# HPLC ON CHIRAL NONRACEMIC SORBENTS WITH CIRCULAR DICHROISM DETECTION: STEREOISOMERS FORMED BY 1,3-DIPOLAR CYCLOADDITIONS

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## 1. Introduction

In this paper we would like to deal with the application of chiral nonracemic sorbents in HPLC separation of stereoisomers using a circular dichroism detector.

Sorbents for separation of stereoisomers by HPLC, in addition to possible achiral constituents like silica, are chiral nonracemic[1], i.e., they are composed of one type of chiral molecules, the excess of which exhibits one sense of chirality. Thus, (+)-poly(trityl methacrylate) on $SiO_2$ is an example of such a sorbent. It contains molecules of the chiral polymer, the excess of which exhibits one sense of chirality, namely the one corresponding to dextrorotation. These polymer molecules are on a matrix of $SiO_2$ fixed only by physical interactions but not chemically. Contrary to pure silica, chiral nonracemic sorbents may retain one of the enantiomers more strongly than the other one, thus causing their separation. Such a property found many applications[1,2], particularly in chemistry and pharmacy.

In addition to the usual UV detector, polarimeters[3,4,5] and, to some extent, circular dichrographs[6,7,8] have been used in HPLC when optically active components of a substrate mixture were analysed. Although polarimetry is preferred in preparative separations[3], circular dichroism (CD) has the advantage of furnishing on-line spectra[9] in analytical HPLC, the latter method being practically excluded[9] for polarimetry. Usually CD and UV detections use the same flow-through cell to monitor differential absorbance $\Delta A$ (the differential absorbance is defined by the equation $\Delta A = A_l - A_r$, where $A_l$ and $A_r$ are absorbances for left (l) and right (r) circularly polarized light) and absorbance $A$ at the same time at the same wavelength. The circular dichrograph provides additionally both qualitative and quantitative information since only chiral components are monitored, including their signs of $\Delta A$ at the chosen wavelength.

This article presents the above analytical methods by applying them to several mixtures of stereoisomers formed by 1,3-dipolar cycloadditions[10–12]. Thus, base-line separated and overlapped peaks as well as on-line CD spectra were measured and evaluated with respect to chemical purity, the number of stereoisomers, their relative proportions and their overall characterization.

## 2. Experimental part

HPLC was carried out using the system LC-10AD from Shimadzu Austria. Columns Chiralpak OT(+) (250 mm length, 4.6 mm in diameter) and Chiracel OD-H (150 mm length, 2.1 mm in diameter) were from Diacel Co., Himeji, Japan. The temperature of the columns was +4 °C. The detector[13] Jasco CD-1595 of Jasco International Co., Tokyo, Japan, provided UV (absorbance $A$) and circular dichroism (differential absorbance $\Delta A$) intensities in arbitrary units at a fixed wavelength between 220 and 420 nm. The flow-through cell has a path length of 25 mm and a volume of approximately 20 µl. The connection to a computer (Fig.1) was accomplished using the Chromatography Data System CLASS-VD 5.02 of Shimadzu Austria.

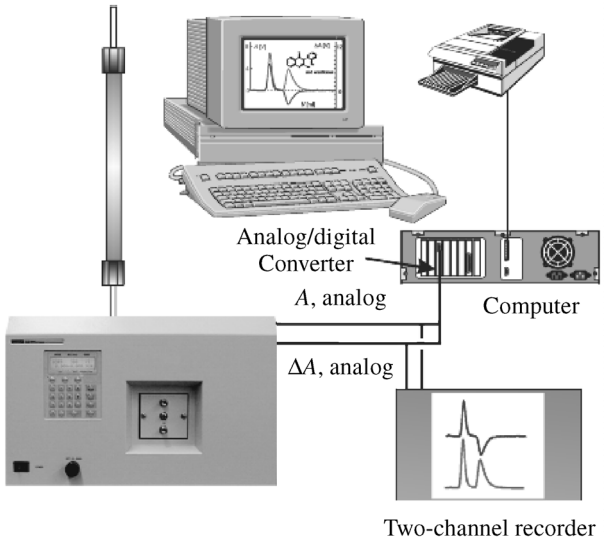



Fig.1. **Experimental set-up for HPLC with UV and circular dichroism detection**. The output of absorbance $A$ and differential absorbance $\Delta A$ as a function of retention time or retention volume is fed to a two-channel recorder and/or to a computer

The cycloadducts[14] investigated were: *IIIa*, yellowish crystals, m.p. 166–168 °C; *IIIb,b'* white crystals, m.p.132–137 °C, after recrystallization from petroleum ether/ethyl acetate at –20 °C, m.p. 135–136 °C; *IIIc,c' + IIId,d'*, yellow-orange oil.

However, for the investigation of cycloadduct[15] *Va,a'*; weakly yellow crystals, m.p. 125–126 °C, recrystallized from diethyl ether, a pump L 6000A, Merck-Hitachi, Darmstadt, Germany, was used and the temperature of the Chiralpak OT(+) column was adjusted to +15 °C. In this particular case, the computer program SEPP for WINDOWS[6,16,17] served to record the experimental chromatograms.

The retentions of the stereoisomers are given as retention factors $k = (t - t_0)/t_0$, where $t$ and $t_0$ are the retention time and the dead time, or $k = (v - v_0)/v_0$, where $v$ and $v_0$ are the retention volume and the dead volume.

Dependences of differential absorbance $\Delta A$ on retention factors $k$ and differential absorbance $\Delta A$ on wavelengths $\lambda$ (ranging between 220 and 420 nm) are shown in Figure 2.

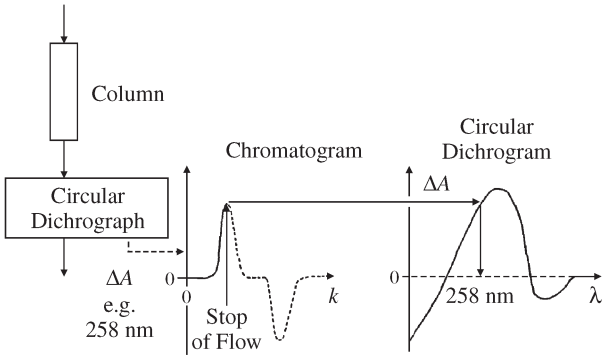



Fig. 2. **Measurement of differential absorbance $\Delta A$ as a function of retention factor $k$ and differential absorbance $\Delta A$ as a function of wavelength $\lambda$, using the set-up of Fig.1**. The column contains a nonracemic sorbent; the injected substrate may be racemic (as shown) or nonracemic. See the text for procedure

The diagram $\Delta A = f(A)$ and the deconvolution of the experimental chromatogram $\Delta A = f(v)$ (Fig. 3) were obtained by the computer program SEPP for WINDOWS[6,16,17].

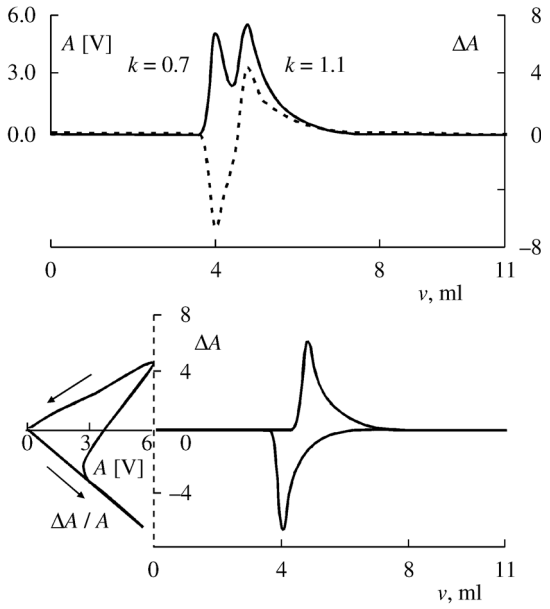



Fig. 3. **Separation of cycloadducts from Scheme 3** (3 μg) in *n*-hexane:propan-2-ol, 9:1, on (+)-poly(trityl methacrylate)/$SiO_2$ (Chiralpak OT(+)). Flow 0.5 ml/min, wavelength of detection 250 nm. Upper part: Experimental chromatograms $A = f(v)$ and $\Delta A = f(v)$ (full line UV detection, dotted line CD detection). Lower left part: Diagram $\Delta A = f(A)$, resulting in the ratio $\Delta A / A$. Lower right part: Computer deconvolution (see text) of the experimental chromatogram $\Delta A = f(v)$, using the ratio $\Delta A / A$ obtained

Approximate on-line CD spectra $\Delta A = f(\lambda)$ (Fig. 4) were obtained by stopping the chromatographic flow on the peaks of stereoisomers.

Figures 3 and 4 will be further discussed later in this paper.

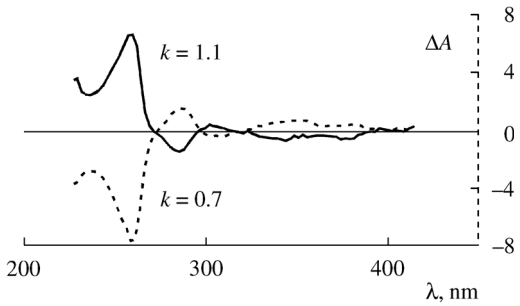



Fig. 4. **On-line CD spectra of the cycloadducts Va,a'** from Scheme 3, obtained for peaks at $k = 0.7$ and 1.1 in Fig. 3 (upper part) after flow stop

## 3. Results and discussion

Two base-line separated peaks

In the case of intramolecular 1,3-dipolar cycloaddition, an azomethine ylide is generated by the reaction of aldehyde *Ia* with amine *IIa* to give only racemic cycloadduct *IIIa* (Scheme 1). This was reflected by two HPLC peaks with opposite signs in the CD detection (Fig. 5). Although the presence of other probable stereoisomers peaks hidden in the main peaks cannot be easily excluded, the above stated assumption of the single racemate existence is correct, given by the excellent sensitivity[6] of the detector, because the crystals used for analysis represented the whole crop of the cycloaddition reaction and had not been worked up in any respect. From Fig. 5 it follows that optical purity can be determined if a nonracemic instead of a racemic sample is investigated.

In some cycloaddition reactions, (–)-menthol was used as a chiral auxiliary because it was assumed that its steric requirements cause shielding of one side of the dipole (Fig. 6) and the reaction therefore shows facial selectivity.

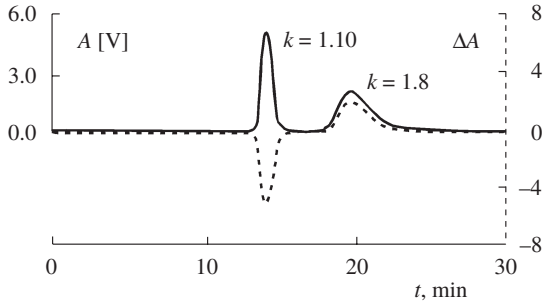
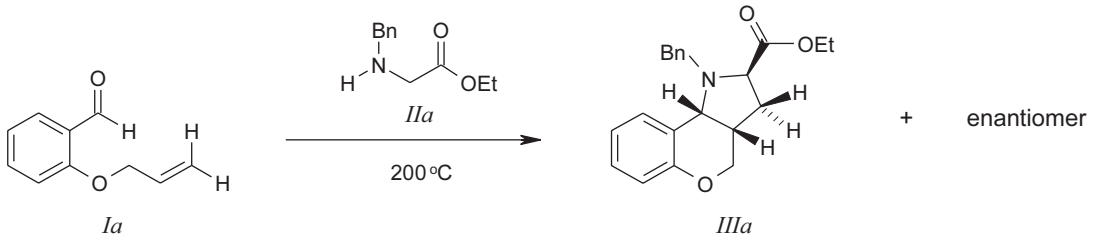



Fig. 5. **Separation of enantiomers of cycloadduct *IIIa*** (12 μg) in *n*-hexane:propan-2-ol, 9:1, on (+)-poly(trityl methacrylate)/$SiO_2$ (Chiralpak OT(+)). Flow 0.5 ml/min, wavelength of detection 267 nm, full line UV detection, dotted line CD detection




Scheme 1

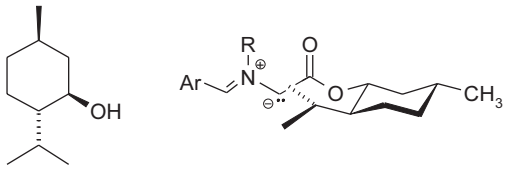



Fig. 6. **Structures of (–)-menthol and the related azomethine ylide**

However, $^1$H NMR and HPLC showed that the selectivity is very low, probably due to the rather long distance between the introduced auxiliary and the generated azomethine ylide. The reaction of aldehyde *Ia* with amine *IIb* affords peaks of two diastereomers *IIIb,b'* (Scheme 2) in a UV intensity ratio of 1.08:1 (Fig. 7, upper part; peaks at $k = 0.1$ and 1.6, respectively).

In principle, the UV absorption coefficients ε of diastereomers may be unequal; in the present case, however, the intensities were taken as good approximations for the relative concentrations. With reference to any other stereoisomers, the statements given above for *IIIa* are also true for the crystals of *IIIb,b'* because these had not been worked up before the first measurement. Recrystallization, however, yields one of the two diastereomers only (Fig. 7, lower part).

## Two overlapped peaks

The intermolecular 1,3-dipolar cycloaddition of the azomethine ylide generated from the phenanthridinium salt *IV* in the presence of base with dimethyl fumarate results in only two diastereomers *Va* and *Va'* (Scheme 3).

They were found as the product after recrystallization as shown by $^1$H NMR and HPLC (Fig. 3, upper part). In this case partial overlap of the HPLC peaks was observed. It has been

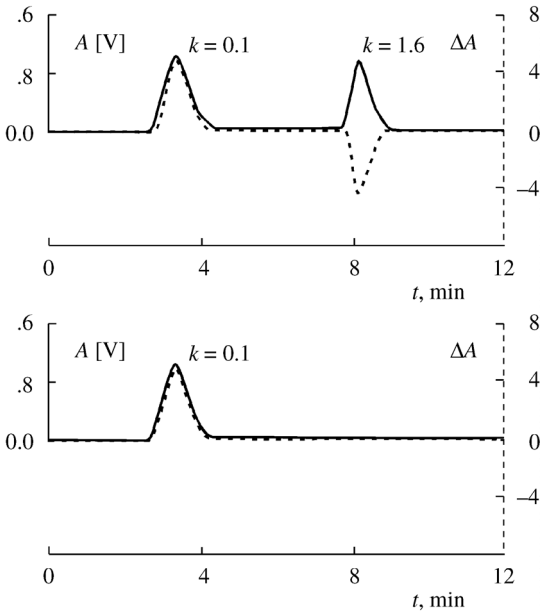
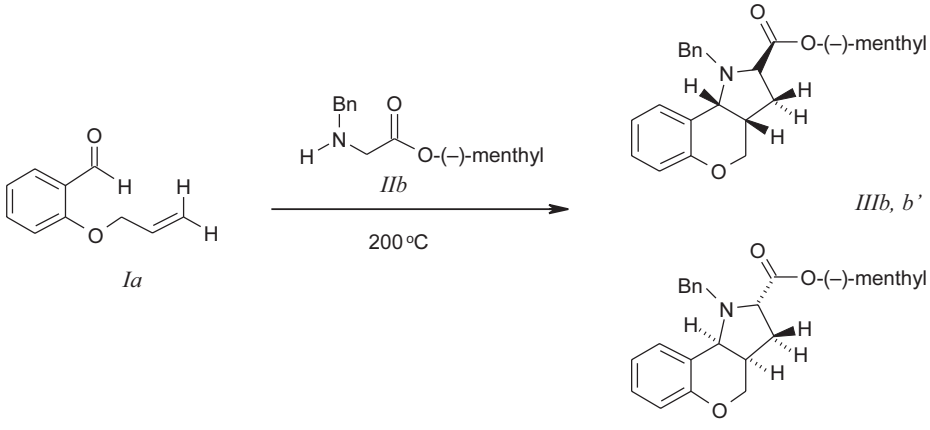
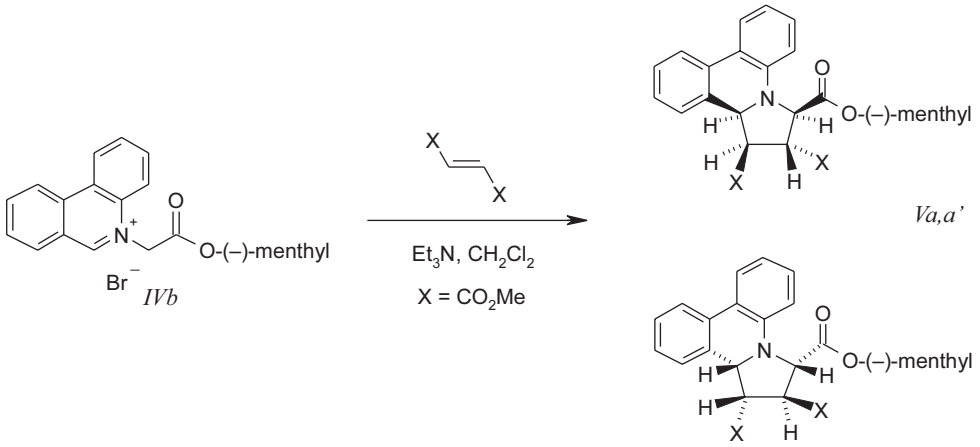



Fig. 7. **Separation of cycloadducts according to Scheme 2** in *n*-hexane: propan-2-ol, 4:1, on tris[*N*-(3,5-dimethylphenyl)carbamoyl]cellulose/$SiO_2$ (Chiracel OD-H). Flow 0.2 ml/min, wavelength of detection 267 nm. Upper record: 9 μg of cycloadducts before recrystallization, lower record: pure diastereoisomer after recrystallization from petroleum ether/ethyl acetate at –20 °C (full line UV detection, dotted line CD detection)

shown[3,6,7,16] that overlapped peaks of enantiomers can be deconvoluted by using double detection, i.e. by UV and CD. This approach treats overlapped peaks as the sum of their two




Scheme 2




Scheme 3

components (which means their difference upon CD detection because of the unequal signs of the present enantiomers). In addition, the ratio $\Delta A/A$ at the wavelength of detection is required for such deconvolutions. These ratios can be determined from a plot of a chiroptical property, e.g. $\Delta A$, versus the absorbance[3,6,16]. We obtained a $\Delta A = f(A)$ plot (Fig. 3, lower left part) and its computed deconvolution of the experimental chromatogram $\Delta A = f(v)$ for *Va* and *Va'* (Fig. 3, lower right part). This deconvolution shows that the HPLC technique described in the present article is not only useful in base-line separations such as in Fig. 5 and 7 but can be applied to overlapped peaks, too.

The diastereomers *Va* and *Va'* behave in a way similar to enantiomers, apparently, because the remote (–)-menthyl substituent has no significant influence on the UV and CD absorptions.

## On-line circular dichroism spectra

Upon UV detection at one wavelength in HPLC, it is possible to obtain spectra for some wavelength region during the whole chromatographic run by a technique called UV diode-array detection. In a similar way, circular dichrograms $\Delta A = f(\lambda)$ have been measured[9] (Fig. 2) during stops of the chromatographic flow (or even without such a stop). This on-line procedure can be accomplished by injection of a racemate, whereas the usual measurement of CD spectra ("off-line" technique) requires a pure or enriched enantiomer. Using a mixture of diastereomers *Va* and *Va'*, we obtained their on-line spectra (Fig. 4). They show better characterizations of stereoisomers than the signs of differential absorbances or angles of rotation obtained in HPLC separations, both of which refer to a single wavelength only. Together with further CD information on this class of compounds, these CD spectra may serve for the determination of relative configurations. Their

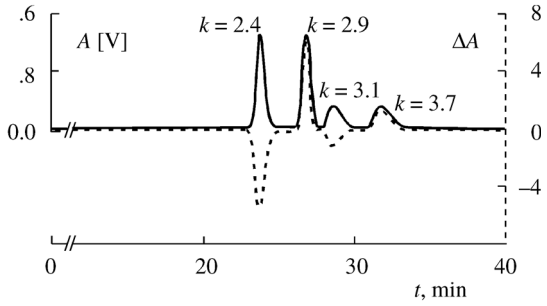
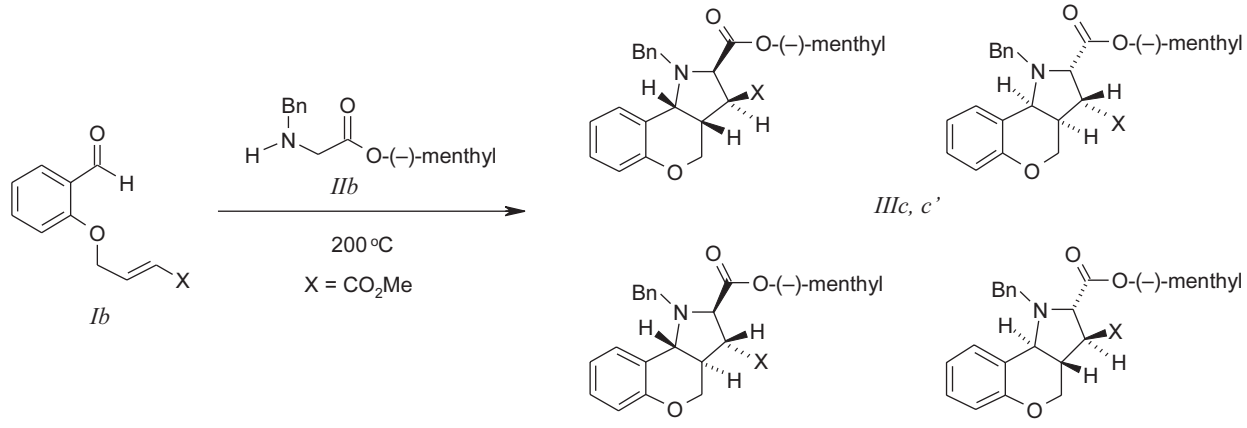



Fig. 8. **Separation of cycloadducts** *IIIc,c'* **+** *IIId,d'* (11 μg) in *n*-hexane:propan-2-ol, 9:1, on (+)-poly(trityl methacrylate)/$SiO_2$ (Chiralpak OT(+)). Flow 0.5 ml/min, wavelength of detection 267 nm (full line UV detector, dotted line CD detector)




Scheme 4

mirror image relationship again shows that the diastereoisomers *Va* and *Va'* behave in a way similar to enantiomers.

Four base-line separated peaks

An intramolecular 1,3-dipolar cycloaddition of aldehyde *Ib* with amine *IIb* resulted in four stereoisomers *IIIc,c',d,d'* (Scheme 4).

They were detected by $^1$H NMR and HPLC (Fig. 8). The intensities of their peaks are in the ratio 4.06:4.01:1.03:1 (from low to high retention factors in Fig. 8). No other stereoisomers were detected; the oil prepared by this cycloaddition reaction was measured immediately without any work-up. The tentative assignments given were obtained in the following way: Diastereomers *IIIc* and *IIIc'* show similar intensities because, disregarding the remote (–)-menthyl substituent, they are enantiomers; the same is true for diastereomers *IIId* and *IIId'*. The higher intensities at $k$ = 2.4 and 2.9 were assigned to *IIIc,c'*, because we managed to separate them from *IIId,d'* and identify them by NMR.

## 4. Conclusion

HPLC is shown to be useful for the analysis of the stereoisomers formed by 1,3-dipolar cycloadditions. NMR spectroscopy usually furnishes more information on stereostructural details than HPLC, particularly *via* vicinal coupling constants and the nuclear Overhauser effect. On the other hand, HPLC on chiral nonracemic sorbents, unlike usual NMR, distinguishes between enantiomers and, using CD detection, permits structure assignment. In particular, the HPLC technique was applied to cycloadducts with the following results:

No impurities, e.g. regioisomers, were detected in the products of synthesis.

The number of stereoisomers was determined and their relative proportions were measured *via* HPLC intensities.

Each stereoisomer was characterized by its retention factor, the sign of its CD differential absorbance at one wavelength or its on-line CD spectrum between 220 and 420 nm. All the information was obtained by using synthetic mixtures of the stereoisomers. However, pure or enriched compounds are required when CD is measured without a combination with HPLC.

The present experiments were carried out with a commercial UV/CD instrument connected to HPLC, which means that the concentration in the detector cell is normally unknown. Therefore, the differential absorption coefficient $\Delta\varepsilon = \Delta A/c.l$ ($l$ – path length in the cell, $c$ – concentration) cannot easily be determined. $\Delta\varepsilon$ is usually obtained with CD spectrometers if the sample in known concentration is filled into the cell directly ("off-line" technique), not *via* chromatographic flow. However, most common CD instruments can be adapted for HPLC (on-line technique) using a commercial kit. Therefore, the instrumental situation and the results presented above might encourage organic stereochemists to add HPLC with CD detection to the analytical tools they already use.

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REFERENCES

1. Eliel E. L., Wilen S. H.: *Stereochemistry of Organic Compounds,* p. 5, 214 and 249. Wiley, New York 1994.
2. Ahuja S.: *Chiral Separations by Chromatography*. Oxford University Press, Oxford 2000.
3. Brandl F., Pustet N., Mannschreck A.: J. Chromatogr., A *909*, 147 (2001).
4. Mannschreck A., Schinabeck M., Brandl F.: Chem. Listy *92*, 261 (1998).
5. Bobbitt D. R., Linder S. W.: Trends Anal. Chem. *20*, 111 (2001).
6. Brandl F., Pustet N., Mannschreck A.: Int. Lab. *29*(2), 10C (1999).
7. Gergely A., Horváth P., Noszál B.: J. Chromatogr. Sci. *38*, 425 (2000).
8. Salvadori P., Di Bari L., Pescitelli G., in: *Circular Dichroism: Principles and Applications* (Berova N., Nakanishi K., Woody R. W., Eds.), p. 797. Wiley, New York 2000.
9. Mannschreck A.: Trends Anal. Chem. *12*, 220 (1993).
10. Potáček M., Topinka T., Dostál J., Humpa O.: Collect. Czech. Chem. Commun. *60*, 1191 (1995).
11. Trávníček M., Pospíšil J., Potáček M.: Collect. Czech. Chem. Commun. *64*, 1993 (1999).
12. Trávníček M., Potáček M., Mannschreck A.: Heterocycl. Commun. *7*, 213 (2001).
13. Jasco International Co., Ltd., Tokyo, Japan: *Model CD--995 Circular Dichroism Chiral Detector*. Information Sheet, 1998.
14. Pospíšil J.: *MSc Thesis*. Masaryk University, Brno 2002.
15. Trávníček M.: *PhD Thesis*. Masaryk University, Brno 2002.
16. Mannschreck A.: Chirality *4*, 163 (1992).
17. Schinabeck M.: *PhD Thesis*. University of Regensburg, Regensburg 1998.

**J. Pospíšil[a], M. Trávníček[a], A. Mannschreck[b], and M. Potáček[a]** *([a]Department of Organic Chemistry, Masaryk University, Brno, Czech Republic, [b]Department of Organic Chemistry, University of Regensburg, Regensburg, Germany):* **HPLC on Chiral Nonracemic Sorbents with Circular Dichroism Detection: Stereoisomers Formed by 1,3-Dipolar Cycloadditions**

The stereoisomers of several 1,3-dipolar cycloadducts were analysed without previous preparative separation by HPLC on chiral nonracemic sorbents. This technique contributed significantly to the chemical purity checking, to the determination of the number of stereoisomers present, to the measurement of their relative proportions and to their characterization by retention factors and circular dichroism (CD) properties. In addition to a usual UV photometer, the detection was carried out with a CD instrument at a fixed wavelength or, on stopping the chromatographic flow, between 220 and 420 nm. The present method is proposed as a supplementary analytical tool in organic stereochemistry.