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Effect of mobile phase amine additives on enantioselectivity for phenylalanine analogs

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Abstract

The use of basic mobile phase additives enhances the chiral separation of underivatized phenylalanine analogs on a common amylosic column. These additives appear to exert their effect through differential disruption of hydrogen binding involved in the recognition process. Several examples of amine increasing retention of the second eluting enantiomer while decreasing retention of the other enantiomer were observed. This gave dramatically increased selectivity and was most commonly observed with cyclopropylamine and cyclobutylamine. The effect was attributed to steric factors involved in the elution process.

Keywords: Enantiomer separation; Mobile phase composition; Phenylalanine analogues; Amine additives

1. Introduction

Underivatized amino acids possess both free amine and free carboxylic acid groups typically resulting in excessive retention on normal-phase chiral columns. In a recent publication [1], we reported the separation of underivatized amino acids on a polysaccharide-based chiral stationary phase made possible through the incorporation of various acidic mobile phase additives. While mobile phase additives are often used to minimize peak broadening arising from unwanted interactions between polar solutes and the stationary phase, it was found that acidic additives could be used to effect elution of strongly retained amino acids and enhance enantioselectivity. The proposed mode of action invoked the weakening of hydrogen bond interactions involved in chiral recognition, allowing elution and an increase in the relative strength of the discriminating $\pi-\pi^*$ interaction.

Basic mobile phase additives are also often used to minimize peak broadening of acidic molecules. In this work the effect of basic additives on the chiral separation of underivatized phenylalanine analogs is examined with some intriguing results of dramatically increased selectivity. Techniques developed to separate underivatized amino acids on polysaccharide stationary phases should be applicable to other charged analytes as well.

2. Experimental

2.1. Reagents

All reagents used in this study were reagent grade

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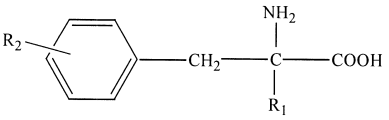
Table 1
Structures of phenylalanine analogs used in this study



Analog	R1	R2
1	Me	H
2	H	*p*-OCH_3
3	Me	*m*-OH
4	Me	*p*-OH
5	H	H
6	H	*p*-OH
7	H	*o*-OH
8	H	*m*-OH
9	H	*m*-F
10	H	*o*-F
11	H	*p*-F
12	H	*p*-CL
13	H	*p*-Br
14	H	*p*-I
15	H	*p*-NO_2

or better. Trifluoroacetic acid and all the amines used in this study were obtained from Sigma–Aldrich (St. Louis, MO, USA), and were used without further purification. HPLC-grade hexane was purchased from EM Sciences (Gibbstown, NJ, USA). Absolute ethanol was obtained from Aaper Alcohol and Chemical (Shelbyville, KY, USA). Phenylalanine analogs used in this study (Table 1) were purchased from Sigma–Aldrich. Separate solutions of racemic mixtures and individual enantiomer of each phenylalanine analog were prepared according to the sample preparation procedures described below, at a final concentration of about 2 mg/ml.

2.2. Sample preparation procedure

Phenylalanine analogs were weighed into 10-ml volumetric flasks, dissolved with sonication in about 5 ml ethanol–trifluoroacetic acid (9:1, v/v) and diluted to volume with ethanol.

2.3. Chromatography

Chromatographic studies were performed on a HP 1100 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a vacuum degasser, a quaternary pump, an autosampler, a thermostated-column device and a variable-wavelength UV detector. The chromatographic data were acquired and processed with computer-based HP Chemstation

Table 2
The effect of various amine additives on enantioselectivity for analogs in Table 1

Amine	Probe														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	1.00	1.49	1.43	1.16	1.23	1.49	1.17	1.00	1.14	1.28	1.32	1.35	1.38	1.41	1.33
DEA	1.00	1.45	1.31	1.16	1.22	1.86	1.15	1.00	1.15	1.28	1.31	1.34	1.36	1.39	1.35
TEA	1.09	1.53	1.22	1.00	1.23	1.60	1.17	1.06	1.11	1.31	1.35	1.39	1.40	1.41	1.38
DiPrA	1.00	1.46	1.39	1.22	1.22	1.67	1.16	1.00	1.15	1.28	1.33	1.36	1.38	1.41	1.35
PrA	1.09	1.99	1.17	1.03	1.42	1.40	1.15	1.08	1.38	1.38	1.63	2.13	2.38	2.65	2.46
iPrA	1.09	2.08	1.20	1.09	1.45	1.43	1.16	1.08	1.42	1.42	1.63	2.22	2.47	2.73	2.60
cPrA	1.17	2.43	1.15	1.00	1.62	1.53	1.19	1.13	1.63	1.54	1.90	2.72	3.10	3.44	3.15
BA	1.07	1.92	1.20	1.05	1.38	1.38	1.15	1.00	1.35	1.36	1.59	2.00	2.26	2.50	2.30
iBA	1.08	1.60	1.18	1.00	1.26	1.34	1.12	1.00	1.19	1.26	1.37	1.56	1.66	1.75	1.66
tBA	1.00	2.00	1.30	1.16	1.43	1.43	1.15	1.00	1.39	1.43	1.63	2.09	2.29	2.48	2.43
cBA	1.17	2.33	1.11	1.00	1.56	1.50	1.19	1.13	1.52	1.49	1.87	2.57	2.89	3.13	3.00
AmA	1.09	1.92	1.20	1.00	1.40	1.43	1.19	1.08	1.36	1.39	1.61	2.02	2.21	2.39	2.21
iAmA	1.08	1.82	1.21	1.00	1.36	1.41	1.06	1.18	1.31	1.37	1.55	1.86	2.02	2.16	2.07
HexA	1.06	1.72	1.21	1.00	1.30	1.35	1.15	1.06	1.24	1.29	1.45	1.74	1.89	2.04	1.88
cHexA	1.11	1.80	1.18	1.00	1.32	1.44	1.19	1.08	1.29	1.36	1.52	1.76	1.88	1.97	1.90
HeptA	1.00	1.71	1.28	1.11	1.30	1.34	1.15	1.00	1.25	1.29	1.46	1.66	1.88	2.02	1.87

DEA, Diethyl amine; TEA, triethylamine; DiPrA, diisopropyl amine; PrA, *n*-propylamine; iPrA, isopropylamine; cPrA, cyclopropylamine; BA, *n*-butylamine; iBA, isobutylamine; tBA, *tert*.-butyl amine; cBA, cyclobutylamine; AmA, amylamine; iAmA, isoamylamine; HexA, *n*-hexylamine; cHexA, cyclohexylamine; HeptA, *n*-heptylamine

Table 3
The effect of various amine additives (see Table 2 for identification) on the retention factor for the second eluting enantiomer (k'_2) for analogs in Table 1

Amine	Probe														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	1.58	3.93	3.92	5.44	2.30	10.9	2.82	4.34	2.13	2.61	2.56	2.77	3.02	3.12	6.46
DEA	1.25	2.89	2.87	3.82	1.71	9.44	2.00	3.05	1.62	1.91	1.93	2.08	2.27	2.52	5.39
TEA	1.66	3.83	3.43	4.38	2.17	10.9	2.70	3.84	2.01	2.47	2.54	2.71	2.93	3.23	7.26
DiPrA	1.31	3.20	3.13	4.42	1.88	9.58	2.25	3.36	1.76	2.11	2.09	2.25	2.45	2.72	5.70
PrA	1.28	4.03	2.54	3.59	2.02	5.24	2.03	3.08	2.00	2.09	2.40	3.30	3.96	4.81	9.71
iPrA	1.32	4.35	2.66	3.83	2.17	5.64	2.13	3.24	2.13	2.25	2.52	3.60	4.30	5.17	10.8
cPrA	1.47	5.16	2.84	3.71	2.39	5.85	2.12	3.25	2.42	2.39	2.91	4.40	5.36	6.52	12.8
BA	1.23	3.82	2.48	3.53	1.94	5.09	1.98	3.36	1.91	2.02	2.29	3.31	3.67	4.41	8.77
iBA	1.50	3.87	3.03	4.25	2.18	6.23	2.48	3.36	2.08	2.31	2.45	2.92	3.32	3.82	7.97
tBA	1.30	4.24	2.86	4.19	2.14	6.03	2.16	3.25	2.08	2.27	2.50	3.38	3.98	4.67	9.89
cBA	1.66	5.51	3.16	4.34	2.53	6.52	2.47	3.78	2.46	2.55	3.13	4.50	5.43	6.41	13.6
AmA	1.35	3.89	2.77	3.67	2.00	5.53	2.11	3.12	1.96	2.11	2.36	3.10	3.66	4.35	8.56
iAmA	1.22	3.32	2.48	3.20	1.76	4.74	2.79	2.30	1.71	1.88	2.06	2.59	3.04	3.59	8.22
HexA	1.52	4.27	3.11	4.38	2.32	6.55	2.57	3.91	2.25	2.46	2.65	3.34	3.87	4.53	9.27
cHexA	1.34	3.47	2.67	3.41	1.87	5.13	2.01	2.89	1.75	1.97	2.17	2.63	3.03	3.50	7.09
HeptA	1.21	3.48	2.58	3.79	1.87	5.27	2.04	3.01	1.82	2.00	2.15	2.56	3.10	3.62	7.33

software. A ChiralPak AD column (250×4.6 mm) was purchased from Chiral Technologies (Exton, PA, USA) and was used as received. Unless otherwise noted, chromatographic studies were performed at 40°C with a 1.0 ml/min flow-rate. The mobile phase consisted of hexane–ethanol (95:5, v/v) containing 0.2% (v/v) trifluoracetic acid and 0.1% (v/v) basic additive. After equilibrium had been achieved, 5-μl of sample solution was injected. Detection was carried out at 210 nm. The dead time was estimated by the retention time of the first solvent disturbance peak.

Table 4
The effect of various amine additives (see Table 2 for identification) on the retention factor for the first eluting enantiomer (k'_1) for analogs in Table 1

Amine	Probe														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	1.58	2.64	2.74	4.69	1.87	7.31	2.40	4.34	1.87	2.04	1.94	2.04	2.25	2.21	4.85
DEA	1.25	1.99	2.18	3.29	1.40	5.09	1.74	3.05	1.41	1.50	1.47	1.55	1.67	1.82	4.00
TEA	1.53	2.50	2.81	4.38	1.77	6.77	2.30	3.61	1.80	1.89	1.88	1.95	2.09	2.29	5.26
DiPrA	1.31	2.18	2.25	3.62	1.53	5.75	1.94	3.36	1.53	1.65	1.57	1.65	1.78	1.93	4.21
PrA	1.17	2.02	2.18	3.47	1.43	3.74	1.76	2.85	1.44	1.51	1.48	1.55	1.67	1.82	3.94
iPrA	1.21	2.10	2.21	3.51	1.49	3.95	1.85	2.99	1.50	1.58	1.54	1.62	1.74	1.90	4.14
cPrA	1.26	2.13		3.71	1.48	3.83	1.78	2.88	1.48	1.55	1.54	1.61	1.73	1.90	4.06
BA	1.15	1.99	2.07	3.36	1.40	3.69	1.73	3.36	1.41	1.49	1.44	1.65	1.62	1.76	3.81
iBA	1.39	2.42	2.56	4.25	1.73	4.66	2.22	3.36	1.75	1.84	1.79	1.87	2.00	2.18	4.81
tBA	1.30	2.11	2.19	3.62	1.49	4.21	1.88	3.25	1.50	1.59	1.53	1.62	1.74	1.89	4.07
cBA	1.43	2.37	2.85	4.34	1.63	4.33	2.08	3.34	1.62	1.72	1.67	1.75	1.88	2.04	4.51
AmA	1.24	2.03	2.31	3.67	1.43	3.86	1.77	2.89	1.44	1.52	1.47	1.53	1.65	1.82	3.88
iAmA	1.13	1.82	2.05	3.20	1.30	3.37	2.62	1.95	1.31	1.38	1.33	1.39	1.50	1.66	3.98
HexA	1.43	2.48	2.57	4.38	1.79	4.86	2.24	3.69	1.81	1.90	1.83	1.92	2.05	2.22	4.93
cHexA	1.21	1.93	2.26	3.41	1.41	3.55	1.69	2.67	1.35	1.44	1.43	1.49	1.61	1.78	3.72
HeptA	1.21	2.03	2.02	3.40	1.44	3.92	1.78	3.01	1.45	1.54	1.47	1.54	1.65	1.80	3.92

3. Results and discussion

3.1. Effect of structure

Chromatographic results are given in Tables 2–4. The variety of phenylalanine probes chromatographed on the AD column allows some generalizations about separation mechanisms. Methyl substitution on the chiral carbon (probes 5→1; 6→4; 8→3) results in lower retention for all three pairs. Decreased selectivity was observed for the phenylalanine and tyrosine substitutions. Selectivity for the methyl substituted, *m*-hydroxy analog was much larger (1.43 vs. 1.00) than for the unsubstituted *m*-hydroxy analog. Together these results indicate that the presence of a methyl group on the chiral carbon alters the fit of the probe into a binding pocket. Results with the *m*-hydroxy analog suggest that the methyl group forces binding in an orientation that can more easily accommodate the *m*-hydroxy group.

Hydroxyl substitution of the aromatic ring gives pronounced effects on retention. Substitution at the *p*-position (Phe→Tyr; 1→4) gives dramatically increased retention of both enantiomers and slightly increased selectivity. At the *o*-position (Phe→7) the effect is minimal while at the *m*-position (Phe→8; 1→3) retention is increased but selectivity varies. These results suggest that the *p*-OH of tyrosine can form an additional hydrogen bond with the stationary phase which increases retention but does little to differentiate enantiomers. The *o*-OH cannot form this bond and a *m*-OH appears to fit into the binding pocket differently, possibly due to steric interferences. Probes 9–11 are substituted at the *o,m,p*-positions with fluorine which cannot form hydrogen bonds with the stationary phase. The effect of these substitutions is minimal.

Seven of the analogs represent substitution at the *p*-position. As discussed above a hydroxyl group in this position appears to participate in an additional hydrogen bond with the stationary phase. For the rest of the analogs, retention and selectivity appear to increase with size of the substituent (Phe→11→12→13→14→2→15) except for the nitro-substituted analog. The increased retention of this analog suggests that this group also forms an additional hydrogen bond. The Phe→11→12→13→14→15 series also tracks π acidity which could account for enhanced binding with aromatic regions of the stationary phase. The position of analog 2, *p*-methoxy phenylalanine, in this series suggests a greater importance of steric factors relative to electronic effects.

The structural effects discussed above depict a complex binding scenario with contributions of additional hydrogen bonds, steric effects and multiple possible orientations of analytes. In a recent review, Yashima [2] represents the stationary phase as a chiral helix with internal carbamate hydrogen bonding sites and external aromatic groups. The aromatic groups may participate in π–π* interactions, as postulated for aromatic regions of Pirkle-type selectors, or may act as steric barriers. Changes in the aromatic group such as changing the electronic nature and positions of the substitutions do not have as dramatic effect as expected except when these changes affect the helicity of the polymer. A recent report by Kubota et al. [3] shows that aromaticity is not a requirement for enantioselectivity on a polysaccharide stationary phase, suggesting that steric effects may be more important than π–π* interactions.

Booth and Wainer [4] further complicate the situation by invoking a two stage process where formation of hydrogen bonds between analyte and carbamate groups is followed by conformational changes resulting in additional interactions. As complex as such representations of chiral binding derived from modeling experiments are, they do not begin to address the additional level of complexity induced by the mobile phase. Mobile phase properties may affect the tertiary structure of the stationary phase. Chromatography requires both binding and elution. Elution requires access of displacing modifier to the hydrogen bonding sites. Mobile phase properties may affect the tertiary structure of the stationary phase. With this level of complexity, interpretation of the effects of mobile phase additives will be limited to observation and conjecture.

3.2. Effect of additives on selectivity

All probes were affected by the incorporation of amine additives into the mobile phase. Nearly all show an increase in selectivity in response to one or more additive. Probes 1 and 8 went from no selec-

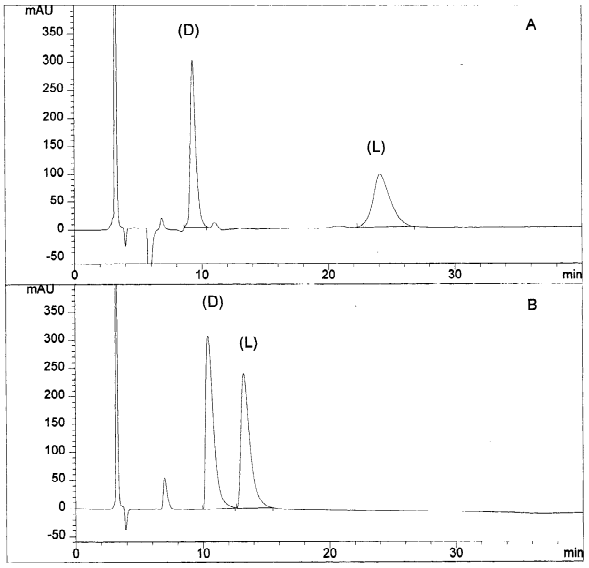


Fig. 1. Chromatogram showing the effect of amine additive on the separation of *p*-iodophenylalanine on an AD column. Mobile phase was ethanol–hexane (5:95, v/v) containing 0.2% (v/v) of trifluoroacetic acid and (A) 0.1% (v/v) of cyclopropylamine or (B) no amine. Flow-rate is 1 ml/min at 40°C with UV detection at 210 nm.

tivity without additive to respectable values of 1.17–1.18. Selectivity for probe 14 increased from 1.41 without additive to 3.44 with cyclopropylamine (Fig. 1). Additive effects may be attributed to three possible mechanisms. Amine additives may act by disrupting hydrogen bonds involved in analyte binding, resulting in decreased retention. Additives could form an ''ion-pair'' complex with analytes which may show different selectivity in interacting with the stationary phase. Formation of an ion-pair complex with the analyte likely results in a complex of lower polarity and higher solubility in the mobile phase. Increased solubility in the mobile phase is unlikely to increase selectivity. It is also possible that mobile phase additives alter the tertiary structure of the stationary phase. These additives are present at low levels and it is unlikely that they exert much influence on this tertiary structure.

Some conclusions may be made upon review of the 225 probe/additive combinations presented in Tables 2–4. Secondary and tertiary amines have little effect on selectivity. Diethyl amine and dipropyl amine decrease both k_1' and k_2' with little differentiation, except for tyrosine. Selectivity for tyrosine is

Table 5
Selectivity obtained from amine additives (see Table 2 for identification) showing decreased or no effect on enantioselectivity for analogs in Table 1

Amine	Probe														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	1.00	1.49	1.43	1.16	1.23	1.49	1.17	1.00	1.14	1.28	1.32	1.35	1.38	1.41	1.33
DEA	1.00	1.45	1.31	1.16	1.22		1.15	1.00	1.15	1.28	1.31	1.34	1.36	1.39	1.35
TEA		1.53	1.22	1.00	1.23		1.17		1.11	1.31	1.35	1.39	1.40	1.41	1.38
DiPrA	1.00	1.46	1.39	1.22	1.22		1.16	1.00	1.15	1.28	1.33	1.36	1.38	1.41	1.35
PrA			1.17	1.03		1.40	1.15								
iPrA			1.20	1.09		1.43	1.16								
cPrA			1.15	1.00			1.19								
BA			1.20	1.05		1.38	1.15	1.00							
iBA			1.18	1.00		1.34	1.12	1.00		1.26					
tBA	1.00		1.30	1.16		1.43	1.15	1.00							
cBA			1.11	1.00		1.50	1.19								
AmA			1.20	1.00		1.43	1.19								
iAmA			1.21	1.00		1.41	1.06								
HexA			1.21	1.00		1.35	1.15			1.29					
cHexA			1.18	1.00		1.44	1.19								
HeptA	1.00		1.28	1.11		1.34	1.15	1.00		1.29					

increased with both of these amines, presumably through their attenuation of the non-discriminating *p*-OH hydrogen bond. Triethylamine, one of the most commonly used amine additives, has very little effect on retention or selectivity. Tertiary amines cannot act as hydrogen bond donors and secondary amines are considerably poorer donors than primary amines.

Among primary amine additives there are examples in Tables 2–4 of decreased or unchanged selectivity (see Table 5). These include hydroxy substituted analogs which show sharp, non-discriminating drops in retention in response to inclusion of primary amines. This suggests a facile disruption of hydrogen bonds external to the binding cavity.

Table 6 shows examples of increase enantioselectivity arising from primary amine additives that derive from a greater decrease in retention of the first-eluting enantiomer relative to the second. There are 62 such examples with selectivity increases ranging from moderate to 1.4×. If amine additives act through disruption of hydrogen bonds involved in attaching analytes to the carbamate cleft, it is reasonable to expect that the additive will have less access to dislodge the more tightly bound second eluting enantiomer. The decline in k_2' will be less than the decline in k_1' and selectivity will increase. Some of these examples show very little decline in k_2'. Fig. 2 shows the effect various amine additives have on k_1' and k_2' for phenyalanine.

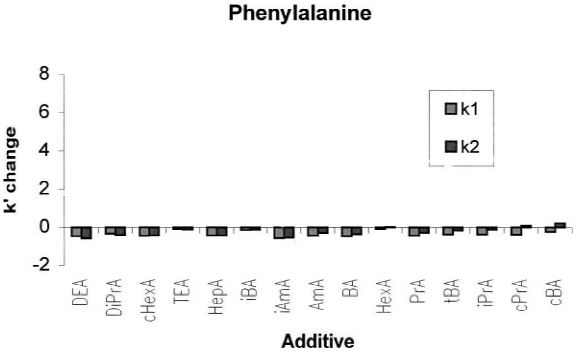


Fig. 2. Plot of capacity factor changes for phenylalanine in response to various amine additives. See Table 2 for identification of the amines.

There are also numerous (61) examples where k_2' actually increases with the addition of amine additive. Fig. 3 shows the effect of additives on k_1' and k_2' for *p*-nitrophenyalanine. Coupled with a decreased k_1', selectivity increases dramatically (Table 7), up to 2.4×. These examples include the larger *p*-substituted probes. The structure of the amine additive appears to impact this effect with cyclopropylamine

Table 6
Selectivity obtained from amine additives (see Table 2 for identification) with increased enantioselectivity arising from larger decrease in k_1' relative to k_2' for analogs in Table 1

Amine	Probe														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	1.00	1.49	1.43	1.16	1.23	1.49	1.17	1.00	1.14	1.28	1.32	1.35	1.38	1.41	1.33
DEA						1.86									
TEA		1.53			1.23	1.60		1.06							
DiPrA				1.22	1.22	1.67									
PrA	1.09				1.42			1.08	1.38	1.38	1.63				
iPrA	1.09				1.45			1.08	1.42	1.42	1.63				
cPrA	1.17					1.53		1.13		1.54					
BA	1.07	1.92			1.38				1.35	1.36	1.59				
iBA	1.08	1.60			1.26				1.19		1.37				
tBA					1.43				1.39	1.43	1.63				
cBA								1.13		1.49					
AmA	1.09	1.92			1.40			1.08	1.36	1.39	1.61				
iAmA	1.08	1.82			1.36			1.18	1.31	1.37	1.55	1.86			
HexA	1.06							1.06							
cHexA	1.11	1.80			1.32			1.08	1.29	1.36	1.52	1.76			
HeptA		1.71			1.30				1.25		1.46	1.66			

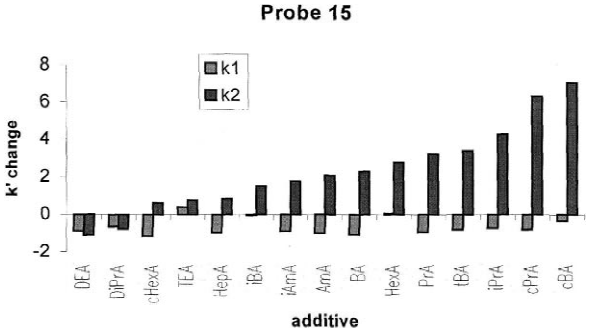


Fig. 3. Plot of capacity factor changes for *p*-nitrophenylalanine in response to various amine additives. See Table 2 for identification of the amines.

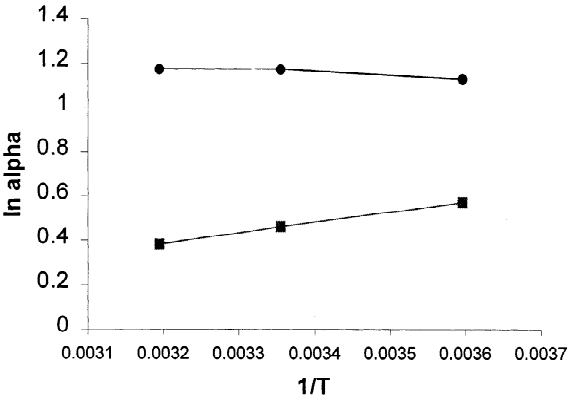


Fig. 4. Van't Hoff plot showing the effect of cyclopropylamine on the thermodynamic behavior of probe 14 on an AD column. The closed circles represent selectivity obtained at different temperatures with cyclopropylamine in the mobile phase while closed squares represent data generated without additive.

and cyclobutylamine being the most successful in inducing this effect. The explanation for this phenomenon is not readily apparent. If the additive were to induce a change in the tertiary structure of the amylosic polymer it is plausible that k_2' could increase. Increased retention could arise from better access to hydrogen bonding sites. This would not explain the role of substitute size in enhancing the effect or the importance of the small ring portion of the amine additive. Any change in the tertiary structure should be in response to a bulk property of the additive. Occasional increases in k_1' would also be expected.

This phenomenon was investigated further by varying the separation temperature to determine the effect of additive on thermodynamic parameters. Fig. 4 shows Van't Hoff plots of the results for probe 14, with and without cyclopropylamine additive. Such plots make use of the relationship described by Koppenhoefer and Bayer [5] for chiral GC separations:

Table 7
Selectivity obtained from amine additives (see Table 2 for identification) with increased enantioselectivity arising from a decrease in k_1' and an increase in k_2' for analogs in Table 1

Amine	Probe														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	1.00	1.49	1.43	1.16	1.23	1.49	1.17	1.00	1.14	1.28	1.32	1.35	1.38	1.41	1.33
DEA															
TEA	1.09														
DiPrA															
PrA		1.99										2.13	2.38	2.65	2.46
iPrA		2.08										2.22	2.47	2.73	2.60
cPrA		2.43			1.62				1.63		1.90	2.72	3.10	3.44	3.15
BA												2.00	2.26	2.50	2.30
iBA												1.56	1.66	1.75	1.66
tBA		2.00										2.09	2.29	2.48	2.43
cBA	1.17	2.33			1.56				1.52		1.87	2.57	2.89	3.13	3.00
AmA												2.02	2.21	2.39	2.21
iAmA													2.02	2.16	2.07
HexA		1.72			1.30				1.24		1.45	1.74	1.89	2.04	1.88
cHexA													1.88	1.97	1.90
HeptA													1.88	2.02	1.87

$$\ln \alpha = -\delta\Delta H^0/RT + \delta\Delta S^0/R \quad (1)$$

Plotting ln α against inverse absolute temperature is expected to give a straight line with a slope of $-\delta\Delta H^0$ and a y-intercept of $\delta\Delta S^0$. This relationship assumes that the phase ratios is the same for each enantiomer (common binding site), that $\delta\Delta H^0$ does not change with temperature (constant heat capacity) and ignores that the observed (or apparent) α differs from true α due to the unavoidable inclusion of non-specific retention. This means that $\delta\Delta H^0$ and $\delta\Delta S^0$ values as usually determined also include non-specific enthalpic and entropic contributions. Despite these limitations, Van't Hoff plots usually behave as predicted in Eq. (1). The plots shown in Fig. 4 are representative of results obtained for all probes with this additive. In the absence of additive, a typical straight line is obtained yielding an apparent $\delta\Delta H^0$ value of -947 cal/mol and an apparent $\delta\Delta S^0$ value of -2.26 cal/mol/K. With additive, a flat response is observed with selectivity not changing between 40 and 25°C and then declining slightly as temperature is lowered to 5°C. The additive does not change elution order so the additive has not induced an entropically driven separation. Flat, curved Van't Hoff plots are typically attributed to competing binding sites. Such behavior could also be expected from a dynamic two stage binding process as suggested by Booth and Wainer [4].

Extending the argument that elution needs to be considered as part of chiral recognition allows for steric contributions of mobile phase components. In empirical modeling studies, Blackwell et al. [6] found that mobile phase modifier characteristic volume was an important determinant of selectivity for phenyalanine analogs on an amylosic (AS) column. This suggests that a modifier's ability to displace analytes may be impacted not only by its hydrogen bonding properties but by its ability to penetrate the selector–analyte complex to disrupt the hydrogen bonds holding the complex together. The second-eluting enantiomer would have a tighter complex and be harder for large modifiers to disrupt than the first eluting enantiomer. It may be postulated that additives could further restrict access into the k_2' complex by hydrogen bonding with portions of the stationary phase around the complex. In essence, the additive would be wedging in the more retained enantiomer. This representation accommodates the unexpected increase in k_2' plus steric factors of the probes and additives.

References

[1] Y.K. Ye, R.W. Stringham, J. Chromatogr. A (2001) in press.
[2] E. Yashima, J. Chromatogr. A 906 (2001) 105.
[3] T. Kubota, C. Yamamoto, Y. Okamoto, J. Am. Chem. Soc. 122 (2000) 4056.
[4] T.D. Booth, I.W. Wainer, J. Chromatogr. A 737 (1996) 157.
[5] B. Koppenhoefer, E. Bayer, Chromatographia 19 (1984) 123.
[6] J.A. Blackwell, R.W. Stringham, D. Xiang, R.E. Waltermire, J. Chromatogr. A 852 (1999) 383.