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Resolution of enantiomers of uridine analogs, potential antiviral agents

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Abstract

Racemic mixtures of 5-substituted carbocylic analogs of uracil nucleosides, 2″- and 3″-furyl, 2″- and 3″-thienyl and 2″-selenienyl, potentially anti-viral agents, were resolved using amylose tris(3,5-dimethylphenyl)carbamate as the stationary phase. The mobile phase was *n*-hexane with ethanol or 2-propanol. Effects of some structural features on the extent of discrimination between the enantiomers were examined through the selectivity and resolution factors as well as the elution order. The structural features were as follows; the type and position of the hetero-atom O, S or Se, in the cyclopentadienyl substituent 5 of the uracil and hydroxymethyl vs. acetoxymethyl groups on the cyclopentene moiety of the uridine analogs. It appeared that effects of the structural features on the separation were solvent dependent, with some very unusual solvent effects. For example, average retention, which did not follow the polarity of the mobile phase modifiers, was much higher when ethanol was used compared to 2-propanol. Also, the elution order of the two enantiomers of several pairs was reversed when ethanol was changed to 2-propanol. In general, ethanol affected higher selectivity and resolution of all the enantiomeric pairs.

Keywords: Enantiomer separation; Uridine; Uracil nucleosides

1. Introduction

Carbocyclic analogs of nucleosides have shown antiviral properties and can potentially serve as candidates for anti-human immunodeficiency virus (HIV) drugs [1,2]. Many of these analogs are chiral and can exhibit stereoselectivity in their biological activity [3]. A well known example of stereoselectivity in drug action is exhibited by the drug Carbovir (CBV), a carbocyclic analog of 2′,3′-dideoxyguanosine that exhibits potent and selective in vitro activity against HIV. It has been shown [4,5] that its antiviral activity is associated with only the (−)-enantiomer. These findings imply that other enantiomers of the carbocyclic analogs of nucleosides are expected to differ in their biological activity and that just one of the enantiomers would be therapeutic. The enantiomeric purity of the therapeutic form of the candidate drug should be established before using it for anti-HIV purposes, to prevent possible side effects. Any future development of these compounds as antiviral or anti-HIV drugs would require that it is possible to analyze them, as enforced by health regulatory agencies for newly developed chiral drugs [6–8].

A literature search linking chiral carbocyclic ana-

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logs of nucleosides to chromatographic separations of the enantiomers produced works describing asymmetric synthesis. In these works, the products were analyzed for optical purity by chiral chromatography. For example, cyclobutyl analogs of guanine were resolved by ligand exchange [9,10]. Enantiomeric analogs of Neplanocin C were resolved on a Chiralcel OD column [11]. Enantiomers of 2′,3′-dideoxy-3′-thiacytidine [12] and 1,3-oxathiolanyl-cytosine [13] were resolved using acetyl derivative of cyclodextrin, Cyclobond AC.

The principle underlying chiral recognition is not quite understood as yet, therefore, the appropriate chiral chromatographic system could not be readily chosen based on an educated guess. We screened a few chiral stationary phases and found that ChiralPAK AD, the tris(3,5-dimethylphenyl) carbamoylated amylose, resolved the enantiomers of a group of 5-substituted carbocyclic analogs of uridines. The prediction of whether enantiomers of a given chiral compound could be resolved by this polysaccharide stationary phase, as well as their elution order, is not possible yet due to the probable multiple recognition mechanisms in the macromolecular stationary phase. The effects of the mobile phase additives on the steric environment of the chiral cavities in the stationary phase are also not very clear. The rationalization of the mechanism of chiral discrimination requires a comparative investigation of the chromatographic behavior of families of solutes. A good strategy would be to use families of chiral compounds with diverse structural features. Chiral carbocyclic analogs of nucleoside can be viewed as such a family [14–16]. We observed the effects of the following structural features of the carbocyclic analogs of uridines on the selectivity and resolution between their enantiomers: type and position of the hetero-atom, O, S, and Se, in the cyclopentadienyl hetero-ring in position 5 of the uracil; CH_2OH compared to CH_2OAc group on the 4′-position of the cyclopentene, the carbocyclic analog of the sugar moiety. We examined the effects of these structural features using various compositions of *n*-hexane with ethanol or 2-propanol and observed that they were solvent-dependent. Understanding the solvent effect on the selectivity and resolution is important for enhancing the optimization possibilities.

2. Experimental

2.1. Instrumentation and materials

HPLC analysis was performed using a HP1050 (Hewlett-Packard, Palo-Alto, CA, USA) instrument, equipped with a diode array UV-detector, a HPCHEM data station and a ThinkJet printer. A Rheodyne (Cotati, CA) injection valve, equipped with a 20-μl loop was used. The chiral column used was a ChiralPak AD column (250×4.6 mm, 10 μm; Daicel Chemical Industries, Tokyo, Japan). The silica column was a 5-μm Adsorbosphere HS Alltech column (250×4.6 mm; Alltech Associates, Deerfield, IL, USA). The solvents, *n*-hexane and 2-propanol (Lab-Scan, Dublin, Ireland) and ethanol (Carlo-Erba, Milano, Italy) were all of HPLC grade. Circular dichroism (CD) spectra were recorded using a Jobin-Yvon (Longjuneau, France) model CD6.

2.2. Procedure

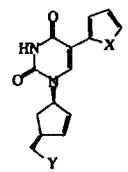
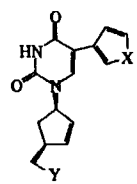
A flow-rate of 0.85 ml/min was used in all experiments and the temperature was maintained at 30±0.1°C using a circulating water bath. Each run was monitored at two wavelengths simultaneously (i.e., 250 and 320 nm). The racemates were injected and the two peaks that were obtained were collected. The two solutions were analyzed by CD to verify that the peaks represent enantiomers. The separated enantiomers were injected following the injection of the racemic mixtures each time during the optimization procedure, in order to identify the enantiomers.

The collection of high quantities of the separate enantiomers (a few milligrams each) required the injection of high sample loads. We observed that under such conditions the system was sensitive to residual adsorption of the enantiomers on the stationary phase. Therefore, after the injection of high concentrations of the enantiomers the column was regenerated with pure ethanol (at a flow-rate of 0.2 ml/min) overnight.

3. Results and discussion

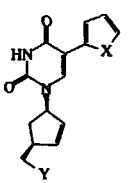
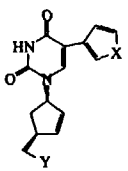
Ten pairs of enantiomers of carbocyclic analogs of

Table 1
Chromatographic parameters of the ten pairs of nucleoside analogs using 2-propanol in the mobile phase

Compound		X	Y	30%				25%				20%				15%			
				$k'_{(+)}$[a]	$k'_{(-)}$[a]	α[b]	R_s[c]	$k'_{(+)}$	$k'_{(-)}$	α	R_s	$k'_{(+)}$	$k'_{(-)}$	α	R_s	$k'_{(+)}$	$k'_{(-)}$	α	R_s
	a:	O	OH	1.97	2.41	1.22	1.91	2.76	3.36	1.22	2.11	4.32	5.24	1.21	2.27	7.82	9.36	1.20	2.39
	b:	S	OH	2.43	2.61	1.07	0.42	3.47	3.70	1.07	0.46	5.55	5.85	1.05	0.55	10.28	10.62	1.03	0.41
	c:	Se	OH	2.44	2.51	1.03	0.21	3.47	3.52	1.01	0.13	5.47	5.49	1.00	0.03	9.78	10.00	1.02	0.28
	d:	O	OAc	2.53	2.5	1.01	0.24	3.45	3.38	1.02	0.24	4.5	4.36	1.03	0.22	6.82	6.51	1.05	0.43
	e:	S	OAc	3.32	3.25	1.02	0.21	4.59	4.42	1.04	0.43	6.05	5.79	1.05	0.52	9.21	8.76	1.05	0.68
	f:	Se	OAc	3.70	3.28	1.13	1.37	5.10	4.46	1.14	1.93	6.86	5.82	1.18	1.74	10.26	8.77	1.17	1.81
	g:	O	OH	1.89	2.04	1.08	0.57	2.72	2.92	1.08	0.84	4.37	4.69	1.07	0.65	8.01	8.55	1.07	0.74
	h:	S	OH	2.37	2.91	1.23	2.05	3.41	4.15	1.22	2.12	5.41	6.50	1.20	2.33	9.92	11.75	1.18	2.39
	i:	O	OAc	2.99	3.47	1.16	1.78	3.85	4.44	1.15	1.87	5.36	6.12	1.14	1.88	8.24	9.33	1.13	1.94
	j:	S	OAc	3.68	4.41	1.20	2.16	4.75	5.63	1.19	2.17	6.57	7.73	1.18	2.24	10.11	11.74	1.16	2.24

[a] $k'=(t_R-t_0)/t_0$, where t_R is the retention time and t_0 is the void time.
[b] $\alpha=k'_2/k'_1$, where $k'_2>k'_1$.
[c] $R_s=2(t_{R2}-t_{R1})/(w_2+w_1)$, where w is the peak width at the base.

Table 2
Chromatographic parameters of the ten pairs of nucleoside analogs using ethanol in the mobile phase

Compound		X	Y	40%				35%				30%			
				$k'_{(+)}$ [a]	$k'_{(-)}$ [a]	α [b]	R_s [c]	$k'_{(+)}$	$k'_{(-)}$	α	R_s	$k'_{(+)}$	$k'_{(-)}$	α	R_s
	a:	O	OH	2.21	3.06	1.39	4.15	2.72	3.79	1.39	4.57	3.77	4.98	1.32	4.01
	b:	S	OH	2.25	4.03	1.79	7.37	2.76	4.94	1.79	7.59	3.79	6.49	1.71	7.17
	c:	Se	OH	2.27	4.30	1.89	8.04	2.77	5.27	1.90	8.73	3.76	6.93	1.84	7.22
	d:	O	OAc	4.45	9.05	2.03	11.24	4.68	9.99	2.14	12.67	5.73	12.09	2.11	13.39
	e:	S	OAc	5.87	13.80	2.35	15.39	6.18	14.39	2.33	15.78	7.69	18.84	2.45	15.18
	f:	Se	OAc	6.39	15.08	2.36	13.82	6.69	16.35	2.44	13.59	8.65	20.43	2.36	15.13
	g:	O	OH	1.88	3.05	1.62	6.06	2.30	3.72	1.62	6.54	3.13	4.88	1.56	6.36
	h:	S	OH	2.35	3.45	1.47	4.86	2.85	4.20	1.47	4.61	3.84	5.61	1.46	5.62
	i:	O	OAc	5.98	11.73	1.96	10.09	7.03	13.73	1.95	10.27	9.01	17.49	1.94	11.04
	j:	S	OAc	7.07	11.99	1.70	7.94	8.20	13.89	1.69	8.16	10.44	17.35	1.66	7.70

[a] $k'=(t_R-t_0)/t_0$, where t_R is the retention time and t_0 is the void time.
[b] $\alpha=k'_2/k'_1$, where $k'_2>k'_1$.
[c] $R_s=2(t_{R2}-t_{R1})/(w_2+w_1)$, where w is the peak width at the base.

5-substituted uracil nucleoside were resolved by the amylose tris(3,5-dimethylphenyl)carbamate chiral stationary phase, using ethanol and 2-propanol in *n*-hexane as the mobile phase. The structures of the various enantiomers are shown in Tables 1–3 and in Figs. 1 and 2. There are two chiral centers in the cyclopentene ring, (1′ and 4′) with the two substituents in our group of compounds having a cis conformation. Tables 1 and 2 present the retention, selectivity and resolution factors of the ten enantiomeric pairs using 2-propanol and ethanol, respectively. Table 3 presents the retention data that was observed on a silica column for comparative purposes.

3.1. Solvent effects

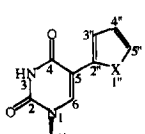
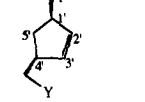
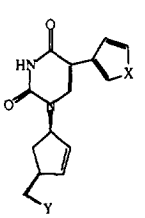
Retention of the ten racemates in a non-chiral normal-phase system was measured using either ethanol or 2-propanol as the modifiers of *n*-hexane in the mobile phase. The results are shown in Table 3. Retention factors obtained with the silica column, using the same percentages of the modifiers in the mobile phase, were relatively small. These small values indicate a weak adsorption on the surface of the silica gel of the stationary phase. In addition, differences between k' of the racemates obtained in ethanol, compared to those obtained in 2-propanol, were insignificant. These small differences indicate that any solvent effect observed in the chiral system should not be attributed to differences in solubility in the two solvent systems.

3.1.1. Retention

The presence of the chiral selector in the stationary phase increased the retention of both enantiomers significantly. The average retention factor of all of the analogs in the chiral system was considerably higher than that observed in the non-chiral system, using 20% and above of 2-propanol or ethanol. Therefore, a relatively high percentage of the alcohol modifiers was needed for the resolution of the enantiomers in this study.

An unusual non-selective retention behavior was observed in the present system for the family of compounds that was studied here. The enantiomers were retained for a significantly longer time in ethanol than in 2-propanol. It is well known that the effect on selectivity and resolution between enantiomers in polysaccharide chiral systems is not necessarily related to the polarity of the two alcoholic modifiers [17,18]. Nevertheless, as far as we know, a higher average retention in ethanol compared to 2-propanol is a rare occurrence when normal phase

Table 3
Retention parameters, k', of the ten pairs of nucleoside analogs, using a silica column

Compound		X	Y	Ethanol			2-Propanol		
				10%	15%	20%	10%	15%	20%
	a:	O	OH	3.47	1.89	1.27	4.75	2.18	1.38
	b:	S	OH	3.14	1.75	1.16	4.44	2.06	1.21
	c:	Se	OH	2.83	1.59	1.15	3.60	1.76	1.06
	d:	O	OAc	1.68	1.11	0.82	1.56	1.02	0.75
	e:	S	OAc	1.88	1.19	0.85	2.08	1.16	0.84
	f:	Se	OAc	1.76	1.12	0.84	1.62	1.06	0.79
	g:	O	OH	3.34	1.79	1.21	4.69	2.14	1.31
	h:	S	OH	2.91	1.65	1.32	4.13	1.97	1.21
	i:	O	OAc	1.88	1.25	0.93	2.34	1.32	0.97
	j:	S	OAc	2.03	1.07	0.90	2.47	1.36	0.97

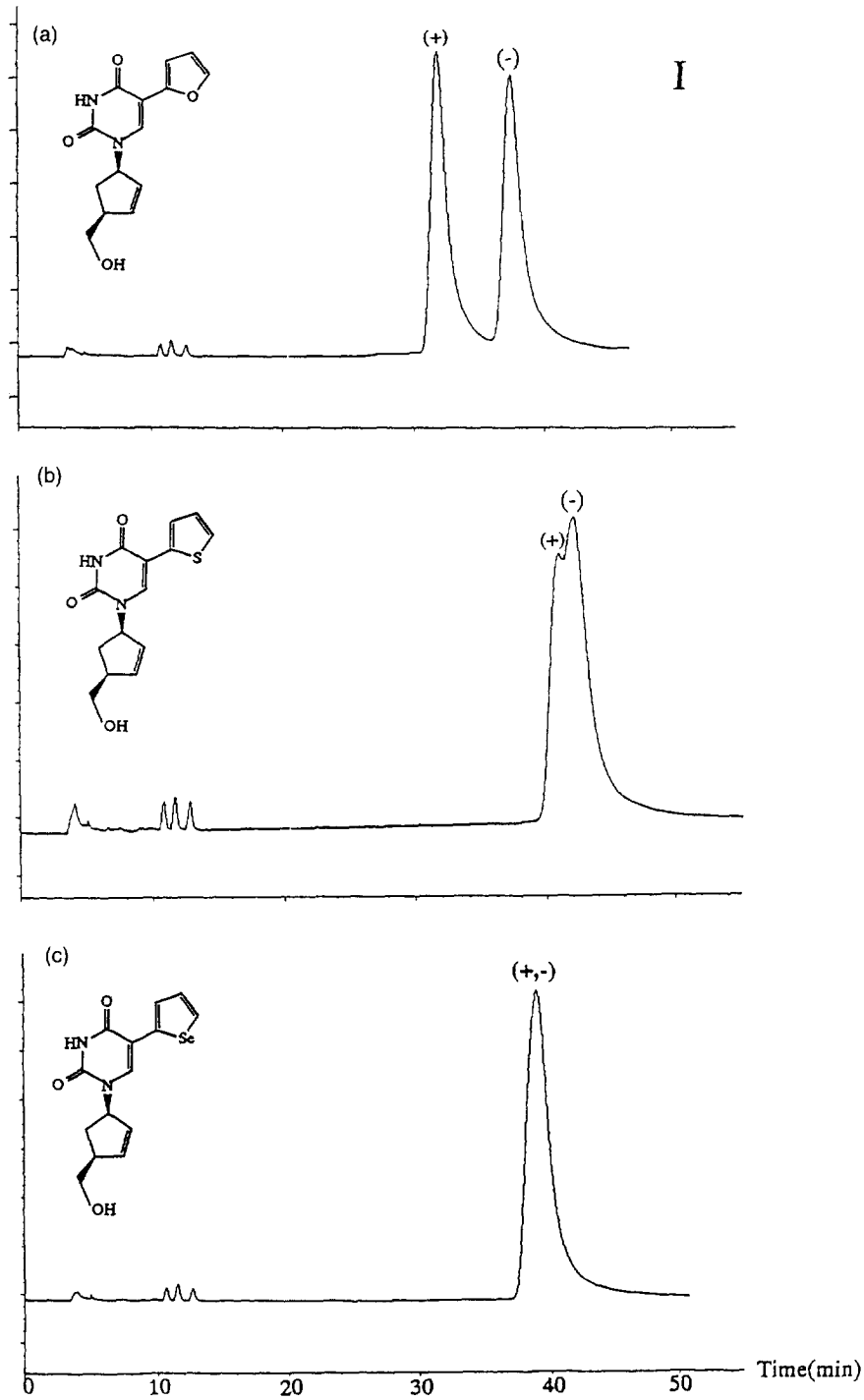


Fig. 1. (*Continued*)

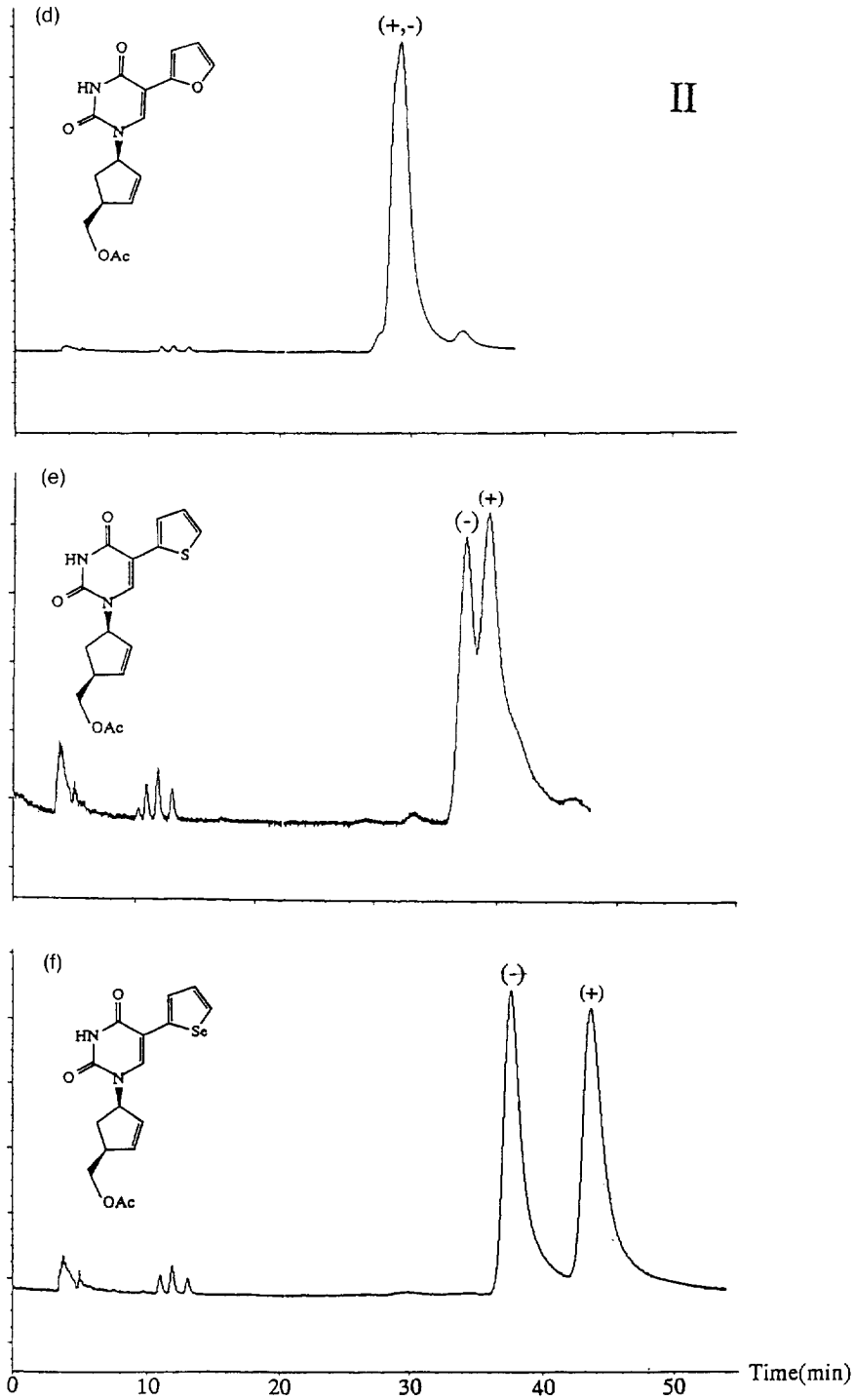


Fig. 1. (*Continued on p. 138*)

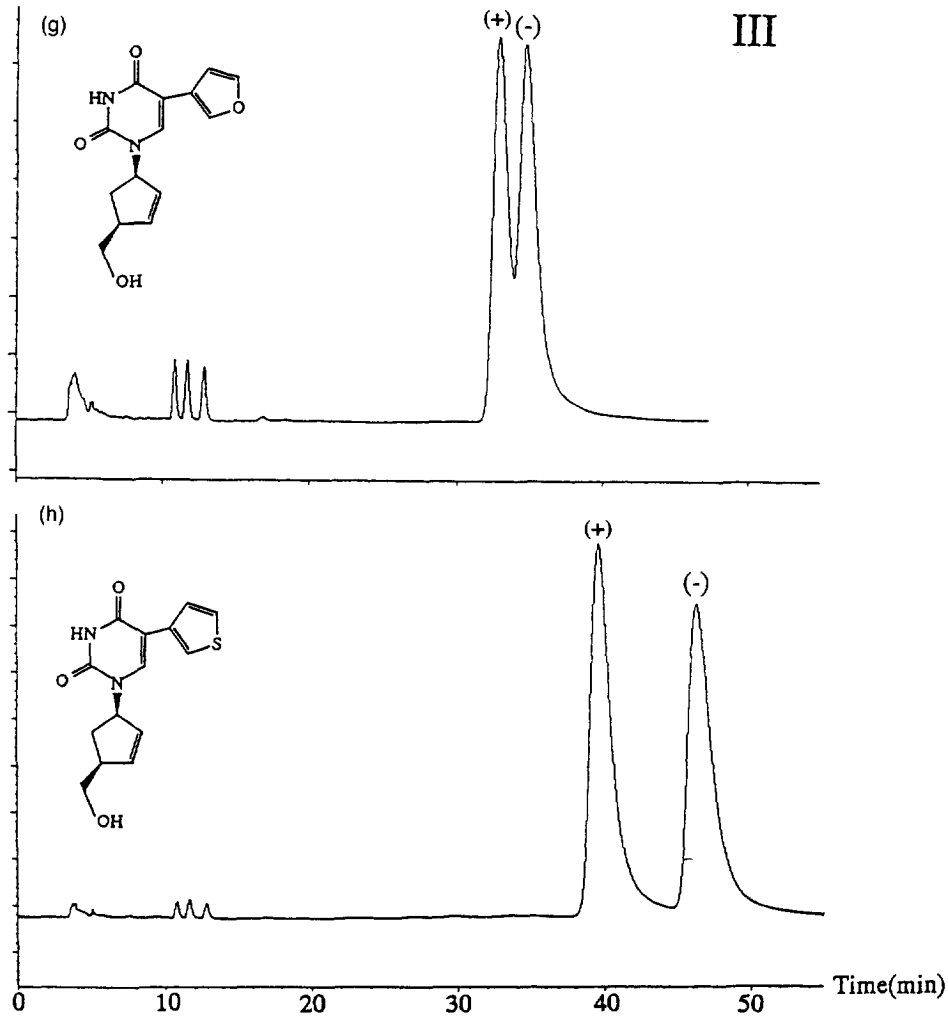


Fig. 1. (*Continued*)

solvents are used in conjunction with a polar stationary phase, even with the polysaccharide stationary phase.

3.1.2. Selectivity and resolution

The differences in the effect of the two alcohol modifiers on the selectivity and resolution factors were striking, as can be seen in Tables 1 and 2 and Fig. 1I–IV Fig. 2I–IV. Very high selectivity and resolution were obtained for all of the enantiomeric pairs using ethanol in the mobile phase, compared to the results obtained when 2-propanol was used. As can be seen in Fig. 1, not all of the ten enantiomeric pairs were resolved, using a practical range of 2-propanol in the mobile phase, whereas all ten enantiomeric pairs were resolved using ethanol as the modifier under all conditions.

The higher values of α and R_s may indicate that ethanol probably affected the conformation of the chiral sites on the stationary phase so that this specific family of enantiomers was strongly affected. Such changes in conformation can lead to significantly higher preferential affinity of the two enantiomers to the stationary phase. The higher resolution obtained using ethanol can be attributed not only to the selectivity, but also to the significantly higher

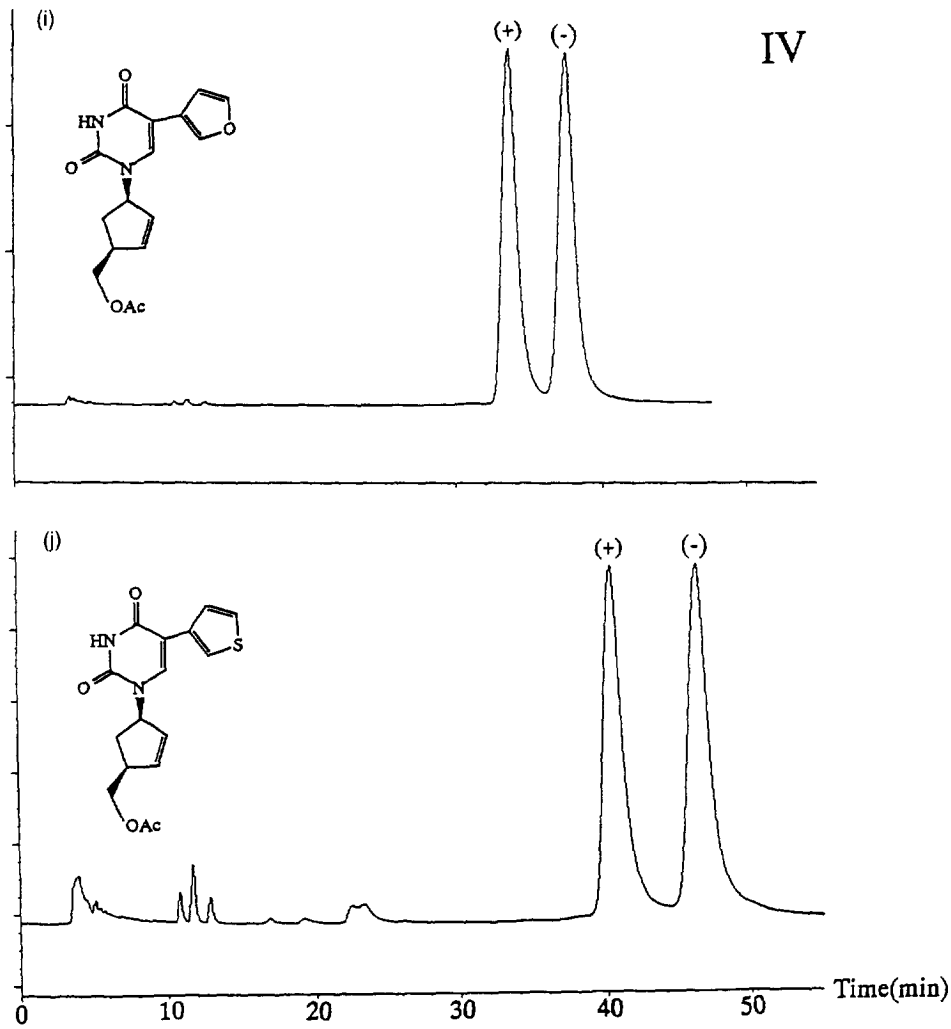


Fig. 1. Chromatograms obtained using 15% 2-propanol in the mobile phase. I. Pairs a, b, c. II. Pairs d, e, f. III. Pairs g, h. IV. Pairs j, i.

efficiency (number of theoretical plates) of the separation. Broader peaks were obtained when 2-propanol was used as the mobile phase modifier.

3.1.3. Elution order

To establish the elution order, the peaks were collected (approximately 100 mg of each peak, adjusted to a volume of 2 ml) and their circular dichroism (CD) was measured. The spectra of the enantiomers, obtained from pairs a–f and g–j, are shown in Fig. 3I–II, respectively. The spectra verify that the separated entities were enantiomers. The direction (negative or positive) of the response (ΔA) in the last peak of the CD absorption curve (λ of around 320 nm) was used to identify the enantiomers as (−) or (+). The elution order was (−/+) enantiomers of all ten racemates using ethanol in the mobile phase. On the other hand, when 2-propanol was the modifier, the order was (+/−) in all of the analogs except d, e and f. The reversal of elution order when ethanol was switched to 2-propanol indicates that the discrimination mechanism was different in the two solvent systems.

3.2. Effects of structural features

Since we studied the chromatographic behavior of

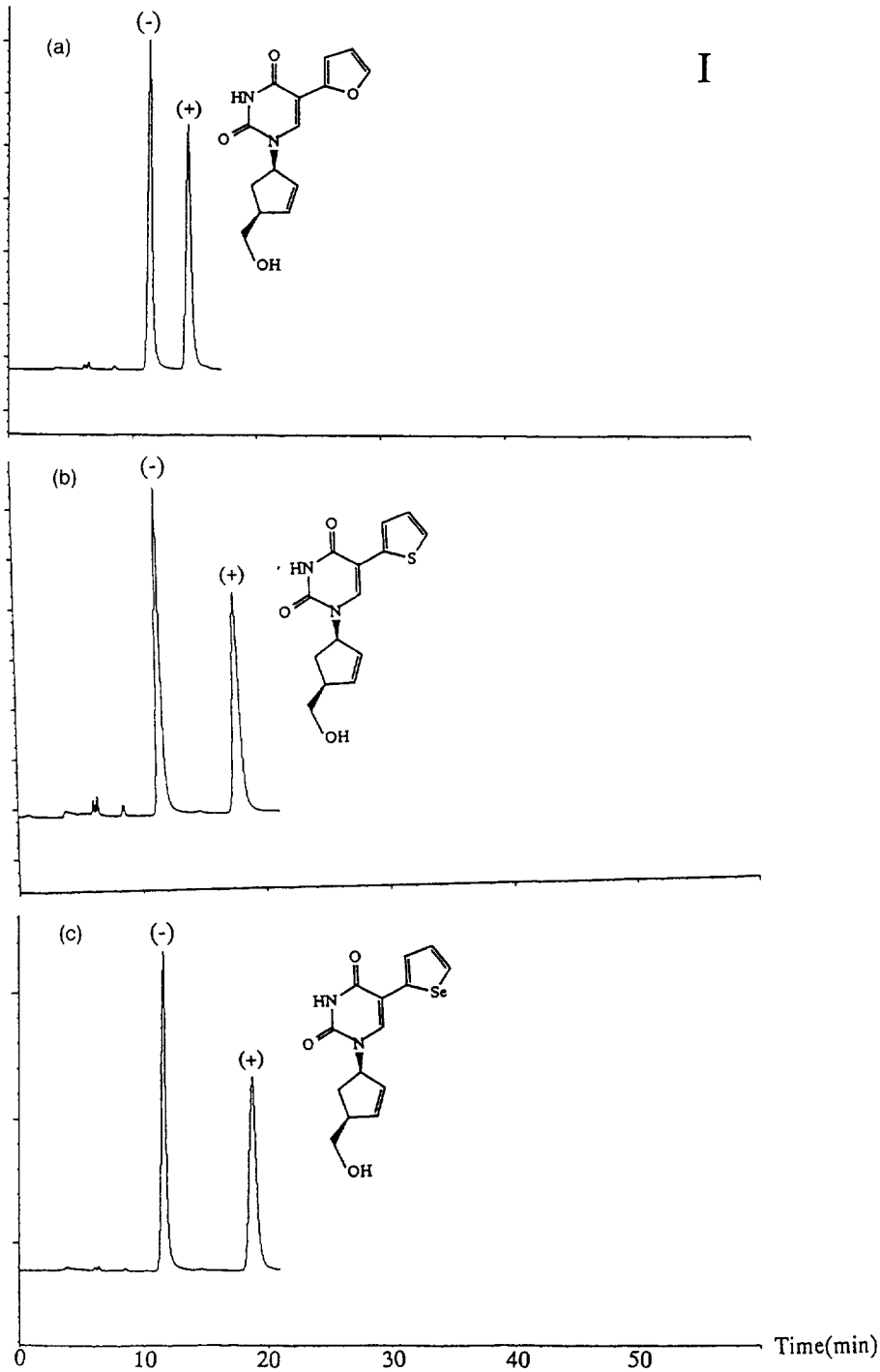


Fig. 2. (*Continued*)

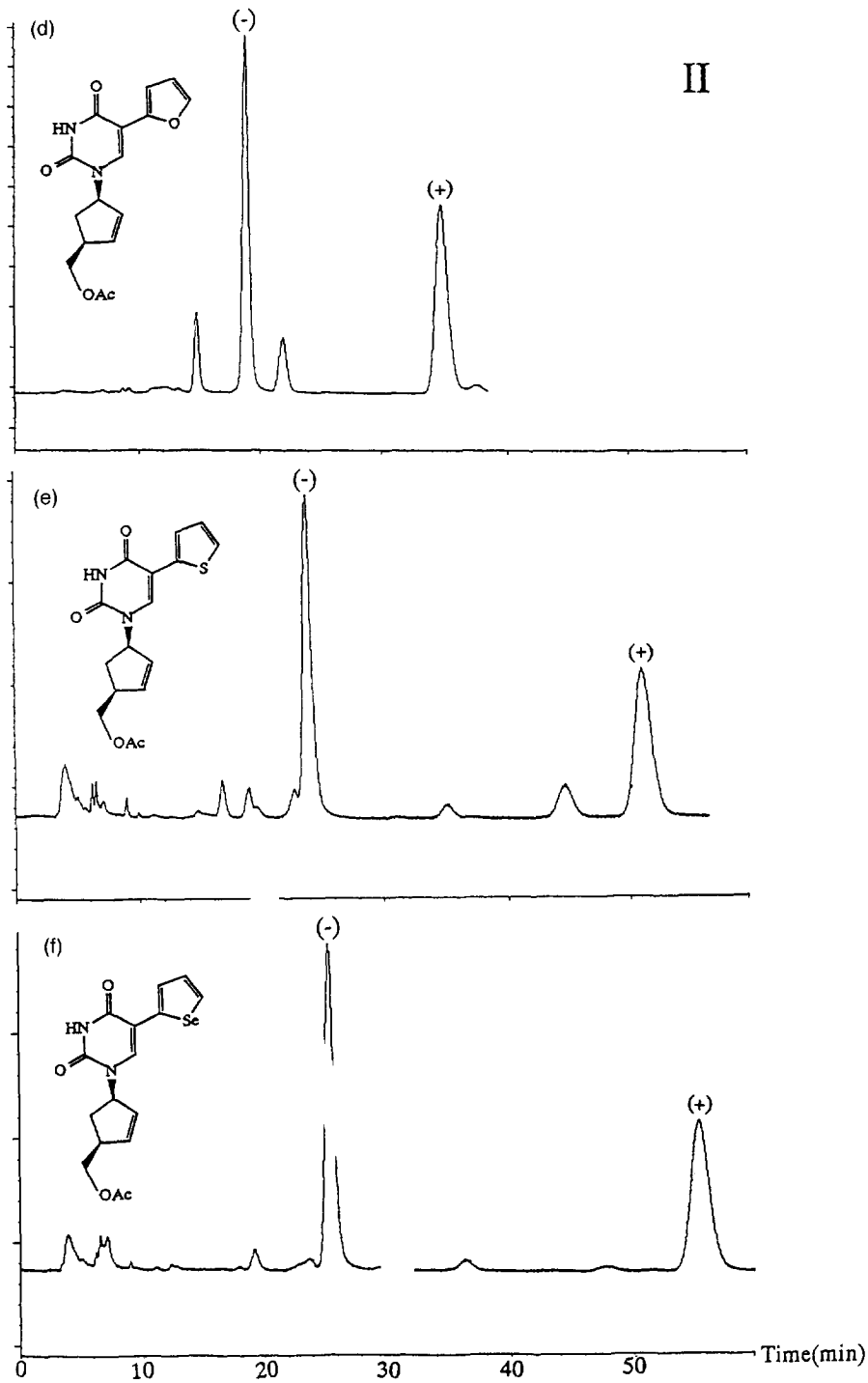


Fig. 2. (*Continued on p. 142*)

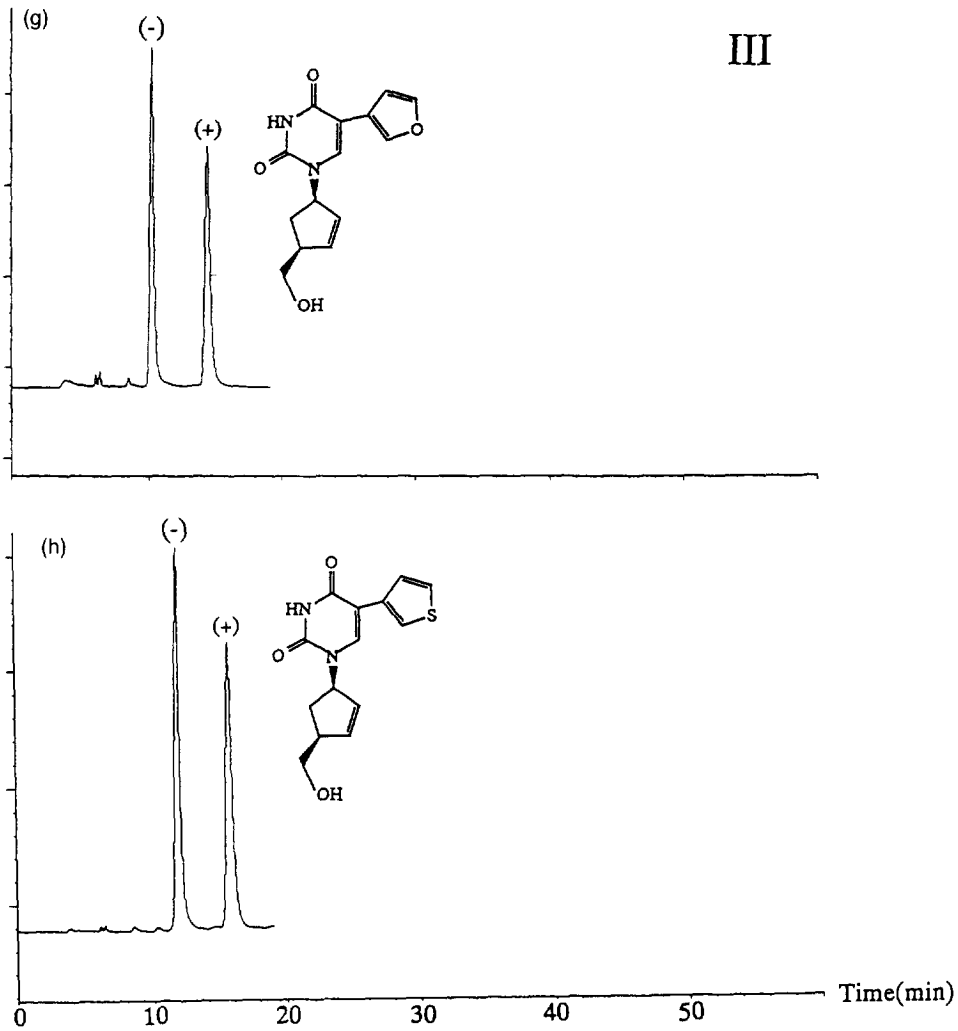


Fig. 2. (*Continued*)

a family of chiral compounds, we examined the effect of their various structural features on this behavior. We compared analogs varying in the type and position of the hetero-atom (marked as X in the tables) and those having hydroxyl vs. acetoxy groups (marked as Y in the tables) using either 2-propanol or ethanol as the mobile phase modifiers. The effects of structural features on the separation were solvent-dependent and indicated also that the mechanism of discrimination was different in the two solvent systems.

3.2.1. Type and position of the hetero-atom in the 5-cyclopentadienyl hetero-ring

The differences among some of the analogs involved the type and position of the hetero-atom, O, S or Se, in the cyclopentadienyl substituent of the uracil. The three atoms differ in their size, (Se>S>

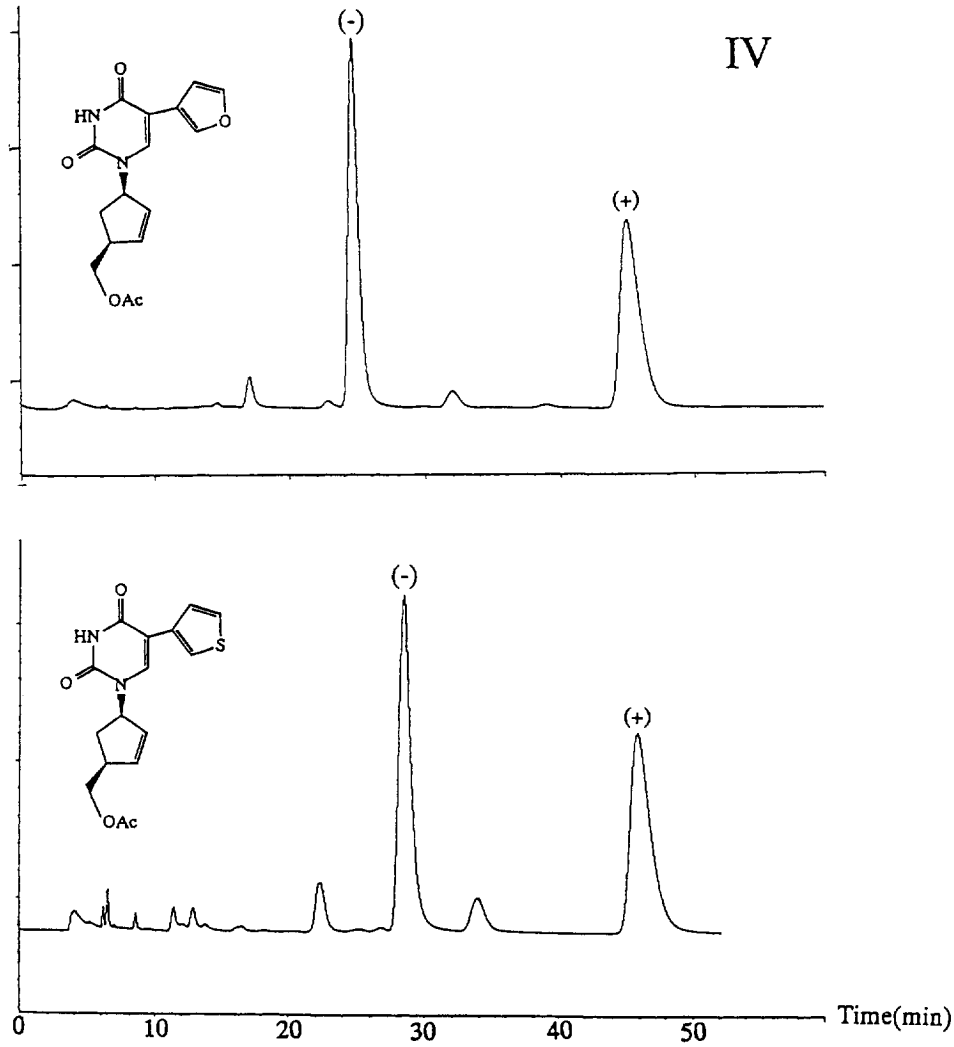


Fig. 2. Chromatograms obtained using 40% ethanol in the mobile phase. I. Pairs a, b, c. II. Pairs d, e, f. III. Pairs g, h. IV. Pairs j, i.

O) and in their ability to form hydrogen bonding (O>S>Se), therefore, they may have different contributions to the discrimination process. The effects were too complex to analyze because they did not have a consistent pattern. In some cases the O analogs were resolved better than the corresponding S or Se analogs and in others they were not. The conclusion from our observations was that any effect that the type or position of the hetero-atom in the cyclopentadienyl hetero-ring might have on the discrimination between the enantiomers is dependent on the type of modifier, and it is not necessarily related to its ability to form hydrogen bonds.

3.2.2. Hydroxyl vs. acetoxy groups

The 4′-CH_2OH (Y=OH) analogs (a, b, c, g, h) and 4′-CH_2OAc (Y=OAc) analogs (d, e, f, i, j) eluted in the non-chiral system as expected in normal phase chromatography (Table 3), i.e., the OH analogs were retained longer than their corresponding OAc analogs. Usually, OH compounds are retained longer than their acetoxy analogs, whenever normal phase solvents are used in conjunction with polar stationary phases (chiral or non-chiral). Nevertheless, this was not the case in our chiral system, i.e., the OAc analogs were retained significantly longer in ethanol, and slightly longer in some concentrations

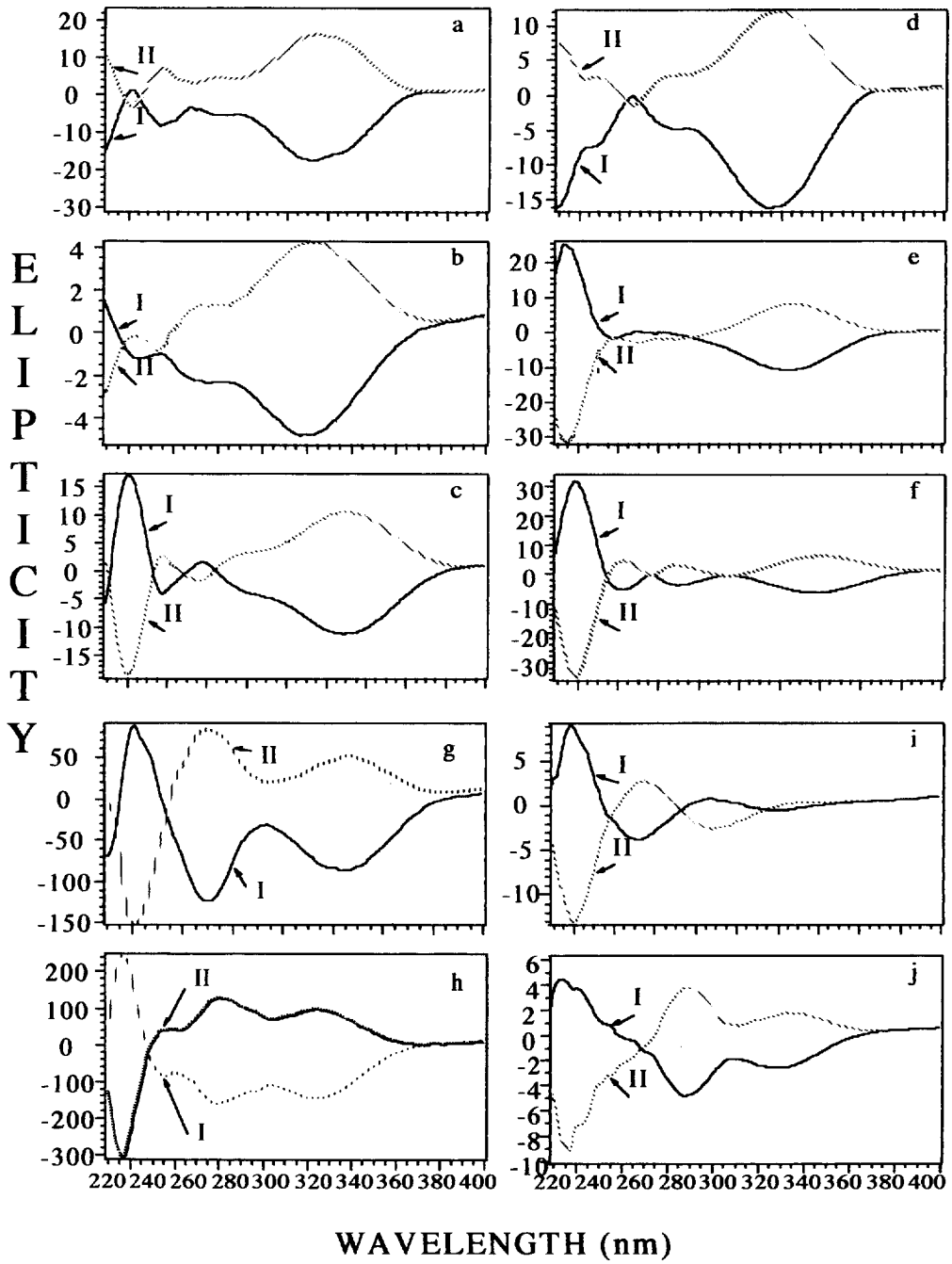


Fig. 3. Circular dichroism spectra of the ten enantiomers that were collected from the effluent. I. The 2″-furyl, thienyl and selenienyl analogs (a–f). II. The 3″-furyl and thienyl analogs (g–j).

of 2-propanol than were the OH analogs (Tables 1 and 2). To the best of our knowledge, this unusual order of retention is a very rare occurrence.

The effect of the OAc group vs. the corresponding OH group on the separation of the enantiomers was also solvent-dependent. In ethanol, the OAc analogs were consistently resolved better, as shown in Fig. 2I and Fig. 2III compared to Fig. 2II Fig. 2IV, respectively. However, in 2-propanol the effect of blocking the 4′-CH_2OH group on the separation of the enantiomers was not consistent enough to draw any conclusions that would help to unveil the mechanism of discrimination. In one instance, for example, the 3″-hetero-ring OH vs. OAc analogs, the order of elution was reversed from (−/+) in the OH analogs to (+/−) in the OAc analogs. A reversal of the elution order clearly indicates that a change in the mechanism of discrimination occurred when the OH groups were blocked. A similar effect was observed in our previous study of cannabidiol enantiomers [19].

We have further compared the effect of blocking the OH group on the separation of the enantiomers of these nucleoside analogs to the same effect on cannabinoid enantiomers, observed in our previous study, using the same amylose stationary phase. Acetylation of OH groups in some of the cannabinoid enantiomers appeared to have been detrimental to their separation, in contrast to the enantiomers of the carbocyclic analogs of the nucleosides studied here [19]. There is an important difference between the cannabinoid enantiomers and the carbocyclic analogs of nucleosides. Blocking OH groups in the nucleoside analogs leaves other hydrogen bonding agents (CO, NH, N) still available for the process of chiral discrimination, whereas in the cannabinoids there were no other remaining strong hydrogen bonding agents available.

An improvement in resolution of various enantiomers upon blocking their OH groups has been noticed before, using various types of chiral stationary phases [20–22].

Such contradictory observations demonstrate dramatically the puzzles frequently encountered in chiral chromatography during the optimization process, while trying to rationalize the chromatographic behavior of the enantiomers.

4. Conclusion

We have resolved enantiomers of carbocyclic analogs of 5-substituted uracil, using a ChiralPak AD chiral column. Preliminary attempts to resolve an additional variety of enantiomers of carbocyclic analogs of 5-thienyl uracil, which are not reported here, were also successful. These preliminary results attest to the great potential of the present method to resolve other chiral carbocyclic analogs of nucleosides.

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