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Design of chiral monochloro-*s*-triazine reagents for the liquid chromatographic separation of amino acid enantiomers ☆

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Abstract

A series of chiral derivatizing reagents (CDRs) was synthesized by nucleophilic replacement of one chlorine atom in cyanuric chloride (2,4,6-trichloro-1,3,5-triazine; *s*-triazine) by alkoxy (methoxy, butoxy, 1,1,1-trifluoroethoxy) or aryloxy groups (phenoxy, nitrophenoxy, phenylphenoxy, 4-methylcoumaryloxy), and displacement of a second chlorine by L-alanine amide, L-phenylalanine amide, L-proline *tert*.-butyl ester, or Boc-L-lysine *tert*.-butyl ester. Further, CDRs were investigated in which two chlorine atoms in cyanuric chloride were substituted consecutively by L-valine amide and L-phenylalanine amide. The resulting CDRs having a remaining reactive chlorine were tested for their capability of derivatizing DL-amino acids followed by liquid chromatographic separation of the resulting diastereomers.

Keywords: Enantiomer separation, indirect; Derivatization, LC; Diastereomer separation; Monochloro-*s*-triazine reagents, chiral; Amino acids

1. Introduction

In recent years cyanuric chloride (2,4,6-trichloro-1,3,5-triazine; trichloro-*s*-triazine) attracted increasing attention for potential use in chromatography. This is attributed to its high reactivity and trifunctionality that allows easy and controlled sequential replacement of the halogens by nucleophiles. This unique feature makes possible design of an abundance of reagents in which, for example, one halogen is substituted by a suitable reporter group and another by a residue that alters the polarity of the molecule, or further increases the reactivity of the remaining monohalo-*s*-triazine reagent, or just serves as blocking group in order to create a monofunctional reagent.

Examples of monosubstituted *s*-triazine reagents with two remaining chlorine are 1-ethoxy-4-(dichloro-*s*-triazinyl)naphthalene used for the detection of corticosteroids [1], dichlorotriazinylaminofluorescein that was employed for the fluorescence labelling of immunoglobulins [2], or a dichloro-*s*-triazine with

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benz[*f*]isoindoyl fluorophor suitable for the detection of bisphenols [3]. In a similar approach 8-(4,6-dichloro-1,3,5-triazinylamino)quinoline was synthesized from cyanuric chloride and 8-aminoquinoline and used for the fluorescence labeling of phenol in wastewater [4].

An example for a reagent in which two chlorine are substituted is 2-chloro-4-methoxy-6-(4-methoxy-1-naphthyl)1,3,5-triazine that was tested for its suitability for amino acid analysis and fluorescence labeling of α-casein [5].

Successive replacement of two halogen atoms in cyanuric chloride with a reporter group and an L-amino acid moiety provided a series of chiral reagents suitable for the derivatization and liquid chromatographic separation of of DL-amino acids as diastereoisomers [6].

Notably, in extension of these approaches, in a disubstituted cyanuric chloride the remaining halogen atom might be replaced by a functional group that reacts selectively with analytes of interest. This approach has been realized with a series of chromogenic or fluorescent triazine-based hydrazines capable of reacting with aliphatic and aromatic aldehydes [7].

The approaches outlined have also been used for the design of stationary phases serving as solid supports in chromatography. Although most of the work focused on immobilization of dyes or biomimetic ligands on suitable supports via *s*-triazine scaffolds [8,9], as early as 1971 L-arginine was bonded through a cyanuric chloride linkage to a dextran gel (Sephadex) yielding a support that was capable of separating D- and L-isomers of β-3,4-dihydroxyphenylalanine [10].

Recently, immobilization of chloro-*s*-triazines bearing amines or amino acid derivatives as chiral selectors on solid supports such as aminopropylsilica provided a series of chiral stationary phases enabling the enantioresolution of preferably amino acid derivatives [11–14]. In extension of this approach bovine serum albumin, serving as chiral selector, was immobilized on 3-aminopropylsilica via cyanuric chloride [15]. Further, a fluorescent dichloro-*s*-triazine reagent, 3-(4,6-dichloro-1,3,5-triazinylamino)-7-dimethylamino-2-methylphenazine, was used for the derivatization of DL-amino acids followed by micellar electrokinetic chromatography with β-cyclodextrin added to the buffer [16].

Peptide derivatized *s*-triazines covalently linked to 3-aminopropyl silica have been reported to separate amino acid enantiomers as well as racemic compounds such as alcohols [17,18]. Notably in this context, triazinyl bonded peptides have been described to serve as chiral solvating agents for the determination by NMR of the enantiomeric composition of 3,5-dinitrophenyl derivatized compounds such as amines and carboxylic acids [19]. It was postulated that this approach will make possible the development of new chiral chromatographic phases.

From the foregoing it is evident that cyanuric chloride (or the more reactive cyanuric fluoride), and multifunctional *s*-triazines derived therefrom, are excellent candidates to be tested for applications in various fields of separation sciences.

In continuation of our previous work we now report on a series of reagents in which one chlorine in cyanuric chloride is substituted by an alkoxy or aryloxy group, whereas the second chlorine is replaced by an L-amino acid derivative (amide or *tertiary* butyl ester). These chiral derivatizing reagents (CDRs) were assessed for their suitability of derivatizing DL-amino acids followed by liquid chromatographic separation of diastereomers formed.

2. Experimental

2.1. Instruments

For HPLC, an instrument consisting of a Model 880 PU reciprocating pump, a Model 880-902 low-pressure gradient former, a Model 801-SC controller and a Model 875-UV variable-wavelength detector were use (Jasco, Kyoto, Japan). For recording chromatograms and data processing a D-2000 ChromatoIntegrator (Merck–Hitachi) was used. Samples were injected manually using a Model 7125 injector (Rheodyne, Cotati, CA, USA). Electron impact mass spectra (70 eV) were measured using a Varian Model 311 A mass spectrometer. Masses of molecular ions and fragments taking ^{35}Cl and ^{37}Cl isotopes into account, and relative intensities are given in below. Elemental analyses were carried out with a Model 1106 CHN elemental analyser (Carlo Erba, Milan, Italy); optical rotations were determined at 25 °C using a Series 241 polarimeter (Perkin-Elmer, Überlingen, Germany) using a 100×3.6-mm

I.D. cuvette; melting points were determined in open capillaries using a Model 520 apparatus (Büchi, Flawil, Switzerland). Molar extinctions ε_{max} (cm l^{-1} mol^{-2}) in MeCN (5×10^{-5} *M*] were determined using a Model 8452 diode array spectrophotometer (Hewlett-Packard, Waldbronn, Germany). All evaporations were carried out in vacuo using a diaphragm pump with digital vacuum gauge and controller (Vacuubrand, Weilheim, Germany) and a rotatory evaporator (Büchi). Toluene was added to organic solvents in order to remove water azeotropically.

2.2. Chromatography

For analytical HPLC, Nucleosil 100 C_{18}, particle size 5-μm, pore size 0.1 nm, and stainless steel columns 250×4 mm I.D. and pre-columns 20×4 mm I.D. were used (Macherey-Nagel, Düren, Germany). A preparative Nucleosil 100 C_{18} column (250×16 mm I.D.) was used for the purification of reagents for elemental analyses. Amounts of 10–30 mg reagents in 50% MeCN (0.2–0.5 ml) were applied, and 25–65% aq. MeCN were used as eluents at flow-rates 5 ml min^{-1}. Suitable fractions were combined, evaporated to dryness and dried over P_2O_5 in vacuo. The course and completeness of the syntheses was followed by TLC and inspection of the plates under an UV-lamp at 254 nm. For TLC pre-coated plates (Kieselgel 60 F_{254}) were used. The solvent systems were as follows (v/v): (I) *n*-hexane–acetone (55:45); (II) *n*-hexane–acetone–dichloromethane (50:30:20); (III) *n*-hexane–acetone–acetic acid (57.5:40:2.5). Retention factors (R_F) for TLC were determined at ca. 21 °C.

2.3. Solvents and chemicals

Methanol (MeOH), and acetonitrile (MeCN) (chromatography grade), 1-butanol (1-BuOH), acetone, ethyl acetate (EtOAc) (synthetic grade), dichloromethane, *n*-hexane, trifluoroethanol, 1,4-dioxane, sodium hydrogen carbonate (p.a.), potassium hydrogen sulfate (p.a.), trifluoroacetic acid (TFA), acetic acid (AcOH), and 2,4,6-collidine were from Merck (Darmstadt, Germany). Dimethylsulfoxide (DMSO) and 4-nitrophenol, were from Fluka (Buchs, Switzerland). Cyanuric chloride, 1-methoxynaphthalene, 4-phenylphenol, and 7-hydroxy-4-methylcoumarin were from Aldrich (Steinheim, Germany). L-Alanine amide hydrochloride (L-Ala-NH_2·HCl), L-valine amide hydrochloride (L-Val-NH_2·HCl), and L-phenylalanine amide (L-Phe-NH_2) were from Novabiochem, Läufelfingen, Switzerland. DL- and L-amino acids serving as analytes were purchased from Fluka or Sigma (St. Louis, MO, USA).

2.4. Derivatization procedures

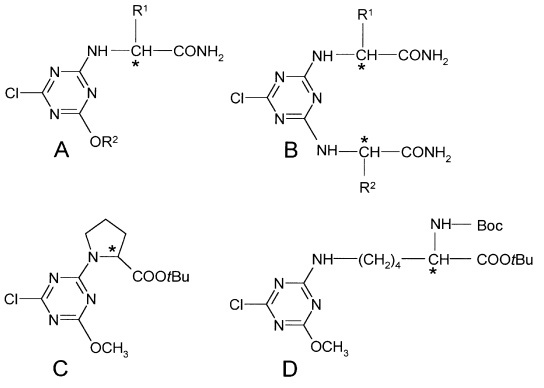
To 30-μl aliquots (3 μmol) of standard mixtures of DL-amino acids (100 m*M* in 1 *M* HCl) were added 1 *M* $NaHCO_3$ (45 μl) and 500-μl aliquots (5 μmol) of chiral derivatizing reagents (10 m*M* in DMSO). The mixtures were heated for 1 h at 80 °C and 0.4–3.0-μl aliquots applied to HPLC.

2.5. Syntheses of chiral derivatizing reagents (CDRs) and intermediates

Synthesis of 2,4-dichloro-6-methoxy-[1,3,5]triazine (DCMT), serving as starting compound for CDRs Nos. 6 and 7 has been described in Ref. [3]. The syntheses of CDRs Nos. 9, 10, and 11, which have previously been used for the design of chiral stationary phases, have been described in Refs. [2,3]. For structures of all CDRs employed in this work, see Fig. 1. In the following, syntheses and characterization of new CDRs and the respective intermediates by consecutive replacement of two chlorine atoms in cyanuric chloride are described. For elemental analyses CDRs were purified by preparative HPLC (see Section 2.2).

2.5.1. 2-Butoxy-4,6-dichloro-[1,3,5]triazine (BCT)

To 18.45 g (100 mmol) cyanuric chloride in acetone (140 ml) were added at 0–5 °C 13.2 ml (100 mmol) 1-BuOH and 13.5 ml (100 mmol) collidine. The reaction solution turned orange and collidine hydrochloride precipitated. After 3 h reaction time the precipitated was filtered off and washed with acetone. The filtrate was evaporated to dryness, toluene (500 ml) was added and the organic phase washed with water (3×250 ml). Then the organic phase was evaporated to dryness and the remaining red oil distilled in vacuo at 19 mbar. The fraction of b.p. 144–150 °C was collected. Yield, 21.0 g (94.6%), colorless oil; R_F (I) 0.89; R_F (II) 0.91; R_F (III) 0.85; MS, *m*/*z* 186 (100%) and 188 (32%) $(M-Cl)^+$.



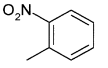
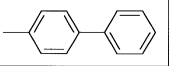
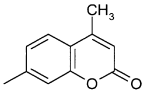
CDR	Structure	R^1	R^2
1	A	$-CH_3$	$-(CH_2)_3-CH_3$
2	A	$-CH_3$	$-C_6H_5$
3	A	$-CH_3$	
4	A	$-CH_3$	
5	A	$-CH_3$	
6	A	$-CH(CH_3)_2$	$-CH_3$
7	A	$-CH_2-C_6H_5$	$-CH_3$
8	A	$-CH_2-C_6H_5$	$-CH_2-CF_3$
9	B	$-CH_2-C_6H_5$	$-CH(CH_3)_2$
10	C		
11	D		

Fig. 1. Structures of chiral derivatizing reagents (CDRs) Nos. 1–11; Boc, *tert.*-butyloxycarbonyl; *t*Bu, *tert.*-butyl; asterisks refer to stereogenic atoms.

2.5.2. N-(4-Butoxy-6-chloro-[1,3,5]triazine-2-yl)-L-alanine amide (CDR-1)

To 1.11 g (5 mmol) BCT in acetone (15 ml) were added 0.62 g (5 mmol) L-Ala-NH_2·HCl in water (10 ml) and 1 *M* $NaHCO_3$ until pH 8 was achieved (ca. 15 ml). After 15 min the formation of a white precipitate was observed. The course of the reaction was monitored by TLC. After 3 h reaction time no more L-Ala-NH_2 and BCT could detected. Water was added, the precipitate formed filtered off, washed with water and dried in vacuo. Yield, 1.02 g (75%), white powder; m.p. 169–170 °C; R_F (I) 0.36, R_F (II) 0.21, R_F (III) 0.47; ε (226 nm) 16 156; MS, *m*/*z* 173 (100%) and 175 (30%) $(M-C_4H_7-CONH_2-H)^+$; $[\alpha]_D^{25} = -29.61$ (*c* 1, dioxane); elemental analysis for $C_{10}H_{16}ClN_5O_2$ (273.72), calc. C 43.88, H 5.85, Cl 12.98, N 25.59; found C 43.77, H 5.79, Cl 13.23, N 25.44.

2.5.3. 2,4-Dichloro-6-phenoxy-[1,3,5]triazine (CPT)

To 3.64 g (20 mmol) cyanuric chloride in acetone (25 ml) were added at 0–5 °C amounts of 2.65 ml (20 mmol) collidin and dropwise 1.88 g (20 mmol) phenol in acetone (10 ml). The reaction temperature did not exceed 10 °C. The course of the reaction was monitored by TLC. After 2 h reaction time no more phenol could be detected. Collidin hydrochloride was filtered off, washed with acetone, and the filtrate was evaporated to dryness. To the remaining residue EtOAc was added and the organic phase was washed with 5% aq. $NaHCO_3$, 5% $KHSO_4$ and water (each 3×250 ml). The organic phase was evaporated to dryness with addition of toluene and the remaining residue crystallized twice from *n*-hexane. Yield, 4.17 g (86%), white powder; m.p. 111.5–113.8 °C; R_F (I) 0.80, R_F (II) 0.85, R_F (III) 0.68; MS, *m*/*z* 206 (100%) and 208 (32%) $(M-Cl)^+$; 241 (15%) and 243 (10%) $(M-H)^+$.

2.5.4. N-(4-Chloro-6-phenoxy-[1,3,5]triazine-2-yl)-L-alanine amide (CDR-2)

To 1.22 (5 mmol) CPT in acetone (26 ml) were added with stirring 0.62 g (5 mmol) L-Ala-NH_2·HCl in water (10 ml) and 1 *M* $NaHCO_3$ (15 ml). The course of the reaction was monitored by TLC. After 3 h water (100 ml) was added, the precipitate formed filtered off and washed with water. To the wet product EtOAc and toluene were added and the organic solvents removed in vacuo. The remaining residue was dissolved at 70 °C in EtOAc (50 ml) and petroleum ether added at r.t. The precipitate formed was filtered off and washed with petroleum ether. Yield, 1.19 g (81%), white powder; m.p. 205–206 °C; R_F (I) 0.29, R_F (II) 0.17, R_F (III) 0.39; ε (226 nm) 22 102; MS, *m*/*z* 249 (100%) and 251

(60%) $(M–CONH_2)^+$; $[\alpha]_D^{25} = -22.91$ (*c* 1, dioxane); elemental analysis for $C_{12}H_{12}ClN_5O_2$ (293.71), calc. C 49.06, H 4.09, Cl 12.10, N 23.85; found C 49.13, H 4.13, Cl 11.81, N 23.60.

2.5.5. 2,4-Dichloro-6-(2-nitrophenoxy)-[1,3,5]triazine (CNPT)

To 2.77 g (15 mmol) cyanuric chloride in acetone (18 ml) were added at 0–5 °C 1.99 ml (15 mmol) collidin and 2.09 g (15 mmol) 2-nitrophenol in acetone (9 ml). The mixture was stirred for 3 h at room temperature and collidin hydrochloride precipitate filtered off and washed with acetone. The combined filtrates were evaporated to dryness and dichloromethane (420 ml) was added. The organic phase was washed with aq. 5% $NaHCO_3$, 5% $KHSO_4$ and water (each 3×300 ml), then the organic phase was evaporated to dryness. The remaining residue was dissolved in a small amount of dichloromethane and *n*-hexane added. The precipitate formed was filtered off and washed with *n*-hexane, dried in vacuo and stored under protection of light under nitrogen. Yield, 2.76 g (64%), yellow powder; m.p. 136–139 °C; R_F (I) 0.73, R_F (II) 0.74, R_F (III) 0.58; MS, *m*/*z* 240 (100%) and 242 (62%) $(M–NO_2)^+$.

2.5.6. N-[4-Chloro-6-(2-nitrophenoxy)-[1,3,5]triazine-2-yl]-L-alanine amide (CDR-3)

To 1.89 g (6.6 mmol) CNPT in acetone (26 ml) 0.82 g (6.6 mmol) L-Ala-NH_2·HCl, water (6 ml) and 1 *M* $NaHCO_3$ (13 ml) were added. After 2 h the mixture was evaporated to dryness in vacuo, and to the remaining residue dichloromethane (220 ml) added. The organic phase was washed with water (1×110 ml), then toluene was added to the organic phase and solvents removed in vacuo. Yield, 1.80 g (81%), yellow powder; m.p. 136–140 °C; R_F (I) 0.19, R_F (II) 0.10, R_F (III) 0.23; ε (226 nm) 19 682; MS, *m*/*z* 294 (100%) and 296 (32%) $(M–CONH_2)^+$; $[\alpha]_D^{25} = -15.15$ (*c* 0.25 dioxane); elemental analysis for $C_{12}H_{11}ClN_6O_4$ (338.71), calc. C 42.54, H 3.25, Cl 10.49 N 24.82; found C 42.62, H 3.30 Cl 10.47, N 24.35.

2.5.7. 2,4-Dichloro-6-(4-phenylphenoxy)-[1,3,5]triazine (CPPT)

To 1.85 g (10 mmol) cyanuric in acetone (24 ml) were added at 0–5 °C 1.33 ml (10 mmol) collidine and 1.71 g (10 mmol) 4-phenylphenol in acetone (15 ml). After stirring at ambient for 2 h a precipitate of collidin hydrochloride was filtered off, the filtrate evaporated to dryness and EtOAc (300 ml) added to the remaining residue. The organic phase was washed with 5% aq. $NaHCO_3$, 5% $KHSO_4$ and water (3×150 ml). Then toluene (30 ml) was added to the organic phase and the mixture evaporated to dryness. Yield, 2.45 g (77%), white powder; m.p. 187–191 °C; R_F (I) 0.80, R_F (II) 0.87, R_F (III) 0.72; MS, *m*/*z* 282 (100%) and 284 (46%) $(M–Cl)^+$, 317 (40%) and 319 (25%) $(M–H)^+$.

2.5.8. N-[4-Chloro-6-(4-phenylphenoxy)-[1,3,5]triazine-2-yl-L-alanine amide (CDR-4)

To 1.27 g (4 mmol) CPPT in dioxane (44 ml) were added 0.50 g (4 mmol) L-Ala-NH_2·HCl in water (6 ml) and 2 *M* Na_2CO_3 (8 ml). The mixture was stirred at ambient for 2 h, then water was added and the precipitate formed removed by filtration. The product was dissolved in acetone, toluene was added and the mixture was evaporated to dryness. The remaining residue was dissolved in chloroform and crystallized by addition of *n*-hexane. Yield, 1.23 g (83%), white powder; m.p. 214–215 °C; R_F (I) 0.32, R_F (II) 0.19, R_F (III) 0.43; ε (230 nm) 21 972; ε (254 nm) 23 610; MS, *m*/*z* 325 (100%) and 327 (38%) $(M–CONH_2)^+$, 369 (15%) and 371 (4%) $(M)^+$; $[\alpha]_D^{25} = -10.29$ (*c* 1, dioxane); elemental analysis for $C_{18}H_{16}ClN_5O_2$ (369.81), calc. C 58.46, H 4.33, Cl 9.61, N 18.94; found C 58.38, H 4.42, Cl 9.59, N 18.68

2.5.9. 2,4-Dichloro-6-(4-methyl-7-benzo[b]-α-pyronyl)oxy-[1,3,5]triazine (CBPT)

To 1.84 g (10 mmol) cyanuric chloride in acetone (24 ml) were added at 0–5 °C 1.33 g (10 mmol) collidine and 1.81 g (10 mmol) 7-hydroxy-4-methyl-benzo[*b*]-α-pyrone in acetone (65 ml). The mixture was stirred at ambient for 2 h, collidine hydrochloride formed was filtered off, and the filtrate was evaporated to dryness in vacuo. To the remaining residue dichloromethane (320 ml) was added and the organic phase was washed with 5% $NaHCO_3$, 5% $KHSO_4$ and water (each 3×). Then toluene was added to the organic phase, the organic phase was evaporated to dryness and the remaining residue

crystallized from toluene. Yield, 2.18 g (67%), white powder; m.p. 205–208 °C; R_F (I) 0.43, R_F (II) 0.38, R_F (III) 0.60; MS, m/z 288 (100%) and 290 (31%) $(M–Cl)^+$, 323 (33%) and 325 (20%) $(M–H)^+$.

2.5.10. N-[4-Chloro-6-(4-methyl-7-benzo[b]-α-pyronyl)oxy-[1,3,5]triazine-2-yl]-L-alanine amide (CDR-5)

To 1.70 g (5.24 mmol) CBPT in dioxane (54 ml) were added 0.65 g (5.24 mmol) L-Ala-NH_2·HCl in 1 *M* $NaHCO_3$ (13 ml) and 2 *M* Na_2CO_3 (2 ml). After 3 h at ambient water was added and the precipitate formed removed by filtration and dried over P_4O_{10} under vacuum. Yield, 1.27 g (65%), white powder; m.p. 210–214 °C; R_F (I) 0.15, R_F (II) 0.08, R_F (III) 0.23; ε (272 nm) 11 922, ε (312 nm) 10 491; MS, m/z 331 (100%) and 333 (64%) $(M–CONH_2)^+$, 375 (12%) $(M–H)^+$; $[\alpha]_D^{25} = +42.31$ (*c* 0.125 dioxane); elemental analysis for $C_{16}H_{14}ClN_5O_4$ (375.77), calc. C 51.13, H 3.73, Cl 9.45, N 18.64, found C 51.00, H. 3.77, Cl 9.28, N 18.35.

2.5.11. N-(4-Chloro-6-methoxy-[1,3,5]triazine-2-yl)-L-valine amide (CDR-6)

To 0.9 g (5 mmol) DCMT in acetone (13 ml) were added 0.76 g (5 mmol) L-Val-NH_2·HCl in water (6 ml) and 1 *M* $NaHCO_3$ (12 ml). The mixture was stirred for 3 h, water was added, the precipitate formed filtered off and washed with water. The residue was dissolved in dioxane, toluene added and organic solvents removed in vacuo. The remaining residue was crystallized from EtOAc. Yield, 0.95 g (73%), white powder; m.p. 199–200 °C; R_F (I) 0.34, R_F (II) 0.21, R_F (III) 0.46; ε (226 nm) 16 146; MS, m/z 215 (100%) and 217 (93%) $(M–CONH_2)^+$; $[\alpha]_D^{25} = -63.33$ (*c* 0.5, dioxane); elemental analysis for $C_9H_{14}ClN_5O_2$ (259.69), calc. C 41.62, H 5.39, Cl 13.68, N 26.97; found C 41.88, H 5.41, Cl 13.65, N 26.83.

2.5.12. N-(4-Chloro-6-methoxy-[1,3,5]triazine-2-yl)-L-phenylalanine amide (CDR-7)

To 0.9 g (5 mmol) DCMT in acetone (13 ml) were added 0.82 g (5 mmol) L-Phe-NH_2 in water (7 ml) and 1 *M* $NaHCO_3$ (5 ml). After stirring for 5 h the solution was evaporated to dryness in vacuo and EtOAc (150 ml) added. The organic phase was washed with water (2×) and evaporated to dryness with addition of toluene. The remaining residue was dissolved in a small amount of chloroform and petroleum ether added. The precipitate formed was filtered off, washed with petroleum ether and dried. Yield, 1.32 g (86%), white powder; m.p. 78–81 °C; R_F (I) 0.27, R_F (II) 0.18, R_F (III) 0.42; ε (226 nm) 14 978; MS, m/z 263 (100%) and 265 (33%) $(M–CONH_2)^+$; $[\alpha]_D^{25} = -21.75$ (*c* 1, dioxane); elemental analysis for $C_{13}H_{14}ClN_5O_2$ (307.74), calc. C 50.73, H 4.55, Cl 11.55, N 22.76; found C 50.76, H 4.64, Cl 11.45, N 22.60.

2.5.13. 2,4-Dichloro-6-(1,1,1-trifluoroethoxy)-[1,3,5]triazine (CFET)

To 3.68 g (20 mmol) cyanuric chloride in acetone (25 ml) were added at 0–5 °C 2.6 ml (20 mmol) collidine and 1.33 ml (20 mmol) trifluoroethanol. The mixture was stirred for 3 h at ambient temperature, collidine hydrochloride formed filtered off, washed with acetone, and the filtrate evaporated to dryness. To the remaining residue EtOAc (500 ml) was added and the organic phase washed with aq. 5% $NaHCO_3$, 5% $KHSO_4$ and water (3×). Then the organic phase was evaporated to dryness with addition of toluene. The remaining oil was dissolved in acetone and water was added. The brown–yellow oil that precipitated was dried over P_4O_{10} under high vacuum. Yield, 3.31 g (67%), brown–yellow oil; R_F (I) 0.75; R_F (II) 0.77; R_F (III) 0.74; MS, m/z 247 (24%) and 249 (16%) $(M)^+$.

2.5.14. N-[4-Chloro-6-(1,1,1-trifluoroethoxy)-[1,3,5]triazine-2-yl]-L-phenylalanine amide (CDR-8)

To 1.24 g (5 mmol) CFET in acetone (17 ml) were added 0.82 g (5 mmol) L-Phe-NH_2 and 1 *M* $NaHCO_3$ (5 ml). The mixture was stirred for 1 h, then water was added. The yellow oil that precipitated was dissolved in acetone and evaporated to dryness with addition of toluene. The remaining residue was dissolved in a small amount of chloroform and *n*-hexane was added. The oily product that precipitated was dried under high vacuum. Yield, 1.36 g (72%), light-brown foam; m.p. 66–67 °C; R_F (I) 0.37, R_F (II) 0.23, R_F (III) 0.52; ε (228 nm) 18 068; MS, m/z 331 (100%) and 333 (31%) $(M–CONH_2)^+$; $[\alpha]_D^{25} = -14.72$ (*c* 1, dioxane); elemental analysis for $C_{14}H_{13}ClF_3N_5O_2$ (273.72), calc. C

44.74, H 3.46, Cl 9.45, N 18.64, found C 44.84, H 3.53, Cl 9.69, N 18.41.

3. Results and discussion

3.1. Structure of reagents and derivatization conditions

Chiral derivatizing reagents (CDRs) were synthesized using trifunctional cyanuric chloride as starting material. In a first step one chlorine was replaced by residues methoxy (small residue), butoxy (more lipophilic in comparison to methoxy), 1,1,1-trifluoroethoxy (electron-withdrawing), phenoxy (aromatic), 2-nitrophenoxy (chromogenic), 4-phenylphenoxy (strongly UV absorbing), and 4-methylcoumaryloxy (fluorescent). In a consecutive step a further halogen was replaced in selected compounds by L-Ala-NH_2, L-Val-NH_2, L-Phe-NH_2, Pro*O*t*Bu or Boc-L-Lys-O*t*Bu. The amino acids served as chiral residues.

Syntheses of CDR-9 comprising two chiral amino acids, and of CDR-10 and CDR-11 have been described previously [2,3]. These reagents had been immobilized on aminopropylsilica and tested for their capabilities of separating amino acid enantiomers.

The structures of the chiral CDRs investigated in this work are presented in Fig. 1. These CDRs were used for the derivatization of selected DL-amino acids followed by chromatographic separation of the diastereomers formed. Using isocratic elution conditions retention factors (k_L), separation factors (α) and resolutions (R_s) were determined and compiled in Table 1. From k_L and α, factors for k_D can be calculated, if required. Representative chromatograms are presented in Fig. 2. Highest resolution and separation factors were obtained at pH 4 of eluents.

For derivatization of amino acids an excess of CDRs (1.67-, 5- and 10-fold) was used. It was found that the corresponding peak areas resulting from use of 5- and 10-fold excess of reagents were 2- and 4-fold smaller in comparison to use of 1.67-fold excess. This indicates interaction of reagent molecules such as formation of hydrogen bridges, dipol–dipol and π–π interactions, resulting in competitive hindrance and lower reactivities.

Derivatization reactions were conducted up to 6 h. It was found that almost no increase of peak areas occurred after 2 h reaction time. Thus, neither kinetic nor steric discrimination occurred. Derivatives formed were stable for at least 4 months stored at 5 °C. Derivatization reactions were carried out at 80 °C. Notably, CDR-8 with electron-withdrawing 1,1,1-trifluoroethoxy reacted already at 60 °C, whereas CDR-9 with electron-donating groups –NHR required heating at 100 °C. It had previously been recognized that fluoro-*s*-triazines react much faster in comparison to chloro-*s*-triazine reagents [6]. Taking

Table 1
Retention factors (k_L), separation factors (α), and resolution (R_s) of diastereomers formed from selected DL-amino acids by derivatization with chiral derivatization reagents (CDR) Nos. 1–11

CDR	DL-Arg			DL-Ser			DL-Glu			DL-Ala			DL-Pro			DL-Val			DL-Phe		
	k_L	α	R_s	k_L	α	R_s	k_L	α	R_s	k_L	α	R_s	k_L	α	R_s	k_L	α	R_s	k_L	α	R_s
1	0.87	1.00	0.0	0.87	1.09	0.4	1.26	1.13	1.1	2.48	1.23	2.9	8.09	1.28	4.5	9.29	1.32	4.8	26.24	1.25	4.7
2	0.72	1.00	0.0	0.66	1.11	0.5	0.97	1.16	1.1	1.84	1.30	3.1	4.52	1.38	4.2	6.48	1.41	5.3	18.95	1.29	5.3
3	0.72	1.00	0.0	0.75	1.00	0.0	1.00	1.09	0.5	1.88	1.18	1.9	4.74	1.20	2.5	6.48	1.25	4.7	17.36	1.18	3.6
4	2.09	1.00	0.0	2.10	1.07	0.9	2.68	1.09	1.3	5.12	1.18	2.9	9.85	1.26	4.4	12.91	1.24	4.2	24.63	1.17	3.3
5	1.29	1.00	0.0	1.18	1.08	0.6	1.65	1.13	1.2	3.01	1.23	3.0	7.10	1.28	3.9	9.86	1.33	5.6	25.64	1.24	4.5
6	0.68	1.22	1.4	0.65	1.19	1.1	1.02	1.30	2.0	1.92	1.49	5.2	4.62	1.66	7.1	8.41	1.68	10.1	30.25	1.49	9.3
7	1.20	1.00	0.0	1.20	1.11	0.8	1.54	1.15	1.4	3.00	1.29	4.3	6.53	1.43	6.2	10.09	1.42	7.0	27.77	1.31	6.1
8	0.97	1.00	0.0	1.08	1.00	0.0	1.27	1.00	0.0	2.70	1.15	3.1	5.17	1.27	4.1	7.20	1.20	3.7	13.14	1.14	2.7
9	0.96	1.14	0.9	1.07	1.19	1.3	1.21	1.37	2.8	1.82	1.71	5.2	2.72	1.75	6.9	4.90	2.31	13.0	16.24	2.11	13.3
10	1.62	1.00	0.0	2.03	1.00	0.0	2.49	1.00	0.0	5.80	1.05	1.0	12.64	1.12	2.6	16.19	1.12	2.3	31.26	1.02	0.5
11	1.33	1.00	0.0	1.35	1.00	0.0	1.51	1.00	0.0	3.68	1.00	0.0	7.85	1.00	0.0	8.43	1.00	0.0	11.93	1.00	0.0

Eluents: 0.01 *M* NaOAc (pH 4) with addition of 15% MeCN (CDR-6), 20% MeCN (CDRs Nos. 1, 2, 3, 5, 7, and 9), or 30% MeCN (CDRs Nos. 4, 8, 10, and 11); isocratic elution at 1 ml min^{-1} and 30 °C; Nucleosil C_{18} column (see Section 2).

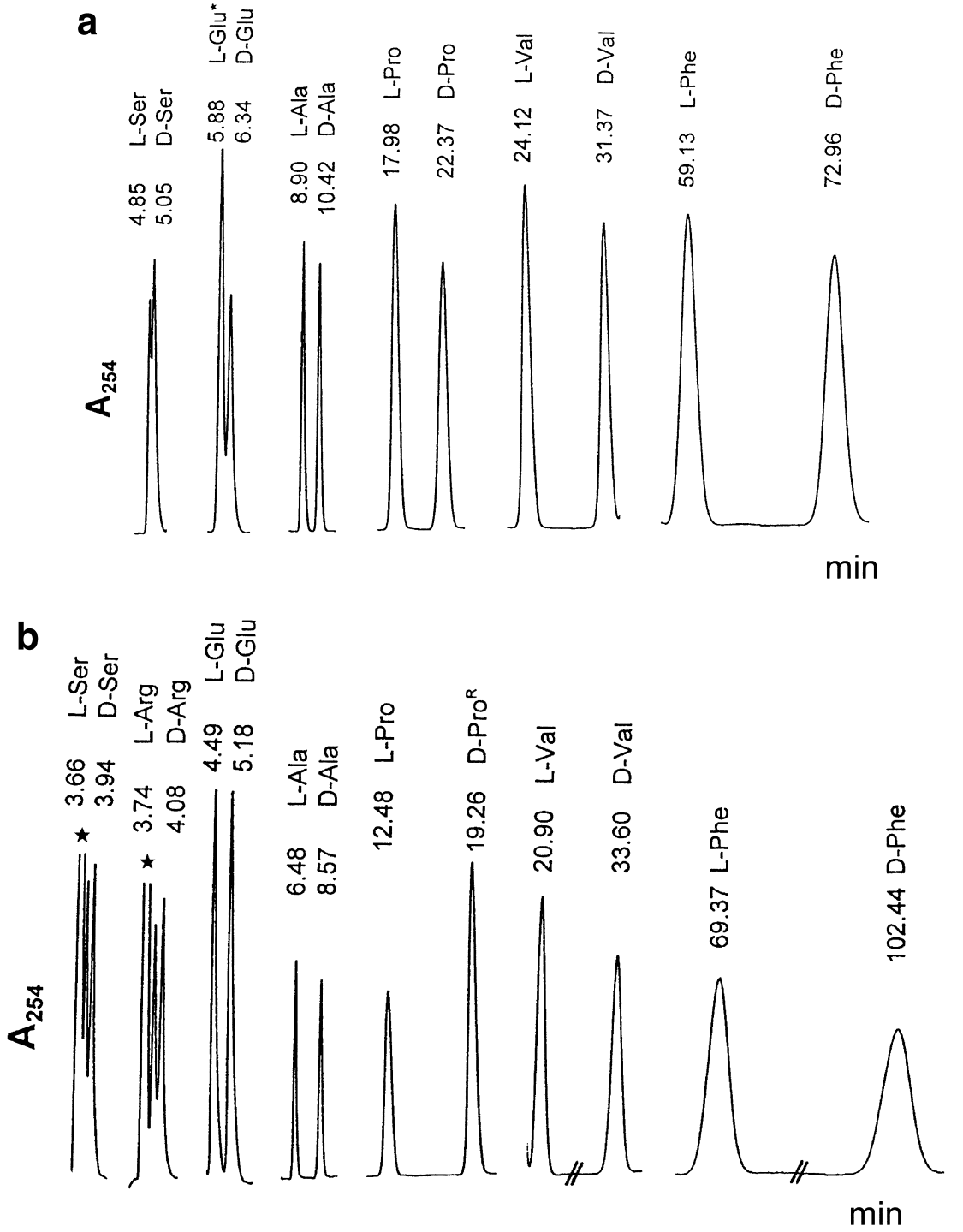


Fig. 2. HPLC of DL-amino acids derivatized with (a) CDR-5, and (b) CDR-6. Asterisk in (a) refers to coelution with peak resulting from reagent, asterisks in (b) refer to peaks resulting from injection of analytes and D-Pro^{R} to coeluting reagent. Chromatographic conditions, Nucleosil 100 C_{18} (analytical column; see Section 2); eluents 0.01 *M* NaOAc (pH 4.0) with (a) 20% MeCN, and (b) 15% MeCN.

these facts into account design of reagents reacting at ambient temperature should be feasible.

Possible racemization of amino acids in the course of the reactions was tested by derivatization of enantiomerically pure L-Val or L-Ala. No diastereomers due to the formation of D-amino acids were detected.

3.2. Evaluation of the suitability of CDRs for separations

In a first series of experiments one chlorine in cyanuric chloride was replaced by an alkoxy or aryloxy group and the second chlorine by L-Ala-NH_2 (CDRs Nos. 1–5).

In a second series of experiments one chlorine was replaced by a methoxy group and the second chlorine by L-Val-NH_2 or L-Phe-NH_2 (CDRs Nos. 6 and 7, respectively), Pro-O*t*Bu (CDR-10) or Boc-Lys-O*t*Bu (CDR-11). For comparison, in CDR-8 the methoxy group of CDR-7 was replaced by a trifluoroethoxy group.

Finally, two chlorine atoms in cyanuric chloride were consecutively replaced by L-Val-NH_2 and L-Phe-NH_2, resulting in CDR-9 having two chiral residues.

From the data compiled in Table 1 it is evident that highest resolution was achieved by CDRs Nos. 6 and 9. Comparison of these reagents shows that CDR-6 provided higher resolution for DL-Arg, DL-Ala, and DL-Pro, whereas CDR-9 provided higher resolution for DL-Ser, DL-Glu, DL-Val, and DL-Phe.

Comparison of separation factors shows that CDRs with methoxy residues (MeO–) at triazine rings show best resolutions. Substitution of the methoxy group with other residues lead to decreased resolution for diastereomers formed from reagents bearing carboxamide groups.

Although CDRs described here show structural relationship to Marfey's reagent (see Ref. [6] and the literature cited therein), diastereomeric derivatives resulting from the latter are best resolved. The trifunctionality of *s*-triazines, however, offers a multitude of analytical aspects.

It is also worth of note in this context that the CDRs investigated enable the (indirect) enantiomeric resolution of free DL-amino acids whereas stationary phases resulting from chiral selectors triazine-linked to aminopropylsilica in many cases require dansylation or dinitrobenzoylation of DL-amino acids in order to achieve (direct) enantiomeric resolution.

4. Perspectives

The methods outlined for the syntheses of CDRs, together with the data and features of reagents, are expected to stimulate further development and exploration of reagents for the chiral as well as nonchiral derivatization of amino acids—or nucleophilic analytes in general—using trifunctional cyanuric halides as starting compounds. For example, reactivity of reagents might be enhanced by using cyanuric fluoride as starting material. Sensitivity and selectivity of detection might be increased by employing reporter groups with high molar extinction coefficients, luminophores, near infrared chromophores, or laser excitable fluorescent groups yielding high quantum yields. An abundance of potentially useful reporter groups can be found in the literature [20,21]. Water solubility and polarity of reagents can be altered by use of ionizable compounds or by introducing long-chain alkoxy groups. Notably, halogens in triazine reagents might be replaced by other reactive residues capable of reacting selectively with analytes [7]. The advantages and perspectives of triazine-based reagents and chiral selectors are now generally recognized [7,19] but their scope and potential in separation sciences have by far not yet been explored.

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