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Diastereomeric resolution of nucleoside analogues, new potential antiviral agents, using high-performance liquid chromatography on polysaccharide-type chiral stationary phases

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Abstract

This paper describes the separation of the four sets of stereoisomers of nucleoside analogs, new potential antiviral agents by direct analytical HPLC methods using derivatized cellulose and amylose chiral stationary phases. The resolution was made using normal-phase methodology with a mobile phase consisting of *n*-hexane-alcohol (ethanol or 2-propanol) in various percentages, and a silica-based cellulose tris-3,5-dimethylphenylcarbamate (Chiralcel OD-H), or tris-methylbenzoate (Chiralcel OJ) and a silica-based amylose tris-3,5-dimethylphenylcarbamate (Chiralpak AD) or tris-(*S*)-1-phenylethylcarbamate (Chiralpak AS). The effects of structural features on the extent of discrimination between the stereoisomers were examined through the retention, the selectivity and the resolution factors as well as the elution order. Baseline separation (R_s>1.5) was easily obtained in many cases. The resolution results were complementary between the different columns.
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1. Introduction

Because of the current interest in the development of antiviral drugs, considerable attention has been focused on the synthesis of new molecules. Carbocyclic analogs of nucleosides have shown antiviral properties and can potentially serve as selective inhibitors of the human immunodeficiency virus (HIV) [1,2]. A number of 2′,3′-dideoxynucleosides, such as AZT (Zidovudine, Retrovir®) and ddI (Didanosine, Videx®) or 2′,3′-dideoxy-2′,3′-didehydronucleosides (d4Ns), corresponding to the introduction of a double bond at the 2′,3′ position, such as d4G (2′,3′-dideoxy-2′,3′-didehydroguanosine—Carbovir), d4C (2′,3′-dideoxy-2′,3′-didehydrocytosine), d4T (2′,3′-dideoxy-2′,3′-didehydrothymidine—Stavudine, Zerit®) (Fig. 1), possessing β-D configuration, have been approved for the treatment of

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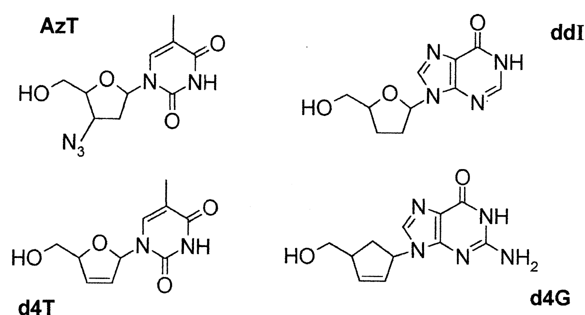

Fig. 1. Chemical structures of anti-HIV molecules.

individuals with AIDS [3–5,8]. Several nucleosides with the unnatural β-L configuration have emerged as antiviral agents against HIV which include 3TC (Lamivudine, Epivir®), FTC, LFMAU [6]. The β-D and β-L nucleosides can be prepared by classical ways starting from the D- and L-monosaccharides respectively.

In the present work a new glycone has been designed in which the 2′,3′ double bond is incorporated into a benzene ring to give a derivative of the benzo[c]furan system: those molecules correspond to homologous of d4T. Compounds of this type **4** have the potential to exercise biological activity in a similar manner to dideoxynucleosides [7]. Furthermore some of the chemical intermediates as **3** also present an interesting biochemical activity [7]. Those compounds **3** and **4** have been prepared according to previously described synthetic routes [3–5] (Fig. 2). They have two chiral centers in the 1,3-dihydrobenzo[c]furan (C-1′ and C-3′). We obtained either the four stereoisomers (1′*S*, 3′*S*);(1′*R*, 3′*R*) (*trans* isomers) and (1′*R*, 3′*S*);(1′*S*, 3′*R*) (*cis* isomers) through non-asymmetric synthesis or the two couples of diastereomers (1′*S*, 3′*S*) (1′*R*, 3′*S*) or (1′*R*, 3′*R*) (1′*S*, 3′*R*) through asymmetric synthesis (Admix α or Admix β inducers). The absolute configuration of the different stereoisomers **3** and **4** has been unequivocally established by ^{1}H NMR spectroscopy, the shifts δ and the coupling constants $J_{1'-3'}$ of the H1′ and the H3′ protons are different for *cis* and *trans* compounds according to previously detailed studies [3,5].

Many of these analogs can exhibit stereoselective biological activity [9]. It has been shown that the antiviral activity of the drug carbovir is associated with only the (−)-enantiomer [10]. These findings imply that other stereoisomers of the carbocyclic analogs of nucleosides are expected to differ in their biological activity and that just one would be therapeutic. Attempts to resolve the diastereomeric couples **3** (1′*S*, 3′*S*), (1′*R*, 3′*S*) and **3**(1′*R*, 3′*R*);(1′*S*, 3′*R*) by conventional achiral analytical HPLC were generally unsuccessful or led to poor separations. The scaling-up to preparative HPLC cannot be generalized to afford sufficient quantities for biological studies. So in order to obtain a more rapid method the direct resolution on chiral stationary phases, without any prederivatization, of the diastereomeric components **3** has been studied. This analytical chiral chromatography will also permit to establish the enantiomeric purity of each isomer. Separation of isomers by chiral HPLC is now well established with over 50 different chiral phases (CSP) commercially available. Among them cellulose and amylose esters and carbamate derivatives coated onto a large pore silica gel backbone have proved to be useful stationary phases and are used in the normal-phase mode [11–17]. To the best of our knowledge only a limited amount of work using liquid chromatography for the analytical or preparative resolution of nucleosides analogs has been published [18,19].

In the continuity of our work on the separation of racemates with potent biological activity [20] we examined in this study the direct separation of **3** on different chiral stationary phases of polysaccharide-derived types and particularly on cellulose (Chiralcel OD-H, Chiralcel OJ) and amylose (Chiralpak AS, Chiralpak AD). The good resolution factor and the high loadability of those CSPs afforded a preliminary preparative separation of the isomers by repeated 50 μl injections of the racemic mixture on an analytical column. The collection of the eluates from the chromatographic peaks afforded milligram amounts sufficient to determine the configuration, *cis* or *trans*, of each component by ^{1}H NMR spectroscopy.

2. Experimental

2.1. Chromatography

Chromatography was carried out on a Chiralcel OD-H column (cellulose tris-3,5-dimethylphenyl-

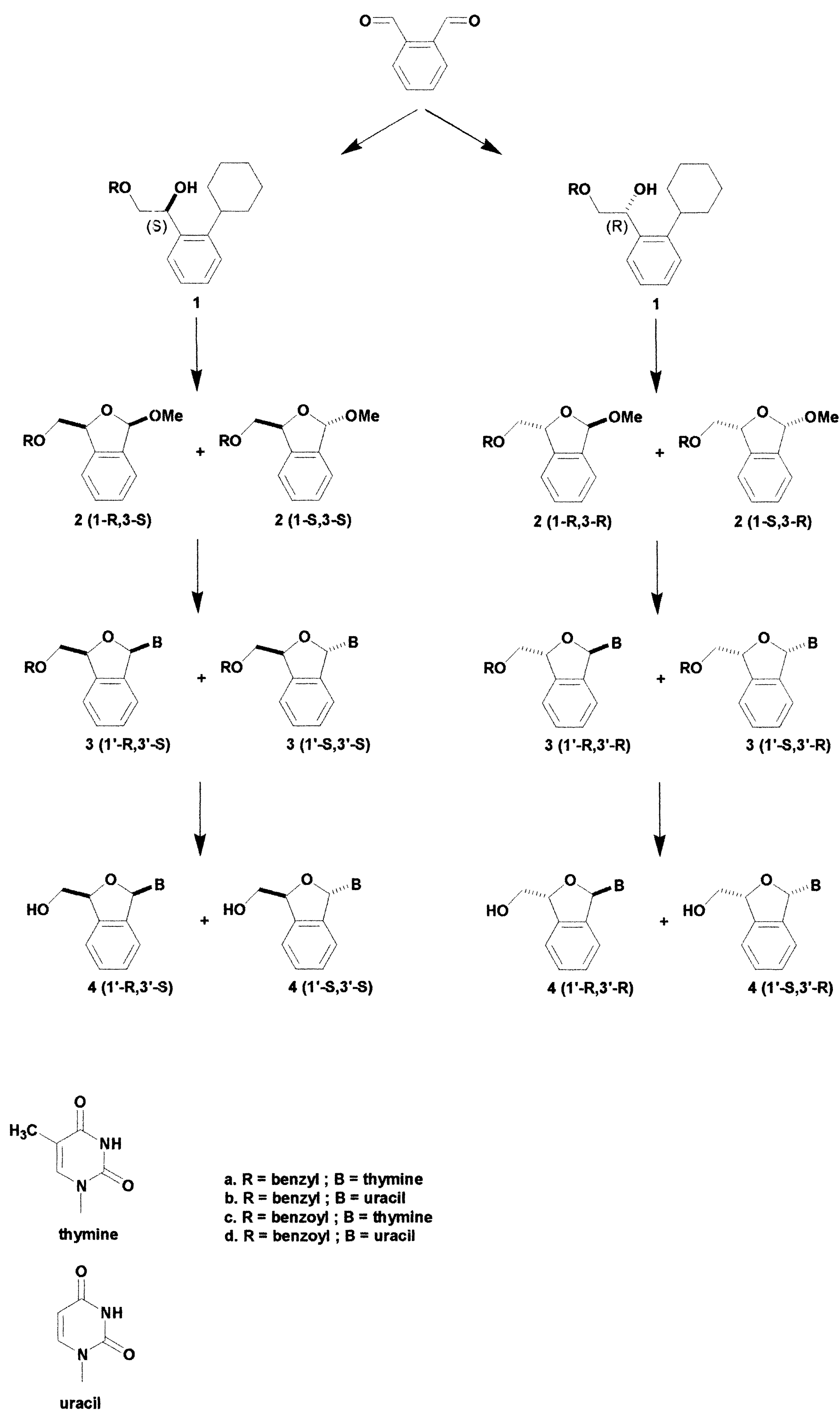

Fig. 2. Chemical structures and synthetic pathways of compounds **3** and **4**.

carbamate; 250×4.6 mm I.D.; 5 μm); a Chiralcel OJ column (cellulose tris-methylbenzoate; 250×4.6 mm I.D.; 10 μm); a Chiralpak AD column (amylose tris-3,5-dimethylphenylcarbamate; 250×4.6 mm I.D.; 10 μm), and on a Chiralpak AS column (amylose tris-(*S*)-1-phenylethylcarbamate; 250×4.6 mm I.D.; 10 μm) (Daicel Chemical Industries, Baker France) using a gradient Waters 600E metering pump model equipped with a Waters 996 photodiode array spectrophotometer. Chromatographic data were collected and processed on a Digital computer running with Millennium 2010. The column eluate was monitored at 210; 210; 230; 275 nm. The sample loop was 20 μl (Rheodyne 7125 injector). A polarimetric HPLC detector (Jasco OR 990) which utilises a Xe–Hg lamp as a light source at full 350–900 nm range with the strong mercury line emission at 365 nm, a cell pathlength 0.25 dm and volume 40 μl was coupled in series. An integrator (Merck 7500) was used to record the output from the polarimetric detector. Mobile phase elution was made isocratically using *n*-hexane and a modifier (ethanol or 2-propanol) at various percentages. The flow-rate was 1.0 ml min^{-1}. The peak of the solvent front was considered to be equal to the dead time (t_0=3.5 min) and was taken from each particular run. Retention times were mean values of two replicate determinations. All separations were carried out at 30°C.

2.2. Reagents

The synthesis of the mixtures was described previously [3–5,8]. Ethanol, 2-propanol and *n*-hexane were HPLC grade from Merck or Baker. All the solutions were filtered (0.45 μm), degassed with a Waters in-line degasser apparatus. The mobile phases used were A: *n*-hexane/ethanol 80/20; B: *n*-hexane/ethanol 90/10; C: *n*-hexane/2-propanol: 80/20; D: *n*-hexane/2-propanol: 90/10. Compounds were chromatographed by dissolving them in the corresponding alcohol to a concentration of about 0.75 m*M* (which corresponds to 15 nmoles injected) and passed through a 0.45-μm membrane filter prior to loading the column.

3. Results and discussion

Tables 1–4 present the retention, selectivity and resolution factors of the pairs of diastereoisomers of carbocyclic analogs of nucleosides, using several kinds of mobile phase by changing the nature of the alcohol and the percentage. Baseline separations were generally obtained. The structures of the analyzed compounds **3** are shown in Fig. 2.

3.1. Type of alcohol modifier

Two kinds of mobile phase were investigated by changing the modifier from ethanol (Eluent A) to 2-propanol (Eluent C). The alcohol modifier can affect the retention of the solutes in different ways. First by improving the solvation in the mobile phase, and secondly by competing for the H-bonding sites in the stationary phase. We observed the increase of the retention factors k' of all compounds by changing the modifier from ethanol to 2-propanol, as generally expected, due to the higher polarity of the ethanol. This behaviour is illustrated in Figs. 3 and 4 (Chiralcel OD-H) for compounds **3c**, **3d** respectively.

On Chiralcel OD-H, ethanol is better adapted for resolution of compounds **3b**-(3′*R*); **3c**-(3′*R*); **3c**-(3′*S*); **3d**-(3′*S*), whereas 2-propanol is better for compounds **3a**-(3′*R*); **3a**-(3′*S*); **3b**-(3′*S*); **3d**-(3′*R*). For example for compound **3b**-(3′*R*) ($k'_1\alpha$; R_s) were (2.98;1.37;3.34) and (4.51;1.38;3.18) with Eluents A and C respectively.

The molecules recognition process reflects the sum of all interactions between the solute and the CSP. These interactions include hydrogen bonding, dipole–dipole interaction, π–π interaction and inclusion phenomenon [21]. In OD-H CSP, regarding the mechanism of isomers discrimination, the most important docking sites on these types of columns are considered to be the polar carbamate residue which can interact through H bondings with the corresponding groups on the analyte molecule [13]. It has also been reported that solvents as alcohol compete more effectively for the recognition sites than the analyte, so the diastereomeric resolution increased as the size of the alcohol increased [16]. But here a maximum of R_s is obtained either with ethanol or with 2-propanol.

This suggests that hydrogen bonding interactions are probably not the predominant type but separations might involve more π–π interactions between the aromatic moiety of the analyte and the

Table 1
HPLC resolution: retention factors (k') separation factor (α) and resolution (R_s) of **3** diastereomers and absolute configuration; Chiralcel OD-H

Compound	Eluent	k'_1	k'_2	α	R_s	1st	2nd Eluted isomer[b]
3a-(3′*R*)	A	2.37 (1′*S*,3′*R*)	2.99 (1′*R*,3′*R*)	1.26	1.05[a]	[−]	[+]
	B	6.28 (1′*S*,3′*R*)	7.58 (1′*R*,3′*R*)	1.21	1.04	[−]	[+]
	C	3.88 (1′*S*,3′*R*)	4.43 (1′*R*,3′*R*)	1.14	1.10	[−]	[+]
3a-(3′*S*)	A	2.17 (1′*S*,3′*S*)	2.47 (1′*R*,3′*S*)	1.09	>0.5[a]	[−]	[+]
	B	5.54 (1′*S*,3′*S*)	6.67 (1′*R*,3′*S*)	1.20	1.25	[−]	[+]
	C	3.27 (1′*S*,3′*S*)	3.88 (1′*R*,3′*S*)	1.19	1.30	[−]	[+]
3b-(3′*R*)	A	2.98 (1′*R*,3′*R*)	4.09 (1′*S*,3′*R*)	1.37	3.34	[−]	[+]
	B	7.36 (1′*R*,3′*R*)	10.15 (1′*S*,3′*R*)	1.38	3.92	[−]	[+]
	C	4.51 (1′*R*,3′*R*)	6.23 (1′*S*,3′*R*I	1.38	3.18	[−]	[+]
3b-(3′*S*)	A	3.43	–	1.00	n.r.	–	–
	B	8.35	–	1.00	n.r.	–	–
	C	5.40 (1′*R*,3′*S*)	5.97 (1′*S*,3′*S*)	1.11	1.15	[−]	[+]
3c-(3′*R*)	A	2.74 (1′*R*,3′*R*)	3.19 (1′*S*,3′*R*)	1.16	1.50	[+]	[−]
	B	7.00 (1′*R*,3′*R*)	7.74 (1′*S*,3′*R*)	1.10	1.12	[+]	[−]
	C	4.35	–	1.00	n.r.	–	–
3c-(3′*S*)	A	2.64(1′*S*,3′*S*)	3.15(1′*R*,3′*S*)	1.19	1.68	[−]	[+]
	B	6.79 (1′*S*,3′*S*)	7.84 (1′*R*,3′*S*)	1.15	1.56	[−]	[+]
	C	4.54 (1′*S*,3′*S*)	5.11 (1′*R*,3′*S*)	1.12	1.03	[−]	[+]
3d-(3′*R*)	A	4.06 (1 ′*S*,3′*R*)	4.92 (1′*R*,3′*R*~	1.21	1.93	[−]	[+]
	B	10.08 (1′*S*,3′*R*)	12.82 (1′*R*,3′*R*)	1.27	2.87	[−]	[+]
	C	5.06 (1′*S*,3′*R*)	7.50 (1′*R*,3′*R*)	1.48	3.60	[−]	[+]
3d-(3′*S*)	A	4.20 (1′*S*,3′*S*)	5.36(1′*R*,3′*S*)	1.27	2.51	[−]	[+]
	B	11.16(1′*S*,3′*S*)	13.78(1′*R*,3′*S*)	1.23	2.38	[−]	[+]
	C	6.52 (1′*S*,3′*S*)	8.49 (1′*R*,3′*S*)	1.30	2.42	[−]	[+]

n.r. unresolved; [a] overlapping; [b] polarimetric detection (Hg lamp); Eluents A: *n*-hexane/ethanol: 80/20; B: *n*-hexane/ethanol: 90/10; C: *n*-hexane/2-propanol: 80/20.

phenyl group of the stationary phase. This suggests also that the inclusion site should change geometry and/or change of size, which are caused by the kind of alcoholic modifier [22–24].

On Chiralpak AS, α and R_s parameters increase with changing from ethanol to 2-propanol for (3′*S*) compounds. On the other hand, for the (3′*R*) compounds, α and R_s parameters decrease. On Chiralpak AD, α and R_s parameters decrease also with changing from ethanol to 2-propanol. These phenomenon

Table 2
HPLC resolution: retention factors (k') separation factor (α) and resolution (R_s) of **3** diastereomers and absolute configuration; Chiralcel OJ

Compound	Eluent	k'_1	k'_2	α	R_s	1st	2nd Eluted isomer[b]
3a-(3′*R*)	A	7.94 (1′*S*,3′*R*)	9.64 (1′*R*,3′*R*)	1.21	2.04		
	B	20.18 (1′*S*,3′*R*)	24.64 (1′*R*,3′*R*)	1.22	2.50	[−]	[+]
3a-(3′*S*)	A	6.91 (1′*R*,3′*S*)	14.37 (1′*S*,3′*S*)	2.08	7.81		
	B	17.52 (1′*R*,3′*S*)	32.58 (1′*S*,3′*S*)	1.86	9.70	[−]	[+]
3b-(3′*R*)	A	13.94 (1′*R*,3′*R*)	14.83 (1′*S*,3′*R*)	1.06	0.7[a]	[−]	[+]
3b-(3′*S*)	A	10.75 (1′*S*,3′*S*)	12.55 (1′*R*,3′*S*)	1.17	1.84	[−]	[+]
3c-(3′*R*)	A	13.58 (1′*S*,3′*R*)	22.73 (1′*R*,3′*R*)	1.67	5.49	[−]	[+]
3c-(3′*S*)	A	12.54 (1′*S*,3′*S*)	22.16 (1′*R*,3′*S*)	1.76	6.12	[−]	[+]
3d-(3′*R*)	A	18.75 (1′*R*,3′*R*)	27.43 (1′*S*,3′*R*)	1.46	4.51	[+]	[−]
3d-(3′*S*)	A	14.48 (1′*S*,3′*S*)	23.36 (1′*R*,3′*S*)	1.61	5.54	[−]	[+]

n.r. unresolved; [a] overlapping; [b] polarimetric detection (Hg lamp); Eluents A: *n*-hexane/ethanol: 80120; B: *n*-hexane/ethanol: 90/10.

Table 3
HPLC resolution: retention factors (k') separation factor (α) and resolution (R_s) of **3** diastereomers and absolute configuration: Chiralpak AS

Compound	Eluent	k'_1	k'_2	α	R_s	1st	2nd Eluted isomer[b]
3a-(3′*R*)	A	1.84 (1′*R*,3′*R*)	2.21 (1′*S*,3′*R*)	1.20	1.85	[+]	[−]
	B	4.31 (1′*R*,3′*R*)	7.01 (1′*S*,3′*R*)	1.63	6.08		
	D	7.43 (1′*R*,3′*R*)	10.13 (1′*S*,3′*R*)	1.36	3.44		
3a-(3′*S*)	A	1.42 (1′*S*,3′*S*)	2.97 (1′*R*,3′*S*)	1.34	7.10	[−]	[+]
	B	3.79 (1′*S*,3′*S*)	5.24 (1′*R*,3′*S*)	1.38	3.85		
	D	5.92 (1′*S*,3′*S*)	15.71 (1′*R*,3′*S*)	2.65	8.83		
3b-(3′*R*)	A	3.43 (1′*R*,3′*R*)	8.47 (1′*S*,3′*R*)	2.47	10.02	[+]	[−]
	B	9.11 (1′*R*,3′*R*)	21.57 (1′*S*,3′*R*)	2.36	11.18		
	C	6.14 (1′*R*,3′*R*)	14.27 (1′*S*,3′*R*)	2.32	9.08		
3b-(3′*S*)	A	3.35 (1′*R*,3′*S*)	5.93 (1′*S*,3′*S*)	1.77	6.54	[−]	[+]
	B	9.02 (1′*R*,3′*S*)	16.00 (1′*S*,3′*S*)	1.77	7.92		
	C	5.47 (1′*R*,3′*S*)	12.29 (1′*S*,3′*S*)	2.25	8.80		
3c-(3′*R*)	A	3.91 (1′*S*,3′*R*)	11.53 (1′*R*,3′*R*)	2.95	9.73	[−]	[+]
	B	10.02 (1′*S*,3′*R*)	31.38 (1′*R*,3′*R*)	3.13	11.87	[−]	[+]
	C	6.33 (1′*S*,3′*R*)	17.38 (1′*R*,3′*R*)	2.75	10.31	[−]	[+]
3c-(3′*S*)	A	2.92 (1′*S*,3′*S*)	4.58 (1′*R*,3′*S*)	1.56	4.97	[−]	[+]
	B	7.63 (1′*S*,3′*S*)	11.43 (1′*R*,3′*S*)	1.50	5.27	[−]	[+]
	C	5.42 (1′*S*,3′*S*)	8.65 (1′*R*,3′*S*)	1.60	4.87	[−]	[+]
3d-(3′*R*)	A	5.40 (1′*S*,3′*R*)	7.05 (1′*R*,3′*R*)	1.30	2.92	[−]	[+]
	B	16.45 (1′*S*,3′*R*)	21.89 (1′*R*,3′*R*)	1.33	3.76	[−]	[+]
	C	12.59 (1′*S*,3′*R*)	13.66 (1′*R*,3′*R*)	1.08	<0.5[a]		
3d-(3′*S*)	A	5.31	–	1	n.r.		
	B	15.09 (1′*R*,3′*S*)	16.22 (1′*S*,3′*S*)	1.13	<0.5[a]	[+]	[−]
	C	12.40	–	1	n.r.		

n.r. unresolved; [a] overlapping; [b] polarimetric detection (Hg lamp); Eluents A: *n*-hexane/ethanol: 80/20; B: *n*-hexane/ethanol: 90/10; C: *n*-hexane/2-propanol: 80/20; D: hexane/2-propanol: 90/10.

Table 4
HPLC resolution: retention factors (k') separation factor (α) and resolution (R_s) of **3** diastereomers and absolute configuration; Chiralpak AD

Compound	Eluent	k'_1	k'_2	α	R_s	1st	2nd Eluted isomer[b]
3a-(3′*R*)	A	4.56 (1′*S*,3′*R*)	15.33 (1′*R*,3′*R*)	3.36	14.57	[−]	[+]
	B	10.34 (1′*S*,3′*R*)	33.32 (1′*R*,3′*R*)	3.22	17.40		
	C	11.92 (1′*S*,3′*R*)	22.35 (1′*R*,3′*R*)	1.87	11.74		
3a-(3′*S*)	A	4.89 (1′*R*,3′*S*)	9.25 (1′*S*,3′*S*)	1.89	8.35	[−]	[+]
	B	11.21 (1′*R*,3′*S*)	19.20 (1′*S*,3′*S*)	1.71	6.04		
	C	15.18 (1′*R*,3′*S*)	17.58 (1′*S*,3′*S*)	1.16	2.71		
3b-(3′*R*)	A	7.56 (1′*R*,3′*R*)	18.55 (1′*S*,3′*R*)	2.45	14.63	[+]	[−]
3b-(3′*S*)	A	5.88 (1′*R*,3′*R*)	12.71 (1′*R*,3′*S*)	2.16	13.89	[−]	[+]
3c-(3′*R*)	A	11.98 (1′*S*,3′*R*)	14.81 (1′*R*,3′*R*)	1.23	3.29	[−]	[+]
3c-(3′*S*)	A	10.07 (1′*S*,3′*R*)	29.04 (1′*S*,3′*S*)	2.62	15.72	[+]	[−]
3d-(3′*R*)	A	>64					
3d-(3′*S*)	A	>64					

n.r. unresolved; Concentration ca 0.75 m*M*; [a] overlapping; [b] polarimetric detection (Hg lamp); Eluents A: *n*-hexane/ethanol: 80/20; B: *n*-hexane/ethanol: 90/10; C: *n*-hexane/2-propanol: 80/20.

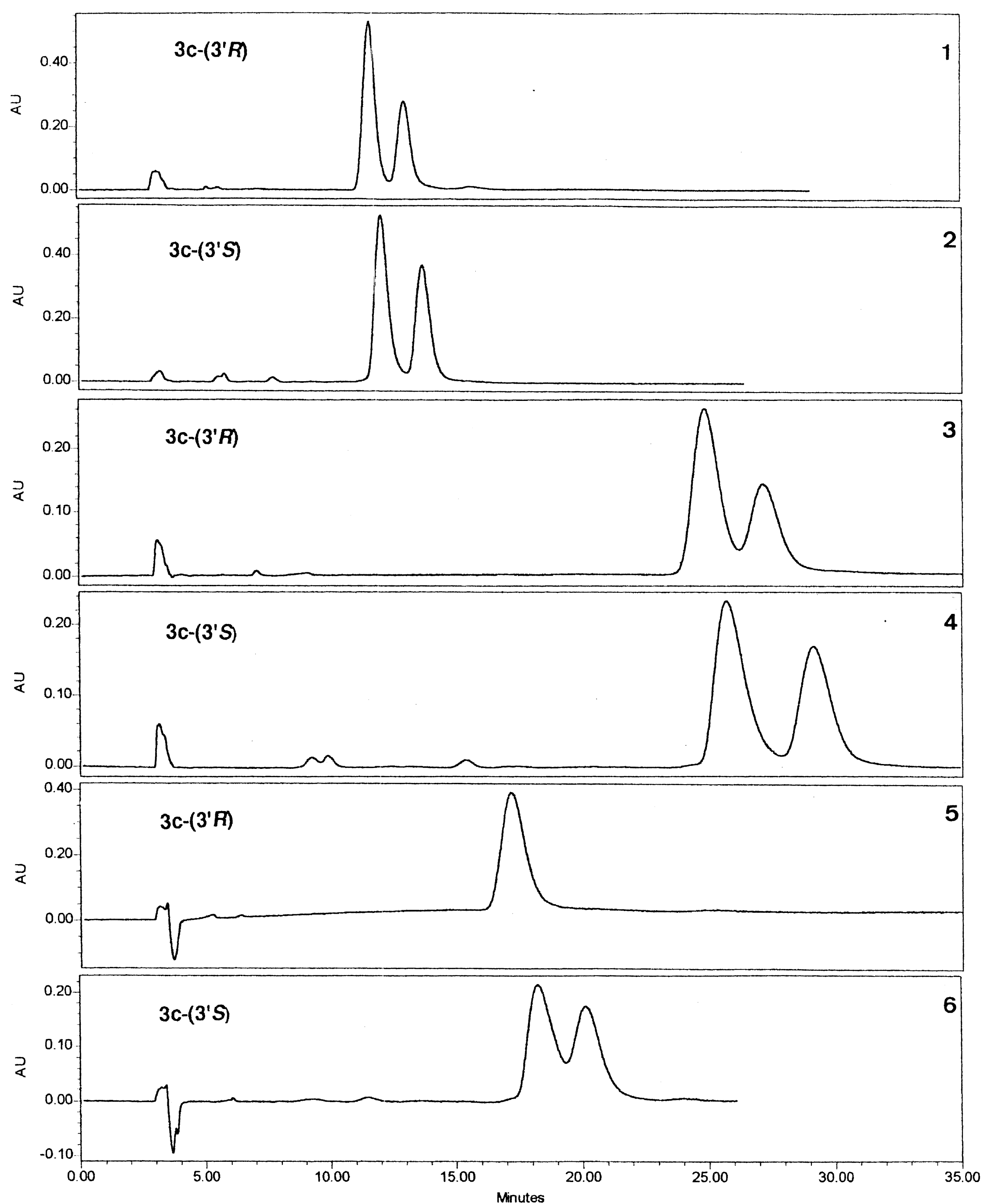

Fig. 3. Overlay plot for the separations of **3c**-(3′*R*) and **3c**-(3′*S*) on Chiralcel OD-H at λ=210 nm with 20% ethanol (Eluent A) (curves 1,2); 10% ethanol (Eluent B) (curves 3,4); 20% 2-propanol (Eluent C) (curves 5,6).

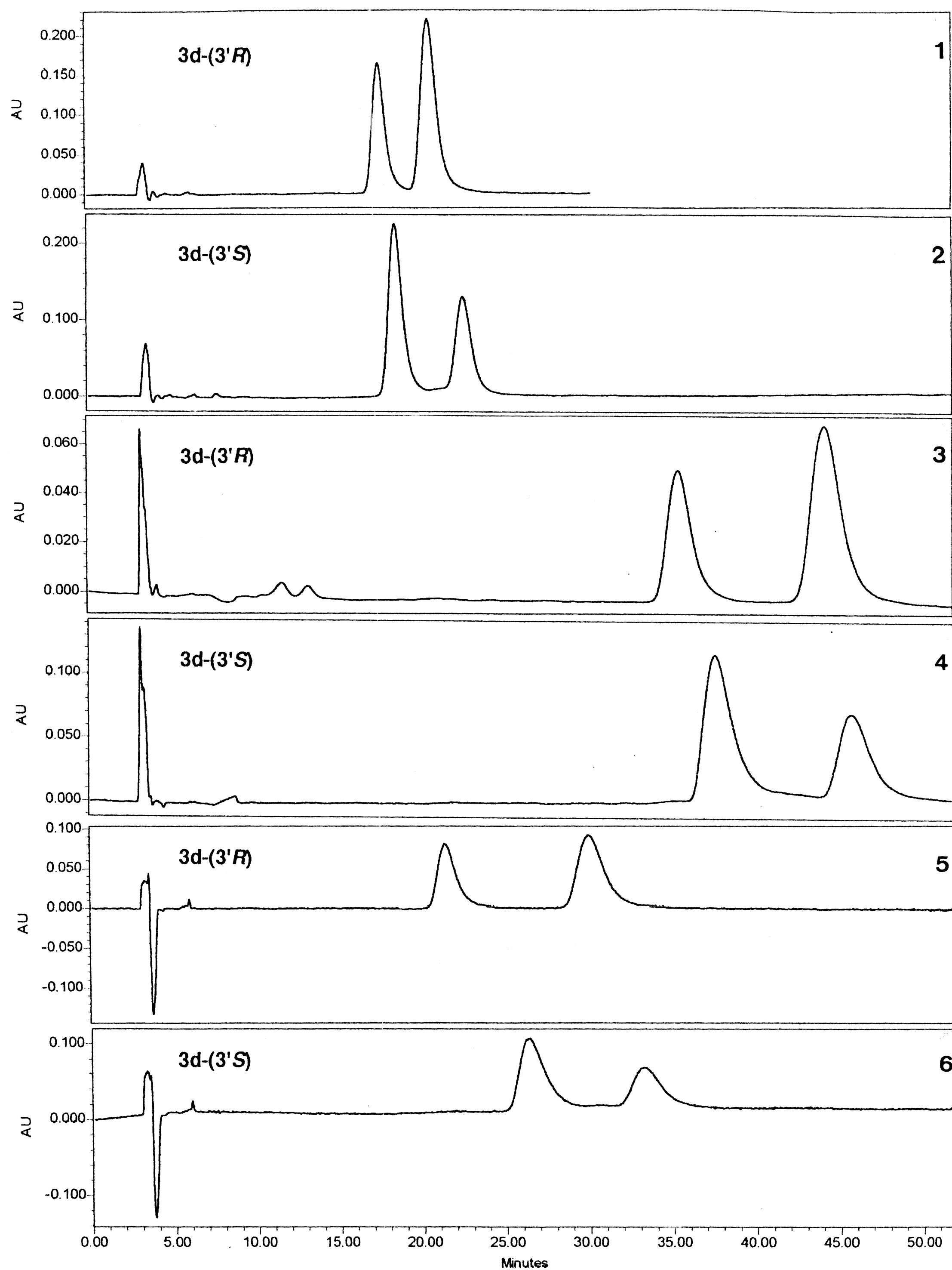

Fig. 4. Overlay plot for the separations of **3d**-(3′*R*) and **3d**-(3′*S*) on Chiralcel OD-H at λ=210 nm with 20% ethanol (Eluent A) (curves 1,2); 10% ethanol (Eluent B) (curves 3,4); 20% 2-propanol (Eluent C) (curves 5,6).

confirms that for this family of (3′*R*) compounds, hydrogen bonding interactions are not the predominant one.

In terms of resolution (R_s), the separation is quite different between the two types of stationary phases (Chiralcel OD-H and Chiralpak AD). On Chiralpak AD, best resolutions are obtained with et 2-propanol gives excellent results. As the derivatization of both cellulose and amylose is the same, the difference in molecules recognition of cellulose and amylose derivatives must be due to the different configuration of the glucose residue and higher order structure of the stationary phases.

3.2. Percentage of alcohol modifier

It can be seen that polar modifier concentration decrease (ethanol: eluents A, B) in the mobile phase, leads to a large increase of the retention factors k'. This behaviour is observed for all compounds on OD-H and AS CSPs and for compound **3a** on OJ and AD CSPs (Tables 1–4).

3.3. Steric hindrance substitution by uracil or thymine group

With solvent A, on Chiralcel OD-H we observe that compounds substituted by a uracil group are more retained and better resolved than compounds substituted by a thymine group, but not compounds **3a**-(3′*S*) and **3b**-(3′*S*). The thymine may present a steric hindrance too important to enter completely into the chiral cavity of the cellulose (tris-3,5-dimethylphenylcarbamate) column (Table 1) and (curves 1,2 Fig. 3; curves 1,2 Fig. 4). With solvent A, on Chiralcel OJ, on Chiralpak AS, and on Chiralpak AD, we observe that uracil-substituted compounds are more retained (but not compounds **3a**-(3′*S*) and **3b**-(3′*S*) on OJ, and compounds **3c**-(3′*R*) and **3d**-(3′*R*) on AS), while thymine-substituted compounds are better resolved (but not compounds **3a**-(3′*R*) and **3b**-(3′*R*) on AS, and compounds **3a**-(3′*S*) and **3b**-(3′*S*) on AD) (Tables 2,3 and 4).

3.4. Substitution by benzyl or benzoyl group

With solvent A, on columns OD-H and OJ, the benzoyl compounds are all more retained and in a general manner better resolved (but not compound **3b**-(3′*R*) (benzyl) better resolved than **3d**-(3′*R*) (benzoyl) on Chiralcel OD-H and, **3a**-(3′*S*) (benzyl) better resolved than **3c**-(3′*S*) (benzoyl) on Chiralcel OJ). The benzoyl group may involve more H bondings than the benzyl group (Tables 1 and 2). Concerning columns Chiralpak AS and AD, benzoyl compounds are more retained than benzyl compounds, (Tables 3 and 4).

3.5. Elution order

The elution order was determined by polarimetric detection. We observed that couple *cis* show a weaker [α] signal than couple *trans*. This observation has been confirmed and completed with the determination of specific polarimetric rotations of each pure isomer (see Conclusion) and was helpful for the determination of the *cis*/*trans* configuration. On cellulose type column (OD-H and OJ) the elution order was (−)/(+) for the four benzyl compounds (**3a**-(3′*R*); **3a**-(3′*S*); **3b**-(3′*R*); **3b**-(3′*S*)). There is no inversion of elution by changing configuration from (3′*R*) to (3′*S*). For the benzoyl compounds **3c**-(3′*R*); **3d**-(3′*R*) the elution order was (+)/(−) and becomes (−)/(+) by changing the configuration from (3′*R*) to (3′*S*) respectively. On this type of CSP the type of alcohol doesn't influence the elution order. The same elution order is observed between the two columns but not compound **3d**-(3′*S*).

On the amylose-type columns (AS and AD) with solvent A, there is inversion of the elution order, for all compounds **(3a–3d)** by changing the configuration from (3′*R*) (+)/(−) to (3′*S*) (−)/(+).

3.6. Preparative separation

The good resolution factor obtained for **3d** on Chiralcel OD-H (eluent C) and the high loadability of this CSP afforded a preliminary preparative separation of its isomers by repeated 50-μl injections containing 150 μg of the racemic mixture on an analytical column. The collection of the eluates from the chromatographic peaks afforded milligram amounts of compounds sufficient to determine the *cis*/*trans* configuration of each component by ^{1}H NMR spectroscopy [3,5] and to measure specific polarimetric rotations (c=1.0%, $CHCl_3$): **3d**(3′*R*)

$[\alpha]_D^{20}=+26$ and $+77$ for the first (**3d**, (1′*S*, 3′*R*), *cis* form) and second (**3d**, (1′*R*, 3′*R*) *trans* form) isomers; **3d** (3′*S*) $[\alpha]_D^{20}=-77$ and -25 for the first (**3d**, (1′*S*, 3′*S*), *trans*-form) and second (**3d**, (1′*R*, 3′*S*), *cis*-form) isomers. The signs and the relative values of those specific polarimetric rotations are in acordance with values observed in polarimetric detection.

4. Conclusion

The resolution results of compounds described above indicated that the CSPs perform in a complementary fashion. The Chiralcel OD-H and Chiralpak AS phase provided more baseline separations. The good separation of diastereomers of **3** (i) makes this chromatographic method suitable for quantifying enantiomeric purity and for studies in pharmacological distribution and (ii) opens the way to the rapid preparative HPLC isolation of individual isomers. Further work is now under investigation to develop the preparative chromatography on higher quantities and complete biochemical studies.

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