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Journal of Chromatography A, 664 (1994) 169–176

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatography of diamine enantiomers as Schiff bases on a chiral stationary phase☆

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(First received July 12th, 1993; revised manuscript received December 14th, 1993)

Abstract

A high-performance liquid chromatographic method for the enantiomeric analysis of chiral 1,2-diamines is described. By derivatization with benzaldehyde, aliphatic and aromatic 1,2-diamines were converted into Schiff base derivatives. Separation of the enantiomeric derivatives was performed on a commercially available cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase. To investigate the effect of the reagent structure on enantioseparation, a series of twenty ring-substituted benzaldehydes were reacted with an aliphatic and an aromatic diamine. The influence of the type of substituent of the benzaldehyde reagent on the enantioselectivity obtained for Schiff base derivatives is discussed.

1. Introduction

Enantiopure diamines are important intermediates for the production of several pharmaceuticals [1]. One of the routes to obtain these compounds is by reduction of enantiopure amino acid amides with lithium aluminium hydride in tetrahydrofuran [2]. In conjunction with this synthesis, analytical methods are required for the control of the enantiopurity of the reaction products. Preliminary experiments in our laboratory showed that by means of high-performance liquid chromatography (HPLC) on a crown ether chiral stationary phase and ligand-exchange chromatography, using N,N-dipropyl-L-alanine as chiral selector, no enantioseparation could be achieved for series of aliphatic 1,2-diamines. Recently, an HPLC method was described for the enantioseparation of chiral 1,2-diamines [3]. However, the Ni(II) chelates that are used as chiral selectors in the mobile phase are not commercially available.

In this paper, we describe an HPLC method for the enantioseparation of a series of chiral 1,2-diamines as their Schiff bases on a cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase (OD-CSP). For Schiff base formation, benzaldehyde was used as a reagent. Further, a series of twenty-ring-substituted benzaldehydes were reacted with an aliphatic and an aromatic diamine in order to investigate the effect of ring substitution on the enantioselectivity of the diamines.

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☆ Parts of this paper were presented at the *4th International Symposium on Chiral Discrimination, Montreal, September 19–22, 1993.*

0021-9673/94/$07.00
SSDI 0021-9673(93)E0061-X

2. Experimental

2.1. Materials

(±)- and (*R*)-1,2-diamino-3-methylbutane, (±)- and (*R*)-1,2-diamino-4-methylpentane, (±)-1,2-diamino-3,3-dimethylbutane, (±)- and (*S*)-1,2-diamino-2-phenylethane and (±)-1,2-diamino-2,3-dimethylbutane were prepared according to ref. 2.

(±)-1,2-Diaminopropane, benzaldehyde, 2-methylbenzaldehyde, 3-methylbenzaldehyde, 4-methylbenzaldehyde, 1-naphthaldehyde, 2-naphthaldehyde, 2-methoxybenzaldehyde, 3-methoxybenzaldehyde, 4-methoxybenzaldehyde, 4-ethoxybenzaldehyde, 4-butoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, 3-methoxy-4-ethoxybenzaldehyde, 3-phenoxybenzaldehyde, 4-cyanobenzaldehyde, 2-chlorobenzaldehyde, 3-chlorobenzaldehyde, 4-chlorobenzaldehyde and 3,4-dichlorobenzaldehyde were obtained from Janssen (Beerse, Belgium) and 4-ethylbenzaldehyde from Aldrich (Milwaukee, WI, USA). HPLC-grade methanol, 2-propanol and *n*-hexane were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade.

2.2. Instrumentation

The chromatographic system consisted of a Gilson (Villiers-le-Bel, France) Model 302 pump and a Spark (Emmen, Netherlands) Marathon autosampler for injection. The injection loop had a 20-μl capacity. The column used was a Daicel Chiralcel OD (250 × 4.6 mm I.D., 10 μm) from J.T. Baker (Deventer, Netherlands). The flow-rate was 1.0 ml/min and the column was operated at ambient temperature. The column effluent was monitored with a Linear Instruments (Reno, NV, USA) Model 204 absorbance detector set at 245 nm. UV spectra of Schiff base derivatives were recorded with a Hewlett-Packard (Palo Alto, CA, USA) Model 1040A diode-array detector. ^{13}C NMR analysis of Schiff base derivatives was performed with a Bruker (Karlsruhe, Germany) AM 400 instrument. Spectra were recorded at 100 MHz. For LC–mass spectrometry (MS), a Finnigan MAT (San José, CA, USA) TSQ-70 triple quadrupole mass spectrometer equipped with a thermospray interface (Finnigan MAT) was used. The chromatographic conditions for LC–MS analysis were identical with those for HPLC–UV analysis. MS analysis was performed using chemical ionization with gaseous ammonia [4].

2.3. Derivatization

About 5 mg of the diamine were dissolved in 1 ml of methanol and the solution was adjusted to pH 12 with 1 *M* sodium hydroxide solution. Benzaldehyde (0.25 mmol) was added, after standing for 20 min at 40°C in a thermostated mixing bath methylamine (8 mmol) was added and the reaction mixture was allowed to stand for another 20 min at 40°C. A 50-μl volume of the reaction mixture was diluted with *n*-hexane (1 ml) and an aliquot of this solution was injected into the HPLC system.

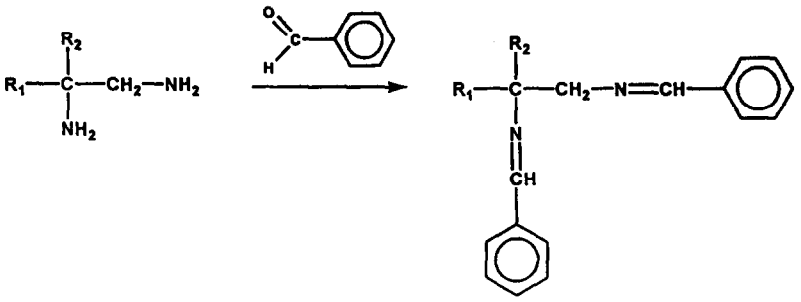
For ring-substituted benzaldehydes, the same derivatization procedure was applied.

3. Results and discussion

3.1. Schiff base derivatization

The products of the reaction of diamines with benzaldehyde were studied by means of ^{13}C NMR spectrometry and LC–MS. The NMR data showed that Schiff base formation occurred at both amino groups of the diamines. No *syn–anti* isomers could be observed by means of NMR. A reaction scheme for Schiff base formation of chiral diamines with benzaldehyde is given in Eq. 1.

To validate the enantioseparation obtained by HPLC–UV analysis, Schiff base derivatives of racemic diamines were analysed by means of LC–MS. For the racemic diamines studied, the mass chromatograms showed both enantiomers whose mass spectra were in accordance with the Schiff base structure shown in Eq. 1.



(1)

UV spectra were recorded for the Schiff bases of the aliphatic diamines studied. From these spectra, an absorbance maximum of 245 nm was selected for detection of the derivatives.

The rates of derivative formation of diamines with benzaldehyde were studied as a function of the reaction time at 40°C. As an example, the formation of the Schiff base of 1,2-diamino-3,3-dimethylbutane is given in Fig. 1.

Complete derivatization of the diamines studied was accomplished within 2 h at 40°C. A good compromise between yield and derivatization times was found by choosing 20 min as the reaction time, which resulted in a Schiff base yield of 75 ± 5% for the compounds studied.

Methylamine was added to the reaction mixture to convert the excess aldehyde into the corresponding Schiff base. Whereas in several instances the benzaldehydes studied interfered with the chromatographic separation of diamine enantiomers, the Schiff bases of the aldehydes with methylamine eluted with a larger k' than the diamine derivatives. For further optimization of a particular separation, the use of other alkylamines may be considered.

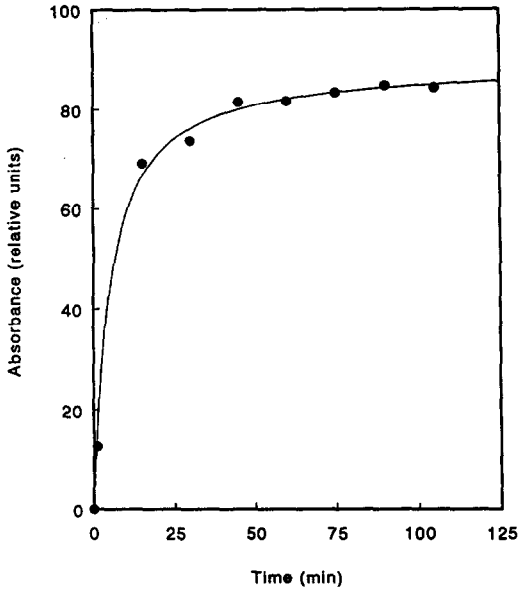


Fig. 1. Absorbance response of the Schiff base derivatization of 1,2-diamino-3,3-dimethylbutane with benzaldehyde as a function of reaction time at 40°C. The molar excess of benzaldehyde was tenfold relative to the amine studied.

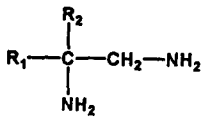
3.2. Enantioselective analysis

Using benzaldehyde as a reagent, the enantioselectivity of a series of 1,2-diamines as their Schiff bases was examined on an OD-CSP. The chromatographic data for the diamines studied are given in Table 1.

It can be seen that the type of substituent (R_1, R_2) on the chiral carbon has a marked effect on the enantioselectivity and resolution. The highest α values were obtained for the diamines with R_1 = H and R_2 = Me or iPr. With respect to the structural isomers studied, *i.e.*, R_1, R_2 = (H, iBu), (H, tBu) and (Me, iPr), it appears that (H, tBu) substitution gives the best selectivity. In comparison with the aliphatic substituents, the phenyl-substituted diamine shows a lower α value. Typical chromatograms of the Schiff base derivatives are shown in Fig. 2.

On the basis of the availability of one of the enantiopure forms, the elution order of the enantiomers of 1,2-diamino-3-methylbutane, 1,2-diamino-4-methylpentane and 1,2-diamino-2-

Table 1
Capacity factors (k'), selectivities (α) and resolution (R_s) of Schiff bases derived from benzaldehyde and chiral diamines with the structure shown



R_1	R_2	k'^a	α	R_s
H	Me	2.04	5.75	18.21
H	iPr	0.87	6.43	21.55
H	iBu	1.27	2.19	9.62
H	tBu	0.49	5.44	15.62
H	Ph	1.24	1.49	4.76
Me	iPr	0.61	2.56	9.20

Mobile phase: *n*-hexane–2-propanol (90:10). For other conditions, see Experimental.
[a] Capacity factor of the first-eluted enantiomer.

phenylethane was determined. In all instances the *R*-form eluted before the *S*-form.

To investigate the effect of ring-substituted benzaldehydes on the chromatographic behaviour of the corresponding Schiff base derivatives of 1,2-diamines, a series of twenty ring-substituted benzaldehydes were reacted with 1,2-diamino-2-phenylethane (DAPE) and 1,2-diamino-4-methylpentane (DAMP). The results are presented in Table 2.

For the enantioseparation of both DAPE and DAMP enantiomers, sixteen out of the twenty ring-substituted benzaldehydes gave successful results. In general, the enantioselectivities obtained for the DAMP derivatives were higher than those for DAPE derivatives. Only three reagents gave higher α values for DAPE than for DAMP. With DAPE, the highest enantioselectivity was obtained for 3-phenoxybenzaldehyde ($\alpha = 3.27$). 3-Methylbenzaldehyde gave the highest α value (2.69) for DAMP. Large differences were found for the structural isomers of the benzaldehydes studied. For the methyl-substituted benzaldehydes, substitution at the 3- or 4-position resulted in higher enantioselectivities than for the 2-substituted analogue. The same phenomenon was observed with the structural isomers of chloro-, methoxy- and naphthyl-substituted benzaldehydes. With respect to the k'

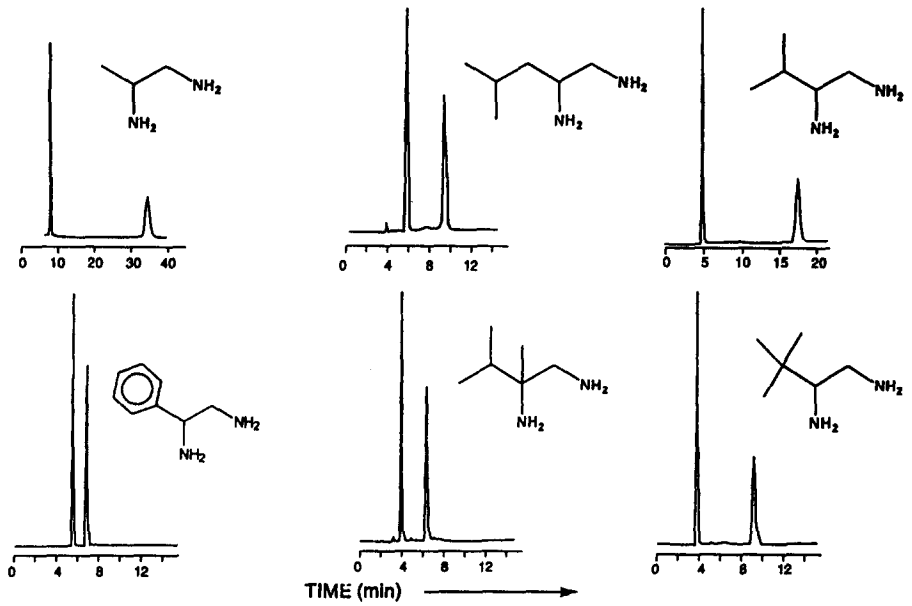
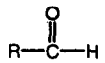
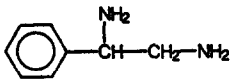
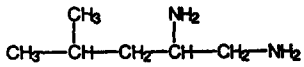
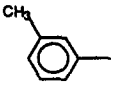
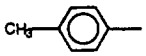
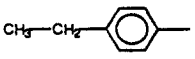
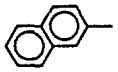
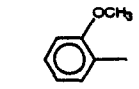
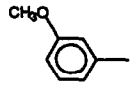
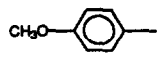
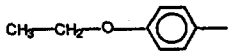
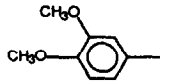
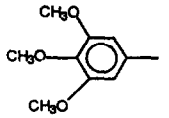


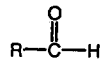
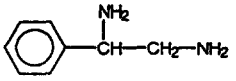
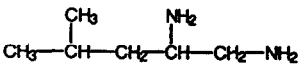
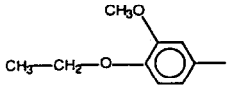
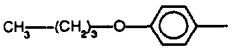
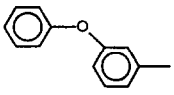
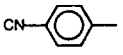
Fig. 2. Separation of the enantiomers of 1,2-diamines after derivatization with benzaldehyde. Mobile phase: *n*-hexane–2-propanol (90:10). Except for 1,2-diaminopropane [t_R (benzaldehyde) = 5 min], the excess benzaldehyde was reacted with methylamine [t_R (Schiff base) = 24 min]. For other conditions, see Experimental.

Table 2
Capacity factors (k'), selectivities (α) and resolution (R_s) of 1,2-diamino-2-phenylethane and 1,2-diamino-4-methylpentane enantiomers after derivatization with several ring-substituted benzaldehydes

Reagent	Compound						
							
R	k'^a	α	R_s	pK_a^b	k'^a	α	R_s
	1.53	1.55	4.80	6.00 5.79	1.86	2.31	10.60
	0.84	1.28	2.21	6.28 6.07	0.63	1.83	5.03
	1.54	1.62	5.67	6.12 5.91	1.69	2.69	11.50
	1.49	1.57	4.57	6.28 6.07	1.54	2.24	10.70
	1.69	1.15	1.40	6.30 6.09	1.92	1.43	4.20
	2.83	1.16	1.79	6.00 5.79	1.88	1.13	1.47
	4.16	2.66	11.70	6.00 5.79	3.43	2.68	12.00
	0.98	1.00	0	6.56 6.35	0.97	1.00	0
	4.18	1.55	5.48	5.78 5.57	5.31	2.34	11.20
	6.10	1.38	4.49	6.56 6.38	7.02	1.72	7.15
	2.95	1.42	3.86	6.48 6.27	3.02	2.07	8.20
	1.65	1.00	0	6.34 6.16	1.65	1.00	0
	1.54	1.00	0	6.12 5.94	1.55	1.00	0

(Continued on p. 174)

Table 2 *(continued)*

Reagent	Compound						
							
R	k'^a	α	R_s	$pK_a{}^b$	k'^a	α	R_s
	1.19	1.00	0	6.26 6.05	1.21	1.00	0
	2.07	1.49	4.27	6.64 6.43	2.01	2.66	10.20
	8.55	3.27	13.10	5.50 5.29	3.44	1.78	6.86
	12.50	1.06	0.84	4.60 4.39	4.30	1.06	1.57
	0.76	1.10	0.68	5.52 5.31	0.49	1.18	1.05
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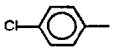
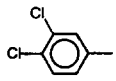
	1.08	1.18	1.60	5.52 5.31	0.57	1.27	1.76
	1.50	1.28	2.75	4.78 4.57	0.67	1.14	1.04

Mobile phase: *n*-hexane–2-propanol (95:5). For other conditions, see Experimental.

[a] Capacity factor of the first-eluted enantiomer.

[b] Calculated pK_a values [11] for both nitrogens of the Schiff base derivatives of 1,2-diamino-2-phenylethane. For the corresponding Schiff bases of 1,2-diamino-4-methylpentane the pK_a values were $pK_{a_{1(DAPE)}} + 0.04$ and $pK_{a_{2(DAPE)}} + 0.03$.

values, 2-substituted benzaldehydes showed lower values than the corresponding 3- or 4-analogues. These effects occurred for both DAMP and DAPE enantiomers. For 4-alkoxy-substituted benzaldehydes, an increase in enantioselectivity was observed on going from methoxy, to ethoxy and to butoxy substituents. However, the retention decreased in this series (for both DAPE and DAMP). In contrast to the mono-substituted alkoxybenzaldehydes, no chiral recognition was obtained for the di- or tri-substituted analogues.

In the following, the results obtained will be discussed in terms of chiral recognition mechanisms. In general, chiral recognition on cellulose carbamate phases is believed to occur mainly

through hydrogen bond formation between the urethane groups of the CSP and functional groups of the solutes capable of forming hydrogen bonds [5–10]. More particularly, the NH and C = O groups of the urethane moiety may act as hydrogen bond donor and acceptor, respectively, for the solutes. All the Schiff base derivatives in this study have sites for hydrogen bond acceptance, *i.e.*, the imine nitrogens. Consequently, the main hydrogen bond is expected to be formed between the imine nitrogens of the solute and the hydrogen atom on the carbamate nitrogen. In addition to the interaction via hydrogen bonds, the aromatic groups in the Schiff bases might be expected to participate in $\pi-\pi$ interactions with the CSP.

With regard to the different types of substituted benzaldehydes used, it is of interest to investigate the effect of the electron-donating and electron-withdrawing power of the substituents on the basicity of the imine nitrogens. A change in the basicity of the imine nitrogens is expected to cause a change in the capability for hydrogen bond formation of the derivatives with the CSP, which in turn might influence chiral recognition. In order to investigate possible relationships between the basicity of the Schiff bases and their retention and enantioselectivity, pK_a values were calculated using the program pKalc 2.0 [11] for the derivatives studied (Table 2).

Regression analysis was used to correlate the pK_a values of the Schiff bases with each of the following chromatographic data: k'_{DAPE}, k'_{DAMP}, k'_{DAMP}/k'_{DAPE}, α_{DAPE} and α_{DAMP}. Of these, only the relationship between pK_a and the ratio k'_{DAMP}/k'_{DAPE} was found to be statistically significant (confidence level > 99.99%). For $pK_{a_1(DAPE)}$, the relationship is shown in Fig. 3. The explained variance (r^2) was 62%. When not taking 3-methoxybenzaldehyde into account, r^2 increased to 75%. The straight line in Fig. 3 shows the result of the fit between the data. From the plot it can be seen that the retention of the DAPE derivative compared with that of the corresponding DAMP derivative increases with decreasing basicity of the derivative. This may be explained by an additional $\pi-\pi$ association of

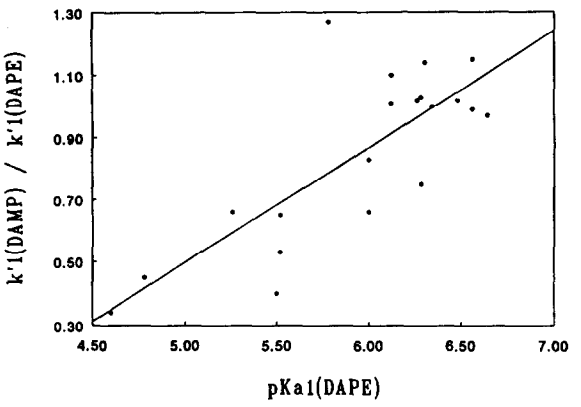


Fig. 3. Plot of $k'_{1(DAMP)}/k'_{1(DAPE)}$ *versus* $pK_{a_1(DAPE)}$.

the phenyl group of DAPE with the CSP in the case of Schiff bases with low basicity.

For all the Schiff base derivatives studied, except that derived from 1,2-diamino-4-methylpentane and 4-cyanobenzaldehyde, the *R*-enantiomer eluted before the *S*-form.

4. Conclusions

Derivatization of chiral 1,2-diamines with benzaldehyde yields the corresponding Schiff base enantiomers, which can readily be resolved on a cellulose tris(3,5-dimethylphenylcarbamate) CSP. The enantioseparation of an aliphatic and an aromatic 1,2-diamine with twenty ring-substituted benzaldehydes revealed that the enantioselectivity depended greatly on the type of substituent and the position and number of substituents on the phenyl rings. For the benzaldehydes tested, substitution at the 2-position showed lower enantioselectivities than with the same substituent in the 3- or 4-position. Statistical analysis showed that there was no significant correlation between the pK_a values and α values of the Schiff base derivatives.

5. Acknowledgements

We thank Mr. G. Kwakkenbos for the LC–MS analysis and Dr. N.K. de Vries for the ^{13}C NMR measurements.

6. References

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