ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1295–1303

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

High performance liquid chromatography enantioseparation of chiral pharmaceuticals using tris(chloro-methylphenylcarbamate)s of cellulose[1]

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Received for review 13 September 1995; revised manuscript received 4 December 1995

Abstract

Chiral recognition abilities of a recently developed new type of cellulose phenylcarbamates were studied. These chiral stationary phases (CSPs) simultaneously contain both electron-withdrawing (Cl) and electron-donating (CH_3) substituents on the phenyl moiety. Chiral pharmaceuticals which belong to the various pharmacological groups (sedatives, hypnotics, anticonvulsants, Ca^{2+} channel blockers, β-blockers, antitusives, antihystaminics, choleretics, diuretics, antimycotics, etc) were resolved to enantiomers. These new CSPs sometimes exhibit alternative chiral recognition ability to that most successful commercially available cellulosic CSP Chiralcel-OD and can be used as a good complement to it in analytical and preparative scale enantioseparations.

Keywords: Chiral drugs; Enantioseparations; Polysaccharide chiral stationary phases

1. Introduction

Marked progress has been achieved in the separation of optical antipodes within the last decade. Nonetheless, this field still remains one of the most exciting in the separation sciences [1,2]. More than 100 chiral stationary phases (CSPs) for gas chromatography (GC), high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) and a considerable number of chiral selectors for capillary electrophoresis (CE) have been described. CE is still considered to be a useful complement to chromatographic techniques due to the orthogonality of separation principles on which these techniques are based. Various modes of CE, such as capillary electrokinetic chromatography [3] and capillary electrochromatography [4], successfully use the chromatographic separation principle. This means that CE will become a competitive technique rather than a complementary one in the near future. The opportunities are obvious for CE in

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[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

PII S0731-7085(96)01748-7

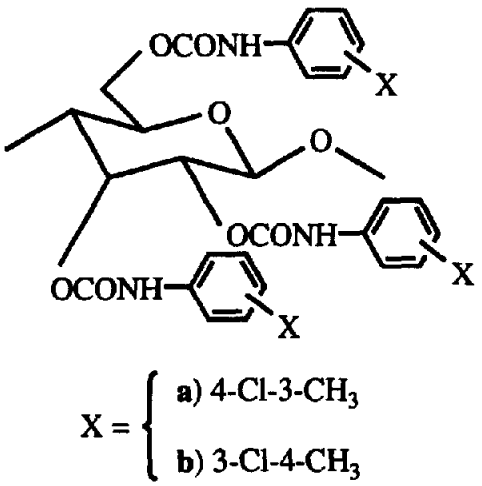

Fig. 1. Structures of tris(4-chloro-3-methylphenylcarbamate) of cellulose (CSP 1) and tris(3-chloro-4-methylphenylcarbamate) of cellulose (CSP 2).

analytical scale enantioseparations, whereas no competition from CE to HPLC is anticipated at all in preparative scale enantioseparations. The commercially available CSPs can be classified into the following groups: (a) ligand exchange [5]; (b) brush type [6]; (c) inclusion [7–9]; (d) protein- and peptide types [10]; and (e) natural and synthetic polymers and their derivatives [1,11]. However, only a few of these CSPs, such as polysaccharide phenylcarbamates, are suitable for preparative scale enantioseparations. The most important advantages of polysaccharide-type CSPs for preparative scale enantioseparations are: (i) universality, which means that they are useful for enantioseparation of almost any type of chiral compound ranging from small nonaromatic pharmaceuticals to chiral clefts [12]; (ii) they are compatible with both organic and aqueous mobile phases; (iii) their natural sources are available in large quantities; and (iv) they have a high chiral selector content which permits usage under overloaded conditions.

Extensive development of simulated moving bed and closed loop recycling technologies for preparative scale enantioseparations can substantially contribute to a financial preferance for separation techniques over synthetic ones in large scale production of pure enantiomers [13–16].

In order to further enhance chiral recognition ability and stability of polysaccharide phenylcarbametes, chloromethylphenylcarbamates of cellulose and amylose were synthesized recently [17–19]. These derivatives differ from previously synthesized polysaccharide phenylcarbamates [1] as they simultaneously contain both electron withdrawing and electron-donating substituents on the phenyl moieties. Spectroscopic studies (^{1}H-NMR, IR and circular dichroism) showed that simultaneous introduction of substituents of opposite nature substantilally modified the physical and chemical properties as well as the chiral recognition abilities of synthesized CSPs [18,19]. Application of these materials for enantioseparation of selected chiral pharmaceuticals using narrow-bore HPLC was described recently [20]. A more detailed study of the potential of tris(chloromethylphenylcarbamate)s of cellulose for the separation of chiral drugs using conventional size columns was performed in the present work.

Table 1
Enantioseparation of *N*-protected racemic amino acid derivatives using CSPs 1 and 2

Racemic compound	CSP 1				CSP 2			
	k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
Benzoylphenylglycine ethyl ester	2.7	1.5	4.0	[a]	1.7	1.6	2.5	[a]
Benzoyltyrosine ethyl ester	4.3	1.6	2.3	[a]	3.2	1.8	3.0	[a]
Benzoylalanine ethyl ester					1.6	1.3	2.0	[a]
Benzoyltyrosinamide	18.2	1.2	1.5	[a]	8.0	1.8	4.0	[a]

[a] *n*-Hexane/2-propanol (85/15, v/v); flow rate 1.0 ml min^{-1}.

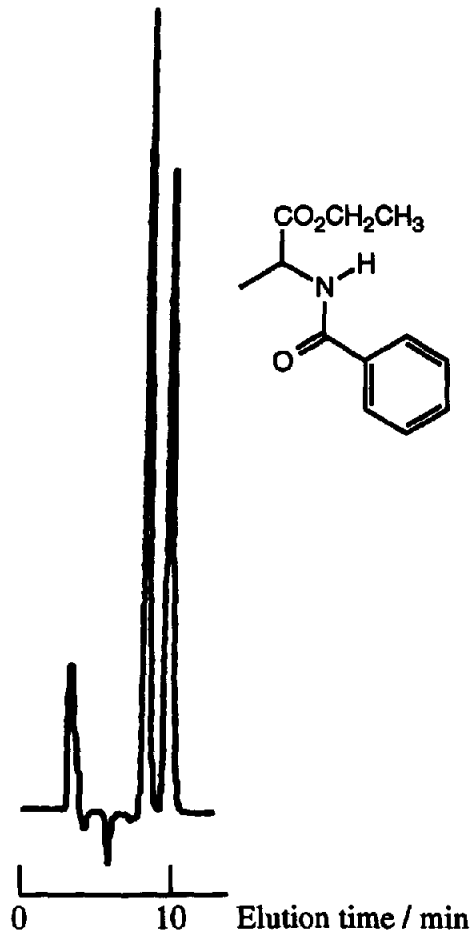

Fig. 2. Enantioseparation of DL-*N*-benzoylalanine ethyl ester on CSP 2. Eluent [a] of Table 1; flow rate 1.0 ml min^{-1}.

2. Experimental

2.1. Materials

Tris(4-chloro-3-methylphenylcarbamate) (CSP 1) and (3-chloro-4-methylphenylcarbamate) (CSP 2) (Fig. 1) of cellulose were synthesized as described previously [18,19] and packed into 25 cm × 0.46 cm i.d. stainless-steel tubes by a conventional high pressure slurry packing technique. The commercially available column Chiralcel-OD used as reference in selected experiments was from Daicel (Daicel Chem. Co., Tokyo, Japan). Racemic compounds were from different sources and were used without further purification. HPLC-grade solvents (*n*-hexane, 2-propanol) were from J.T. Baker (Deventer, The Netherlands).

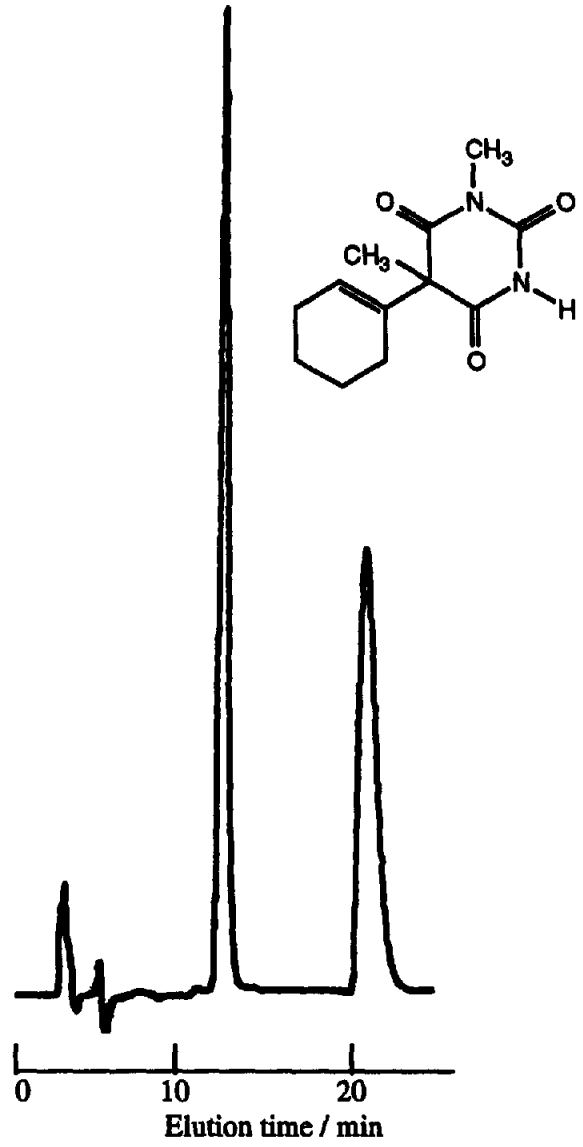

Fig. 3. Enantioseparation of *RS*-hexobarbital on CSP 2. Eluent [a] of Table 1; flow rate 1.0 ml min^{-1}.

Table 2
Enantioseparation of racemic barbiturates using CSPs 1 and 2

Racemic compound	CSP 1				CSP 2			
	k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
Hexobarbital	6.5	1.7	5.0	[a]	7.2	1.6	5.4	[a]
1-Methylphenobarbital	2.4	1.7	3.23	[b]	3.4	1.9	5.6	[b]
1-Methyl-5-ethyl-5-propylbarbital	1.0	1.3	1.2	[c]	1.2	1.1	0.8	[c]
Pentobarbital	1.6	1.1	1.0	[b]	1.6	1.0	–	[b]
Thiopental	4.7	1.1	0.7	[c]	5.0	1.1	0.9	[c]

[a] see Table 1.
[b] *n*-Hexane/2-propanol (90/10, v/v); flow rate 1 ml min^{-1}.
[c] *n*-Hexane/2-propanol (92.5/7.5, v/v); flow rate 1 ml min^{-1}.

Table 3
Enantioseparation of racemic diazepine derivatives using CSPs 1 and 2

Racemic compound	CSP 1				CSP 2			
	k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
3-Methylbenzodiazepine	4.0	1.1	0.5	[c]	1.3	1.6	2.0	[a]
Oxazepam	18.4	1.3	1.2	[a]	9.4	1.3	2.0	[a]
Lorazepam	19.3	1.0	–	[a]	11.4	1.4	3.0	[a]
Lopirazepam	14.5	1.5	3.0	[a]	11.1	2.0	4.0	[a]
Camazepam	9.5	1.3	2.0	[a]	6.3	1.6	3.5	[a]
Cloxazolam	5.6	1.0	–	[c]	2.0	1.7	3.0	[a]
Ketazolam					3.8	1.6	2.0	[b]
Oxazolam[d]	3.8	1.1	0.5	[a]	1.7	1.1	0.5	[b]
	5.0	1.2	1.0		2.6	1.1	–	

[a–c] See Tables 1 and 2.
[d] Both diastereomers of oxazolam were resolved to enantiomers in a single run.

2.2. Apparatus

HPLC separations were carried out using a Beckman Model 110a isocratic pump, a Beckman Model 332 analytical optical unit (both from Beckman, Palo Alto, CA) and a model 7125 injector with a 20 μl loop (Rheodyne, Cotati, CA). All chromatographic parameters (k', α, R_s) were calculated using equations commonly used in chromatography.

3. Results and discussion

3.1. Enantioseparation of racemic N-protected amino acid derivatives

The ability of polysaccharide phenylcarbamates to resolve enantiomers of various *N*-protected amino acid esters has already been described [21]. The usefulness of CSPs 1 and 2 for the enantioseparation of *N*-protected amino acid deriva-

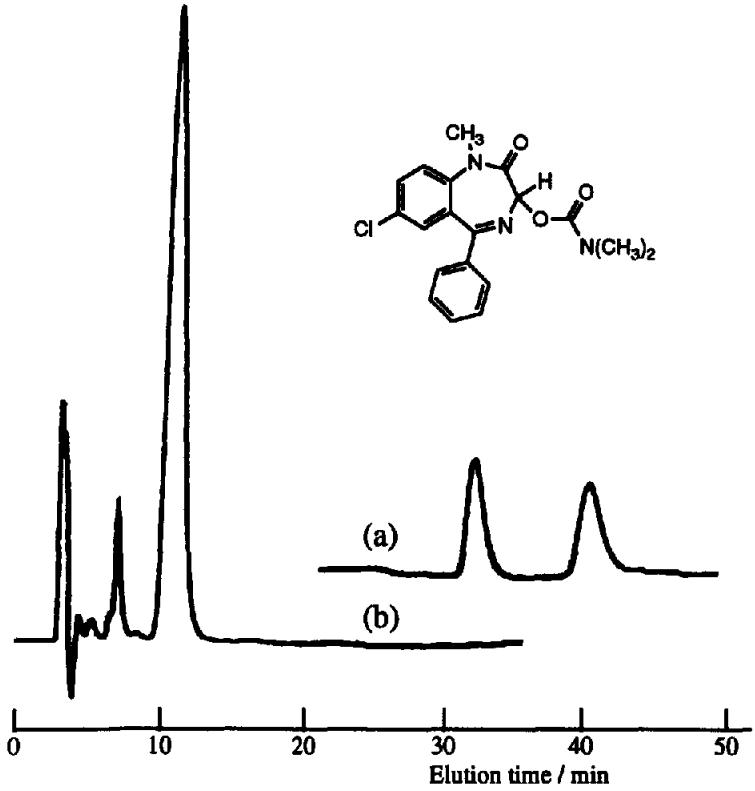

Fig. 4. Enantioseparation of *RS*-camazepam on CSP 1 (a) and on Chiralcel-OD (b). Eluent [a] of Table 1; flow rate 1.0 ml min^{-1}.

tives is exemplified using ethyl esters of racemic *N*-benzoylphenylglycine, *N*-benzoyltyrosine and *N*-bensoylalanine, as well as with *N*-benzoyltyrosinamide (Table 1, Fig. 2). High values of selectivity (α) in some cases show the potential of these CSPs for preparative scale enantioseparations.

Table 4
Enantioseparation of racemic β-blockers using CSPs 1 and 2

Racemic compound	CSP1				CSP2			
	k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
Pindolol	2.1	1.7	1.2	[d]	13.2	1.6	1.3	[b]
Propranolol	3.4	1.1	1.0	[e]	1.9	1.3	1.5	[e]
Sotalol	7.6	1.2	1.0	[d]	7.1	1.2	0.8	[d]
Alprenolol	1.0	1.2	0.8	[f]	3.6	$\cong 1$	–	[f]
Bupranolol	1.2	1.1	0.6	[f]	1.2	1.1	0.6	[g]
Acebutolol	25.7	1.1	1.0	[e]	5.0	1.0	–	[e]
Penbutolol	2.3	1.1	0.8	[g]	2.3	1.2	1.1	[g]
Toliprolol	2.8	1.0	–	[g]	5.7	1.2	1.3	[g]

[a–c] See Tables 1 and 2.
[d] *n*-Hexane/2-propanol/diethylamine (80/20/0.1, v/v/v); flow rate 1.0 ml min^{-1}.
[e] *n*-Hexane/2-propanol/diethylamine (90/10/0.1, v/v/v); flow rate 1.0 ml min^{-1}.
[f] *n*-Hexane/2-propanol/diethylamine (95/5/0.1, v/v/v); flow rate 1.0 ml min^{-1}.
[g] *n*-Hexane/2-propanol/diethylamine (98/2/0.1, v/v/v); flow rate 1.0 ml min^{-1}.

3.2. Enantioseparation of racemic barbiturates

Barbituric acid derivatives are widely used as hypnotics, sedatives, analgesics, anticonvulsants, etc. Many clinically used barbiturates are chiral and their enantiomers exhibit different, sometimes even opposite, pharmacological effects. Enantioseparation of several chiral barbiturates has been described using cellulose tris (4-methylphenylbenzoate) (Chiralcel OJ) [22] but not with cellulose phenylcarbamates. Both CSPs 1 and 2 can successfully be used for the enantioseparation of chiral barbiturates containing a chiral carbon atom in a barbiturate heterocycle (hexobarbital (Fig. 3), 1-methylphenobarbital, 1-methyl-5-ethyl-5-propylbarbital) or in a side-chain (pentobarbital, thiopental) (Table 2).

3.3. Enantioseparation of racemic diazepines and related compounds

Commercially available polysaccharide derivatives are not optimal for enantioseparation of chiral diazepine derivatives. As Table 3 shows, CSPs 1 and 2 are very suitable where commercial CSPs are not. Enantioseparations of racemic camazepam using Chiralcel-OD and CSP 1 are shown in Fig. 4 as a representative example.

3.4. Enantioseparation of racemic β-blockers

Enantioseparation of racemic β-blockers has been extensively studied using commercially available polysaccharide derivatives and a number of interesting effects, for example the temperature- and/or organic modifier-dependent reversal of the enantiomer elution order, have been observed [23,24]. Although commercially available CSPs exhibit high and almost universal chiral recognition ability for this group of pharmaceuticals, there are a few substances in this group (for example acebutolol) which are not resolved sufficiently to enantiomers using commercial CSPs.

Table 5
Enantioseparation of racemic imidazole derivatives using CSPs 1 and 2

Racemic compound	CSP1				CSP2			
	k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
Enilconazole	3.5	1.3	2.0	[a]	2.6	1.4	2.0	[a]
Econazole	4.5	1.7	3.5	[a]	3.9	1.7	2.5	[a]
Miconazole	4.2	1.5	2.0	[a]	3.9	1.5	1.8	[a]
Bifonazole	3.6	1.6	2.0	[a]	4.6	1.8	2.0	[b]
Ornidazole	4.3	1.1	1.0	[a]	2.8	1.5	1.5	[a]
Bayleton	1.1	1.3	1.5	[a]	1.0	1.2	1.4	[a]
Metomidate	3.8	1.6	4.0	[c]	4.3	2.1	4.0	[c]

[a–c] See Tables 1 and 2.

Table 6
Enantioseparation of racemic dihydropyridine derivatives using CSPs 1 and 2

Racemic compound	CSP1				CSP2			
	k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
Nisoldipine	4.3	1.2	1.2	[c]	1.2	1.2	1.2	[a]
Nimodipine	6.4	1.1	0.6	[c]	1.6	1.0	–	[a]
Isradipine	3.5	1.2	0.8	[c]	0.9	1.0	–	[a]
Nicardipine	5.9	1.1	0.9	[b]				
Bay K 8644	8.7	1.1	0.7	[c]	2.4	1.2	1.0	[a]

[a–c] See Tables 1 and 2.

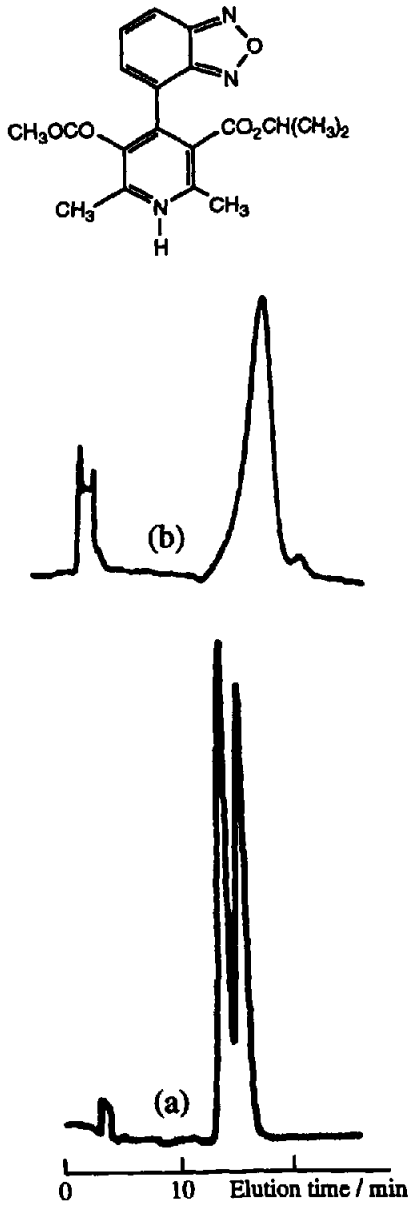

Fig. 5. Enantioseparation of *RS*-isradipine on CSP 1 (a) and on Chiralcel-OD (b). Eluent [c] of Table 2; flow rate 1.0 ml min^{-1}.

CSPs 1 and 2 are suitable for enantioseparation of chiral β-blockers (Table 4). Moreover, using CSP 1, acebutolol can also be resolved to enantiomers [18]

3.5. Enantioseparation of chiral imidazole derivatives

Chiral imidazole derivatives are widely used or recommended for pharmaceutical use as antimycotics (for example, bifonazole, miconazole, enilconazole), antiepileptics (denzimol), cytostatics (erbulozol) and so on. As Table 5 shows, CSPs 1 and 2 are also well suited for enantioseparation of this group of pharmaceuticals.

3.6. Enantioseparaton of chiral dihydropyridine derivatives

4-Aryl-1,4-dihydropyridine calcium antagon-ists are important peripherial vasodilators and are widely used in the treatment of cerebrovasculatory disorders and hypertension [25]. It has been established that in many instances chiral dihydropyridines, such as nitrendipine and nicardipine, are superior to the corresponding symmetrically substituted derivatives such as nifedipine [26,27]. Detailed pharmacological studies revealed that enantiomers of chiral dihydropyridines have different, in some cases even opposite, vasodilating and hyptensive activities and toxicities [25]. Many chiral dihydropyridines are difficult to resolve using commercially available polysaccharide CSPs. For example, chiral nicardipine, isradipine and nimodipine are not resolvable on Chiralcel-OD [28]. All these and some other chiral dihydropyridines can be resolved to enantiomers using CSPs 1 and 2 (Table 6, Fig. 5).

3.7. Enantioseparation of chiral antihystaminic drugs

Antihystaminic drugs cover substances of various chemical groups, many of them chiral. The chiral compounds of this pharmacological group resolvable on CSPs 1 and 2 are summarized in Table 7.

3.8. Other pharmaceuticals resolved to enantiomers using CSPS 1 and 2

A number of randomly chosen drugs and drug candidates were resolved to enantiomers using CSPs 1 and 2 (Table 8). There are several racemic

Table 7
Enantioseparation of racemic antihystaminic drugs using CSP 1 and 2

Racemic compound	CSP1				CSP2			
	k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
Phenyramine	3.0	1.1	1.1	[g]	2.9	1.1	1.0	[g]
Doxylamine	0.9	1.9	2.0	[b]	1.0	1.2	1.0	[b]
Chlorphenoxamine	2.3	1.2	1.5	[g]	0.4	1.9	2.0	[g]
Carbinoxamine	7.0	1.1	0.6	[h]	7.7	1.2	0.7	[b]
Azelastine	6.3	1.4	1.0	[a]	10.6	1.0	–	[g]
Mequitazine	13.1	1.1	1.2	[g]	17.0	1.0	–	[g]

[a–g] See Tables 1–4.
[h] *n*-Hexane/2-propanol (98/2, v/v); flow rate 1.0 ml min^{-1}.

compounds included in this table for which no acceptable enantioseparation has been reported yet.

In conclusion, tris(chloromethylphenylcarbamate)s of cellulose show a broad spectrum of chiral recognition abilities and can be used for chiral HPLC analysis. The important result from a mechanistic point of view seems to be that CSPs 1 and 2, which differ only slightly in terms of structure, exhibit a marked difference in chiral recognition ability with regard to some racemic compounds.

Acknowledgements

The authors thank Professor G. Blaschke (Institute of Pharmaceutical Chemistry, University of Münster, Münster, Germany) for generous gifts of some racemic compounds.

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Table 8
Enantioseparation of various chiral drugs and drug candidates using CSPs 1 and 2

Racemic compound	Pharmaceutical activity	CSP1				CSP2			
		k'_1	α	R_s	Eluent	k'_1	α	R_s	Eluent
Paramethadione	Antiepileptic	0.9	1.1	0.7	[a]	2.2	1.1	0.5	[g]
Norgestrel	Progestin	17.1	1.1	1.2	[c]	14.8	1.3	2.0	[b]
Tesicam	Antiinflammatory	8.0	1.7	4.0	[a]	6.1	1.6	2.8	[a]
Mesuximide	Antiepileptic	4.1	1.1	1.2	[c]	2.0	1.4	2.2	[a]
Metofoline	Opioid analgesic	1.2	1.3	1.2	[c]	1.2	2.1	4.0	[b]
Tramadol	Analgesic	2.0	1.2	2.0	[g]	1.7	1.5	3.0	[g]
Clofedanol	Antitussive	1.9	1.1	1.0	[g]	1.4	1.0	–	[g]
Lofexidine	α_2-Receptor antagonist	8.5	1.2	2.0	[g]	7.0	1.3	1.5	[g]
Clenbuterol	β_2-Sympathomimetic	6.1	1.1	0.7	[g]	5.0	1.0	–	[g]
Piprozolin	Choleretic	8.7	2.4	6.0	[c]	4.0	3.4	7.0	[a]
Etozolin	Diuretic	11.9	2.9	5.0	[c]	6.3	4.5	8.0	[a]
Doxapram	Analeptic	9.0	1.2	1.5	[c]	6.6	2.1	5.0	[b]
Chlormezanone	Tranquillizer	7.4	1.2	0.7	[d]				
Aminogluthethimide	Antineoplastic	25.0	1.2	1.2	[d]	16.3	1.2	1.5	[d]
Articain	Local anesthetic	5.0	1.5	4.0	[f]	4.1	1.4	2.5	[f]
Etidocain	Local anesthetic	2.1	1.2	1.0	[f]	1.1	1.2	1.2	[f]
Nefopam	Analgesic	1.2	1.2	1.0	[f]	1.7	1.0	–	[g]

[a–g] See Tables 1–4

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