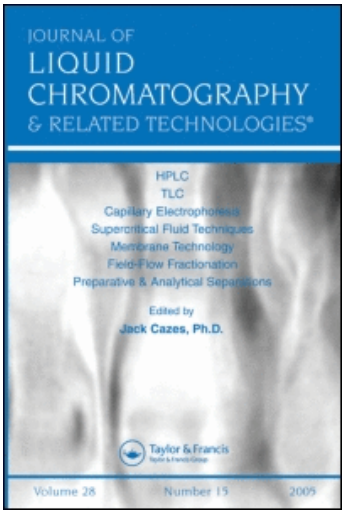

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HPLC ENANTIOMERIC SEPARATION OF o,o-DIALKYL-2-BENZYLOXYCARBONYL-AMINOARYLMETHYL-PHOSPHONATES ON CELLULOSE TRIS(3,5-DIMETHYLPHENYL CARBAMATE) AND N-(3,5-DINITROBENZOYL) LEUCINE CSPs

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HPLC ENANTIOMERIC SEPARATION OF o,o-DIALKYL-2-BENZYLOXYCARBONYL-AMINOARYLMETHYL-PHOSPHONATES ON CELLULOSE TRIS(3,5-DIMETHYLPHENYL CARBAMATE) AND N-(3,5-DINITROBENZOYL) LEUCINE CSPs

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ABSTRACT

A series of racemic nine o,o-dialkyl-2-benzyloxycarbonyl-aminoarylmethyl-phosphonate enantiomers has been separated by high performance liquid chromatography on cellulose tri(3,5-dimethyl-phenylcarbamate) and N-(3,5-dinitrobenzoyl) leucine (DNB-leu) chiral stationary phases. The chromatographic parameters, the capacity factor (k), separation factor (α), and the resolution factor (Rs) of all solutes are presented. The influence of the

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mobile phase composition on the enantioselectivity has been discussed.

INTRODUCTION

Numerous chiral stationary phases (CSPs) had been developed in the last decade to provide an efficient method for separation of optical isomers.(1-2) Among these CSPs, Pirkle's and derivative cellulose CSPs, have proved to be very effective for the determination of the purity, absolute configuration of a variety of enantiomers.(3-4) Recently, the chiral separations of some organo-phosphorus compounds were reported on different Pirkle's CSPs, for example, the separation of the enantiomers of a series of diethyl *N*-(aryl)-1-arylmethanephosphonates on a WHELK-O-column.(5) It showed that the C-aryl substituents play an important role on both the retention and the enantioselectivity.

Selim(6) separated a series of enantiomers of dimethyl N-3,5-dinitrobenzoyl-α-amino substituted-benzyl phosphonate derivative on the (R)-2,2,2-trifluoro-1-(9-anthryl) ethanol derivative chiral stationary phase. Pirkle also reported the separation of the enantiomers of a variety of N-DNB-amino-phosphonic acids derivatives on the (R)-2,2,2-trifluoro-1-(9-anthryl) ethanol derivative,(7) (R)-*N*-(2-naphthyl)-D-alanine,(8-9) (S)-*N*-(1-naphthyl)-leucine(9-10) and N-[11-(dimethylethoxysilyl) undecanoyl]-L-proline-3,5-dimethyl-anilide(11-12) chiral stationary phases. The separation of several 3,5-dinitrobenzenzyloxycarbonyl-amino-phosphonic acids derivatives were successfully resolved on the quinine carbamate CSP.(13) Liu reported the separation of the enantiomers of α-amino-alkylphosphonic acids derivatives on the (L,L) valinyl-valine-tert-butylamide CSP.(14) Grassert(15) described the separation of the enantiomers of α-amino-phosphonic acids derivatives on polysaccharide-types, namely Chiralpak AD and Chiralcel OD-H CSPs. Kuwano reported the separation of the enantiomers of dimethyl-1(N-acetylamino)-2-oxo-2-phenyl-1-E-3-phenyl-2-propenyl-ethyl phosphonate on Chiralpak AD.(16)

We also reported the resolution of a series of rancemic o,o-diethyl, (p-methyl-benzenesulfonamido), aryl(alkyl)-methylphosphonates, and o,o-dialkyl-1-benzyloxycarbonyl-aminoarylmethyl phosphonates on the N-(3,5-dinitrobenzoyl) leucine (DNB-leu) CSP.(17-19) In this paper, we describe the separation of a series of racemic o,o-dialkyl-1-benzyloxycarbonyl-aminoarylmethyl phosphonates enantiomers on cellulose tri(3,5-dimethylphenyl-carbamate) known as Chiralcel OD and a Pirkle-type column, namely DNB-leu CSPs. The influences of the mobile phase composition and column temperature on the retention and enantioselectivity have been also investigated. The chiral recognition mechanisms involved between those analytes and the chiral selectors used in this study is discussed.

EXPERIMENTAL

Materials

A series of nine dialkyl-benzyloxycarbonyl-aminoaryl methylphosphonate compounds were synthesized by the National Laboratory of Elemento-Organic Chemistry, Nankai University.(20) The general structure of the compounds is shown in Figure 1. These compounds were dissolved in ethanol and then diluted with the eluent solvent to the concentration of 1mg mL^{-1}. All solvents were filtered by a 0.5 μm filter and degassed in vacuum before use.

Apparatus

The chromatography was performed on a Shimadzu (Japan) modular liquid chromatography equipped with CR-6A integrator, SPD-10A UV-Vis detector, and LC-10AD solvent delivery system.

Chromatography Conditions

The cellulose tris(3,5-dimethylphenyl carbamate) chiral stationary phase was synthesized according to ref.(21-22) and packed into a 250 × 4.6 mm I.D. stainless steel column. The mobile phase compositions were 2, 5, 7 and 10% of 2-propanol in n-hexane. The flow-rate was maintained at 1 mL min^{-1}. The column temperatures were 10, 20, 30, and 40°C, respectively. Detection was set at 230 nm.

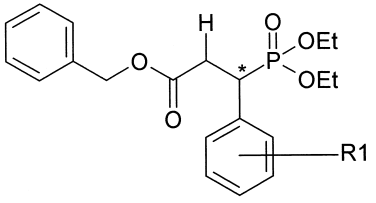

Figure 1. The general structure of compounds used in this study: o,o-dialkyl-1-benzyloxycarbanyl-aminoarylmethyl phosphonates. Aterisk * denotes the position of the chiral carbon.

RESULTS AND DISCUSSION

Chiral Separation on Cellulose tris(3,5-Dimethylphenyl Carbamate) CSP

In order to optimize the separation, the effects of different ratios between 2-propanol and n-hexane in the mobile phase on the chiral separation have been investigated. The chromatographic parameters namely the capacity factor (*k)*, separation factor (α), and resolution factor (Rs) are shown in Table 1. It is shown that the *k* values are decreased with increasing the concentration of 2-propanol in mobile phase for all compounds. The α values varied by the different substituents in different positions on the phenyl group attached to the chiral center.

The Influence of the Position of Substituents on the Chiral Separation

When the substituents are in the para- position of the benzene ring, the chiral separation was worse than no substituent, no matter whether the substituent was a π-donor or π-acceptor group. As shown in Figure 2, this is verified by the worst separation of the compound 6 substituted by a $-NO_2$ group (strong π-acceptor group) in the para position. However, with the weak π-acceptor, a substituent such as –Cl, was better than the nitro substituent compound, yet it was poorer than the compounds with π-donor substituents. The π-donor compounds 2 and 4, substituted by –CH3 and –OCH3 in para position, show similar behavior, i.e., the chiral separation of the weak π-donor compounds is better than that of stronger π-donor compounds. Accordingly, the order of chiral separation of the para- substituted phosphonate analogs studied was $\alpha_{p\text{-}H} >> \alpha_{p\text{-}CH3} > \alpha_{p\text{-}OCH3} > \alpha_{p\text{-}Cl} >> \alpha_{p\text{-}NO2}$.

When the same substituent is in different positions of the benzene ring, it shows that the chiral separation the otho- position substituted analogs are much better than that in the meta- or para- position, whether the substituent was π-donor or π-acceptor group. The order of the chiral separation was $\alpha_{o\text{-}Cl} >> \alpha_{p\text{-}Cl} > \alpha_{m\text{-}Cl}$ and $\alpha_{o\text{-}OCH3} >> \alpha_{p\text{-}OCH3}$ as shown in Figures 3 and 4. The chiral separation of compounds with substituents in the para- position, are slightly better than that in the meta position. The chiral separation orders were $\alpha_H >> \alpha_{m\text{-}NO2} > \alpha_{p\text{-}NO2}$ and $\alpha_H > \alpha_{o\text{-}Cl} >> \alpha_{p\text{-}Cl} > \alpha_{m\text{-}Cl}$.

The Influence of the Properties of Substituents on Chiral Separation

When the substituents are in the same position, the π-donor substituted compounds get better chiral separation than that of π-acceptor compounds. It is

Table 1. k_1, k_2 and α Values of the Chiral Separation of Nine Organic Phosphonate Compounds Using Different Mobile Phase Compositions on the tris(3,5-Dimethylphenyl Carbamate) Cellulose CSP

		2% 2-PrOH			5% 2-PrOH			7% 2-PrOH			10% 2-PrOH		
No.	R_1	k_2	α	Rs	k_2	α	Rs	k_2	α	Rs	k_2	α	Rs
1	H	12.9	2.30	2.07	5.04	2.31	1.87	3.70	2.41	1.82	2.46	2.29	1.58
2	p-CH_3	8.98	1.75	1.52	3.63	1.85	1.42	2.63	1.87	1.33	1.76	1.83	1.14
3	o-OCH_3	42.6	4.59	3.02	18.3	5.30	3.04	11.6	5.15	2.93	8.36	5.36	2.87
4	p-OCH_3	16.8	1.69	1.52	6.63	1.79	1.52	4.36	1.79	1.42	2.88	1.78	1.29
5	m-NO_2	17.0	1.18	0.57	5.10	1.17	0.48	4.38	1.23	0.60	2.84	1.22	0.53
6	p-NO_2	22.6	1.19	0.60	6.61	1.19	0.55	5.95	1.30	0.78	3.79	1.30	0.72
7	o-Cl	12.6	2.15	1.96	5.23	2.24	1.84	3.76	2.23	1.72	2.49	2.22	1.55
8	m-Cl	7.48	1.57	1.27	3.36	1.65	1.20	2.26	1.59	1.02	1.58	1.58	0.89
9	p-Cl	9.00	1.68	1.44	3.87	1.75	1.35	2.60	1.73	1.20	1.79	1.69	1.04

Column temperatures: 20°C, flow rate: 1.0 mL min^{-1}.

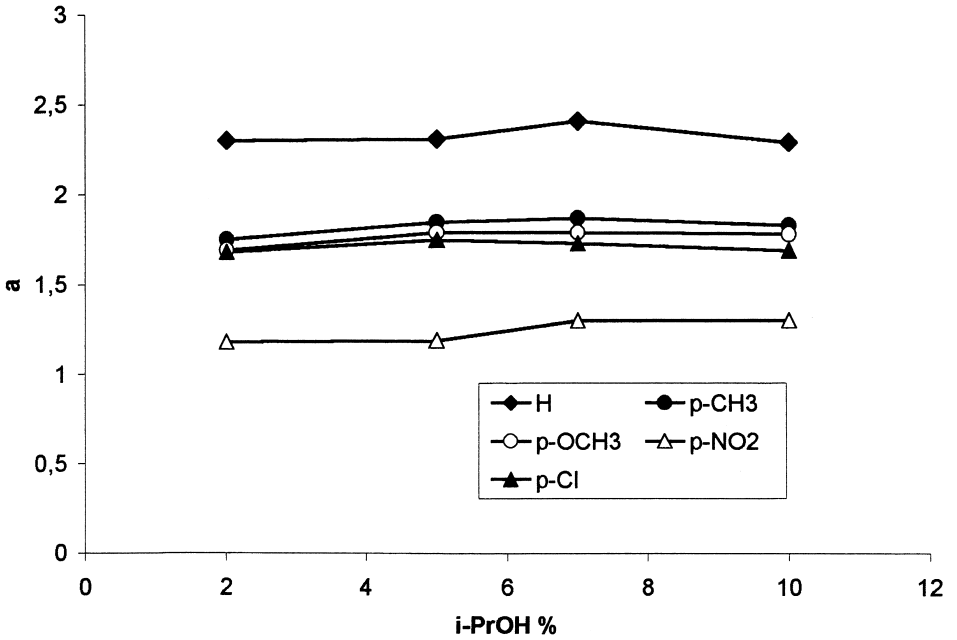

Figure 2. The substituents in the opposite position of benzene ring.

of interest to note, that the compound substituted with weak π-acceptor or π-donor groups are resolved better than those compounds substituted with strong π-acceptor or π-donor groups. The orders of chiral separation of substituent in the same position are: $\alpha_{p\text{-}CH3} > \alpha_{p\text{-}OCH3} > \alpha_{p\text{-}Cl} >> \alpha_{p\text{-}NO2}$, $\alpha_{m\text{-}Cl} >> \alpha_{m\text{-}NO2}$, $\alpha_{o\text{-}OCH3} >> \alpha_{o\text{-}Cl}$.

The Chiral Discrimination on Cellulose tris(3,5-Dimethylphenyl Carbamate) CSP

The position of the two methyl substituents in the benzene ring of the Chiralcel OD-CSP seems to be important in chiral separation of those phosphonates analytes on cellulose tris(3,5-dimethylphenyl carbamate) CSP. The chiral discrimination of the cellulose tris(3,5-dimethylphenyl carbamate) CSP also depends on the stereogenic fit of the enantiomer on the groove on the CSP.

The chiral separation results in compounds which the study slightly changed, with exception of compound 3, from the change the composition of mobile phase. The separation factors (α), some of which are almost the same (the change of α between ±0.01), resulted with the change of 2-propanol in the mobile phase.

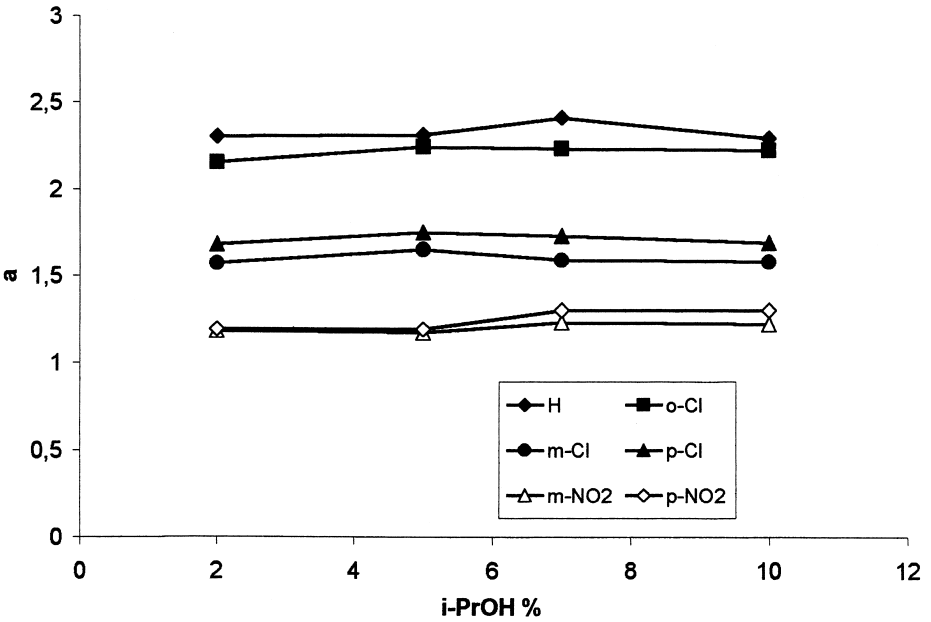

Figure 3. The substituents –Cl and –NO2 in the different positions of benzene ring.

Chiral Separation on N-(3,5-Dinitrobenzoyl) Leucine (DNB-leu) CSP

The separation data by N-(3,5-dinitrobenzoyl) leucine (DNB-leu) CSP, capacity factor *k*, α values, and the resolution factor Rs are indicated in Table 2. The *k,* α, and Rs values decreased with increasing the concentration of 2-propanol in the mobile phase for all compounds. All of the compounds can be baseline separated easily. The order of chiral separation of substituent in para position of the benzene ring was $\alpha_{p\text{-}OCH3} > \alpha_{p\text{-}NO2} > \alpha_{p\text{-}CH3} > \alpha_{p\text{-}H} > \alpha_{p\text{-}Cl}$. When the same substituent is in a different position of the benzene ring, the orders are: $\alpha_{m\text{-}Cl} > \alpha_{o\text{-}Cl} > \alpha_{p\text{-}Cl}$, $\alpha_{p\text{-}OCH3} > \alpha_{o\text{-}OCH3}$, $\alpha_{m\text{-}NO2} \cong \alpha_{p\text{-}NO2}$. From the results, it can be noticed that in N-(3,5-dinitrobenzoyl) leucine (DNB-leu) CSP, the position of substituents in the benzene ring was less important than that in cellulose tris(3,5-dimethylphenyl carbamate) CSP. The chiral discrimination of N-(3,5-dinitrobenzoyl) leucine (DNB-leu) CSP was based on π-π interaction and H-bond interaction, while in the cellulose CSP the chiral discrimination was based on π-π interaction, H-bond interaction, and the stereogenic fit in the chiral groove of the cellulose.

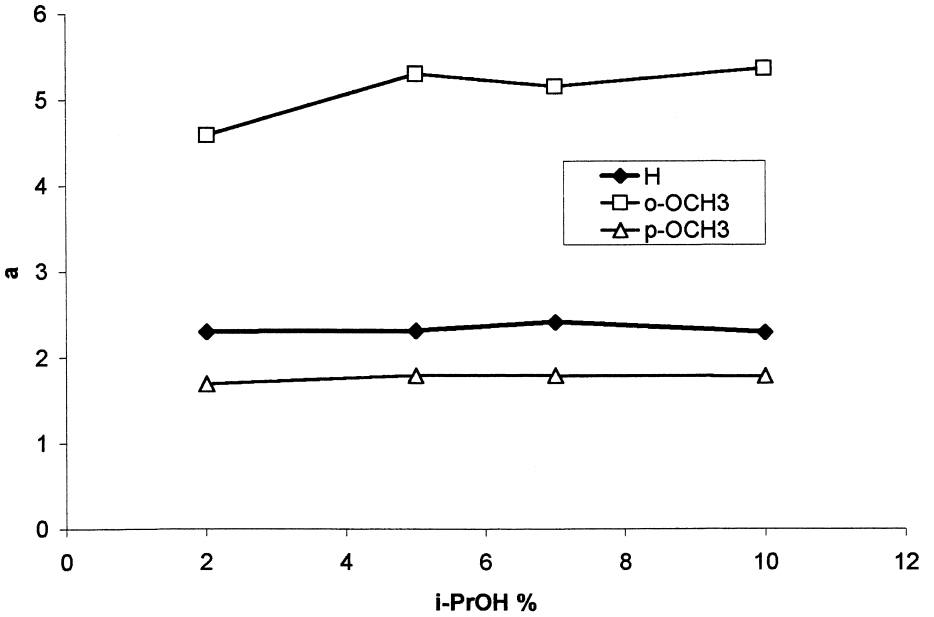

Figure 4. The substituent of –OCH3 in the different positions of benzene ring.

Table 2. k_1, k_2 and α Values of the Chiral Separation of Nine Organic Phosphonate Compounds Using Different Mobile Phase Compositions on DNBleu CSP

		5% 2-PrOH			10% 2-PrOH			15% 2-PrOH		
No.	R_1	k_2	α	Rs	k_2	α	Rs	k_2	α	Rs
1	H	1.27	1.43	1.33	0.81	1.27	0.75	3.27	1.47*	1.49
2	p-CH_3	2.92	1.65	2.33	1.48	1.51	1.59	1.00	1.45	1.23
3	o-OCH_3	5.17	1.35	1.72	2.31	1.28	1.21	1.77	1.26	1.04
4	p-OCH_3	7.26	2.01	3.49	3.06	1.78	2.61	1.89	1.69	2.11
5	m-NO_2	9.29	1.69	2.91	3.78	1.54	2.19	2.89	1.51	1.98
6	p-NO_2	7.70	1.67	2.81	3.19	1.52	2.06	2.84	1.49	1.92
7	o-Cl	3.02	1.42	1.76	1.49	1.34	1.20	1.05	1.28	0.84
8	m-Cl	3.12	1.57	2.17	1.40	1.44	1.41	1.20	1.43	1.30
9	p-Cl	1.12	1.35	1.08	0.74	1.25	0.67	3.20	1.47*	1.93

*The composition of mobile phase was 2% 2-propanol and 98% hexane.
Column Temperatures: 20°C, Flow Rate: 1.0mL min^{-1}.

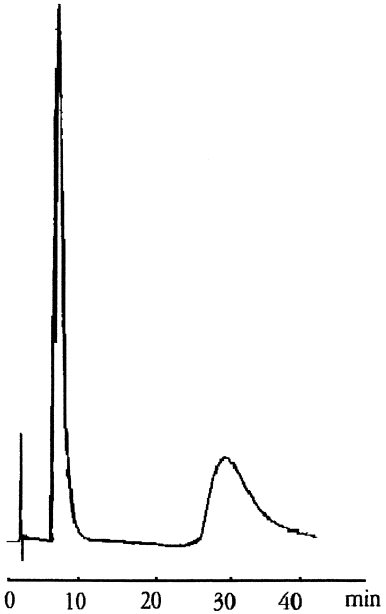

Figure 5. Compound No.3; mobile phase, n-hexane: 2-propanol (93:7), temperature 20°C, on cellulose tris(3,5-dimethylphenyl carbamate) CSP.

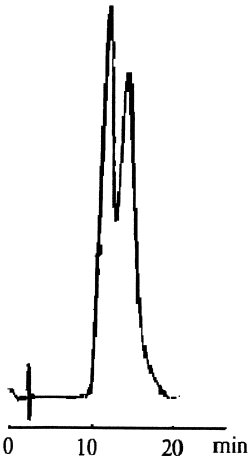

Figure 6. Compound No.5; mobile phase, n-hexane: isopropanol (93:7), temperature 20°C, on cellulose tris(3,5-dimethylphenyl carbamate) CSP.

Table 3. k_1, k_2 and α Values of the Chiral Separation of Nine Organic Phosphonate Compounds at Different Column Temperature Compositions on the tris(3,5-Dimethylphenyl Carbamate) Cellulose CSP

		10°C			20°C			30°C			40°C		
No.	R_1	k_2	α	Rs	k_2	α	Rs	k_2	α	Rs	k_2	α	Rs
1	H	3.77	2.48	1.86	2.73	2.19	1.57	2.03	1.95	1.29	1.69	1.74	1.06
2	p-CH_3	2.76	1.92	1.39	2.05	1.75	1.14	1.49	1.54	0.83	1.23	1.38	0.60
3	o-OCH_3	12.1	5.35	2.95	8.87	4.68	2.79	5.58	3.77	2.46	4.24	3.11	2.17
4	p-OCH_3	4.59	1.88	1.52	3.28	1.69	1.24	2.42	1.53	0.97	1.98	1.34	0.67
5	m-NO_2	4.47	1.27	0.69	3.52	1.23	0.58	2.58	1.16	0.39	2.23	1.00	0
6	p-NO_2	6.05	1.36	0.90	5.00	1.30	0.76	3.27	1.18	0.46	2.71	1.00	0
7	o-Cl	4.00	2.37	1.83	2.98	2.10	1.53	2.17	1.87	1.26	1.79	1.64	0.99
8	m-Cl	2.40	1.66	1.11	1.94	1.60	0.98	1.39	1.41	0.67	1.15	1.23	0.40
9	p-Cl	2.73	1.89	1.36	2.21	1.70	1.12	1.57	1.50	0.80	1.29	1.34	0.56

Mobile Phase: 8% Isopropyl Alcohol, Flow Rate: 1.0 mL min^{-1}.

The above results indicated that using cellulose tri(3,5-drimethylphenylcarbamate CSP, provide resolution better than that obtained by the Pirkle's type *N*-(3,5-dinitrobenzoyl) leucine (DNB-leu) CSP, expect for the compounds with the nitro substitution. The position of the substituent group plays an important role in the chiral separation. The compounds with ortho substituent are better resolved than their para- substituent analogs. It is of interest to note, that the separation factor of compound 3, substituted with o-OCH_3, is larger than 5.

The influence of temperature on the chiral separation is well documented.(23,24) In this paper, the effects of column temperatures on the chiral separation have been investigated in the range of 10-40°C for all the compounds. The results are shown in Table 3.

CONCLUSION

The resolution of nine racemic o,o-dialkyl-benzyloxycarbonyl-aminoarylmethyl-phosphonates is achieved by HPLC on cellulose tris(3,5-dimethylphenylcarbamate) and DNB-leu CSPs. The chiral separation depends on the type of substituents on those compounds. The chiral separation of organophosphonates is also influenced more by the position of substituent of the phosphonate esters, rather than that of the nature of the substituents on the benzene ring being a π-donor or a π-acceptor on cellulose tris(3,5-dimethyl-phenylcarbamate) CSP. In N-(3,5-dinitrobenzoyl) leucine (DNB-leu) CSP, the position of substituents in the benzene ring was less important than that in cellulose tris(3,5-dimethylphenyl carbamate) CSP. The chiral discrimination of N-(3,5-dinitrobenzoyl) leucine (DNB-leu) CSP was based on π-π interaction and H-bond interaction.

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