta\varepsilon'$ values. These $\Delta\varepsilon'$ values are calculated in the usual way as $\Delta\varepsilon' = \Delta A/c \times d$, where c is the molar concentration of the chiral ligand, assuming 100% complexation (A = absorption; d = path length of the cell).
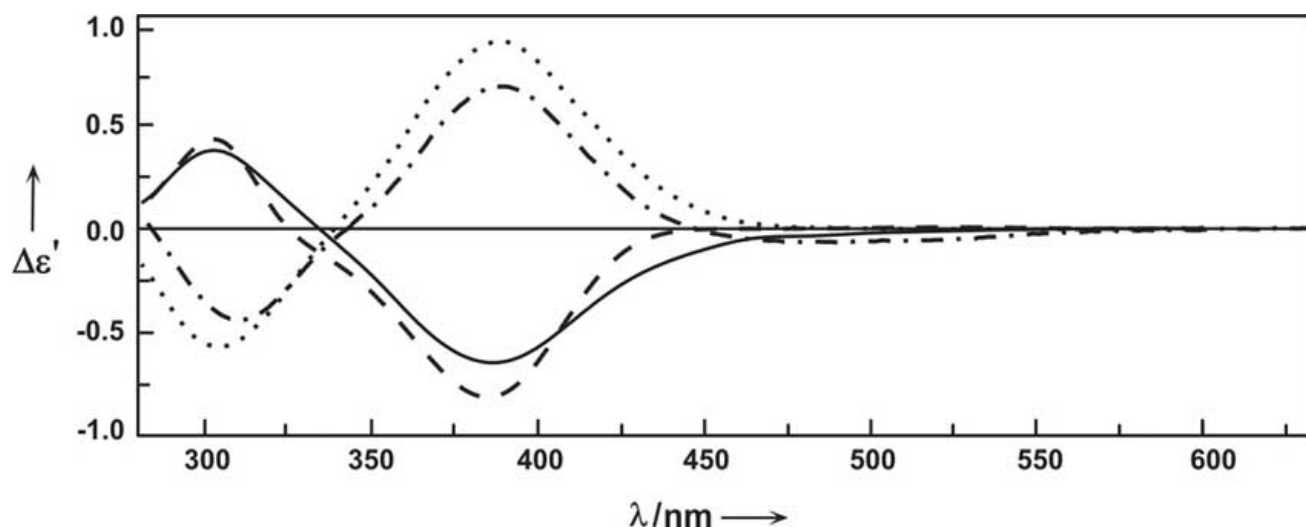
Fig. (4). CD spectra of *in situ* formed Mo-complexes of L-lysine (**2**) (———), α-L-amino butyric acid (**3**)(– – – –), D-valine (**4**) (· · · · · ·), and D-phenylalanine (**5**)(–·–·–·–·).

This, however, creates no disadvantage for the method because for the purpose of absolute configuration determination, only the sign of the appropriate Cotton effects is important and the magnitude of $\Delta\varepsilon$ is irrelevant.

Structure – CD Spectra Relationship

α-Amino Acids

In most cases, carboxylic acids give up to four Cotton effects in the range of 250 - 600 nm with $[Mo_2(OAc)_4]$. The most intense and therefore the most suitable peaks to determine the absolute configuration at the α-carbon atom of the amino acids appear in the spectral range between 400 and 300 nm, see Fig. (**4**) [29, 30].

Based on the literature data [27] we can assume that α-amino acids ligate to the stock complex via the carboxylate group. To correlate correctly the signs of the CEs with the stereostructure of the α-amino acid under investigation, the amino acid in the complexed form should adopt such a conformation in which the C_α–N bond is in a *syn*-periplanar

Fig. (5). Preferred conformation of Mo-complexes with bridging L-α-amino acid. The C–N bond is arranged *syn*-periplanar to one of the carboxylate bonds. Projection perpendicular to the Mo-Mo axis (left) and along the same axis (right). The sector signs refer to Cotton effect at 380 nm and dashed lines to nodal planes.

arrangement with one of the carboxylate bonds (Fig. **5**). Such a conformation resembles the one for Mo-complexes of α-amino acids observed in the crystalline state [31, 32]. Fig. (**5**) shows a schematic projection of the preferred conformation of the Mo-complex with an L-α-amino acid.

In addition, the X-ray data indicate that this conformation may be further stabilized by an intramolecular hydrogen bond, see Fig. (**5**). In such a conformational arrangement a bulky R group is located in the left upper sector for which the hexadecant rule predicts a negative sign of the CE around 400 nm [20]. According to the rule, the sign of the 300 nm CE should be positive. An opposite pattern of sign sequence for 300 nm and 400 nm CEs, respectively, predicts the hexadecant rule for D-α-amino acids.

Most importantly, in such complexes the conformational mobility of ligand molecule is substantially reduced because of the presence of other bidentate carboxylate ligands on both sides of the chiral one [26, 29]. Thus, the conformation of the flexible amino acid molecule becomes fixed to some extent after ligation to the Mo_2-core and thereby the relative configuration of the amino acid unit is established. In the next step, the sign of the particular CEs obtained from the CD spectrum allows to determine unequivocally the absolute configuration. Consequently, the determination of the absolute configuration becomes possible on the basis of the chiroptical data alone. In addition, due to this reduced conformational flexibility, the induced CEs are usually particularly intense and therefore the interpretation of the CD spectra becomes straightforward. This is indeed the case, as can be seen from Fig. (**4**) presenting CD spectra of *in situ* formed Mo-complexes of L-lysine (**2**), α-L-amino butyric acid (**3**), D-valine (**4**), and D-phenylalanine (**5**). The conclusive Cotton effects at around 380 – 390 nm are negative for L-amino acids and positive for their D counterparts, in accordance with the hexadecant rule. In
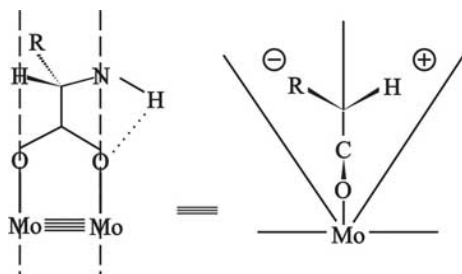
addition, the intensity of particular CD bands is strong enough to enable unequivocal interpretation of the results. On the basis of the above mentioned results and measurements of a variety of other α-amino acids the sector rule for the correlation between the signs of particular CEs and configuration of α-amino acids can be formulated as follows:

"All α-L-amino acids give with $[Mo_2(OAc)_4]$ a negative Cotton effect of around 380 nm and a positive one near 300 nm. On the contrary, α-D-amino acids display with Mo_2-core an opposite pattern of signs in this spectral range."

A more complex situation, however, is observed for the cases of α-amino acids possessing other functional groups, which might also ligate with the Mo_2-core (*e.g.*, OH, SH or NH_2). The shape of the CD spectrum of Mo-complex of D-arginine (**6**) differs from those presented in Figure (**4**) (Fig. **6**). The positive 380 nm CD-band is red shifted but still clearly detectable. The negative 300 nm CE is smaller and also shifted towards longer wavelengths. This may be due to a perturbing effect of the guanidyl unit present in the molecule. The guanidyl unit obviously takes part in the ligation to the Mo_2-core. Still more drastic is the influence of an additional OH- or SH-groups, as *e.g.*, in D-threonine (**7**) and L-cysteine (**8**). The 1,2-amino alcohol and amino thiol moieties, respectively, seem to be better bidentate ligands then the carboxylate ones. The positive sign of the CE at around 290 nm in D-threonine is consistent with a positive sign of the N−C−C−O torsional angle, whereas a negative sign of the CE at around 310 nm in L-cysteine corresponds to a negative sign of the S−C−C−N moiety. Thereby both compounds follow the helicity rule proposed for 1,2-amino alcohols and 1,2-mercaptoalcohols [29, 33] rather than the acids hexadecant rule. For comparison, Fig. (**6**) presents the spectrum of L-lysine (**2**), exhibiting typical behavior for Mo-complexes of α-amino acids with a negative CE at around 380 nm and a positive one at around 300 nm. As can be seen, the extra amino group in the ε-position of L-lysine has no influence on the spectrum.

To establish the limits of applicability of the proposed method, the CD spectra at very low concentrations of the ligand and with the ligand-to-metal ratio of 1:1 were measured after 1 h of components mixing. In general, to obtain well-developed CEs, about 1 mg of the ligand is sufficient. In many cases, however, as little as 0.1 mg ($\sim 1 \times 10^{-4}$ M/L) of the ligand is adequate to record the CD spectrum with good signal/noise (S/N) ratio. In one extreme case it was possible to obtain a satisfactory spectrum of D-phenylglycine (**1**) at concentration of approx. $\sim 8 \times 10^{-6}$ M/L (with 0.006 mg of the ligand). However, in that case only the 380 nm band was well developed.

III. NEWEST APPLICATIONS AND DEVELOP-MENTS

Diversely Substituted α-Amino Acids

Recently, we have investigated chiroptical properties of a variety of diversely *N*-substituted α-amino acids in order to prove the applicability of the hexadecant rule to this class of compounds. As exemplified in Fig. (**7**), the introduction of substituents on nitrogen atom of the amino group may cause changes in the observed spectra. CD spectra of Mo-complexes of D-alanine (**9**) and its Boc-derivative **10** (Boc=*tert*-butoxycarbonyl) considerably differ in the magnitude of their Cotton effects. Also in the case of *N*-Boc-L-alanine (*ent*-**10**) the amplitude is substantially lower than the one for *N*-Me-L-alanine (**11**). It means, that protection groups can, and do, influence the CD results.

Decrease of magnitude of particular Cotton effects in compounds **10** and *ent*-**10** can be explained by assumption that atoms from the protection group are located partially or even entirely in a sector(s) with an opposite sign. Therefore,
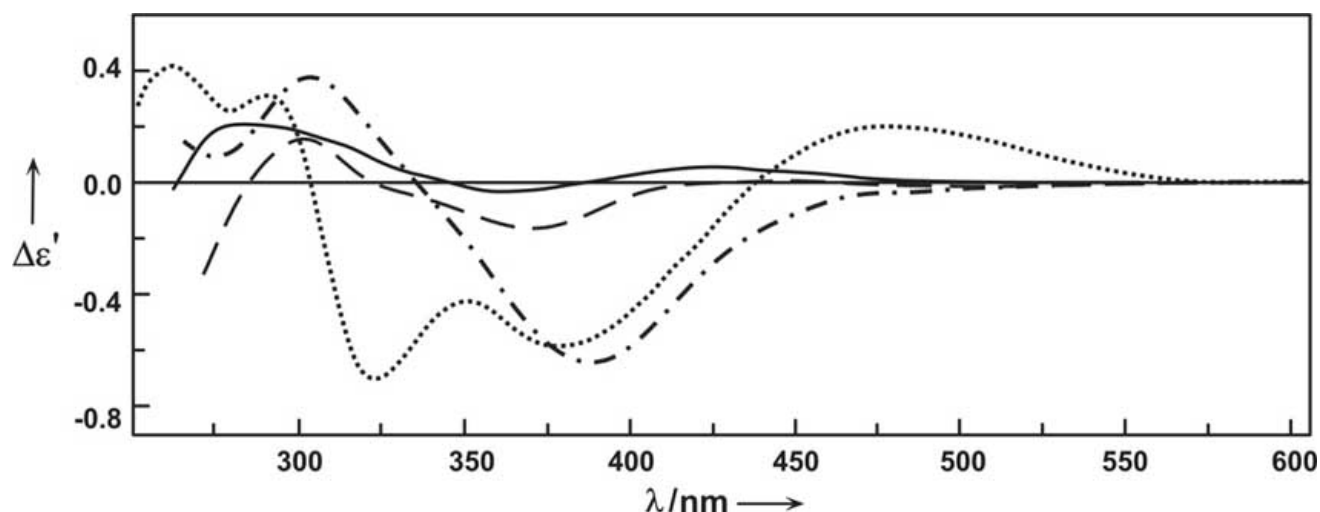

Fig. (6). CD spectra of the *in situ* formed Mo-complexes of D-arginine (**6**) (———), D-threonine (**7**) (− − − −), L-cysteine (**8**) (· · · · · ·), and L-lysine (**2**) (−·−··−·−·). The CD curve for L-cysteine is reduced twice.
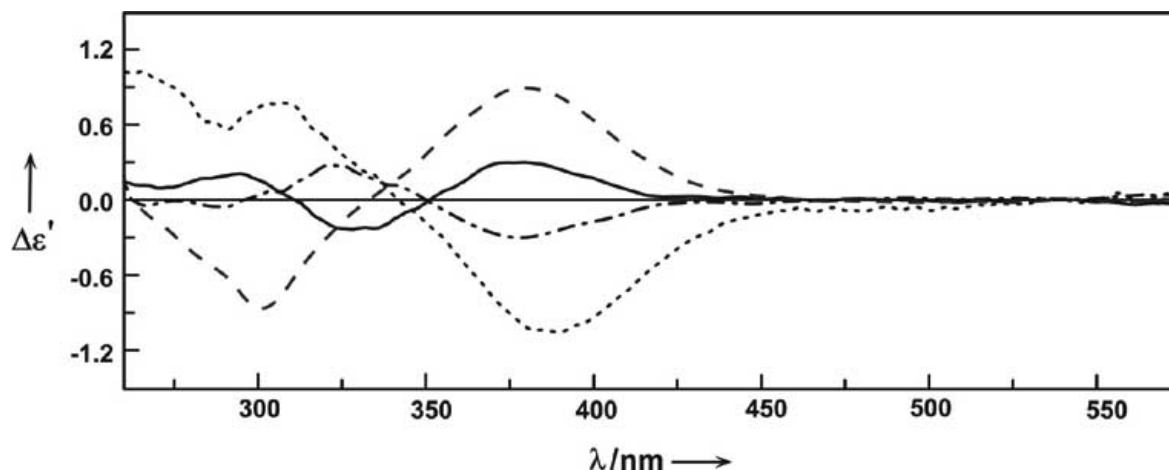
Fig. (7). CD spectra of the *in situ* formed Mo-complexes of D-alanine (**9**) (– – – –), Boc-D-alanine (**10**) (————), Boc-L-alanine (*ent*-**10**) (–·–·–·–·), and *N*-Me-L-alanine (**11**) (· · · · · ·).

their contributions to the net CD cancel to a great extent the initial contributions of substituent R (Fig. **8**). In some cases the contribution to the net CD can cancel each other, as it was observed in the case of *N*-Boc or *N*-acetoxy derivatives of D- and L-alanine and other amino acids. Another example of such behavior was presented by *N*-Fmoc (Fmoc=9-fluorenylmethoxycarbonyl) derivatives of amino acids. On the basis of the results of our measurements performed with a variety of amino acids we can state that *N*-Fmoc protected amino acids do not give CEs with dimolybdenum tetraacetate. Moreover, we have found that *N*-Cbz (Cbz=benzyloxycarbonyl) protected amino acids behave similarly. The experimentally determined CD of Mo-complexes with *N*-Cbz protected, among others, alanine, phenylalanine, glutamine and leucine reveled lack of CEs. On this basis we could conclude that *N*-Cbz protected amino acids can not be studied by means of the dimolybdenum method.
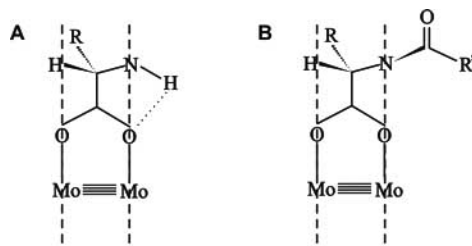

Fig. (8). Spatial distribution of bulky substituents R and R' in *N*-unprotected α-amino acids with R'= H (A) and in *N*-protected α-amino acids (B). In case B both R and R' substituents are located in oppositely signed sectors.

However, if the amino group in the ε-position of L-lysine is Cbz protected (compound **12**) the well-developed CEs are obtained with [Mo₂(OAc)₄]. As can be seen from Fig. (**9**), the CD spectra of both L-lysine and its *N*ᵋ-Cbz-protected derivative **12** differ in magnitude only. The greater

magnitude of the L-lysine compared with **12** is related to the fact that the sector contribution falls off rapidly with increasing distance from the chromophore and from the stereogenic center. Due to its position in the side chain the bulky Cbz-protection group makes a smaller, oppositely signed contribution to the net CD. Therefore, the major contribution in compound **12** appears to be made by the α-amino acid unit coordinated to the Mo₂O₈ core.

In an extreme case, depending upon the bulkiness of the protection group, oppositely signed Cotton effects around 380 and 300 nm can occur. Therefore, the rule for prediction of the Cotton effect sign in the case of *N*-protected amino acids should be applied with care. It should be added that the type of influence on the CD spectra strongly depends on the kind of *N*-substituent. So, the spectra of *N*-methyl substituted amino acids resembles in both sign and magnitude for the ones unsubstituted amino acids. As an example, the CD spectrum of *N*-Me-L-alanine (**11**) *versus* the one of D-alanine (**9**) is shown in Fig. (**7**).

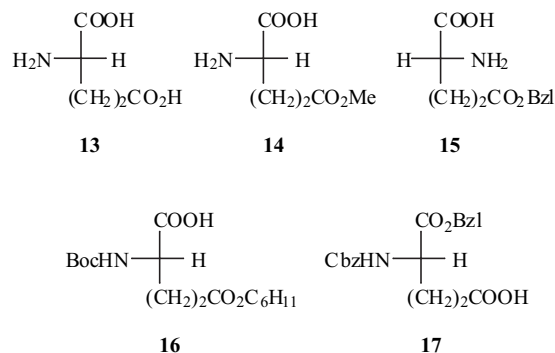What is more complicated is the situation of L-glutamic acid (**13**) and its derivatives **14** – **17** (Chart **2**). Two
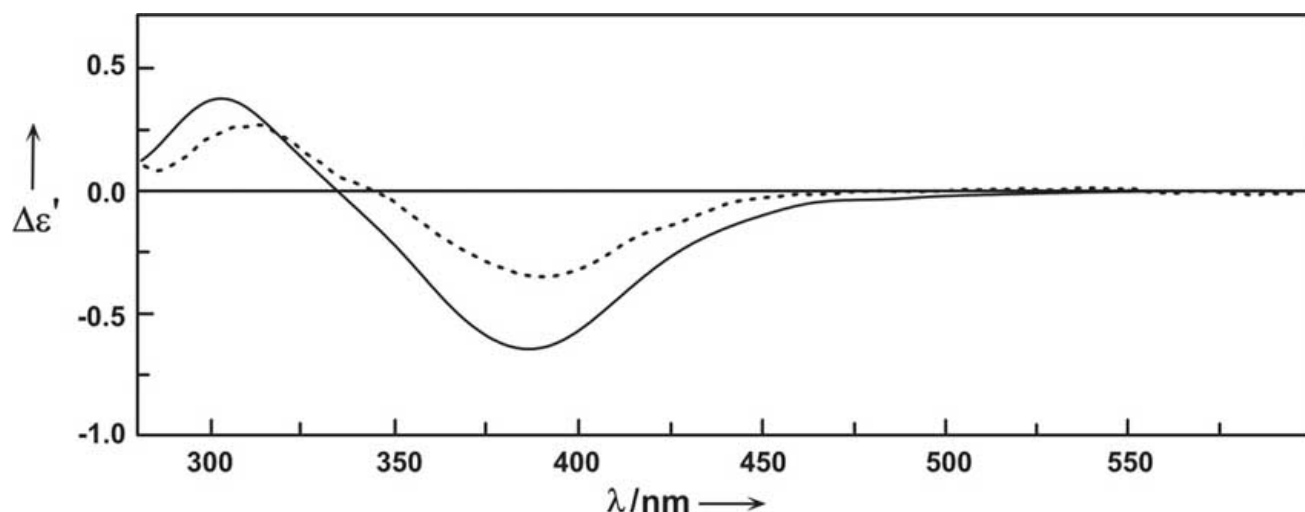

Chart 2.

Fig. (9). CD spectra of the *in situ* formed Mo-complexes of L-lysine (**2**) (————) and its N^{ϵ}-Cbz-derivative **12** (· · · · · ·).

carboxylic groups present in the molecule are able to ligate to the Mo_2-core giving, accordingly to the hexadecant rule, oppositely signed contributions to the net CD spectrum. As a result, no CD should be obtained as it was proven experimentally.

For compounds **14** and **15** with protected carboxylic group in the γ-position, however, a CD curves with well-developed, and typically for α-amino acids, strong Cotton effects at around 380 and 300 nm are obtained (Fig. **9**). The CD curves obtained for Mo-complexes of compounds **16** and **17** with *N*-protected amino group resemble the ones of compounds **10** and *ent-10*, described above. In these cases

again, the intensity of Cotton effects is substantially lower in comparison to the magnitude of CEs in **14** and **15**. However, the sign of the CE at 380 nm is still negative, as predicted by the rule for L-amino acids. It has to be added that in compound **16** the complexation occurs *via* the carboxyl group from the α-amino acid unit whilst in the case of **17** *via* the γ-carboxyl group.

β-Amino Acids

To refine our earlier data and expand the scope of the method to β-amino acids we decided to perform circular dichroic study on this class of compounds with
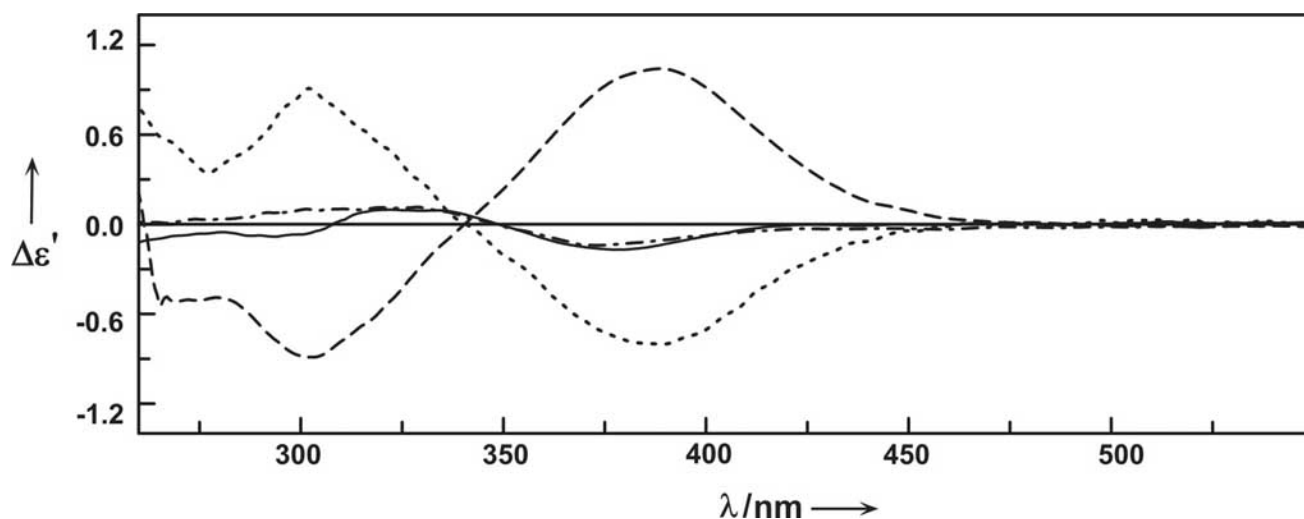

Fig. (10). CD spectra of the *in situ* formed Mo-complexes of **14** (· · · · · ·), **15** (– – – –), **16** (————), and **17** (–·–·–·–·).

18 = 2*S*
19 = 2*R*

20 = 2*S*
21 = 2*R*
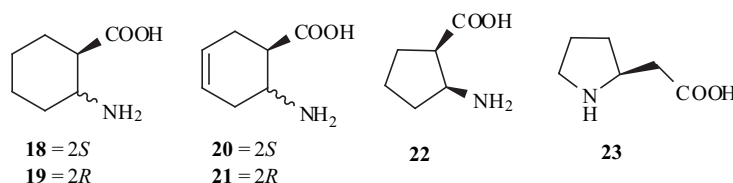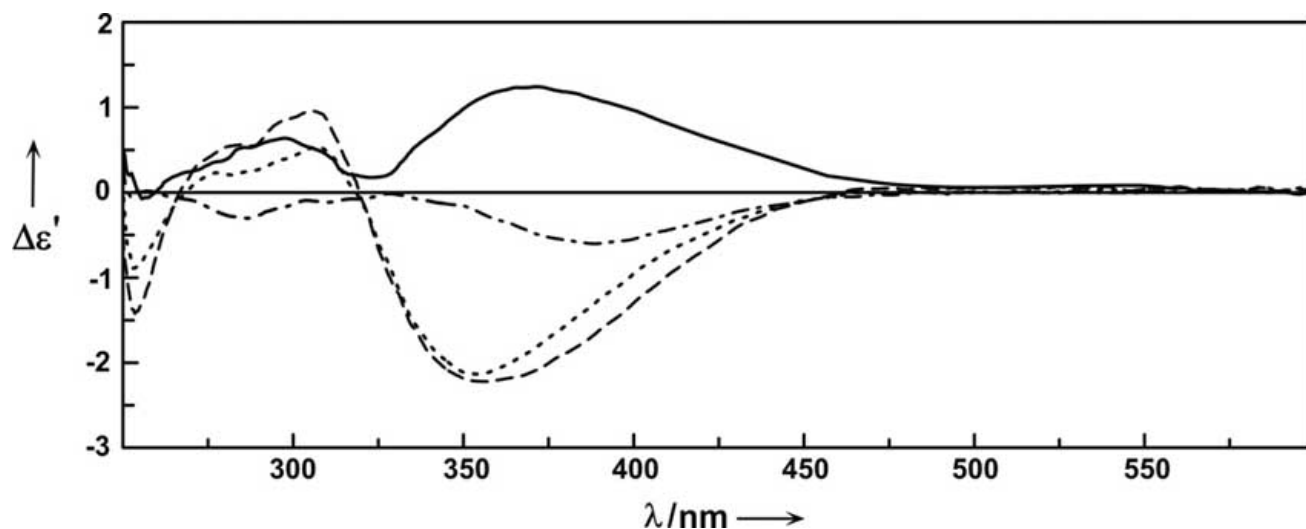
22

23

Chart 3.

Fig. (11). CD spectra of the *in situ* formed Mo-complexes of β-amino acids **18** (– – – –), **19** (———), **20** (· · · · ·), and L-β-homoproline **(23)** (–·–·–·–·).

[Mo$_2$(OAc)$_4$]. As model compounds β-amino acids **18** – **23** (Chart **3**) were chosen [34, 35].

Compounds **18**, **20**, and **22** with (1*R*,2*S*) configuration in the presence of [Mo$_2$(OAc)$_4$] give a negative Cotton effect of around 370 nm and a positive one of around 300 nm. The shape of the CD curve of their (1*R*,2*R*) isomers **19** and **21** is enantiomorphic, and exhibits a positive CE of around 370 nm (Fig. **11**).

It can be assumed that analogously to (*R*)-hydratropic acid [20], the most stable conformation of compounds **18** – **23** in their Mo-complexes is the one with a syn-periplanar arrangement of the C$_\alpha$—C$_\beta$ bond and one of the carboxylate bonds. In addition, this conformation may be stabilized by an intramolecular hydrogen bond to be formed between nitrogen atom of the amino group at C(2) and an oxygen atom of the carboxylate group (Fig. **12**). A direct application of the hexadecant rule to the preferred conformers of compounds **18**, **20**, and **22** demonstrates that the ring carbon atoms are situated in nodal planes. Therefore, these atoms will exert no effect on the CD. In all these compounds, only the amino group situated at C(2) carbon atom occupies a negative sector thus contributing negatively to their CD. A similar situation takes place in the case of L-β-homoproline (**23**). Accordingly, in agreement with the hexadecant rule, a negative sign of the CE of around 380 nm is expected for all of these compounds. This is indeed the case, as can be seen from Fig. (**11**) presenting CD curves of the representatives of this class of compounds.
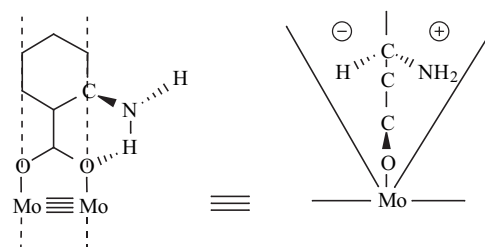

Fig. (12). Preferred conformation of Mo-complexes with bridging D-β-amino acid. The C$_\alpha$–C$_\beta$ bond is arranged *syn-periplanar* to one of the carboxylate bonds. Projection perpendicular to the Mo-Mo axis (left) and along the same axis (right). The sector signs refer to Cotton effect at 380 nm and dashed lines to nodal planes.

An opposite regularity holds, as expected, for compounds **19** and **21** with (1*R*,2*R*) absolute configuration. In these cases once again, the ring carbon atoms are placed in the nodal planes and the amino group at C(2) lies in the positive sector. Thus, a positive sign of the CE of around 380 nm is expected on the basis of the hexadecant rule. The experimental CD curves are in agreement with this prediction (Fig. **11**).

IV. CONCLUSIONS

It has been shown that the dimolybdenum tetraacetate can be advantageously applied in the determination of the

absolute configuration of α- and β-amino acids as an auxiliary chromophoric system. The results presented above confirm the validity of the hexadecant rule to configurational assignment of α-amino acids and β-amino acids as well. It can be stated that, similarly to the α-amino acids, the sign of the 380 nm CE can also be used for the determination of the absolute configuration of β-amino acids. However, this method should be applied with care for *N*-protected amino acids. Depending upon the bulkiness of the protection group the particular Cotton effects can even change their signs.

The application of dimolybdenum tetraacetates as an auxiliary chromophore is especially useful in the case of flexible molecules. The stock complex reduces the conformational freedom of the ligands and forces flexible molecules into only one single conformation. This leads to an easier interpretation of CD spectra and often also to the larger CEs. In addition, it is noteworthy that such a restriction of the conformational freedom makes possible the assignment of the absolute configuration on the basis of the chiroptical data alone.

The fact that no quantitative values are obtained could be regarded as a disadvantage of the proposed method. However, for the purpose of determination of the absolute configuration only the signs and the relative magnitudes of the CEs are important, and not their absolute values. On the other hand, this disadvantage is compensated by the fact that there is no need to synthesize, isolate and purify any derivatives before obtaining the CD spectrum. It is worthy to consider that, in general, less than 1 mg of the potential ligand is sufficient to obtain a very good and reproducible CD spectrum. We have demonstrated that in an extreme case the determination of absolute configuration can be successfully carried out for the ligand concentration of around 8×10^{-6} M/L.

In conclusion, the *in situ* method can be regarded as a straightforward and versatile method for the determination of the absolute configuration of α- and β-amino acids, supplementary to other methods, e.g. exciton chirality method.

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