Journal of Chromatography, 627 (1992) 97–105
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 595

Use of chiral monohalo-*s*-triazine reagents for the liquid chromatographic resolution of DL-amino acids

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(First received June 19th, 1992; revised manuscript received August 27th, 1992)

ABSTRACT

Nucleophilic replacement of one halogen atom (chlorine, fluorine) in the trihalo-*s*-triazines (2,4,6-trihalo-1,3,5-triazines), cyanuric chloride or cyanuric fluoride by reaction with either methanol, 2-naphthol, 1-methoxynaphthalene, or 4-aminoazobenzene furnished ultraviolet-absorbing, fluorescent of chromogenic dihalo-*s*-triazines. Substitution of a further halogen atom in these compounds by reaction with L-alanine amide provided chiral monohalo-*s*-triazines. The remaining halogen atom was substituted by reaction with selected D- or L-amino acids to form diastereomeric derivatives which were separated by reverse-phase (C_{18}) high-performance liquid chromatography using mixtures of water, acetonitrile and trifluoroacetic acid as eluents. Because of its possibilities for selection among a large number of detection groups in combination with various chiral moieties, the approach is considered to be a general method for the design and construction of tailor-made reagents suitable for precolumn derivatization and indirect liquid chromatographic separation of amino acid enantiomers.

INTRODUCTION

A number of chiral reagent suitable for precolumn derivatization have been described for the indirect, liquid chromatographic resolution of DL-amino acids (DL-AA) as diastereoisomers. Some examples are naphthylethyl isocyanate [1], 9(+)-fluorenylethyl chloroformate [2], chiral thiols together with *o*-phthaldialdehyde [3], N^2-(5-fluoro-2,4-dinitrophenyl)-L-alanine amide (Marfey's reagent) [4] or structurally related chiral variants of Sanger's reagent [5,6]. The latter reagents deserve attention because of the large differences in the retention times of the diastereomers formed. This observation has been explained in terms of the planar configuration of the dinitrobenzene moiety and the spatial arrangement of the AA units in the diastereomers, which make the formation of an intramolecular hydrogen bond more favourable in the L–L diastereomer than in the L–D diastereomer (the first letter refers to the configuration of the AA in the reagent, *cf.*, Fig. 1).

On the basis of the structural similarity of the diastereomers formed by reactions of DL-AA with chiral fluorodinitrobenzenes and chiral monohalo-*s*-triazines (Fig. 1), it was assumed that the latter derivatives might also be resolvable by high-performance liquid chromatography (HPLC). Owing to the trifunctionality of the starting compound trichloro-*s*-triazine, or the more reactive trifluoro-*s*-triazine, it should be possible to design reagents containing a large selection of detectable groups in addition to chiral moieties.

In principle, the general chemistry used for the syntheses of triazine derivatives used as biocides [7,8] and of reactive dyes with heterocyclic reactive systems [9] is applicable; this also indicates the potential scope of the approach described in this paper. Although triazine derivatives are important fungicides and herbicides [7,8] and triazine reactive dyes are of paramount importance for the dying of wool and cellulose fibres [9–11], a number of

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0021-9673/92/$05.00

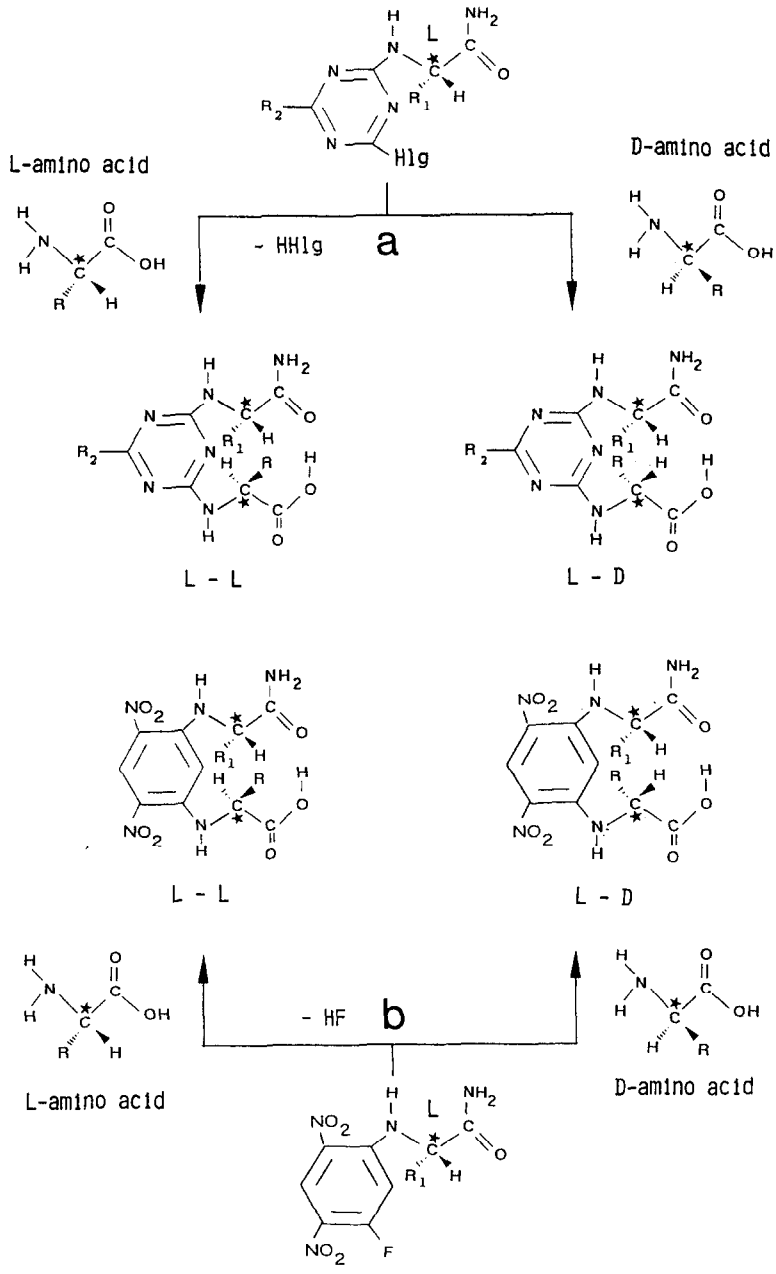

Fig. 1. Structures of diastereomers obtained by derivatization of DL-amino acids with (a) chiral monohalo-*s*-triazines (Hlg = halogen, Cl or F) in comparison with diastereomers formed by reaction with (b) chiral N^2-(5-fluoro-2,4-dinitrophenyl)-L-amino acid amides. R = side-chains of chiral amino acids to be analyzed, R_1 = methyl in alanine amide; R_2 = detectable or otherwise suitable group in (a); asterisks indicate chiral centres.

applications have also been reported in chromatography. Chromogenic reactive dyes are employed in affinity chromatography of proteins [12], dichlorotriazinylaminofluorescein has been used as a fluorescence label for immunoglobulins [13], 1-ethoxy-4-(dichloro-*s*-triazinyl)naphthalene was described as a fluorescent reagent for corticosteroids [14] and chiral *s*-triazines have been used a chiral stationary phases in gas–liquid chromatography [15].

EXPERIMENTAL

Instruments

A JASCO (Kyoto, Japan) instrument consisting of a Model 880 PU reciprocating pump with active damper, a Model 880-02 low-pressure gradient former, a Model 801-SC controller and a Model 875-UV variable-wavelength UV detector or a Model RF-535 fluorescence detector (Shimadzu, Kyoto, Japan) was used for HPLC. A Chromato-Integrator D-2500 (Merck–Hitachi, Darmstadt, Germany) was used for data processing and samples were injected on to the column by means of a Model ISS-100 autosampler (Perkin-Elmer, Überlingen, Germany).

Chromatography

Nucleosil 100 C_{18}, particle size 5 μm (Macherey–Nagel, Düren, Germany), was packed into 250 mm × 4 mm I.D. steel columns equipped with guard cartridges (20 × 4 mm I.D.) packed with the same stationary phase.

Eluents for HPLC are specified in Fig. 3; the water used was deionized by reverse osmosis with a Milli-Q water purification system (Millipore, Bedford, MA, USA); the eluents were filtered through a 0.45-μm filter (HVL P04700; Millipore) and degassed by sonication before used.

Chemicals

Chemicals and solvents used for HPLC were of analytical-reagent or for chromatography grade from Merck (Darmstadt, Germany). Light petroleum was of b.p. 50–70°C. Amino acids were purchased from Sigma (St. Louis, MO, USA) or Fluka (Buchs, Switzerland). L-Alanine amide hydrochloride was obtained from Novabiochem (Läufelfingen, Switzerland), cyanuric chloride and 1-methoxynaphthalene from Aldrich (Steinheim, Germany) and cyanuric fluoride and 4-phenylazoaniline (4-aminoazobenzene) from Fluka.

Derivatization of standard amino acid solutions

Standard solutions of selected DL-amino acids (100 m*M* in 1 *M* HCl) were prepared. To 30 μl (3 μmol) of standard solution, 45 μl of 1.0 *M* $NaHCO_3$ solution and 500 μl (5 μmol) of the reagent solution were added and the mixture was heated for 1 h at 80°C (for FAZT-Ala-NH_2, CNOT-Ala-NH_2 and CMOT-Ala-NH_2) or at 100°C (for CAZT-Ala-NH_2 and CMNT-Ala-NH_2). Then 30 μl of 1.0 *M* HCl and 1.4 ml of dimethyl sulphoxide (DMSO) were added, the mixture was filtered by means of a 0.2-μm disposable filter (Anotop 10, pore size 0.2 μm; Merck) and 1-μl aliquots were injected on to the HPLC column, with the exceptions of CNOT-Ala-NH_2 and CMOT-Ala-NH_2, for which 5 μl were injected.

Syntheses and characterization of dihalo-s-triazines and chiral monohalo-s-triazines

Thin-layer chromatography, mass spectrometry, UV–Vis and fluorescence spectrometry. For thin-layer chromatography (TLC), precoated plates (Kieselgel 60 F_{254}, size 20 × 20 cm; Merck) were used; R_F values were determined at 21°C in glass chambers (Desaga, Heidelberg, Germany) coated with filter-paper; the distance from the start to the front of the TLC plates was 10 cm. The solvent systems were as follows (v/v): (I) light petroleum–acetone (55:45); (II) light petroleum–chloroform–methanol (40:30:30); and (III) ethyl acetate–light petroleum (60:40). Spots were made visible by inspection of the TLC plates under UV light at 254 nm.

Electron impact mass spectrometry was performed at an ionizing energy at 70 eV using a Varian 311 A mass spectrometer. Mass spectra of compounds containing chlorine were calculated for the most abundant isotopes ^{35}Cl and ^{37}Cl; int. refers to intense ions in the mass spectra. Melting points (m.p.) or decomposition temperatures (decomp.) of compounds were determined in open capillaries using a Model 520 melting point apparatus (Büchi, Flawil, Switzerland) and are not corrected.

Spectra in the ultraviolet (UV) and visible (Vis) ranges were taken using a Model DU 64 spectrophotometer (Beckman Instruments); a Model LS 50 luminescence spectrometer with a xenon pulsation lamp (Perkin-Elmer) was used for measurement of the fluorescence spectra. For the measurement of the UV–Vis spectra, 0.1 mmol of the reagents was dissolved in 10 ml of DMSO in volumetric flasks and the solutions were made up to 100 ml by addition of eluent B. The solutions were then further diluted with these eluents until maxima of extinctions in the range 0.5–0.9 were obtained. Spectra were recorded at 20°C. Fluorescence spectra were recorded for 10^{-5} *M* solutions in the eluents used for HPLC.

Molar absorption coefficients (ε) are given in 1 mol^{-1} cm^{-1}.

*Synthesis of N^2-[2-(4-chloro-6-methoxy-1,3,5-triazinyl)]-L-alanine amide (CMOT-Ala-NH_2) (**a**).* 2,4-Dichloro-6-methoxy-1,3,5-triazine (DCMT) was synthesized according to ref. 7; yield, 3.37 g (30%); m.p., 87°C (lit. [7] m.p., 88–89°C); R_F (I), 0.79; MS, *m/z* 179 and 181 (M^+), 149 and 151 (M − OMe + H), intense.

For the synthesis of **a**, DCMT (0.9 g, 5 mmol) and L-Ala-NH_2 · HCl (623 mg, 5 mmol) were dissolved in a mixture of acetone (15 ml) and water (10 ml) and 1.0 *M* $NaHCO_3$ (15 ml) was added. The mixture was stirred for 2 h at room temperature (r.t.), then neutralized by addition of 0.1 *M* HCl and evaporated to dryness *in vacuo*. The residue was dissolved in ethanol and *n*-hexane was added at 0°C. The precipitate was filtered, the filtrate was evaporated to dryness and the procedure was repeated twice. Total yield, 295 mg (25%), colourless crystals; m.p., 180°C; R_F (I), 0.39; R_F (II), 0.64; MS, *m/z* 187 and 189 (M − $CONH_2$), very intense.

*Synthesis of N^2-[2-(4-chloro-6-(2-naphthoxy)-1,3,5-triazinyl)]-L-alanine amide (CNOT-Ala-NH_2) (**b**).* 2,4-Dichloro-6-(2-naphthoxy)-1,3,5-triazine (DNOT) was synthesized according to ref. 7; yield, 3.9 g (67%), colourless, amorphous powder; m.p., 150°C (lit. [8] m.p., 145–154°C); R_F (I), 0.77; MS, *m/z* 291 and 293 (M^+), 256 and 258 (M − Cl), int., 127 ($C_{10}H_7^+$), intense.

For the synthesis of **b**, 1 *M* $NaHCO_3$ (3 ml) was added to DNOT (292 mg, 1 mmol) and L-Ala-NH_2 · HCl (125 mg, 1 mmol), dissolved in a mixture of acetone (13 ml) and water (2 ml). The mixture was stirred for 12 h, water (20 ml) was added and the white precipitate was filtered, washed with water and dried *in vacuo*. Yield, 293 mg (85%) (amorphous); m.p., 218°C; R_F (I), 0.48; R_F (II), 0.73; R_F (III), 0.11; UV (nm), 232; ε (232 nm), 23 500; MS, *m/z* 343 and 345 (M^+), int., 299 and 301 (M − $CONH_2$), very int., 127 ($C_{10}H_8^+$), intense.

*N^2-[2-(4-Chloro-6-(4-methoxy-1-naphthyl)-1,3,5-triazinyl)]-L-alanine amide (CMNT-Ala-NH_2) (**c**).* 2,4-Dichloro-6-(4-methoxy-1-naphthyl)-1,3,5-triazine (DMNT) was obtained as follows. Solid $AlCl_3$ (2.67 g, 20 mmol) was added to cyanuric chloride (3.69 g, 20 mmol) and 1-methoxynaphthalene (3.16 g, 20 mmol) dissolved in toluene (60 ml) at 5–10°C. The mixture was stirred for 15 h at r.t., the brown precipitate that formed was removed by filtration, washed with toluene and poured into 250 ml of ice-cold 0.1 m*M* HCl. The precipitate was filtered, washed with cold water, dried *in vacuo* and dissolved in boiling toluene. The yellow compound DMNT which precipitated at r.t. was filtered off and dried *in vacuo*. The filtrate was evaporated to dryness and the procedure was repeated twice. Total yield, 3.21 g (52%), amorphous powder that exhibits blue fluorescence in organic solvents; m.p., 128°C; R_F (II), 0.88; MS, *m/z* 305 and 307 (M^+), very intense.

For the synthesis of **c**, DMNT (306 mg, 1 mmol) was dissolved in acetone (200 ml) at 40°C and L-alanine amide · HCl (125 mg, 1 mmol) and 1 *M* $NaHCO_3$ (3 ml) were added. After 90 min at 40°C a precipitate had formed which was filtered off; the filtrate was reduced to about half its volume by evaporation, light petroleum (200 ml) was added and the mixture was stirred for 30 min at 0°C. The colourless precipitate was filtered, washed with light petroleum and water and dried *in vacuo*. Yield, 227 mg (64%), amorphous powder that exhibits blue fluorescence in organic solvents; m.p., 232°C (decomp.); R_F (I), 0.43; R_F (II), 0.75; R_F (III), 0.12; UV (nm), 241, 340; ε (241), 45 000; ε (340), 13 500; MS, *m/z* 358 and 360 (MH^+), int., 314 and 316 (M − $CONH_2$ + H), very intense.

*N^2-[2-(4-Chloro-6-(4-phenylazoanilino)-1,3,5-triazinyl)]-L-alanine amide (CAZT-Ala-NH_2) (**d**).* 2,4-Dichloro-6-(4-phenylazoanilino)-1,3,5-triazine (DAZT) was obtained as follows. 4-Aminoazobenzene (1.97 g, 10 mmol) was dissolved in 120 ml of dioxane and 40 ml of water and a solution of cyanuric chloride (1.88 g, 10 mmol) in 20 ml of dioxane and 5 ml of water was added at 0–5°C. The mixture was maintained at pH 6–7 by addition of 2 *M* Na_2CO_3. The organic solvent was removed *in vacuo*, the precipitate formed was filtered off, dissolved in dioxane (200 ml) and water (250 ml) was added. The orange precipitate was filtered off, washed with water and dried *in vacuo*. Yield, 1.09 g (28%); m.p., 216°C (decomp.) (lit. [8] m.p., 211–213°C), orange, amorphous powder; R_F (I), 0.81; MS, *m/z* 344 and 346 (M^+), int., 239 and 241 (M − $N_2C_6H_5$), very intense.

For the synthesis of **d**, L-Ala-NH_2 · HCl (249 mg, 2 mmol) and 1 *M* $NaHCO_3$ (6 ml) were added to a solution of DATZ (690 mg, 2 mmol) in acetone (60 ml) and water (10 ml). After 1 h at 40°C the

orange precipitate that formed was filtered off, washed with water and dried *in vacuo*. Yield, 656 mg (83%); m.p., 226°C (decomp.); R_F (I), 0.44; R_F (II), 0.75; R_F (III), 0.10; UV (nm), 367; ε (367 nm), 32 700; MS, *m/z* 397 and 399 (MH^+), int., 353 and 355 ($M - CONH_2 + H$), int., 320 ($M - C_6H_5$), int., 292 ($M - N_2C_6H_5$), intense.

*N^2-[2-(4-Fluoro-6-(4-phenylazoanilino)-1,3,5-triazinyl)]-L-alanine amide (FAZT-Ala-NH_2) (**e**).* 2,4-Difluoro-6-(4-phenylazoanilino)-1,3,5-triazine (DAZT) was obtained as follows. To 4-phenylazoaniline (789 mg, 4 mmol) in dioxane (40 ml) and water (20 ml), cyanuric fluoride (341 μl, 4 mmol) was added within 20 min at −5 to 0°C, and pH 4–4.5 was maintained by addition of 2 *M* Na_2CO_3. After 20 min at 0°C and 1 h at r.t., the orange precipitate that formed was filtered off, washed with water and dried *in vacuo*. Yield, 966 mg (77%), orange amorphous powder; m.p., 188°C (decomp.); R_F (I), 0.80; MS, *m/z* 312 (M^+), int., 207 ($M - N_2C_6H_5$), very intense.

For the synthesis of **e**, DAZT (312 mg, 1 mmol) and L-Ala-NH_2 · HCl (125 mg, 1 mmol) were dissolved in dioxane (30 ml) and water (10 ml), and the pH was adjusted to 7.5–8 by addition of 1 *M* $NaHCO_3$. After 10 min at r.t. water (20 ml) was added, the precipitate that formed was filtered off, washed with water and dried *in vacuo*. Yield, 241 mg (63%); m.p., 276°C (decomp.); R_F (I), 0.39; R_F (II), 0.70; R_F (III), 0.10; UV (nm), 343; ε (343 nm), 31 300; MS, *m/z* 380 (M^+), int., 336 ($M - CONH_2$), int., 303 ($M - C_6H_5$), 275 ($M - N_2C_6H_5$), intense.

RESULTS AND DISCUSSION

The chiral monohalo-*s*-triazine reagents shown in Fig. 2 were synthesized from cyanuric chloride (2,4,6-chloro-1,3,5-triazine) and in one instance from cyanuric fluoride (2,4,6-fluoro-1,3,5-triazine) via replacement of one halogen atom by reaction with selected nucleophiles. The substituents thus introduced serve either as a blocking group (methoxy in CMOT-Ala-NH_2), a UV-absorbing moiety (2-naphthoxy in CNOT-Ala-NH_2), a fluorophore (1-methoxynaphthyl in CMNT-Ala-NH_2), or a chromogenic group (4-phenylazoanilino in CAZT-Ala-NH_2 or FAZT-Ala-NH_2). In a second derivatization step, one further halogen was substituted by reaction with L-alanine amide, yielding the chiral

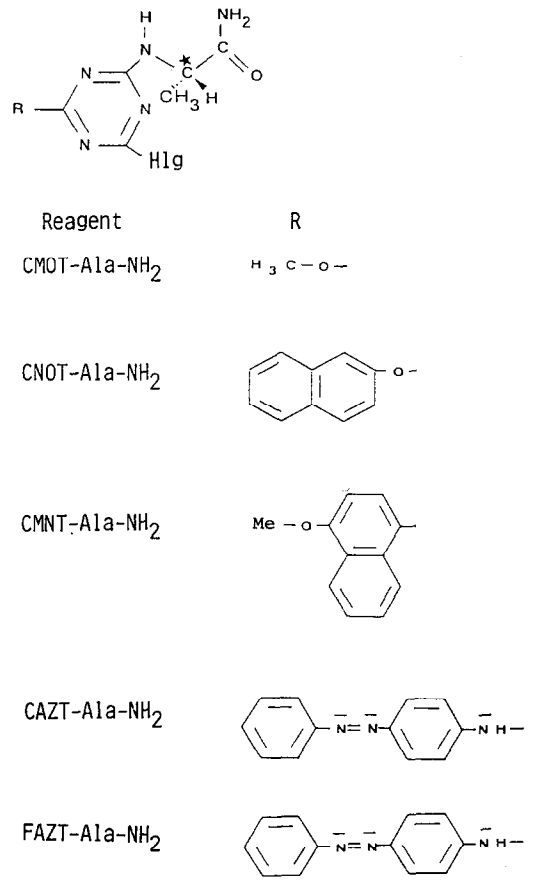

Fig. 2. General structure of chiral monohalo-*s*-triazines and of groups R in reagents abbreviated as follows: (**a**) CMOT-Ala-NH_2; (**b**) CNOT-Ala-NH_2; (**c**) CMNT-Ala-NH_2; (**d**) CAZT-Ala-NH_2; and (**e**) FAZT-Ala-NH_2; the chiral moiety consists of L-alanine amide (Ala-NH_2) and an asterisk indicates the chiral centre; Hlg = Cl in **a**–**d** and F in **e**; Me = methyl; for systematic names of the reagents **a**–**e**, see Experimental.

monohalo-*s*-triazine derivatives shown in Fig. 2. Dihalo- and monohalo-*s*-triazines were isolated and characterized as described under Experimental. In principle, the derivatization reactions may also be carried out in the reverse order. The monohalo-*s*-triazine reagents furnished diastereomeric derivatives on reaction with DL-AA under alkaline conditions with heating at 80–100°C for 1 h. No racemization of the AA to be analysed or of AA in the reagents was observed under these conditions (detection limit *ca.* 0.5% D-AA in an excess of L-AA). Investigation of

TABLE I

NET RETENTION TIMES OF FIRST- ($t'_{R(L-L)}$) AND SECOND- ($t'_{R(D-L)}$) ELUTED DIASTEREOMERS FORMED BY REACTION OF SELECTED DL-AMINO ACIDS WITH CHIRAL MONOHALO-*s*-TRIAZINES, AND DIFFERENCES IN ELUTION TIMES (Δt_R)

The first letter in diastereomers L–L and D–L refers to the configuration of the amino acids to be analysed and the second to that of the amino acid in the chiral reagents.

Reagent[a]	Net retention time (min)[b]														
	Amino acid to be analysed[c]														
	DL-Ala			DL-Val			DL-Phe			DL-Glu			DL-Arg		
	$t'_{R(L-L)}$	$t'_{R(D-L)}$	Δt_R	$t'_{R(L-L)}$	$t'_{R(D-L)}$	Δt_R	$t'_{R(L-L)}$	$t'_{R(D-L)}$	Δt_R	$t'_{R(L-L)}$	$t'_{R(D-L)}$	Δt_R	$t'_{R(L-L)}$	$t'_{R(D-L)}$	Δt_R
CMOT-Ala-NH_2	2.80	3.67	0.87	12.69	19.64	6.95	68.55	99.35	30.80	2.12	2.49	0.37	1.65	1.93	0.28
CNOT-Ala-NH_2	16.31	16.56	0.25	18.32	18.82	0.50	21.25	21.25	0.00	14.80	14.80	0.00	13.35	13.35	0.00
CMNT-Ala-NH_2	15.85	15.85	0.00	16.91	17.41	0.50	19.51	19.79	0.28	14.01	14.01	0.00	12.99	12.99	0.00
FAZT-Ala-NH_2[d]	19.32	19.71	0.39	21.34	22.32	0.98	24.61	25.39	0.78	18.08	18.08	0.00	16.49	16.49	0.00

[a] For abbreviations and structures, see Experimental and Fig. 2.

[b] t_0(DMSO) = 2.16 min.

[c] DL-Ser was not resolved by derivatization with the four reagents.

[d] Diastereomers formed by reaction of DL-amino acids with FAZT-Ala-NH_2 are identical with those formed with CAZT-Ala-NH_2 and therefore show the same retention times.

the derivatization by TLC and spraying with ninhydrin reagent showed >97% completeness of the reactions and no chiral discrimination was detectable under these conditions, as indicated by the equal peak areas of the diastereomers formed from DL-AA. The DL-AA used were selected according to representative side-chain features, Ala (neutral), Val (neutral, sterically hindered), Ser (hydroxymethyl), Phe (aromatic), Glu (acidic) and Arg (basic).

The diastereomers formed were subjected to HPLC using octadecylsilylsilica as the stationary phase and acidic eluents of pH 1.9–2.0. As with diastereomers formed by reaction of DL-AA with chiral variants of Sanger's reagent [5,6], L–L diastereomers eluted before L–D diastereomers (the first letter refers to the configuration of the AA in the reagent). Net retention times and differences in net retention times of the diastereomeric derivatives of DL-AA formed with the reagents are given in Table I and sections of chromatograms of those diastereomers which display resolution are shown in Fig. 3. For the diastereomers formed with CMOT-Ala-NH_2, the ab-

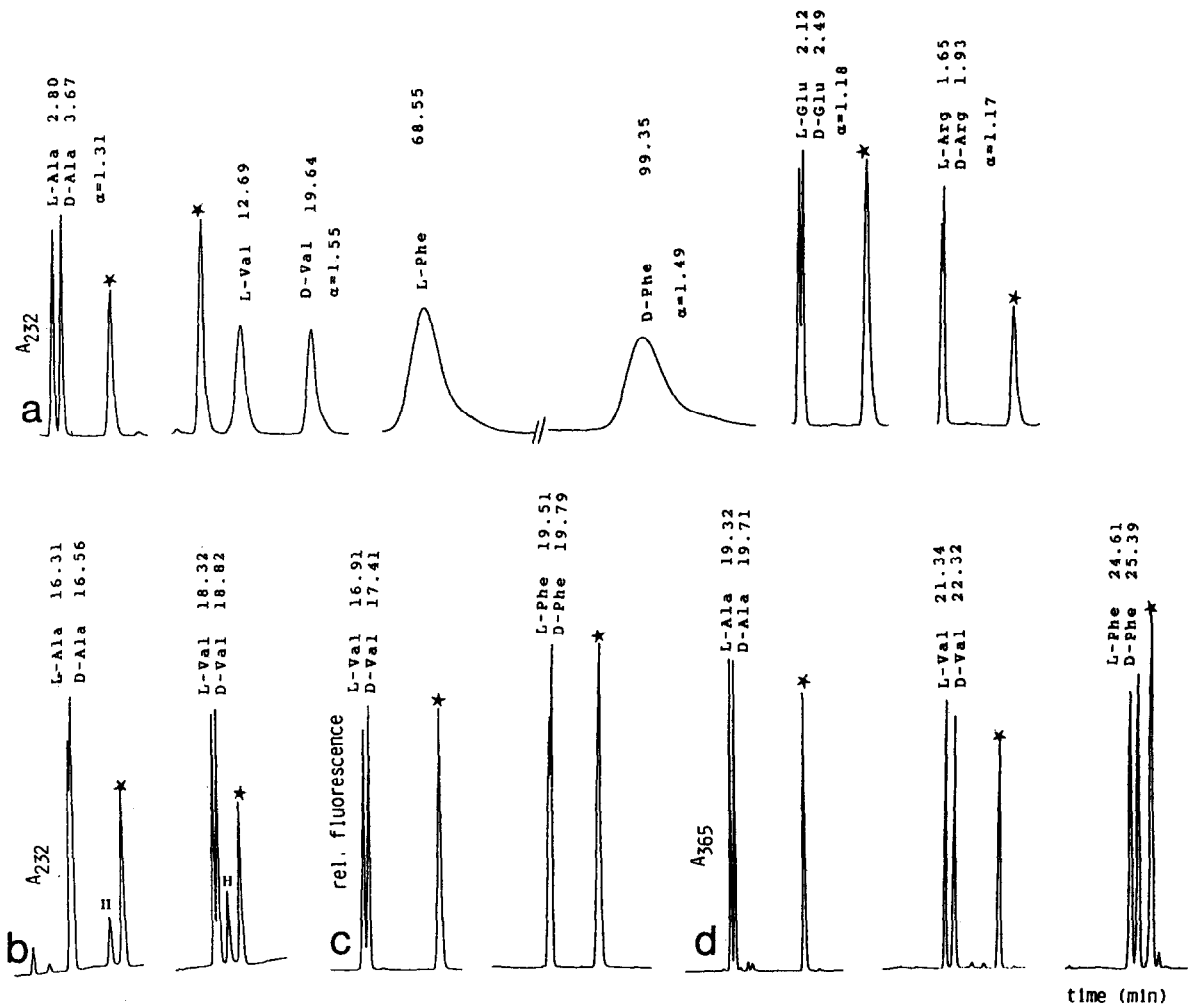

Fig. 3. Sections of chromatograms of diastereomers of selected amino acids (AA) formed by reaction with (a) CMOT-Ala-NH_2, (b) CNOT-Ala-NH_2, (c) CMNT-Ala-NH_2 and (d) FAZT-Ala-NH_2. Designation of the AA to be analysed refers to their configuration in diastereomers; asterisk = reagent peak, H = hydrolysed reagent. Elution conditions: (a) eluent A, isocratic; (b)–(d) 100% A to 100% B in 40 min; eluent A = 100 ml of acetonitrile (MeCN) + 900 ml of water + 1 ml of trifluoroacetic acid (TFA); eluent B = 800 ml of MeCN + 200 ml of water + 1 ml of TFA; flow-rate (a)–(d), 1 ml min^{-1}. A_{232} and A_{365} refers to absorbance at 232 and 365 nm, respectively; the relative fluorescence was measured at 340 nm (excitation) and 415 nm (emission).

sorption of the triazine ring at 232 nm was used for detection. For the detection of diastereomers formed with CNOT-Ala-NH_2, either the absorption maximum of the 2-naphthoxy group at 232 nm or its fluorescence at 352 nm with excitation at 232 nm can be used. The reagent CMNT-Ala-NH_2 and the diastereomers formed therefrom show blue fluorescence in acetone solution when inspected under daylight, and bright blue, intense fluorescence on radiation with a UV lamp at 366 nm. This reagent has favourable fluorescence properties as it has two absorption maxima, at 241 and 340 nm, and shows fluorescence at 415 nm on excitation at the absorption maxima (Fig. 4).

The orange chromogenic reagent CAZT-Ala-NH_2 and its more reactive analogue FAZT-Ala-NH_2 show absorption maxima in the visible range at 367 and 343 nm, respectively. Among the reagents described, CMOT-Ala-NH_2 gave very high resolution for DL-Phe and DL-Val, baseline resolution for DL-Ala and partial resolution for DL-Glu and DL-Arg. None of the other reagents was capable of resolving DL-Glu, DL-Arg and DL-Ser; DL-Ser was also not resolved after reaction with CMOT-Ala-NH_2.

The results demonstrate that the chiral monohalo-*s*-triazines described are capable of resolving certain DL-AA under the conditions used. The resolution of diastereomers is lower in some instances in comparison with those obtained by reaction of the same DL-AA with chiral Sanger-type reagents [5,6]. This suggests a possible contribution of the nitro groups to the resolution of the respective diastereomers (*cf.*, structures shown in Fig. 1). From the inspection of the structure of the reagents (*cf.*, Fig. 1) and the data of Table I, it appears also that increasing bulkiness of the substituents R in the monohalo-*s*-triazine reagents leads to a decrease in resolution.

An important aspect of the use of cyanuric halides as starting materials for the synthesis of chiral reagents is their trifunctionality, which allows the design of tailor-made reagents using the combination of a large number of chiral moieties and of detectable groups. The former can be amino acid derivatives, the latter UV-absorbing, chromogenic or fluorescent groups, or those having favourable

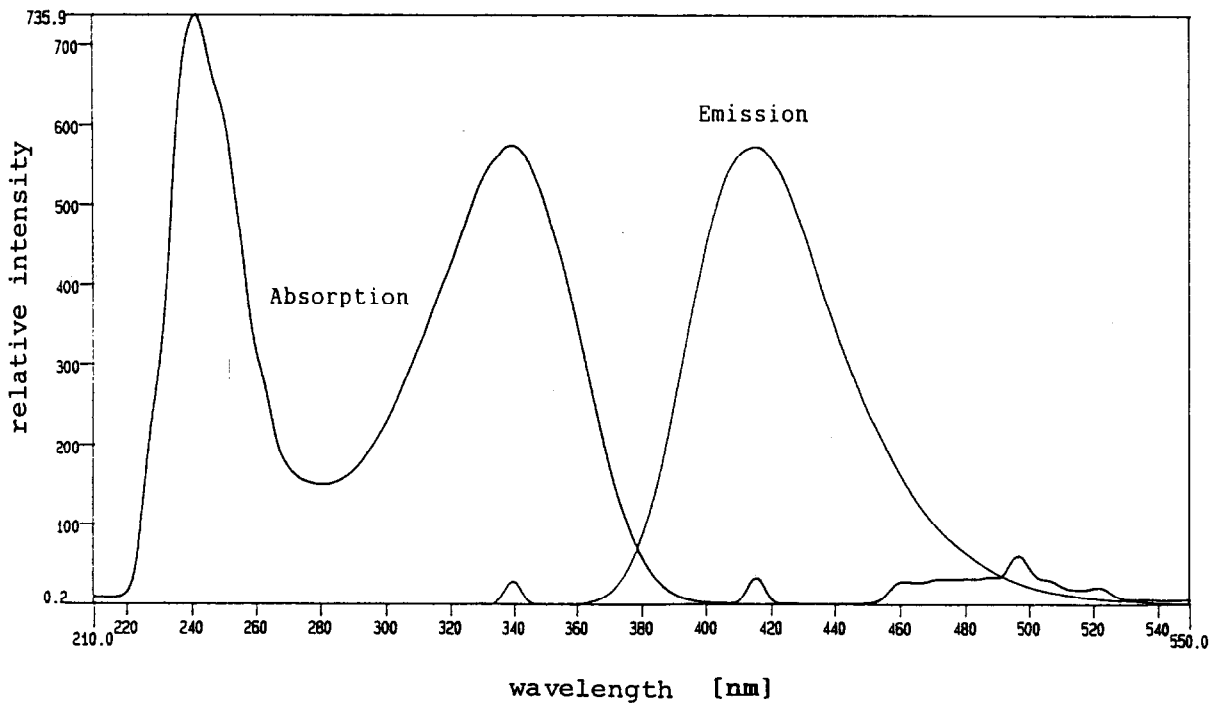

Fig. 4. Fluorescence spectrum of the chiral reagent CMNT-Ala-NH_2. The absorption spectrum was taken with emission at 415 nm and the emission spectrum with excitation at 340 nm.

hydrophobic, hydrophilic, electrochemical or otherwise suitable properties (for potential compounds, see also refs. 7 and 8). From this point of view the approach described is unique among the various methods for the indirect liquid chromatographic resolution of AA enantiomers [17].

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