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Liquid Chromatographic Direct Resolution of Flecainide and its Analogs on a Chiral Stationary Phase Based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic Acid

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ABSTRACT Flecainide, an antiarrythmic agent, and its analogs were resolved on a high performance liquid chromatographic chiral stationary phase (CSP) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid with the use of a mobile phase consisting of methanol-acetonitrile-trifluoroacetic acid-triethylamine (80/20/0.1/0.3, v/v/v/v). The chiral resolution was quite successful, the separation factors (α) and the resolutions (R_S) for 20 analytes including flecainide being in the range of 1.19–1.82 and 1.73–6.80, respectively. The ortho-substituent of the benzoyl group of analytes was found to cause decrease in the retention times of analytes probably because of the conformational deformation of analytes originated from the steric hindrance exerted by the ortho-substituent. *Chirality 22:693–698, 2010.* © 2009 Wiley-Liss, Inc.

KEY WORDS: chiral stationary phase; chiral resolution; enantiomer separation; flecainide; liquid chromatography; (+)-18-crown-6)-2,3,11,12-tetracarboxylic acid

INTRODUCTION

Flecainide, *N*-(2-piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide, has proven to be an effective antiarrythmic agent used as its acetate salt form in the treatment of cardiac arrythmias.[1] Flecainide is a chiral drug containing one chiral center and consequently it is very important to develop enantioselective analytical methods of separating and quantifying the two enantiomers to elucidate their stereoselective differences in pharmacokinetics and pharmacodynamics.

Among various enantioselective analytical methods, liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has been known to be the most accurate, convenient and economic means for the exact determination of the enantiomeric composition or the enantiomeric purity of chiral compounds.[2] However, only a few efforts have been devoted to the development of enantioselective analytical methods of separating and quantifying the two enantiomers of flecainide on liquid chromatographic CSPs. For example, the recoveries of (*R*)- and (*S*)-flecainide from animal plasma and tissues have been quantitatively determined by HPLC on Chiralpak AD column.[3] Flecainide has also been resolved by supercritical fluid chromatography coupled to electrospray mass spectrometry on Chiralcel OJ column.[4] CSPs based on immobilized proteins were also used in the separation of the two enantiomers of flecainide by HPLC.[5,6] In addition, cation exchange CSPs based on β-amino sulfonic acid-terminated dipeptide derivatives were used to separate the two enantiomers of flecainide by nonaqueous capillary electrochromatography.[7]

Crown ether-based CSPs have been known to be very effective for the resolution of racemic primary amino compounds.[8] Especially CSP **1** (Fig. 1) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**2**, Fig. 1) has been known to be quite effective for the resolution of α-,[9] β-,[10] and γ-amino acids,[11] racemic primary amines, amino alcohols,[12] racemic fluoroquinolone antibacterials[13] and *di*- and *tri*-peptides.[14] While crown ether-based CSPs have been known to be useful only for the resolution of racemic primary amino compounds, CSP **1** was found to be effective for the resolution of secondary amino compounds. For example, methoxyphenamine containing a secondary amino group and secondary amino alcohols related to β-blockers have been resolved on CSP **1**.[15,16] However, racemic cyclic secondary amino compounds have not been resolved on CSP **1**. Flecainide is actually a cyclic secondary amino compound. Consequently, resolution of flecainide on CSP **1** will be the first example for the resolution of cyclic secondary amino compound on crown ether-based CSPs. In this study, we wish to extend the use of CSP **1** to the resolution of flecainide (**3**) and its analogs (**4**–**22**) shown in Figure 2. To elucidate the role of the 2,5-di(2,2,2-trifluoroethoxy)benzoyl group of flecainide in the chiral recognition, analogs containing various benzoyl group were prepared and their chromatographic behaviors on CSP **1** were compared with each other.

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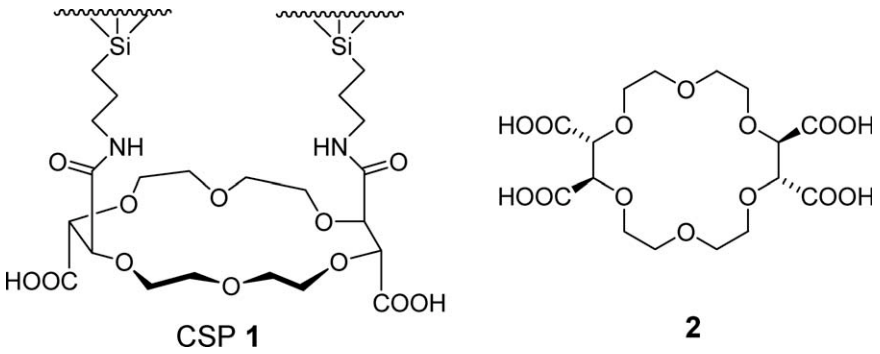

Fig. 1. Structures of CSP **1** and (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (**2**).

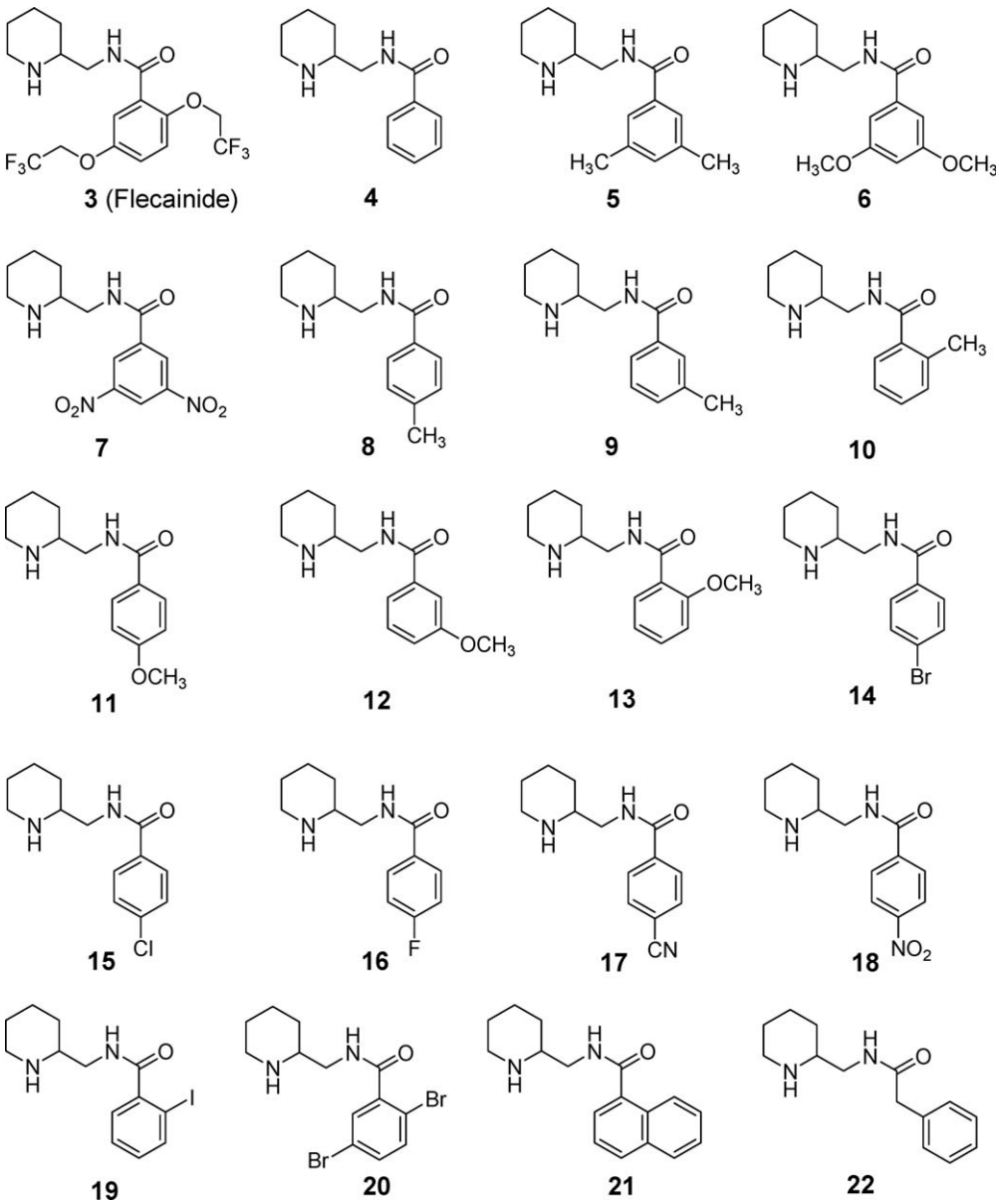

Fig. 2. The structures of flecainide (**3**) and its analogs (**4**-**22**).

TABLE 1. Resolution of flecainide (3) and its analogs (4, 8, 13, and 18) on CSP 1 with the variation of the content of ethanol (EtOH) or methanol (MeOH) in acetonitrile (ACN) as mobile phase containing trifluoroacetic acid (TFA)-triethylamine (TEA) of the constant ratio [EtOH or MeOH-ACN-TFA-TEA, x/(100-x)/0.1/0.5, v/v/v/v][a]

Alcohol content (x) in acetonitrile	3			4			8			13			18		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
30% EtOH	10.26	1.28	2.40	52.79	1.23	1.44	47.19	1.23	1.68	13.77	1.53	2.59	68.95	1.17	1.38
50% EtOH	10.02	1.32	2.80	47.10	1.25	1.54	41.94	1.26	1.77	13.61	1.57	2.74	64.28	1.17	1.44
80% EtOH	14.87	1.38	3.15	51.93	1.29	1.85	43.99	1.31	2.26	18.51	1.63	3.06	79.95	1.21	1.56
30% MeOH	7.52	1.37	3.45	38.02	1.28	2.13	34.66	1.29	2.47	11.52	1.62	3.17	45.55	1.21	1.81
50% MeOH	6.37	1.40	3.91	29.08	1.32	2.38	25.85	1.34	2.69	10.11	1.65	3.40	35.09	1.24	2.04
80% MeOH	8.46	1.48	4.25	31.44	1.38	2.87	27.50	1.41	2.86	12.76	1.73	3.92	41.10	1.29	2.48

[a]Flow rate: 1.0 ml/min. Detection: 254 nm UV. Column temperature: 20°C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: resolution.

EXPERIMENTAL

Chromatography was performed with an HPLC system consisting of a Waters model 510 HPLC pump (Milford, MA), a Rheodyne model 7725i injector (Rohnert Park, CA) with a 20 μl sample loop, a Waters model 484 detector (Milford, MA) and a YongLin Autochro Data Module (software: YongLin Autochro 2000). The temperature of the chiral column was set at 20°C by using a Julabo F30 Ultratemp 2000 cooling circulator (Seelbach, Germany). Chiral column packed with CSP **1** [Chirosil RCA(+); 250 mm × 4.6 mm I.D.] was available from RS tech (Daejeon, Korea).

Flecainide (**3**, Fig. 2) was purchased from Sigma Aldrich. Flecainide analogs (**4–22**, Fig. 2) were prepared by treating 2-(aminomethyl)piperidine with an appropriate acid chloride (1.0 equivalent) in the presence of triethylamine (1.2 equivalent) in methylene chloride at 0°C. The structures of flecainide analogs (**4–22**) thus prepared were identified by ^{1}H NMR spectra. Each of flecainide (**3**) and its analogs (**4–22**) was dissolved in methanol (usually 1.0 mg/ml) and then used for the resolution on CSP **1**. The usual injection volume was 2.0 μl.

RESULTS AND DISCUSSION

Secondary amino alcohols related to β-blockers were reported to be successfully resolved on CSP **1** by using a mixture of ethanol-acetonitrile-trifluoroacetic acid-triethylamine with the ratio of 20/80/0.1/0.5 (v/v/v/v) as a mobile phase.[16] However, the mobile phase utilized for the resolution of secondary amino alcohols related to β-blockers was found not to be the optimal one in the resolution of flecainide (**3**) and its analogs (**4–22**) shown in Figure 2 on CSP **1** and consequently, we tried to find the better mobile phase condition.

As an effort to find the improved mobile phase condition, we selected five analytes including flecainide (**3**, **4**, **8**, **13**, and **18**). As a first step we resolved the five selected analytes on CSP **1** with the variation of the ratio of ethanol-acetonitrile in mobile phase at the constant ratio of trifluoroacetic acid-triethylamine (0.1/0.5, v/v). The chromatographic resolution results are summarized in Table 1. As the content of ethanol in acetonitrile was increased from 30 to 50 and then 80%, the separation factor (α) and the resolution (R_S) improved continuously. However, the retention factors (k_1) decreased as the ethanol content was increased from 30 to 50% and then increased as the ethanol content was increased from 50 to 80%. When the ethanol content was increased further, the retention times increased too much to be useful. The reason for the minimum retention factors at 50% ethanol in acetonitrile is not clear yet. When ethanol in the mobile phase was changed to methanol, retention factors (k_1) decreased and the separation factors (α) and the resolutions (R_S) improved as shown in Table 1. By changing ethanol to methanol, the mobile phase polarity is expected to increase and consequently retention factors (k_1) might decrease, but the reason for the improved chiral recognition efficiency is not clear yet.

The trends of the retention factors (k_1), the separation factors (α) and the resolutions (R_S) for the resolution of selected analytes on CSP **1** with the variation of the methanol content in acetonitrile were exactly consistent with those observed with the variation of the ethanol content in acetonitrile. As an example, the chromatograms for the resolution of flecainide on CSP **1** with the variation of methanol content in acetonitrile at the constant ratio of trifluoroacetic acid and triethylamine (0.1/0.5. v/v) are presented in Figure 3. From Table 1 and Figure 3 it is concluded

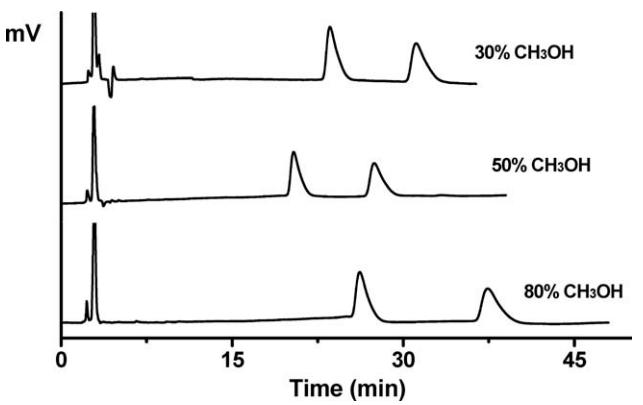

Fig. 3. Chromatograms for the resolution of flecainide (**3**) on CSP **1** with the variation of methanol content in mobile phase consisting of methanol-acetonitrile-trifluoroacetic acid-triethylamine (x/100-x/0.1/0.5, v/v/v/v) at 20°C. Flow rate: 1.0 ml/min. Detection: 254 nm UV.

TABLE 2. Resolution of flecainide (3) and its analogs (4, 8, 13, and 18) on CSP 1 with the variation of the ratio of trifluoroacetic acid (TFA)-triethylamine (TEA) in 80 % methanol in acetonitrile as mobile phase (MeOH-ACN-TFA-TEA, 80/20/x/y, v/v/v/v)[a]

TFA–TEA ratio in mobile phase	3			4			8			13			18		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
0.05–0.5 (v/v)	5.79	1.35	3.02	21.65	1.30	2.27	19.65	1.31	2.49	9.09	1.54	2.79	27.76	1.23	1.84
0.1–0.5 (v/v)	8.46	1.48	4.25	31.44	1.38	2.87	27.50	1.41	2.86	12.76	1.73	3.92	41.40	1.29	2.48
0.3–0.5 (v/v)	8.34	1.39	3.38	23.38	1.32	2.15	20.77	1.34	2.76	9.99	1.56	2.97	35.17	1.25	2.18
0.1–0.4 (v/v)	10.34	1.49	5.00	36.60	1.40	2.73	32.45	1.40	3.22	16.04	1.74	4.38	50.83	1.32	2.48
0.1–0.3 (v/v)	15.37	1.57	5.04	48.58	1.43	3.12	42.59	1.45	3.23	22.77	1.82	6.80	69.70	1.34	2.92

[a]Flow rate: 1.0 ml/min. Detection: 254 nm UV. Column temperature: 20°C. k_1: Retention factor of the first eluted enantiomer. α: Separation.

that methanol is better than ethanol as an alcohol component in mobile phase and the higher content of methanol in acetonitrile is desirable for the better separation factors (α) and the resolutions (R_S), but too much content of methanol in acetonitrile should not be used to maintain the retention times short enough to be useful. Overall the optimal content of methanol in mobile phase for the resolution of flecainide (**3**) and its analogs (**4–22**) on CSP **1** seems to be 80%.

Variation of the ratio of trifluoroacetic acid-triethylamine in the mobile phase consisting of 80% methanol in acetonitrile is also expected to affect the chromatographic behaviors for the resolution of selected analytes on CSP **1**. In Table 2, the chromatographic results for the resolution of selected five analytes on CSP **1** with the variation of the ratio of trifluoroacetic acid-triethylamine in 80% methanol in acetonitrile are summarized. The representative chromatograms for the resolution of flecainide with the variation of the content of trifluoroacetic acid or with the variation of the content of triethylamine in mobile phase on CSP **1** are presented in Figures 4 and 5. When the content of trifluoroacetic acid was decreased from 0.1 to 0.05 % or increased from 0.1 to 0.3% at the constant content of triethylamine (0.5%), the retention factors, the separation factors and the resolutions decreased as shown in Table 2 and Figure 4, indicating that 0.1% trifluoroacetic acid in the mobile phase is the best condition. When the content of triethylamine was decreased from 0.5 to 0.4 and then to 0.3% at the constant content of trifluoroacetic acid (0.1%), the retention factors, separation factors and resolutions improved generally as shown in Table 2 and Figure 5. Decreasing the content of triethylamine in mobile phase further was found to increase the retention times of the two enantiomers too much. From the chromatographic resolution results summarized in Tables 1 and 2, it is concluded that the optimal mobile phase for the resolution of flecainide and its analogs is the mixture of methanol-acetonitrile-trifluoroacetic acid-triethylamine with the ratio of 80/20/0.1/0.3 (v/v/v/v).

The optimum mobile phase consisting of methanol-acetonitrile-trifluoroacetic acid-triethylamine (80/20/0.1/0.3, v/v/v/v) was applied to the resolution of all of the 20 analytes shown in Figure 2 and the chromatographic resolution results are summarized in Table 3. Elution orders of the two enantiomers were not determined because optically active samples were not available. Among the 20 analytes resolved, flecainide (**3**) shows the shortest retention time. Analyte **4** containing simple nonsubstituted benzoyl group shows the highest retention time among 20 analytes except for analytes **7** and **18** containing 3,5-dinitro or 4-

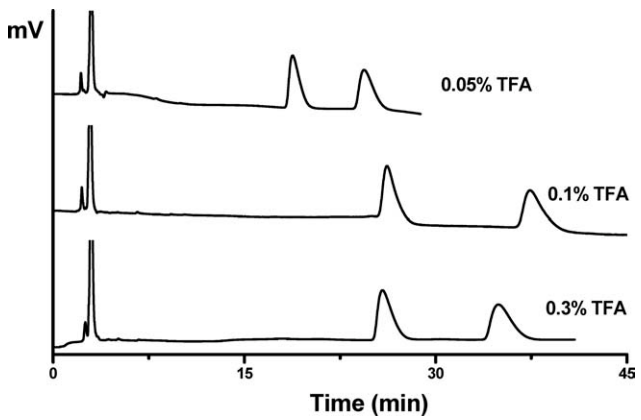

Fig. 4. Chromatograms for the resolution of flecainide (**3**) on CSP **1** with the variation of trifluoroacetic acid content in mobile phase consisting of methanol-acetonitrile-trifluoroacetic acid-triethylamine (80/20/x/0.5, v/v/v/v) at 20°C. Flow rate: 1.0 ml/min. Detection: 254 nm UV.

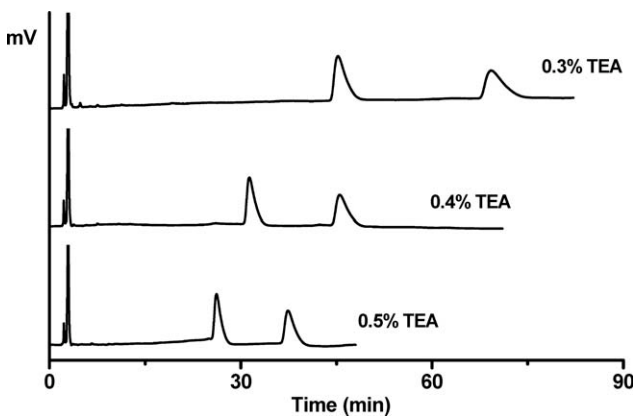

Fig. 5. Chromatograms for the resolution of flecainide (**3**) on CSP **1** with the variation of triethylamine content in mobile phase consisting of methanol-acetonitrile-trifluoroacetic acid-triethylamine (80/20/0.1/x, v/v/v/v) at 20°C. Flow rate: 1.0 ml/min. Detection: 254 nm UV.

TABLE 3. Resolution of flecainide (3) and its analogs (4–22) on CSP 1 with the use of a mixture of methanol-acetonitrile-trifluoroacetic acid-triethylamine (80/20/0.1/0.3, v/v/v/v) as a mobile phase[a]

Analytes	k_1	k_2	α	R_S
3	15.37	24.13	1.57	5.04
4	48.58	69.47	1.43	3.12
5	30.85	42.88	1.39	3.19
6	38.42	50.33	1.31	3.15
7	65.68	74.88	1.14	1.46
8	42.59	61.76	1.45	3.23
9	31.14	41.73	1.34	2.73
10	27.34	33.90	1.24	2.11
11	36.57	50.10	1.37	2.86
12	35.58	46.61	1.31	3.01
13	22.77	41.44	1.82	6.80
14	46.26	60.60	1.31	2.98
15	43.71	57.26	1.31	2.88
16	38.99	51.08	1.31	3.10
17	44.74	56.82	1.27	2.69
18	69.70	93.40	1.34	2.92
19	29.80	37.25	1.25	2.35
20	30.69	36.52	1.19	1.73
21	40.04	48.85	1.22	2.38
22	31.75	39.69	1.25	2.06

[a]Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature, 20°C; k_1, retention factor of the first eluted enantiomer; k_2, retention factor of the second eluted enantiomer; α, separation factor; R_S, resolution.

nitrobenzoyl group. The polar nitro groups of analytes **7** and **18** are expected to induce nonenantioselective interaction with the CSP (for example, hydrogen bonding interaction between the nitro group of analytes and the amide N—H hydrogen of the CSP) and consequently bring about long retention times. Analytes containing 3,5-disubstituted benzoyl group (analytes **5–7**) show relatively large retention factors compared to that of flecainide. Consequently, the shortest retention time of flecainide can be expected to stem from the two 2,2,2-trifluoroethoxy group attached especially to the 2 and 6 position of the benzoyl group. The relatively large size of 2,2,2-trifluoroethoxy group might be responsible for the short retention time. In addition, the ortho-position of 2,2,2-trifluoroethoxy group might be responsible for the short retention time. Among analytes containing para-, meta- or ortho-methylbenzoyl group (analytes **8–10**) and analytes containing para-, meta- or ortho-methoxybenzoyl group (analytes **11–13**), those containing ortho-methyl or ortho-methoxybenzoyl group (**10** and **13**) show the shortest retention times. In addition, analytes **19** and **20**, which contain ortho-substituted benzoyl group also show relatively short retention times compared with those of analytes containing para-substituted benzoyl group (analytes **14–18**). From these results, it is concluded that the ortho-substitution at the benzoyl group of analytes reduces the retention times of analytes. Even though the reason is not clear yet, the conformational deformation of analytes originated from the steric hindrance exerted by the ortho-substitution seems to inhibit the interaction between the CSP and analytes and consequently the retention times are relatively short. However, the interaction between the CSP and analytes affected by the conformational deformation should be nonenantioselective because the enantioselectivities denoted by the separation factors for the resolution of some analytes containing ortho-substituted benzoyl group are not reduced, but even improves especially for the resolution of analytes **3** and **13**.

Electron donating or withdrawing ability of the substituent at the benzoyl group of analytes does not produce notable effects on the resolution of analytes. For example, analytes containing electron donating group at the para-position of benzoyl group (analytes **8** and **11**) and analytes containing electron withdrawing group at the para-position of benzoyl group (analytes **14–18**) do not show significant difference in their retention factors, separation factors and resolutions except for the relatively large retention factor for the resolution of analyte **18**. Resolution of analyte **21** containing 1-naphthoyl group was not quite different from others. Interestingly, even analyte **22** containing phenylacetyl group show the chromatographic resolution behaviors which are quite similar to those for the resolution of other analytes. Consequently, we expected that even analytes containing simple aliphatic acyl group can be resolved on CSP **1** and we concluded that the two 2,2,2-trifluoroethoxybenzoyl groups of flecainide do not play a significant role in the chiral recognition even though the exact chiral recognition mechanism is not clear yet.

In summary, in this study, racemic cyclic secondary amino compounds such as flecainide and its analogs were resolved on CSP **1**. A mixture of methanol-acetonitrile-trifluoroacetic acid-triethylamine (80/20/0.1/0.3, v/v/v/v) was found to be the optimal mobile phase condition. Under the optimal mobile phase condition, 20 analytes including flecainide and its analogs were resolved quite well. The type of the substituent(s) on the benzoyl group of analytes did not affect the chiral recognition behaviors of analytes significantly, but the ortho-substituent was found to decrease the retention times of analytes probably because of the conformational deformation originated from the steric hindrance exerted by the ortho-substituent.

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