Preparation of a New Chiral Stationary Phase Based on Macrocyclic Amide Chiral Selector for the Liquid Chromatographic Chiral Separations

JI YEONG SUNG, SEUNG HYUCK CHOI, AND MYUNG HO HYUN*
Department of Chemistry, Pusan National University, Busan, Republic of Korea

ABSTRACT A new chiral stationary phase (CSP) based on macrocyclic amide receptor was prepared starting from (1R,2R)-1,2-diphenylethylenediamine. The new CSP was successfully applied to the resolution of various N-(substituted benzoyl)-α-amino amides with reasonably good separation factors and resolutions ($\alpha = 1.75 \sim 2.97$ and $R_S = 2.89 \sim 6.82$ for 16 analytes). The new CSP was also applied to the resolution of 3-substituted 1,4-benzodiazepin-2-ones and some diuretic chiral drugs including bendroflumethiazide and methylchlothiazide and metolazone. The resolution results for 3-substituted 1,4-benzodiazepin-2-ones and some diuretic chiral drugs were also reasonably good. *Chirality 28:253–258, 2016.*

KEY WORDS: chiral stationary phase; enantiomer separation; liquid chromatography; macrocyclic amide chiral selector

Liquid chromatographic chiral stationary phases (CSPs) have attracted quite a lot of attention because CSPs can be used in the preparative-scale separation of the two enantiomers and in the exact determination of the enantiomeric composition of chiral compounds in a very accurate, convenient, and cost-effective way.[1–3] Various liquid chromatographic CSPs have been developed by bonding appropriate chiral selectors to column supporting materials. For example, polysaccharide derivatives,[4,5] macrocyclic antibiotics,[6,7] macrocyclic oligosaccharides,[8] chiral crown ethers,[9–14] proteins,[15] and other chiral molecules[16,17] bonded to silica gel have been used as liquid chromatographic CSPs.

Macrocyclic amide chiral selectors bonded to silica gel have also been used as liquid chromatographic CSPs. In particular, a CSP based on a macrocyclic amide receptor prepared from (1R,2R)-1,2-diphenylethylenediamine and 5-allyloxyisophthalic acid was very successful in the resolution of *N*-3,5-dinitrobenzoyl amino acid amides and some other racemic compounds.[18] Another CSP based on a macrocyclic amide receptor prepared from bi-β-naphthol was also utilized for the resolution of some racemic compounds.[19] We also have been interested in the development of CSPs based on a macrocyclic amide receptor. In this study we report the preparation of a new CSP (CSP **1**, Fig. 1) based on a macrocyclic amide receptor prepared from (1R,2R)-1,2-diphenylethylenediamine and its application to the resolution of some chiral compounds.

MATERIALS AND METHODS

Preparation of CSP 1 and Column Packing

CSP 1 was prepared according to the scheme shown in Figure 1.

Dimethyl 5-(4-Pentenyloxy)isophthlatae 1. Dimethyl 5-hydroxyisophthalate (2.0 g, 9.5 mmole) was dissolved in acetonitrile (100 ml) in a 250 ml round bottom flask. To the solution was added K_2CO_3 (2.0 g, 14.5 mmole). After stirring the solution for 30 min, 5-bromo-1-petene (1.35 ml, 11.4 mmole) was added. The whole mixture was heated to reflux for 5 h. After cooling the solution, acetonitrile was removed by using a rotary evaporator. The residue was dissolved in dichloromethane and then the solution was washed with 1 N NaOH solution. The organic solution was dried over anhydrous Na_2SO_4 and then solvent was removed by using a rotary evaporator to afford dimethyl 5-(4-pentenyloxy)isophthalate **1** (2.32 g, 88% yield). ^{1}H NMR ($CDCl_3$) (ppm) 1.83–1.99 (m, 2H), 2.20–2.30 (m, 2H), 3.93 (s, 6H), 4.04 (t, 2H), 4.98–5.11 (m, 2H), 5.75–5.95 (m, 1H), 7.73 (s, 2H), 8.25 (s, 1H).

5-(4-Pentenyloxy)isophthalic Acid 2. Compound **1** (2.0 g, 7.2 mmole) was added to 1 M KOH solution of methanol (100 ml) in 250 ml round bottom flask. The mixture was heated to reflux for 5 h. After cooling the reaction mixture, methanol was removed by using a rotary evaporator. The residue was dissolved in ethyl acetate and then the solution was treated with 1 N HCl solution. The separated organic solution was dried over anhydrous Na_2SO_4 and then solvent was removed by using a rotary evaporator to afford 5-(4-pentenyloxy)isophthalic acid **2** (1.78 g, 99% yield). ^{1}H NMR (Acetone d_6) (ppm) 1.88–1.98 (m, 2H), 2.24–2.34 (m, 2H), 4.16 (t, 2H), 4.97–5.11 (m, 2H), 5.84–5.98 (m, 1H), 7.77 (s, 2H), 8.28 (s, 1H), 11.03 (broad s, 2H).

N-t-Boc-(1R,2R)-Diphenylethylenediamine 3. (1R,2R)-1,2-Diphenylethylenediamine (0.5 g, 2.4 mmole) was dissolved in chloroform (40 ml). To the stirred solution was added di-*tert*-butyl dicarbonate (0.54 ml, 2.4 mmole) through a dropping funnel slowly for 1 h at 0 °C and then the whole mixture was stirred for 4 h at 0 °C. To the reaction mixture was added saturated $NaHCO_3$ solution. After shaking well, the organic layer was separated and then dried over anhydrous Na_2SO_4. Solvent was removed by using a rotary evaporator. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane/methanol: 1/3/0.1) to afford compound **3** (0.36 g, 48% yield). ^{1}H NMR ($CDCl_3$) (ppm) 1.32 (s, 9H), 4.34 (s, 1H), 4.84 (s, 1H), 5.76 (s, 1H), 7.21–7.38 (m, 10H).

Preparation of Compound 4. The mixture of 5-(4-pentenyloxy)isophthalic acid **2** (1.50 g, 6.0 mmole) and thionyl chloride (20 ml) in 100 ml round bottom flask was heated to reflux for 6 h under an argon atmosphere. Excess thionyl chloride was removed by using a rotary evaporator and then the residue was dissolved in methylene chloride (50 mL). The methylene chloride solution was slowly added through a cannula to another methylene chloride solution (90 ml) containing *N*-t-Boc-(1R,2R)-diphenylethylenediamine **3** (3.74 g, 12.0 mmole) and triethylamine (1.82 ml, 13.2 mmole) in 250 ml round bottom flask at –78 °C. The whole mixture was stirred at –78 °C

*Correspondence to: M.H. Hyun, Department of Chemistry, Pusan National University, Busan 609-735, Republic of Korea. E-mail: mhhyun@pusan.ac.kr
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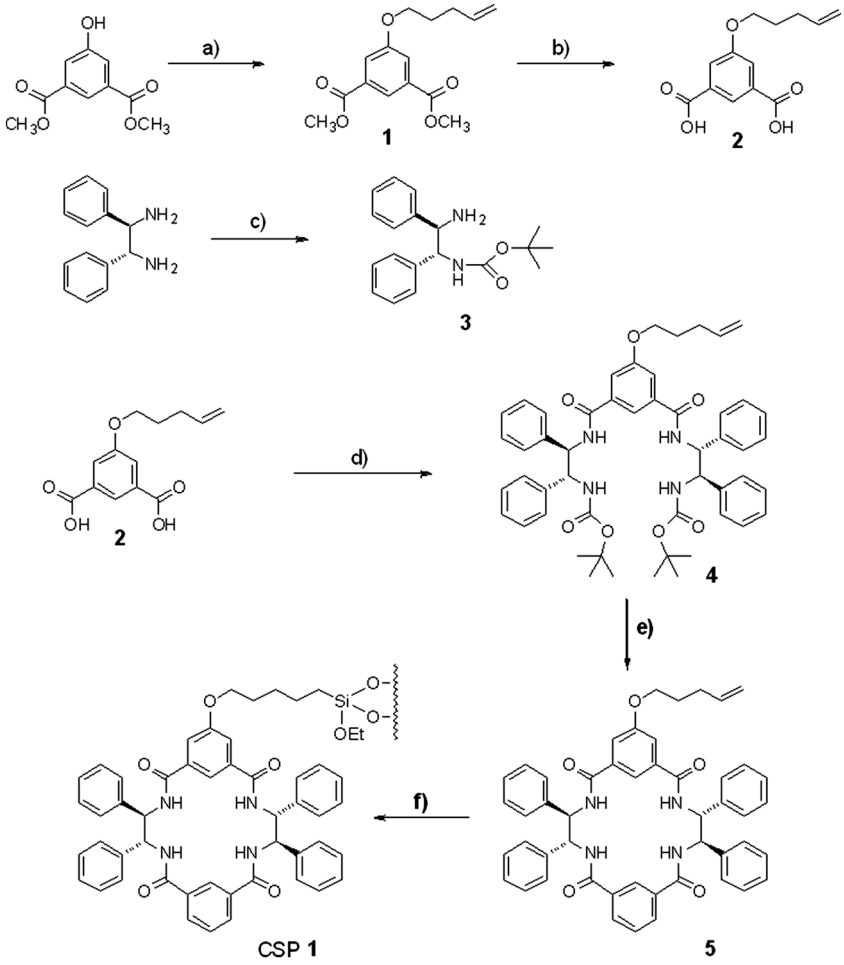

Fig. 1. Scheme for the synthesis of CSP **1**. **a**) 5-Bromo-1-pentene, K_2CO_3, acetonitrile, reflux. **b**) KOH, methanol, reflux. **c**) Di-*tert*-butyl dicarbonate, $CHCl_3$, 0 °C. **d**) (1) $SOCl_2$, reflux. (2) Compound **3**, triethylamine, methylene chloride, –78 °C and then 0 °C. **e**) (1) CF_3COOH, methylene chloride. (2) Isophthaloyl chloride, *N*,*N*-diisopropylethylamine, tetrahydrofuran (THF), 0 °C and then room temperature. **f**) (1) Trichlorosilane, Pt/C (10 wt%), methylene chloride, toluene. (2) Ethanol/triethylamine (1:1, v/v), methylene chloride. (3) Silica gel, toluene, Dean-Stark trap.

for 1 h and then at 0 °C for 10 h. The reaction mixture was washed with 1 N HCl solution and then with 1 N NaOH solution. Finally, the organic solution was dried over anhydrous Na_2SO_4. Solvent was removed by using a rotary evaporator. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane: 1/3) to afford compound **4** (2.67 g, 53% yield). ^{1}H NMR (DMSO d_6) (ppm) 1.23 (s, 18H), 1.81–1.89 (m, 2H), 2.17–2.24 (m, 2H), 3.99–4.03 (m, 2H), 4.98-5.02 (m, 2H), 5.03–5.16 (m, 2H), 5.45–5.51 (m, 2H), 5.80-5.92 (m, 1H), 7.10–7.36 (m, 22 H), 7.64–7.71 (m, 3H), 8.78 (d, 2H).

Preparation of Compound 5. Compound **4** (2.5 g, 3.0 mmole) was dissolved in methylene chloride (80 ml). To the stirred solution was added trifluoroacetic acid (2.23 ml, 30 mmole). The whole mixture was stirred at room temperature for 3 h. Solvent and trifluoroacetic acid were removed by using a rotary evaporator. The residue was dissolved in methylene chloride and then the solution was washed with 1 N NaOH solution. The organic solution was dried over anhydrous Na_2SO_4. Solvent was removed by using a rotary evaporator to afford corresponding diamine compound, N^1,N^3-bis((1R,2R)-2-amino-1,2-diphenylethyl)-5-(pent-4-en-1-yloxy)isophthalamide. Without further purification, the diamine compound was dissolved in tetrahydrofuran (THF) (300 ml). To the solution was added *N*,*N*-diisopropylethylamine (1.15 ml, 6.6 mmole). The whole mixture was stirred for 30 min at 0 °C under an argon atmosphere. To the stirred solution was added a solution of isophthaloyl chloride (0.63 g, 3.1 mmole) in THF (30 ml) through a cannula at 0 °C. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated by using a rotary evaporator. The residue was dissolve in methylene chloride. The methylene chloride solution was washed with 1 N HCl solution and 1 N NaOH solution. The organic solution was dried over anhydrous Na_2SO_4. Solvent was removed by using a rotary evaporator. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane, 1/3, v/v) to afford compound **5** (1.50 g, 65% yield). ^{1}H NMR (acetone d_6) (ppm) 1.76–1.83 (m, 2H), 2.12–2.19 (m, 2H), 3.94–4.05 (m, 2H), 4.89–5.02 (m, 2H), 5.61–5.69 (m, 4H), 5.77-5.87 (s, 1H), 7.17–7.47 (m, 23 H), 7.86–7.89 (m, 2H), 8.20 (s, 1H), 8.58–8.65 (m, 5H).

Preparation of CSP 1 and Column Packing. Compound **5** (1.40 g, 1.8 mmole) was dissolved in the mixed solvent of methylene chloride (20 ml) and toluene (10 ml) in a 100 ml two neck round bottom flask. To the solution was added platinum on activated carbon (Pt/C, 10 wt%) (10 mg). The heterogeneous solution was stirred for 30 min and then tricholosilane (20 ml) was added. The whole mixture was stirred for 30 h in an oil bath maintained at 80 °C. After cooling, solvent and excess trichlorosilane were removed by using a rotary evaporator. The residue was dissolved in methylene chloride (20 ml). To the solution was slowly added 10 mL of the mixed solvent of ethyl alcohol and triethylamine (10 ml, 1:1, v/v) at 0 °C. The whole mixture was stirred at room temperature for 30 min and then evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane, 1/3, v/v) to afford triethoxysilyl compound (1.18 g, 70%). ^{1}H NMR ($CDCl_3$) (ppm) 0.54–0.60 (m, 2H), 1.13 (t, 9H), 1.35–2.02 (m, 6H), 3.73 (q, 6H), 3.82–3.90 (m, 2H), 5.52–5.56 (m, 4H), 7.00 (s, 2H), 7.19–7.31 (m, 20 H), 7.46-7.49 (m, 2H), 7.68 (s, 1H), 8.15 (s, 1H), 8.34–8.42 (m, 5H).

For the preparation of CSP **1**, Kromasil silica gel (4.2 g, 5 μm, 100 Å) available from Eka Chemicals was added to toluene (100 ml) in a 250 ml flask equipped with a Dean-Stark trap, a condenser, and a magnetic stirring bar. The mixture was heated to reflux until the complete azeotropic removal of water. After cooling, triethoxysilyl compound (1.10 g, 1.2 mmole) dissolved in toluene (10 ml) was added. The whole mixture was heated to reflux for 72 h. After cooling, the modified silica gel (CSP **1**) was filtered and then washed successively with toluene, methanol, acetone, ethyl acetate, methylene chloride, hexane, and diethyl ether. Elemental analysis of CSP **1** (Found: C, 7.31%; H, 1.44%; N, 0.59%) showed a loading of 0.12 mmole of selector (based on C) or 0.11 mmole of selector (based on N) per gram of stationary phase. CSP 1 thus prepared was slurried in methanol and packed into a 250 x 4.6 mm I. D. stainless-steel HPLC column using a conventional slurry packing method with an Alltech slurry packer.

Chromatography

An HPLC system consisting of a Waters model 515 HPLC pump (Milford, MA), a Rheodyne model 7125 injector (Rohnert Park, CA) with a 20 μl sample loop, a Waters 2487 tunable absorbance detector and a YoungLin Autochro data module (Software: YoungLin Autochro-WIN 2.0 plus) was used for the liquid chromatography. The chiral column temperature was controlled by using a JEIO TECH VTRC-620 cooling circulator (Daejeon, Korea).

N-(Substituted benzoyl)-α-amino amides **6** (Fig. 2) used as analytes were prepared by treating *N*-(substituted benzoyl)-α-amino acids suspended in methylene chloride with the appropriate alkylamine in the presence of 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) at room temperature as described in the previous report.[20] *N*-(Substituted benzoyl)-α-amino acids were prepared by treating substituted benzoyl chloride with α-amino acids in the presence of propylene oxide in THF at room temperature, as reported.[21] 3-Substituted 1,4-benzodiazepin-2-ones **7** and **8** (Fig. 2) prepared by coupling *o*-aminobenzophenones with *N-t*-Boc-α-amino acids followed by deprotection and cyclization in the presence of trifluoroacetic acid via the known procedure[22] were available from a prior study.[23] Other analytes including bendroflumethiazide (**9**, Fig. 2), methylchlothiazide (**10**, Fig. 2) and metolazone (**11**, Fig. 2) are available commercially from Alfa Chemistry (Stony Brook, NY) or Advanced Technology & Industrial Co. (Hong Kong), but the actual samples were available from a prior study[24] or from laboratory stock. Injection samples were prepared by dissolving each analyte in methanol at a concentration of 1.0 mg/mL and an injection volume was typically 2.0 μl.

RESULTS AND DISCUSSION

CSP **1** contains hydrogen-bonding donor and acceptor sites and aromatic π–π interaction sites. Consequently, CSP **1** is expected to be able to resolve various racemic compounds. At first, CSP **1** was applied to the resolution of various *N*-(substituted benzoyl)-α-amino amides **6** with the use of 20% 2-propanol in hexane as an optimized mobile phase. In the resolution of secondary amide derivatives of *N*-(3,5-dinitrobenzoyl)leucine (analytes **6a–6h** in Table 1) on CSP **1**, the retention factors (k_1) for the first eluted enantiomers decreased continuously as the amide alkyl group (R_2 in Table 1) of analytes increased in length. As the length of the amide alkyl group of analytes is increased, the interaction between the analyte and the CSP decrease and, consequently, the retention of analytes should decrease. However, the separation factors (α) and resolutions (R_S) were found to show the maximum values when the amide alkyl group (R_2 group in Table 1) is the propyl group, indicating the optimum length of the amide alkyl group is the three methylene unit. Other *N*-(3,5-dinitrobenzoyl)-α-amino *n*-propyl amides including valine derivative (analyte **6m** in Table 1), phenylglycine derivative (analyte **6n** in Table 1), phenylalanine derivative (analyte **6o** in Table 1), and tyrosine derivative (analyte **6p** in Table 1) were resolved very well.

In order to see the role of the N-H hydrogen of the secondary amide group of analytes in the chiral recognition, the N-H hydrogen was replaced with n-hexyl or phenyl group and the resulting tertiary amides were resolved on CSP **1**. As shown in Table 1, *N*-(3,5-dinitrobenzoyl)leucine *N*-hexyl *N*-propyl amide (analyte **6i** in Table 1) and *N*-(3,5-dinitrobenzoyl)leucine *N*-hexyl *N*-phenyl amide (analyte **6j** in Table 1) were resolved very well on CSP **1** with similar separation factor for analyte **6i** or even better separation factor for analyte **6j**. These results indicate that the N-H hydrogen of the secondary amide group of analytes does not play any role in the chiral recognition. By replacing the N-H hydrogen of the secondary amide group of analytes with hexyl or phenyl group, the resolution (R_S) is increased quite a lot. While the N-H hydrogen of the secondary amide group of analytes does not play any role in the chiral recognition, it seems to improve only the affinity for the stationary phase and, consequently, leads to peak broadening. However, tertiary amides of *N*-(3,5-dinitrobenzoyl)leucine seem to minimize the peak broadening. In this instance, the resolution (R_S) should be increased.

The role of the *N*-(3,5-dinitrobenzoyl) group of analytes in the chiral recognition was also checked by resolving *N*-(3,5-dimethylbenzoyl)leucine *n*-propyl amide (analyte **6k** in Table 1) and *N*-(3,5-dimethoxybenzoyl)leucine *n*-propyl

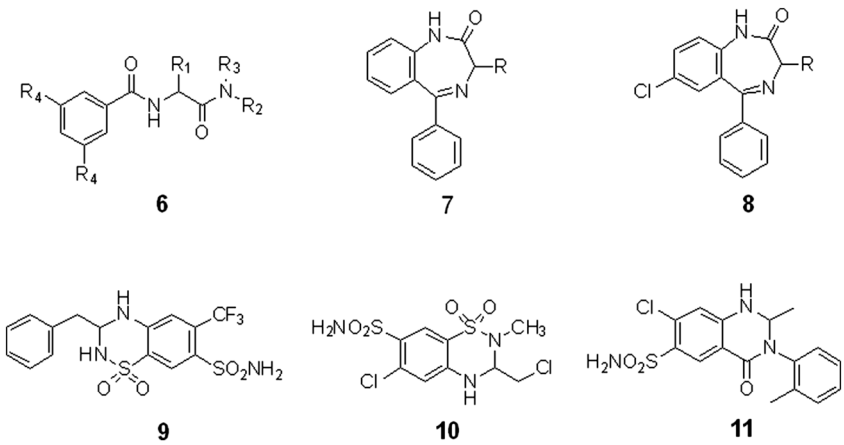

Fig. 2. Structures of *N*-(substituted benzoyl)-α-amino amides (**6**), 3-substituted 1,4-benzodiazepin-2-ones (**7** and **8**), bendroflumethiazide (**9**), methylchlothiazide (**10**), and metolazone (**11**).

TABLE 1. Resolution of *N*-(substituted benzoyl)-α-amino amides 6 on CSP 1[a]

Anal.	R_1	R_2	R_3	R_4	k_1	α	R_S
6a	$(CH_3)_2CHCH_2$	CH_3	H	NO_2	1.46 (D)	1.94	3.26
6b	$(CH_3)_2CHCH_2$	CH_2CH_3	H	NO_2	1.45 (D)	1.94	3.52
6c	$(CH_3)_2CHCH_2$	$(CH_2)_2CH_3$	H	NO_2	0.89 (D)	2.04	3.85
6d	$(CH_3)_2CHCH_2$	$(CH_2)_3CH_3$	H	NO_2	0.82 (D)	1.89	3.69
6e	$(CH_3)_2CHCH_2$	$(CH_2)_4CH_3$	H	NO_2	0.78 (D)	1.76	3.62
6f	$(CH_3)_2CHCH_2$	$(CH_2)_5CH_3$	H	NO_2	0.77 (D)	1.75	3.32
6g	$(CH_3)_2CHCH_2$	$(CH_2)_6CH_3$	H	NO_2	0.75 (D)	1.79	2.89
6h	$(CH_3)_2CHCH_2$	$(CH_2)_7CH_3$	H	NO_2	0.71 (D)	1.79	2.95
6i	$(CH_3)_2CHCH_2$	$(CH_2)_2CH_3$	$(CH_2)_5CH_3$	NO_2	0.93 (D)	1.78	5.04
6j	$(CH_3)_2CHCH_2$	$(CH_2)_2CH_3$	C_6H_5	NO_2	0.46 (D)	2.63	6.82
6k	$(CH_3)_2CHCH_2$	$(CH_2)_2CH_3$	H	CH_3	0.29 (D)	2.29	3.88
6l	$(CH_3)_2CHCH_2$	$(CH_2)_2CH_3$	H	OCH_3	0.74 (D)	2.66	4.74
6m	$(CH_3)_2CH$	$(CH_2)_2CH_3$	H	NO_2	0.91 (D)	1.76	3.25
6n	C_6H_5	$(CH_2)_2CH_3$	H	NO_2	1.96 (D)	1.67	2.97
6o	$(C_6H_5)CH_2$	$(CH_2)_2CH_3$	H	NO_2	1.53 (D)	1.71	3.21
6p	4-OH$(C_6H_5)CH_2$	$(CH_2)_2CH_3$	H	NO_2	2.85 (D)	2.97	4.56

[a]Mobile phase: 20% 2-propanol in hexane. Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature: 20 °C. Column size: 250 x 4.6 mm I.D. k_1: Retention factor of the first eluted enantiomer. In the parentheses, the absolute configuration of the first eluted enantiomer is presented. α: Separation factor. R_S: Resolution.

amide (analyte **6l** in Table 1) on CSP **1**. The separation factors and resolutions for **6k** and **6l** were even greater than those for **6c**. These results indicate that the π–π interaction between the electron-rich N-(3,5-dimethylbenzoyl) or N-(3,5-dimethoxybenzoyl) group of analyte and the aromatic group of the CSP is more significant than that between the electron-deficient *N*-(3,5-dinitrobenzoyl) group of analyte and the aromatic group of the CSP. Consequently, the isophthalamide group of the CSP instead of the phenyl group is expected to be involved in the chiral recognition as an aromatic π–π interaction site.

3-Substituted 1,4-benzodiazepin-2-ones such as camazepam, lorazepam, lormetazepam, and oxazepam have been widely used as anxiolytics and/or tranquilizers and their two enantiomers have been known to show different pharmaceutical activity.[25] In this instance, the exact determination of the enantiomeric composition of 3-substituted 1,4-benzodiazepin-2-ones is important. Previously, Pirkle-type CSPs have been successfully used for the resolution of 3-substituted 1,4-benzodiazepin-2-ones.[23,26] However, CSPs based on macrocyclic amide chiral selectors have not been used. In this study, CSP **1** was successfully applied to the resolution of racemic 3-substituted 1,4-benzodiazepin-2-ones. For the resolution of racemic 3-substituted 1,4-benzodiazepin-2-ones **7** and **8** on CSP **1**, we tested 20%, 10%, and 5% 2-propanol in hexane as a mobile phase. As an example, Figure 3 shows the representative chromatograms for the resolution of analytes **7c** and **8c** on CSP **1** with the variation of the content of 2-propanol in hexane in the mobile phase. As the content of 2-propanol in hexane increased from 5% to 10% and then to 20%, the retention factors were found to decrease significantly. The separation factors (α) were found to decrease slightly as the content of 2-propanol in hexane increased from 5% to 10% and then to 20%, but the resolutions were found to show the maximum value when 10% 2-propanol in hexane was used as a mobile phase. The chromatographic results for the resolution of racemic 3-substituted 1,4-benzodiazepin-2-ones **7** and **8** on CSP **1** with the use of 10% 2-propanol in hexane as a mobile phase are summarized in Table 2. In every case, type **8** analytes containing chloride-substituted benzo ring were found to show higher separation factors and resolutions than the corresponding type **7** analytes containing nonsubstituted benzo ring, but the reason is not clear yet.

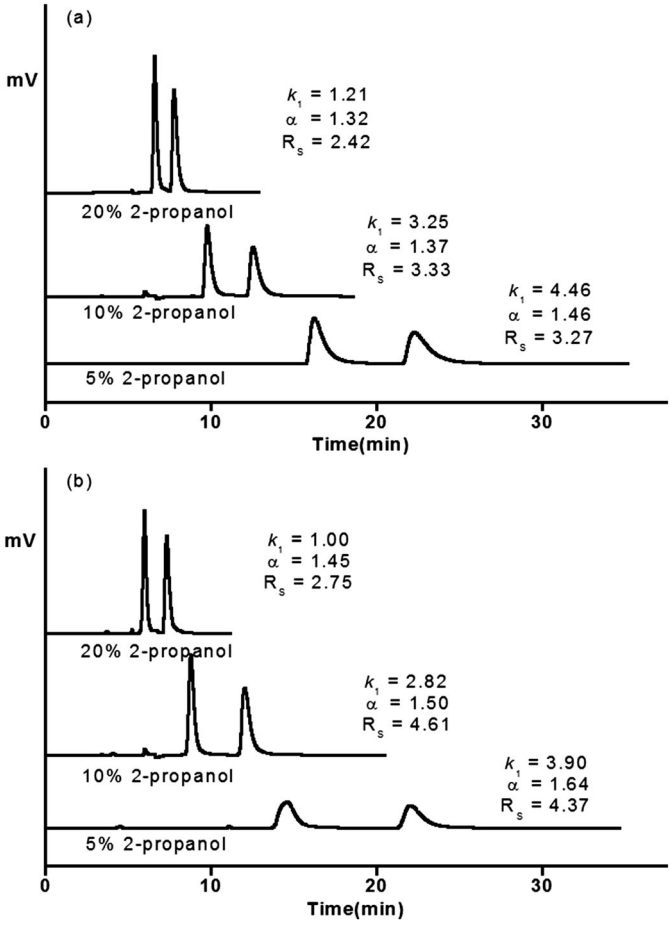

Fig. 3. Chromatograms for the resolution of 3-substituted 1,4-benzodiazepin-2-ones **7c** (**a**) and **8c** (**b**). Mobile phase: 5%, 10% and 20% 2-propanol in hexane. Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature: 20 °C.

TABLE 2. Resolution of 3-substituted 1,4-benzodiazepin-2-ones 7 and 8 on CSP 1[a]

Entry	R	k_1	k_2	α	R_S
7a	CH_3	3.13 (S)	3.51 (R)	1.12	1.57
7b	$(CH_2)_3CH_3$	2.42 (S)	2.73 (R)	1.13	0.94
7c	$(CH_2)_2SCH_3$	3.25 (S)	4.45 (R)	1.37	3.33
7d	$CH(CH_3)_2$	2.16 (S)	2.31 (R)	1.07	0.87
7e	$CH_2CH(CH_3)_2$	2.26 (S)	2.55 (R)	1.13	1.73
7f	$CH_2C_6H_5$	2.86 (S)	3.75 (R)	1.31	2.41
8a	CH_3	2.56 (S)	3.15 (R)	1.23	2.15
8b	$(CH_2)_3CH_3$	1.89 (S)	2.15 (R)	1.14	1.50
8c	$(CH_2)_2SCH_3$	2.82 (S)	4.23 (R)	1.50	4.61
8d	$CH(CH_3)_2$	1.83 (S)	1.92 (R)	1.15	1.06
8e	$CH_2CH(CH_3)_2$	1.91 (S)	2.29 (R)	1.20	2.81
8f	$CH_2C_6H_5$	2.46 (S)	3.99 (R)	1.62	4.70

[a]Mobile phase: 10% 2-propanol in hexane. Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature: 20 °C. Column size: 250 x 4.6 mm I.D. k_1 and k_2: Retention factor of the first and second eluted enantiomer, respectively. In the parentheses, the absolute configuration is presented. α: Separation factor. R_S: Resolution.

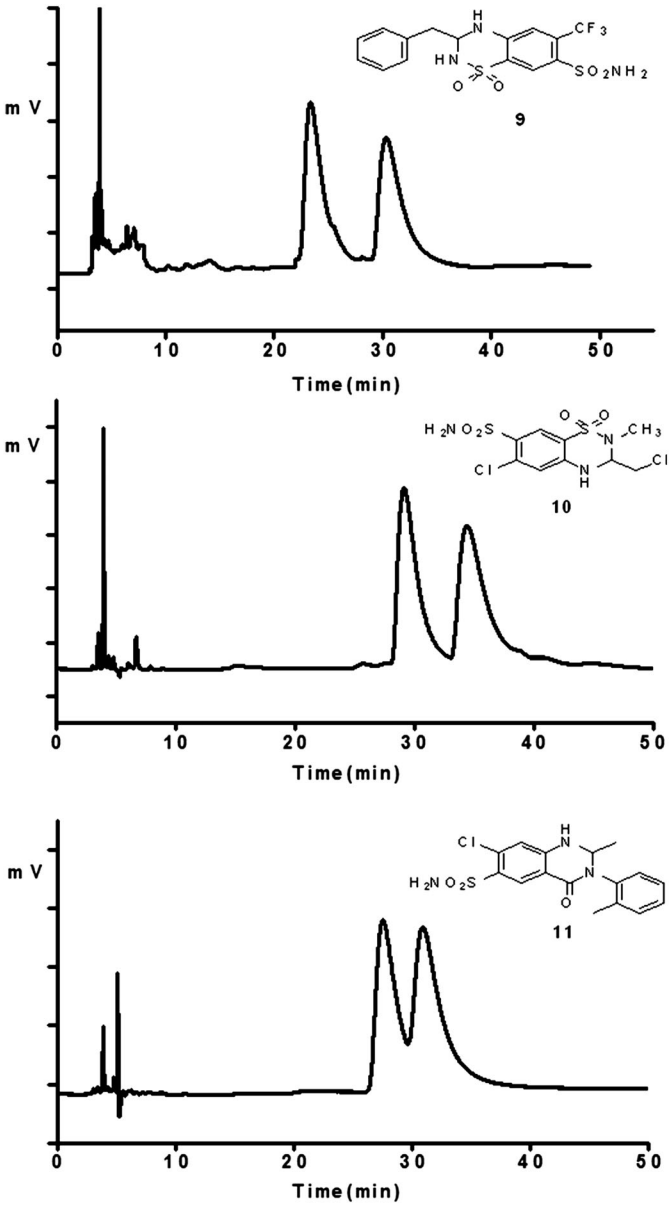

Fig. 4. Chromatograms for the resolution of bendroflumethiazide (**9**) and methylchlothiazide (**10**) and metolazone (**11**). Mobile phase: 20% 2-propanol in hexane. Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature: 20 °C.

As another example for the application of CSP **1**, chiral drugs including thiazide diuretics such as bendroflumethiazide (**9**) and methylchlothiazide (**10**) and thiazide-like diuretic metolazone (**11**), were resolved on CSP **1**. Even though the two enantiomers of thiazide diuretics have been known to show different biological activity,[27] their chiral separations on CSPs are not common. Previously, only Pirkle-type CSPs have been utilized for the separation of the enantiomers of thiazide diuretics.[28,29] However, CSP **1** was found to be also successful for the resolution of thiazide diuretics. The chromatograms for the resolution of bendroflumethiazide (**9**) and methylchlothiazide (**10**) and thiazide-like diuretic metolazone (**11**) on CSP **1** are presented in Figure 4. Bendroflumethiazide (**9**) was resolved quite well with a separation factor of 1.34 and resolution of 2.70 when 20% isopropanol in hexane was used as a mobile phase. Under identical chromatographic conditions, methylchlothiazide (**10**) was also found to be baseline resolved with a separation factor of 1.20 and resolution of 1.50. However, metolazone (**11**) was resolved only slightly, with a separation factor of 1.14 and resolution of 0.84.

CONCLUSION

A new CSP (CSP **1**) based on macrocyclic amide receptor prepared from (1R,2R)-1,2-diphenylethylenediamine was prepared. CSP **1** was successfully applied to the resolution of various *N*-(substituted benzoyl)-α-amino amides, 3-substituted 1,4-benzodiazepin-2-ones, and some diuretic chiral drugs with reasonably good separation factors and resolutions. All of the analytes resolved on CSP **1** contain at least one aromatic functional group. Consequently, CSP **1** is concluded to be useful in the resolution of various racemic compounds containing aromatic functional groups. Further application of CSP **1** is under way in our laboratory.

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