ELSEVIER

Journal of Chromatography A, 972 (2002) 211–219

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of the enantiomeric purity of nucleoside analogs related to d4T and acyclovir, new potential antiviral agents, using liquid chromatography on cellulose chiral stationary phases

Emmanuelle Lipka-Belloli[a], Virginie Glaçon[c], Grahame Mackenzie[c], David Ewing[c], Christophe Len[b], Claude Vaccher[a,*], Jean-Paul Bonte[a]

[a]*Laboratoire de Chimie Analytique, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Lille 2, B.P. 83, 3 Rue du Pr. Laguesse, 59006 Lille Cedex, France*

[b]*Laboratoire des Glucides, Université de Picardie, 80039 Amiens, France*

[c]*Centre for Organic and Biological Chemistry, University of Hull, Hull HU6 7RX, UK*

Received 25 March 2002; received in revised form 9 July 2002; accepted 9 July 2002

Abstract

We reported a method of determination of enantiomeric purity of the new potential antiviral agents by direct analytical HPLC. Those agents are nucleoside analogs, having one chiral center. They are synthesized as a single enantiomer (*R* or *S*) by an asymmetric pathway. The chiral stationary phases chosen are silica-based cellulose tris-3,5-dimethylphenylcarbamate (Chiralcel OD-H), or tris-methylbenzoate (Chiralcel OJ). Resolution was achieved using normal-phase chromatography with a mobile phase consisting of *n*-hexane–alcohol (ethanol or 2-propanol) in various percentages. Furthermore the effects of structural features on retention, selectivity and resolution, as well as on the elution order were thoroughly studied. Differences in the lipophilicity of the compounds were also examined.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Lipophilicity; Nucleosides

1. Introduction

Since the beginning of acquired immunodeficiency syndrome (AIDS) 15 years ago, there has been continued interest in the synthesis of new compounds. Today, a number of 2′,3′-dideoxynucleosides, such as AZT (3′-azido-3′-deoxythymidine—Zidovudine, Retrovir), ddI (2′,3′-dideoxyinosine—Didanosine, Videx) and ddC (2′,3′-dideoxycytidine) or 2′,3′-didehydro-2′,3′-dideoxynucleosides (d4Ns), corresponding to the introduction of a double bond at the 2′,3′ position, such as d4G (2′,3′-didehydro-2′,3′-dideoxyguanosine—Carbovir), d4C (2′,3′-didehydro-2′,3′-dideoxycytosine), d4T (2′,3′-didehydro-2′,3′-dideoxythymidine—Stavudine, Zerit) (Fig. 1a), possessing β-D configuration, have been approved by the US Food and Drug Administration in human immunodeficiency virus (HIV) therapy [1–4]. Several nucleosides with the unnatural β-L configuration have emerged as antiviral agents against HIV including 3TC [β-L-(−)-2′-deoxy-3′-thiacytidine—Lamivudine, Epivir), FTC, L-

*Corresponding author. Tel.: +33-3-2096-4701; fax: +33-3-2095-9009.

E-mail address: cvaccher@phare.univ-lille2.fr (C. Vaccher).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01036-1

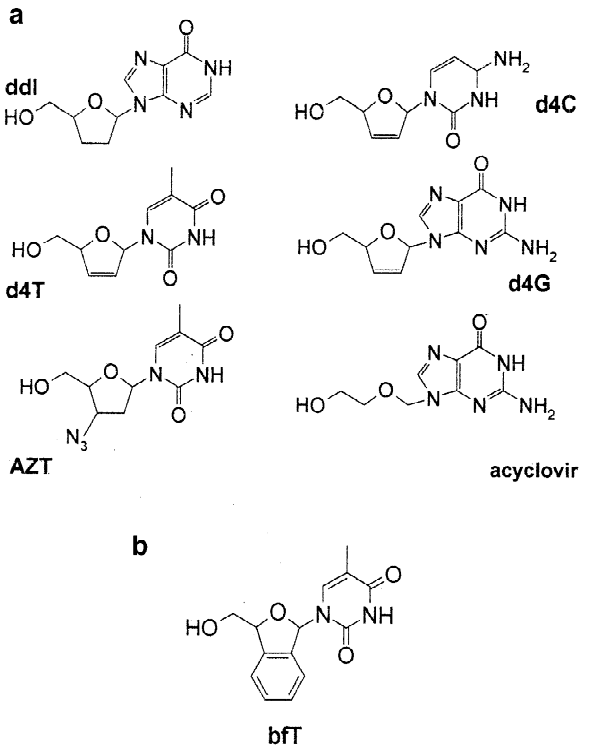

Fig. 1. (a) Formulae of anti-HIV and anti-HSV molecules. (b) Formulae of potential anti-HIV molecule.

FMAU [5]. Lastly, an acyclic nucleoside, such as acyclovir (Fig. 1a) was used in the efficient treatment of herpes simplex virus (HSV) infection [6,7].

In a previous paper [8] we described the diastereoisomeric resolution of nucleoside analogs of d4T based on a benzo[*c*]furan core, e.g., precursors of compounds such as bfT (Fig. 1b). In the continuity of our work and in order to study the structure–activity relationship and to obtain compounds with a higher therapeutic index, five new acyclic nucleoside analogs **1**–**5**, related to d4T and acyclovir have been developed (Fig. 2): 1-{[(2-*O*-benzoyl-1-phenyl)-ethoxy]methyl}thymine, compound **1**; 1-{[(2-*O*-benzoyl-1-phenyl)ethoxy]methyl}uracil, compound **2**; 1-{[(2-*O*-benzoyl-1-phenyl)ethoxy]methyl}cytosine, compound **3**; 1-{[(2-*O*-benzoyl-1-phenyl)ethoxy]-methyl}adenine, compound **4**; 1-{[(2-*O*-benzoyl-1-phenyl)ethoxy]methyl}guanine, compound **5**. All chiral compounds are produced as single enantiomers in the *R* or *S* configuration, in a high yield, by an asymmetric pathway (ADmix α or ADmix β inducers) [9]. Those compounds can be viewed as *seco*-benzo[*c*]furan species. The acyclic nucleoside analogs with one asymmetric carbon atom differ in the biological activity of both (+) and (−) enantiomers. Only the *cis*-(−) enantiomer of Carbovir has antivir-

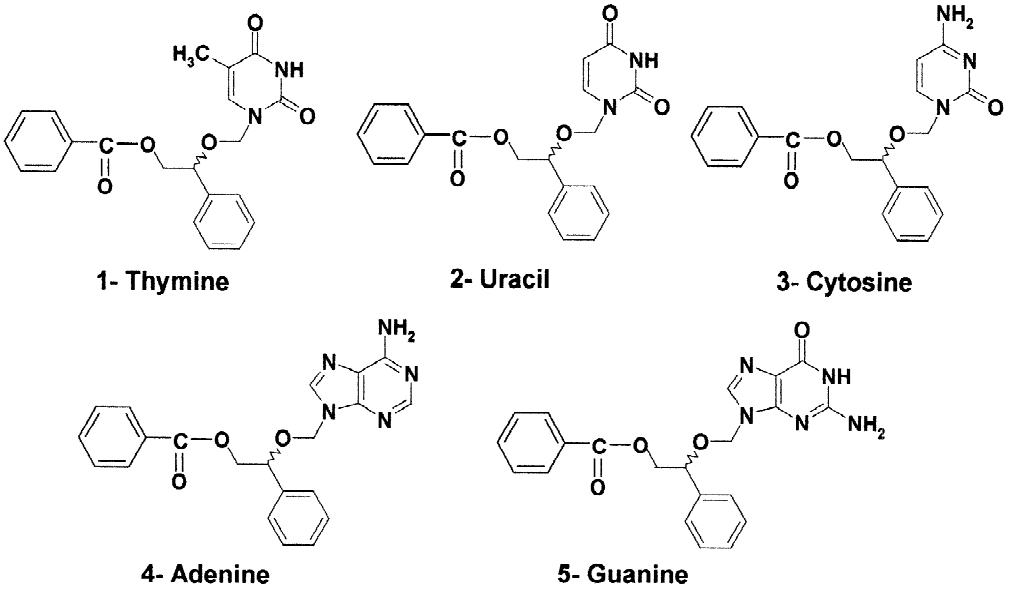

Fig. 2. Formulae of compounds **1**–**5**.

al activity [10]. For this reason each isomer needs to be obtained enantiomerically pure by synthesis or chromatography separation. To the best of our knowledge no work using liquid chromatography for the direct enantiomeric separation of such nucleoside analogs has been published. We develop a method to check the enantiomeric purity and to control any chiral inversion in the substances. Two chiral stationary phases (CSPs) were tested for the high-performance liquid chromatographic (HPLC) separation of the five couples of enantiomers: Chiralcel OD-H and Chiralcel OJ. Silica-based cellulose tris-3,5-dimethylphenylcarbamate (OD-H) and tris-methylbenzoate (OJ) show a particularly high chiral recognition for a variety of racemic compounds and with a wide range of mobile phases. Moreover they exhibit quite excellent enantioselectivity for a variety of racemates [11]. The large usefulness and broad applicability of these polysaccharide bonded stationary phases are related in numerous recent review articles [11–16]. The CSPs were tested with various mobile phases consisting of *n*-hexane and alcohol modifier, to establish the enantiomeric purity of each isomer.

2. Experimental

2.1. Instrumentation

Chromatography was performed on a Waters 600 HPLC system equipped with a Waters 996 photodiode array spectrophotometer. The sample loop was 20 μl (Rheodyne 7125 injector). Chromatographic data were collected and processed on a Digital computer running Millennium 2010. The stainless-steel columns Chiralcel OD-H (cellulose tris-3,5-dimethylphenylcarbamate; 250×4.6 mm I.D.; 5 μm) and Chiralcel OJ (cellulose tris-methylbenzoate; 250×4.6 mm I.D.; 10 μm) were purchased from Daicel Chemical Industries.

2.2. Reagents

The syntheses of the compounds **1**–**5** were described previously [9]. 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 (70:30); B: *n*-hexane–ethanol (80:20); C: *n*-hexane–2-propanol (70:30); D: *n*-hexane–2-propanol (80:20). Compounds were chromatographed by dissolving them in methanol to a concentration of about 0.75 m*M* (which corresponds to 15 nmol injected) and passed through a 0.45 μm membrane filter prior to loading the column.

2.3. Chromatographic conditions and calculations

The column eluate was monitored at 200, 230 and 245 nm. Mobile phase elution was made isocratically using *n*-hexane and a modifier (ethanol or 2-propanol) at various percentages (70:30 or 80:20). The flow-rate was 0.8 ml min^{-1}. Retention times were mean values of two replicate determinations. All separations were carried out at 30 °C. The peak of the solvent front was considered to be equal to the dead time (t_0=4.1 min) and was taken from each particular run. The retention factor *k*, was determined as $k=(t_R-t_0)/t_0$. The resolution was calculated as $R_s=\{[1.18\cdot(t_{R2}-t_{R1})]/(\delta_1+\delta_2)\}$, where t_{Ri} and δ_i are the retention times and the half-widths of each enantiomer, respectively. R_s and α were determined from the two chromatograms of the two pure enantiomers.

3. Results and discussion

3.1. Mobile phase effects on retention and stereoselectivity

The results are summarized in Tables 1 and 2. Fig. 3a and b are representative examples of the separations achieved, respectively, on Chiralcel OD-H (eluent A) and Chiralcel OJ (eluent A).

3.1.1. Structure of mobile phase modifier

Two kinds of mobile phase were investigated by changing the modifier from ethanol (eluents A, B) to 2-propanol (eluents C, D). For the five compounds, both on Chiralcel OD-H and OJ, we observed a decrease in the retention *k* by passing from 2-propanol to ethanol (respectively, from eluent C to A and from eluent D to B). Since the polarity of

Table 1
HPLC resolution on Chiralcel OD-H (λ=200 nm): retention factors (k) separation factor (α) and resolution (R_s) of enantiomers and absolute configuration

Compound	Eluent	k_1	k_2	α [a]	R_s [a]	First eluted isomer [b]
1	A	1.23 (*R*)	1.40 (*S*)	1.26	1.14	[−]
	B	2.00 (*R*)	2.27 (*S*)	1.14	1.35	[−]
	C	1.69 (*R*)	1.98 (*S*)	1.17	1.51	[−]
	D	3.07 (*R*)	3.19 (*S*)	1.04	1.45	[−]
2	A	1.66 (*S*)	2.38 (*R*)	1.43	2.90	[+]
	B	3.23 (*S*)	4.46 (*R*)	1.38	2.94	[+]
	C	3.44 (*S*)	4.50 (*R*)	1.31	1.64	[+]
	D	6.99 (*S*)	9.88 (*R*)	1.41	2.75	[+]
3	A	1.00 (*R*)	1.09 (*S*)	1.09	<0.5	[−]
	B	1.59 (*R*)	1.75 (*S*)	1.10	1.00	[−]
	C	1.38 (*R*)	1.47 (*S*)	1.06	1.19	[−]
	D	2.41 (*R*)	2.76 (*S*)	0.87	0.72	[−]
4	A	0.69 (*S*)	0.72 (*R*)	1.04	<0.5	[+]
	B	1.42 (*S*)	1.43 (*R*)	1.01	<0.5	−
	C	1.36 (*S*)	1.45 (*R*)	1.07	<0.5	[+]
	D	2.91 (*S*)	3.46 (*R*)	1.19	1.34	[+]
5	A	1.90 (*R*)	1.97 (*S*)	1.04	<0.5	[−]
	B	3.15 (*R*)	3.26 (*S*)	1.03	<0.5	[−]
	C	2.65 (*R*)	3.23 (*S*)	1.22	2.18	[−]
	D	5.23 (*R*)	5.75 (*S*)	1.10	1.50	[−]

n.r., Unresolved.
Eluents: A=*n*-hexane–ethanol (70:30); B=*n*-hexane–ethanol (80:20); C=*n*-hexane–2-propanol (70/30); D=*n*-hexane–2-propanol (80:20).
[a] α and R_s determined from the two chromatograms of the pure enantiomers.
[b] Perkin-Elmer 241 polarimeter (Sodium D-line: 689 nm).

ethanol (P' value, 4.3) is larger than that of 2-propanol (P' value, 3.9) [12], it is expected that the k values obtained using ethanol as modifier, would be smaller than values obtained with 2-propanol. On Chiralcel OD-H, except for compound **2**, the resolution R_s is higher with 2-propanol than with ethanol. The use of ethanol as modifier appeared to be better suited for all the compounds using Chiralcel OJ, except for compounds **4** and **5** where better resolution is observed using 2-propanol.

3.1.2. Concentration of mobile phase modifier

The effect of concentration of alcohol modifier (from 20 to 30%) on retention k and resolution R_s was investigated using ethanol and 2-propanol. It can be seen that an increase of the polar modifier concentration in the mobile phase, from eluent B to A and from eluent D to C, leads to a decrease in retention k, for all the five compounds, both on Chiralcel OD-H and OJ. Passing from 30 to 20% of ethanol (eluent A to B) resolution R_s increases or remains slightly constant on the two stationary phases. With changing percentage of 2-propanol from 30 to 20% (eluent C to D) the same behavior is observed on Chiralcel OJ and on Chiralcel OD-H, but only for compounds **2** and **4**.

On the other hand, on Chiralcel OD-H, for compounds **1**, **3** and **5**, we observed an unusual effect: decreasing the concentration of alcohol modifier, leads to a decrease of resolution too.

3.2. Effects of the structure of polysaccharide derivatives on retention and stereoselectivity

Nowadays it is well known, that the chiral recognition process results, firstly, of several interactions of different magnitude involving hydrophobic interaction [13], hydrogen interaction, dipole–dipole in-

Table 2
HPLC resolution on Chiralcel OJ (λ=200 nm): retention factors (k) separation factor (α) and resolution (R_s) of enantiomers and absolute configuration

Compound	Eluent	k_1	k_2	α[a]	R_s[a]	First eluted isomer[b]
1	A	4.28 (*R*)	7.28 (*S*)	1.70	4.94	[−]
	B	7.26 (*R*)	12.29 (*S*)	1.69	5.45	[−]
	C	5.65 (*R*)	6.05 (*S*)	1.07	0.56	[−]
	D	10.94 (*R*)	11.14 (*S*)	1.02	<0.5	[−]
2	A	2.48 (*R*)	5.77 (*S*)	2.33	4.62	[−]
	B	5.05 (*R*)	11.52 (*S*)	2.29	5.50	[−]
	C	3.67 (*R*)	7.02 (*S*)	1.91	2.15	[−]
	D	9.00 (*R*)	17.30 (*S*)	3.95	3.10	[−]
3	A	0.99 (*R*)	1.09 (*S*)	1.10	0.74	[−]
	B	4.95 (*R*)	7.41 (*S*)	1.50	4.40	[−]
	C	2.78 (*R*)	3.47 (*S*)	1.25	1.67	[−]
	D	5.72 (*R*)	6.56 (*S*)	1.15	1.64	[−]
4	A	0.96 (*S*)	1.10 (*R*)	1.14	0.91	[+]
	B	2.13 (*R*)	2.42 (*S*)	1.14	0.80	[−]
	C	2.00 (*R*)	3.12 (*S*)	1.56	2.27	[−]
	D	4.38 (*R*)	7.80 (*S*)	1.78	3.28	[−]
5	A	11.94 (*R*)	15.21 (*S*)	1.27	2.55	[−]
	C	15.86 (*S*)	22.87 (*R*)	1.44	3.46	[+]

n.r., Unresolved.
Eluents: A=*n*-hexane–ethanol (70:30); B=*n*-hexane–ethanol (80:20); C=*n*-hexane–2-propanol (70:30); D=*n*-hexane–2-propanol (80:20).
[a] α and R_s determined from the two chromatograms of the pure enantiomers.
[b] Perkin-Elmer 241 polarimeter (Sodium D-line: 689 nm).

teraction between the electronegative atoms of the solute and the –CO–O– and the –NH–CO– of the CSPs, π–π interaction, between the aromatic ring of the solute and the substituted phenyl moiety of the CSP and secondly, by inclusion phenomenon of the solute into the chiral cavity of the CSP [17]. This second contribution to chiral recognition is due to a regular higher order structure of the chiral sites of the CSP: a left handed threefold (3/2) helicoidal chain conformation for modified cellulose [18]. The results of the investigation of the effect of alcoholic modifier, on retention and stereoselectivity on cellulose tris-3,5-dimethylphenylcarbamate (OD-H CSP) show best results with 2-propanol.

This can be explained by two kind of phenomena: (1) as aforementioned, in the polysaccharides phenyl carbamates, the part of N–H and C=O groups are involved in hydrogen bonding and then responsible of the high order structure of these materials, (2) the steric hindrance of the 2-propanol seems to prevent the alcohol modifier to compete with the solute for hydrogen bonding sites [17].

Concerning cellulose tris-methylbenzoate (OJ CSP), best results are obtained with the lower alcohol (ethanol) added to the mobile phase. Partial resolution or total loss of resolution is observed when using 2-propanol. This suggests that hydrogen bonding interactions are probably not predominant on this kind of CSP but separations might involve more π–π interactions between the aromatic ring of the analyte and the substituted phenyl moiety of the stationary phase.

3.3. Effects of the solute structure on retention and stereoselectivity

We examined the structural features and particularly the lipophilicity (log P) (Table 3) [19] of the molecules on the retention (k) and selectivity (α). Our compounds can be classified into two categories:

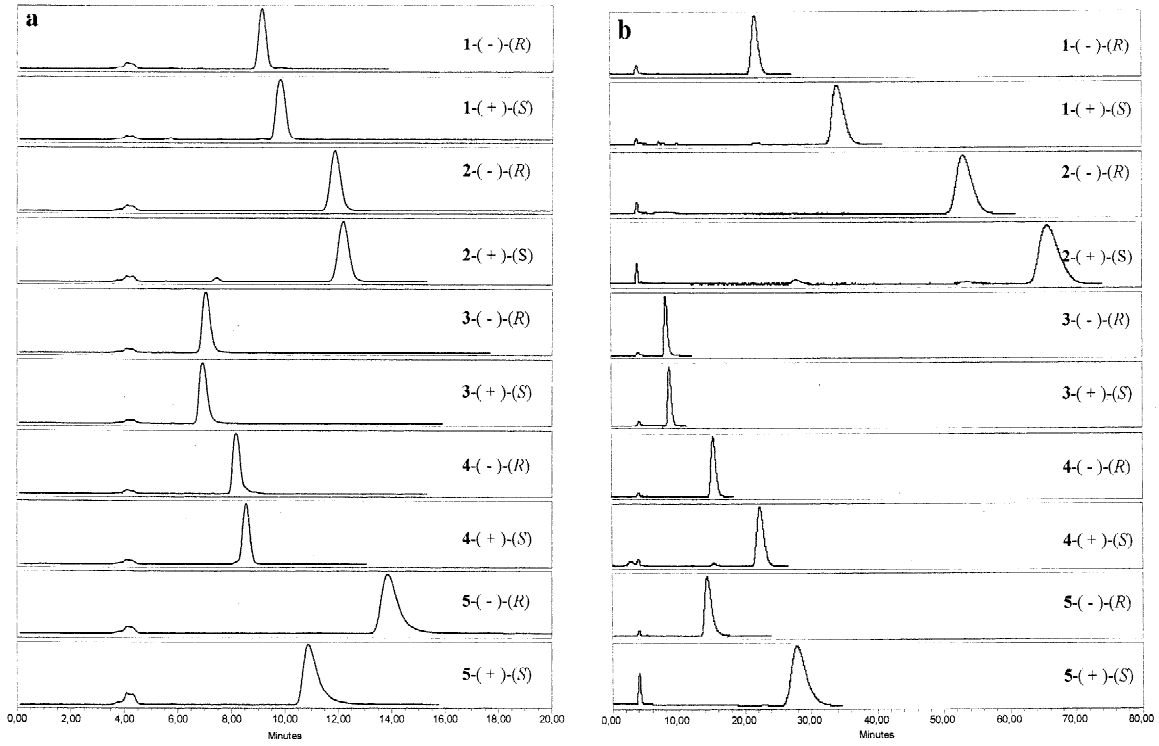

Fig. 3. (a) Chromatograms of compounds **1**-(*R*), **1**-(*S*) to **5**-(*R*), **5**-(*S*), on Chiralcel OD-H stationary phase; eluent A (*n*-hexane–ethanol, 70:30); flow-rate: 0.8 ml min^{-1}; temperature 30 °C; wavelength 200 nm. (b) Chromatograms of compounds **1**-(*R*), **1**-(*S*) to **5**-(*R*), **5**-(*S*), on Chiralcel OJ stationary phase; eluent A (*n*-hexane–ethanol, 70:30); flow-rate: 0.8 ml min^{-1}; temperature 30 °C; wavelength 200 nm.

substituted with a pyrimidine group (compounds **1**, **2** and **3**) and substituted with a purine group (compounds **4** and **5**).

Concerning molecules with a purine group, compound **4** is more lipophilic (i.e., involving more hydrophobic interactions) than compound **5** [20]. Then the retention *k*, selectivity α and resolution R_s of compound **5** are higher than those of compound **4**, both on OD-H (Fig. 3a–b) and OJ. This may be caused by the presence of a supplementary carboxyl group.

Table 3
Log *P* values of the different bases as given in Ref. [19]

Compound	**1**	**2**	**3**	**4**	**5**
Base	Thymine	Uracil	Cytosine	Adenine	Guanine
Log *P*	−1.17	−1.51	−1.77	−0.55	−1.30

Concerning the first series of molecules **1**–**3** (pyrimidine derivatives), we thought that the elution order would be the opposite of the lipophilicity order: a linear correlation has been reported by Roussel et al. [21,22] between lipophilicity and retention factor *k*. Log *k* vs. log *P* and log α vs. log *P* are reported, for the five compounds, on Chiralcel OD-H (Fig. 4a and b, respectively). Firstly, we observed that, for the two enantiomers, *k* decreased when log *P* increased, for all compounds except compound **3** which deviated significantly from the trend as shown in Fig. 4a. Secondly the lower the value of log *P*, the higher is the selectivity α, as shown in Fig. 4b. However compound **3** again showed a deviation from the general trend. This could be due to the bulkiness of the cytosine, which prevents inclusion in the chiral cavity and then the stabilizing interactions of the diastereomeric solute–CSP complex. On Chiralcel OJ, for compounds **1**–**3**

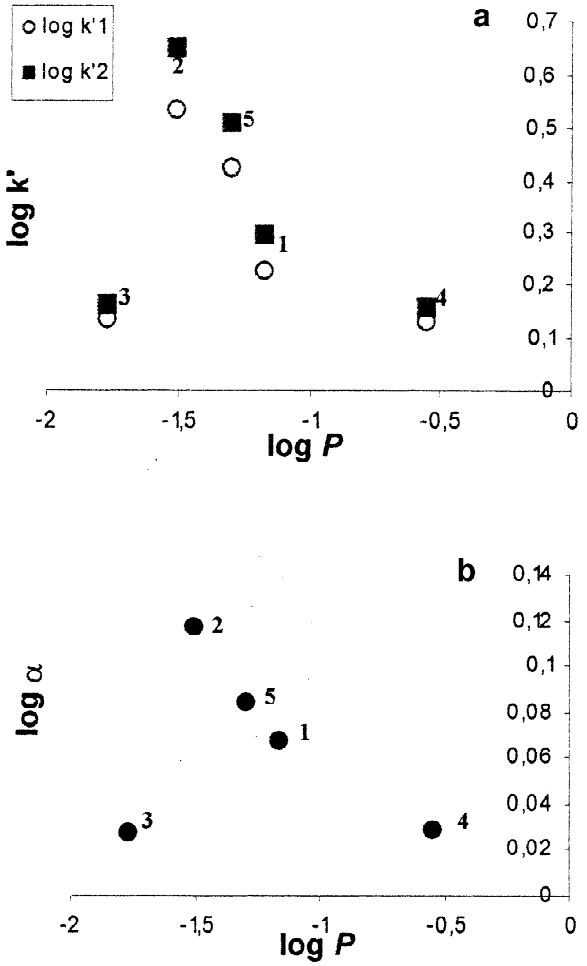

Fig. 4. (a) Log k vs. log P on Chiralcel OD-H stationary phase; eluent C (n-hexane–2-propanol, 70:30); flow-rate: 0.8 ml min^{-1}; temperature 30 °C; wavelength 200 nm. (b) Log α vs. log P on Chiralcel OD-H stationary phase; eluent C (n-hexane–2-propanol, 70:30); flow-rate: 0.8 ml min^{-1}; temperature 30 °C; wavelength 200 nm.

k increases when log P increases. Of course steric hindrance of each solute must also take place [23].

3.4. Enantiomer elution order

On cellulose tris-3,5-dimethylphenylcarbamate (OD-H), the (−)-(R) enantiomer elutes first both with 2-propanol and ethanol as alcohol modifier, for compounds **1**, **3** and **5**. On the other hand the (+)-(S) enantiomer elutes first for compounds **2** and **4** (Table 1). The elution order does not seem correlated with the nature of the base (pyrimidine or purine) in the analyte.

On cellulose tris-methylbenzoate (OJ), for compounds **1**, **2**, **3** and **4**, both using 2-propanol and ethanol as alcohol modifier, the (−)-(R) enantiomer is eluted first. For compound **5**, the same behavior is observed with ethanol, but changing the modifier from ethanol to 2-propanol leads to an inversion of elution order (Table 2). The reversal of the elution order of the two enantiomers, on cellulose-based CSP upon changing the kind of modifier in the mobile phase, has been often reported and was generally related to an alteration in the steric environment of the chiral cavities [12].

3.5. Validation method for the determination of enantiomeric purity

After optimization, the chiral purity of compounds **1**, **4** and **5** has been evaluated using the Chiralcel OD-H with a mobile phase composed of n-hexane–ethanol (70:30) (eluent A). Compound **2** has been tested with a mobile phase consisting of n-hexane–2-propanol (70:30) (eluent C) on Chiralcel OD-H and compound **3** on Chiralcel OJ. The chiral assay for each enantiomer was validated for detection and concentration limits. The limit of detection (LOD) calculated at a signal-to-noise ratio equal to 3 [24] was between 1.25 and 9.34 μmol l^{-1} corresponding to 0.16 and 1.24% minor enantiomer for a major enantiomer target concentration of 0.75 mM corresponding to 100%. Results are given in Table 4. By way of an example, a chromatogram of compound **4**-(R) in the presence of 2.40% of **4**-(S) is presented in Fig. 5. The purity was determined by the relative percentages of the peak areas.

4. Conclusion

We have presented a successful method development for the chiral separation and determination of enantiomeric purity of nucleoside analogs by liquid chromatography. As development in synthesis of

Table 4
Limit of detection and enantiomeric purity of compounds **1**–**5**

Compound	Enantiomer	LOD (%)	Concentration limit (μM)	Enantiomeric purity (%)
1	(*R*)	0.27	2.07	99.00
	(*S*)	0.33	2.50	99.20
2	(*R*)	0.16	1.25	>99.71
	(*S*)	0.29	2.22	97.50
3	(*R*)	2.27	17.10	98.60
	(*S*)	2.62	19.70	99.20
4	(*R*)	0.32	4.89	95.30
	(*S*)	0.85	6.41	97.60
5	(*R*)	0.80	6.00	>98.76
	(*S*)	1.24	9.34	99.75

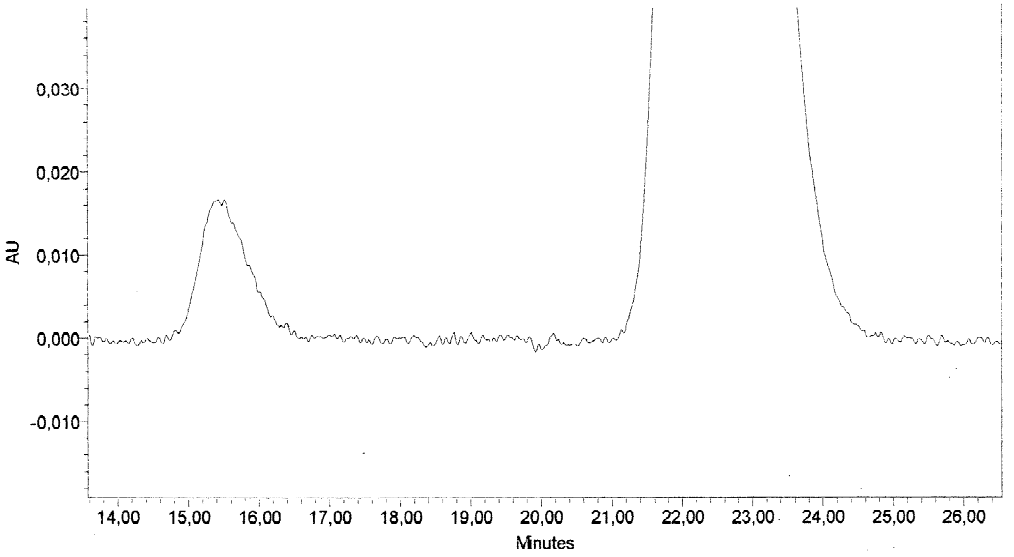

Fig. 5. Chromatogram of a **4**-(*S*) sample in presence of 2.40% of **4**-(*R*), on Chiralcel OJ stationary phase; eluent A (*n*-hexane–ethanol, 70:30); flow-rate: 0.8 ml min^{-1}; temperature 30 °C; wavelength 200 nm.

news antiviral continues, new methodology in HPLC will be necessary in order to understand the impact of chirality on biological activity and host toxicity.

References

[1] L.A. Agrofoglio, S.S. Challand, Acyclic, Carbocyclic and L-Nucleosides, Kluwer Academic, Dordrecht, 1998.
[2] D.F. Ewing, N. Fahmi, C. Len, G. Mackenzie, G. Ronco, P. Villa, G. Shaw, Collect. Czech. Chem. Commun. 61 (1996) S145.
[3] D.F. Ewing, N. Fahmi, G. Mackenzie, A. Pranzo, Nucleosides Nucleotides 18 (1999) 559.
[4] D.F. Ewing, N. Fahmi, C. Len, G. Mackenzie, A. Pranzo, J. Chem. Soc., Perkin Trans I 21 (2000) 3561.
[5] E. De Clerq, J. Med. Chem. 38 (1995) 2491.
[6] G.B. Elion, P.A. Furman, J.A. Fyfe, P. de Miranda, L. Beauchamp, H.J. Schaeffer, Proc. Natl. Acad. Sci. USA 74 (1977) 5716.

[7] K.K. Biron, G.B. Elion, Antimicrob. Agents Chemother. 18 (3) (1980) 443.
[8] E. Lipka-Belloli, C. Len, G. Mackenzie, G. Ronco, J.P. Bonte, C. Vaccher, J. Chromatogr. A 943 (2002) 91.
[9] D.F. Ewing, V. Glaçon, G. Mackenzie, C. Len, Tetrahedron Lett. 43 (2002) 989.
[10] W.B. Mahony, B.A. Domin, S.M. Daluge, W.H. Miller, T.P. Zimmerman, J. Biol. Chem. 267 (1992) 19792.
[11] E. Yashima, J. Chromatogr. A 906 (2001) 105.
[12] T. Wang, Y.W. Chen, J. Chromatogr. A 855 (1999) 411.
[13] B. Chankvetadze, C. Yamamoto, Y. Okamoto, J. Chromatogr. A 922 (2001) 127.
[14] H.Y. Aboul-Enein, I. Ali, Chirality 14 (2002) 47.
[15] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020.
[16] B. Chankvetadze, I. Kartozia, C. Yamamoto, Y. Okamoto, J. Pharm. Biomed. Anal. 27 (2002) 467.
[17] E. Belloli, C. Foulon, S. Yous, M.P. Vaccher, J.P. Bonte, C. Vaccher, J. Chromatogr. A 907 (2001) 101.
[18] E. Belloli, C. Vaccher, J.P. Bonte, Anal. Lett. 33 (14) (2000) 2999.
[19] J. Balzarini, M. Cools, E. De Clercq, Biochem. Biophys. Res. Commun. 158 (1989) 413.
[20] L. Novotny, M. Abdel-Hamid, H. Hamza, J. Pharm. Biomed. Anal. 24 (2000) 125.
[21] C. Roussel, C. Popescu, Chirality 6 (1994) 251.
[22] C. Roussel, C. Popescu, T. Shibata, J. Chromatogr. A 722 (1996) 177.
[23] A. Lambert, J.M. Ballon, A. Nicolas, Pharm. Res. 18 (2001) 886.
[24] U. Selditz, Y. Liao, J.P. Franke, R.A. de Zeeuw, H. Wiktrom, J. Chromatogr. A 803 (1998) 169.