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Chiral separation of amino acids using a chiral crown ether by impregnation on a polymeric support and monoamine modified silica gel

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Abstract—A chiral monoaza-15-crown-5 ether derivative was prepared from L-Leucinol and used as a chiral stationary phase. The new chiral stationary phases CSP-**1** and CSP-**2** were employed in separating the enantiomers of the sodium and potassium salts of amino acids. The sodium and potassium salt of the D-enantiomers of all amino acids (PhyAlaNa, PhyAlaK and PhyGlyNa, PhyGlyK, and TrpNa, TrpK) show higher selectivity than the L-enantiomers for both CSP-**1** and CSP-**2**.
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1. Introduction

Crown ethers are macrocyclic polyethers which form stable selective complexes with suitable cations. They were synthesized for the first time in 1967 with pioneering work of Pedersen.[1] A more advanced generation of crown ethers, including chiral crown ethers, was synthesized by Lehn[2,3] and Cram.[4,5] Host–guest chiral recognition is important in a variety of physical, chemical, and biological processes including sensing, purification, and resolution of enantiomers, asymmetric catalytic reactions and single enantiomeric forms of amino acids and sugars.[6] Therefore, the design, synthesis, and the use of molecules capable of enantiomeric recognition of other molecules are of great interest to workers in these fields. Modern Chemistry utilizes these chemicals in various respects, for instance as enzyme models[7] in biochemistry, for phase transfer catalysis[8] in organic synthesis, and analytical chemistry.

For molecular recognition by artificial chiral crown ether derivates, amino acids, and their salts are the most important target molecules. Recently, chromatographic techniques with chiral stationary phases (CSPs) have been used extensively to achieve enantiomer separation for analytical and preparative purposes. Various CSPs based on native or derivatized chiral amino acids, derivatized carbohydrates, cyclodextrins, and proteins are commercially available.[9] For the separation of amino compounds, chiral crown ethers have been recognized as the most promising selectors in LC chiral stationary phases. Previously,[10] we synthesized chiral amide derivatives of octaester calixresorcarene and used it as a chiral stationary phase.

Herein, we report the preparation of a chiral monoaza-15-crown-5 ether derivative[11] stationary phases (CSPs) starting from L-Leucine (Scheme 1) and show its application to the chiral discrimination of amino acids.

2. Result and discussion

2.1. Synthesis of chiral aza-15-crown-5 ether

The synthesis of L-Leucinol was accomplished in one step from L-Leucine according to a procedure described in the literature.[12] The chiral azacrown ether derivative **4**[11] was prepared from **3** and 1,2-bis-(2-*p*-tolylsulfonyl ethoxy) benzene,[13] using sodium hydride as a base in THF as shown in Scheme 1. The structures proposed for these chiral macro cycles and amino alcohols are consistent with data obtained from ^{1}H NMR, ^{13}C NMR, IR spectra, and elemental analyses.

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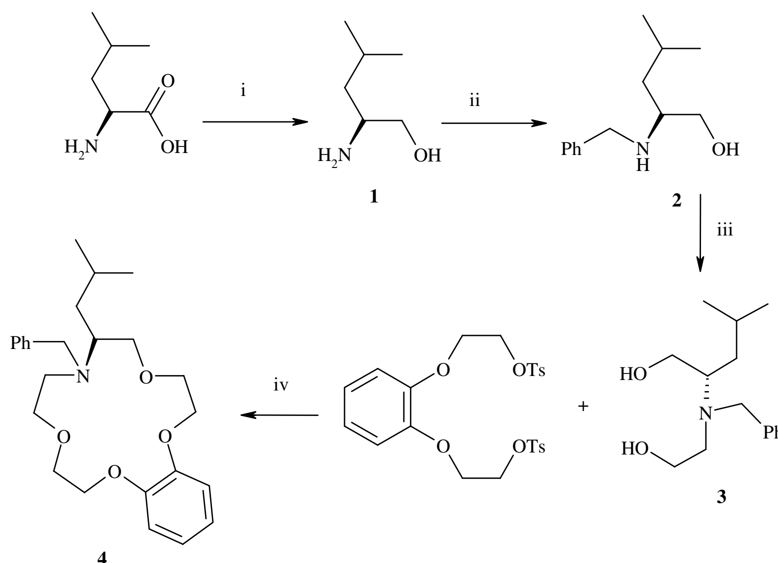

Scheme 1. Reagents and conditions: (i) $NaBH_4$–I_2/THF; (ii) $PhCH_2Cl$, Na_2CO_3, 12 h, 100 °C; (iii) ethylene oxide, MeOH, −20 °C; (iv) NaH, THF, reflux, 50 h.

2.2. Impregnation process

The IR absorption frequencies of XAD-16 and the chiral crown ether impregnated resin are given in Table 1. The IR spectrum of the loaded Amberlite XAD-16 was compared with that of free Amberlite XAD-16. The –C–O– stretching vibration at 1109, 1058 cm^{-1} and the –C–N– stretching vibration at 1175, 1039 cm^{-1} are clearly observed in the chiral crown ether loaded resin.

The IR absorption frequencies of aminopropyl-silica gel and the chiral crown ether impregnated silica gel are given in Table 2. The IR spectrum of the chiral crown ether modified silica gel was compared with that of aminopropyl modified silica gel and frees silica gel. The Si–OH stretching frequency in silica gel at 3460 cm^{-1} is overlapping with the C–NH_2 stretching frequency in aminopropyl modified silica gel at 3454 cm^{-1} and in crown ether modified silica gel at 3438 cm^{-1}.

Table 1. Some fundamental frequencies (in cm^{-1}) of Amberlite XAD-16 and chiral crown ether impregnated resin

Amberlite XAD-16	XAD-16-chiral crown ether	Assignments
3107, 3020	3020, 3010	Aromatic C–H stretching
2925, 2850	2965	Aliphatic C–H stretching
1511, 1489	1510, 1486	C=C ring stretching
—	1109, 1058	C–O stretching
—	1175–1039	C–N stretching

Table 2. Some fundamental frequencies (in cm^{-1}) of aminopropyl modified silica gel and chiral crown ether silica gel

Silica gel	Aminopropyl-silica gel	Silica gel-chiral crown ether	Assignments
3460	—	—	OH stretching
—	3454	3438	NH stretching
	—	3018	Aromatic C–H stretching
—	2938	2927	Aliphatic C–H stretching
	—	1505, 1475	C=C ring stretching
1258	—	—	OH bending
	—	1100, 1053	C–O stretching
	—	1164, 1035	C–N stretching

The C=C ring stretching vibration at 1505, 1475 cm^{-1} and the –C–O– stretching vibration at 1100, 1053 cm^{-1} and the –C–N– stretching vibration at 1164, 1035 cm^{-1} are clearly observed in the chiral crown ether loaded silica gel.

2.3. Molecular recognition

In order to develop a CSP for separation of chiral enantiomers of sodium and potassium salt of amino acids using a normal mobile phase as an eluent, the chiral 15-crown-5 was adopted as a selector, due to the 15-crown-5 ether derivative exhibiting highly enantioselective complexation toward primary organic ammonium cations in organic solvents.[11] Therefore, **4** was designed in which the 15-crown-5 framework is impregnated on a polymeric supported Amberlite XAD-16 (CSP-**1**) and mono amine modified silica gel (CSP-**2**) for chiral separation of amino acids. The results are summarized in Table 3. When CSP-**1** was used as selector the retention times of L- and D-PhyAlaNa were 2.25 and 3.00, while for CSP-**2** these values were 3.00 and 3.75, respectively. Similarly, for CSP-**1** the retention times of L- and D-enantiomers of TrpK salts were 3.25 and 4.50, while for CSP-**2** these were 3.75 and 4.75, respectively (Fig. 1). These results may be caused by the differences in steric hindrance or bulkiness of CSP-**1** and CSP-**2**, and also strong interaction of CSP-**2** with amino acid salts, because it involves aminopropyl modified silica gel.

It can be seen in Table 3 that there is obvious discrimination between retention times of sodium and potassium salts of the L- and D-enantiomers of all amino acids. Thus, sodium or potassium salts of L-enantiomers of amino acids

Table 3. The sorption of amino acids on the sorbents

Amino acids	Amberlite XAD-16 (CSP-**1**)		Aminopropyl modified silica gel (CSP-**2**)	
	Sorption (%)	Stripping volume (mL)	Sorption (%)	Stripping volume (mL)
L-PhyAlaNa	61.15 ± 0.13	2.25	56.22 ± 0.20	3.00
D-PhyAlaNa	62.20 ± 0.10	3.00	57.27 ± 0.18	3.75
L-PhyAlaK	58.45 ± 0.17	2.25	53.23 ± 0.22	3.00
D-PhyAlaK	60.70 ± 0.10	3.00	55.46 ± 0.20	4.00
L-PhyGlyNa	56.62 ± 0.20	1.50	51.67 ± 0.25	2.25
D-PhyGlyNa	57.06 ± 0.15	2.00	52.70 ± 0.22	3.00
L-PhyGlyK	52.25 ± 0.25	1.50	47.23 ± 0.30	2.50
D-PhyGlyK	53.33 ± 0.20	2.00	49.18 ± 0.27	3.25
L-TrpNa	66.22 ± 0.10	3.00	60.09 ± 0.15	3.25
D-TrpNa	68.29 ± 0.10	4.00	62.39 ± 0.13	4.50
L-TrpK	63.60 ± 0.15	3.25	58.33 ± 0.20	3.75
D-TrpK	65.29 ± 0.11	4.50	59.18 ± 0.19	4.75

Experimental conditions: resin 0.1 g; volume of solution passed 10 mL; amino acid pH 10.5.

can be discriminated from those of other amino acids. When CSP-**1** was used for chiral separation, the retention times L-TrpNa and D-TrpNa were 3.00 and 4.00; for CSP-**2** these values were 3.25 and 4.50, respectively (Table 3 and Fig. 1). The same results were observed in the case of the potassium salts of the D- and L-enantiomers of amino acids. There are no signs of discrimination between the sodium and potassium salts of PhyAla and PhyGly for both the L- and D-enantiomers for CSP-**1**. This result showed that the structure of amino acid has a profound effect on the enantiomeric recognition, but there is no effect of the cation in the recognition (Fig. 2).

It has been observed that the sodium and potassium salts of D-enantiomers of all amino acids (PhyAlaNa, PhyAlaK and PhyGlyNa, PhyGlyK, and TrpNa, TrpK) show higher selectivity than the L-enantiomers for both CSP-**1** and CSP-**2**. This indicated that the D-enantiomers of amino acids were interacted more tightly with CSP-**1** and CSP-**2** than the L-enantiomers and as predicted, the L-enantiomers of amino acids provided more steric hindrance than the D-enantiomers. In conclusion, it has been shown that the retention times of L- and D-enantiomers of tryptophan were longer than those of phenylalanine and phenylglycine. In our previous study,[10] the retention time of tryptophan was shorter than others. It can be stated that besides hydrogen bonding, electrostatic interactions between tryptophan and chiral stationary phases, π–π interactions between indol ring and benzo unit of chiral stationary phases are dominant.

In all cases, for tryptophan of D- and L-enantiomer salts, the retention times are significantly longer than in the case of other amino acids. These results can be explained by assuming the favorable π–π interaction between the tryptophan unit and the crown's benzo unit. Such studies have been published.[14,15]

These results show that steric effects or steric repulsion and structural rigidity of the host, hydrogen binding, π–π interaction between host and guest may be the most important factors for the enantioselective recognition of amino acids salts. Additionally, π-stacking interactions between host **4** and the aromatic part of the amino acid may contribute further to the overall binding strength of the receptor. Although the sodium ion forms a stronger complex with 15-crown-5 than its potassium counterpart, it is very unlikely to find a correlation between the cation size and the enantioselectivity.

In can be seen in Table 3, when CSP-**1** and CSP-**2** were compared, that the retention time of CSP-**2** is longer than that of CSP-**1** with respect to its lower sorption percentage in sorption amounts of amino acids. The percentage sorption amounts of amino acids to Amberlite XAD-16 are higher. This is because the sorption amount of the chiral crown ether to Amberlite XAD-16 is higher than the modified silica gel. This may result from the π–π interaction between benzo unit of crown ether and Amberlite XAD-16.

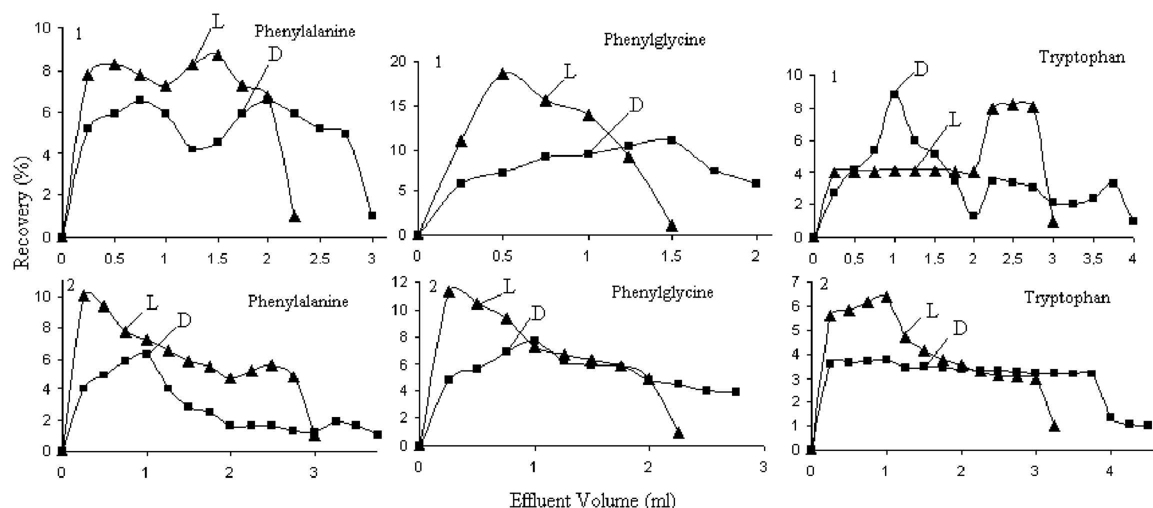

Figure 1. Separation of D- and L-forms of the amino acids sodium salt on the sorbents. (1) Amberlite XAD-16 and (2) aminopropyl modified silica gel.

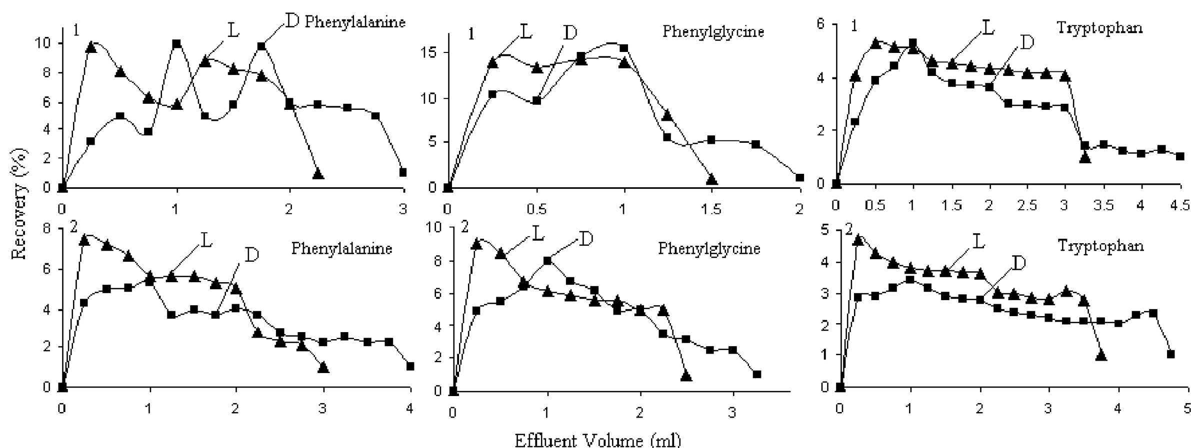

Figure 2. Separation of D- and L-forms of the amino acid potassium salts on the sorbents. (1) Amberlite XAD-16 and (2) aminopropyl modified silica gel.

3. Conclusion

In conclusion, CSP-**1** and CSP-**2** prepared by bonding chiral crown ether **4** to Amberlite XAD-16 and silica gel can be used for chiral separation of amino acids. These results presented here may indicate further developments in practical applications of the crown ether in solving analytical problems, for instance in HPLC method.

4. Experimental

4.1. General information

All chemicals were of reagent grade unless otherwise specified. Standard amino acid solutions (5×10^{-4} mol l^{-1}) were prepared by dissolving the required amount in doubly deionized water. Water was used throughout the work and deionized by a Millpore Milli-Q system.

The Amberlite XAD-16 resin (styrene–divinylbenzene copolymer, surface area: 800 $m^2 g^{-1}$, pore diameter: 10 nm and bead size: 20–60 mesh) was purified with 4 M HCl solution, after elimination of chlorides by washing with distilled water, with an ethanol–water (1:1) solution and finally with water again. Then the resin was dried in a vacuum oven at 60 °C and stored in a polyethylene bottle.

Perkin Elmer Spectrum BX Fourier Transform IR spectrometer was used to record the IR spectra of KBr discs, in the range 4000–700 cm^{-1}; 30 co-added interferograms were scanned at 2 cm^{-1} resolution. UV-1601 model Shimadzu UV–vis spectrophotometer in the range of 200–400 nm with 30 mm quartz cells was used to determine amino acids. For solid phase experiments, Varian cartridge (plastic container, 1.0 cm × 10.0 cm) equipped with 20 μm polypropylene frits was used.

4.2. Synthesis

4.2.1. (*S*)-*N*-Benzyl-2-amino-4-methyl-1-pentanol 2. (*S*)-Leucinol (21 g, 0.181 mol), benzyl chloride (5.72 g, 0.045 mol), and anhydrous Na_2CO_3 (4.65 g, 0.045 mol) were placed in a 250 mL two-necked round-bottomed flask equipped with a Dean Stark apparatus. The mixture was stirred at 110 °C for 12 h under dry N_2. Then the mixture was cooled and $CHCI_3$ (150 mL) was added to the mixture and refluxed for 2 h, and then $CHCl_3$ layer was separated from the solid phase. The solid phase was re-extracted with $CHCI_3$ (3 × 150 mL). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated. The product was distilled and crystallized from petroleum ether–benzene to give 8 g (88%), bp 123–125 °C/0.8 mmHg, mp 72–73 °C; $[\alpha]_D^{20} = +33.5$ (*c* 1, MeOH); IR (KBr) 3294, 3070, 3024, 2960, 2928, 2909, 1503, 1464, 1387, 1348, 1271, 1208, 1092, 1060, 1022, 970, 880, 841, 783, 746, 707, 617 cm^{-1}; ^1H NMR ($CDCI_3$) δ 0.90–0.96 (dd, 6H), 1.25–1.31 (m, 1H), 2.40–1.67 (m, 2H), 2.76–2.78 (m, 1H) 3.28–3.70 (m, 2H), 3.77–3.85 (dd, 2H), 7.26–7.38 (m, 5H); ^{13}C NMR ($CDCI_3$) δ 23.13, 25.37, 41.65, 56.62, 63.58, 77.81, 77.46, 127.53, 128.56, 128.90, 140.46; Anal. Calcd for $C_{13}H_{21}NO$: C, 75.44; H, 10.20; N, 6.60. Found: C, 75.36; H, 10.36; N, 6.76.

4.2.2. (*S*)-*N*-Benzyl-4-hydroxymethyl-3-aza-6-methyl-heptane-1-ol 3. A solution of **2** (10 g, 0.049 mol) in 250 mL methanol was cooled to −20 °C in a 100 mL flask. Ethylene oxide (2.2 mL, 0.049 mol) in 10 mL of methanol was added to the solution dropwise at −20 °C. The mixture was kept at −20 °C during addition in deepfreeze. After addition, the mixture was stirred for 24 h at −20 °C and 24 h at +4 °C. The mixture was kept for 1 day at room temperature in a closed flask. Methanol was evaporated on a rotary evaporator. The product was purified by distillation under reduced pressure to give 10 g (83%), bp 168–170 °C/0.8 mmHg; $[\alpha]_D^{20} = +35.6$ (*c* 1, MeOH), IR (KBr) 3358, 3060, 3024, 2960, 1599, 1496, 1478, 1458, 1362, 1162, 1112, 1066, 918, 867, 732, 701 cm^{-1}; ^1H NMR ($CDCI_3$) δ 0.88–0.92 (dd, 6H), 1.08–1.13 (m, 1H), 1.38–1.44 and 1.52–1.56 (m, 2H), 2.54–2.58 (m, 1H), 2.77–2.87 (m, 2H), 3.37–3.59 (m, 6H) 7.28–7.40 (m, 5H); ^{13}C NMR ($CDCI_3$) δ 22.77, 24.06, 25.82, 35.60, 51.42, 55.19, 59.14, 60.50, 62.37, 127.57, 128.86, 129.24, 140.37; Anal. Calcd for $C_{15}H_{25}NO_2$: C, 71.71; H, 9.96; N, 5.58. Found: C, 71.70; H, 9.89; N, 5.60.

4.2.3. (*S*)-2-Isobutyl-*N*-benzyl-4,7,10,13-tetraoxa-8,9-benzo-1-azacyclopentadec-8-ene 4. To a suspension of NaH (2.15 g, 0.0715 mol, % 80 in mineral oil) in 100 mL dry THF at 0 °C was added a solution of diol **3** (4 g, 0.021 mol) in 250 mL of THF. The reaction mixture was refluxed for 2 h. After cooling to 0 °C, a solution of 1,2-bis-(2-*p*-tolylsulfonyl ethoxy) benzene (10.62 g, 0.021 mol) in 250 mL of THF was slowly added to the reaction mixture. The suspension was refluxed for 50 h. The solvent was evaporated and 100 mL of water was added to the residue. The mixture was extracted with CH_2CI_2 (3 × 150). The combined organic layers were washed with 100 mL water again, dried on anhydrous Na_2SO_4, and the solvent was evaporated. The crude product was purified by flash column chromatography on silica gel (eluent: triethylamine–ethyl acetate–petroleum ether 60–80 = 3:17:80) to give 4 g (61%); $[\alpha]_D^{20} = -11.6$ (c 1.4, $CHCl_3$), IR: ν 3070, 3031, 2940, 2877, 1592, 1503, 1458, 1362, 1259, 1227, 1131, 1053, 938, 783, 739, 707 cm^{-1}; ^1H NMR ($CDCI_3$) δ 0.88–0.95 (m, 6H), 1.21–1.81 (m, 3H), 2.86–3.13 (m, 3H), 3.62–3.88 (m, 10H), 4.12–4.20 (m, 4H), 6.90–6.98 (m, 4H), 7.22–7.40 (m, 5H); ^{13}C NMR ($CDCI_3$) δ 22.99, 23.71, 25.39, 38.47, 50.63, 55.99, 58.34, 69.32, 69.88, 69.97, 70.95, 71.93, 73.80, 113.85, 121.63, 126.99, 128.48, 129.00, 141.71, 149.43; Anal. Calcd for $C_{25}H_{35}NO_4$: C, 72.64; H, 8.47; N, 3.30. Found: C, 72.40; H, 8.10; N, 3.10.

4.2.4. Preparation of monoamine modified silica gel. Ten grams of dry silica gel (70–230 mesh) was mixed with 50 mL dry toluene and 12 mL of 3-aminopropylmethoxysilane in a round-bottomed flask and refluxed overnight. The reaction mixture was left to cool, filtered off, and washed with toluene, ethanol, and diethyl ether and finally dried at 60 °C for 6 h.

4.2.5. Impregnation process. Amounts of 0.1 g portion of dry Amberlite XAD-16 and the aminopropyl modified silica gel were contacted with 25.0 mL of chiral crown ether solution and stirred for 24 h. The impregnated resin and impregnated aminopropyl modified silica gel were separated by filtration through a sintered glass funnel and were washed with water to remove the solvent. The resin and the silica gel were used as air-dried products. The CSP-1 and CSP-2 contents in the impregnated solution were determined gravimetrically, by weighing the dry ligand residue after removing the resin and evaporation of solution. The amounts of impregnated Amberlite XAD-16 (CSP-**1**, 72%) and aminopropyl modified silica gel (CSP-**2** 63%) were calculated from the material balance.

4.2.6. Column experiments. The chiral crown ether (0.1 g) on Amberlite XAD-16 and 0.1 g of the chiral crown ether on derivatized silica gel were firstly wetted with 5 mL methanol and stirred for 10 min, and then 5 mL of doubly deionized water was added and stirred for 10 min again. Lastly, the mixture was transferred to the polyethylene column and 25 mL of methanol–water (10:90) was passed through the column. A sample solution containing amino acid (phenylalanine or phenylglycine or tryptophan as their potassium or sodium salts) was taken and the pH was adjusted to ~10.5 and passed through the above column. Then, the amino acids were stripped from the column with doubly deionized water and determined spectrophotometrically at 253 nm (for phenylalanine and phenylglycine) and 280 nm (for tryptophan). All runs were carried out at ambient temperature (23–25 °C).

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