# Molecular Engineering 4. Chiral Calix[4]crown Having Binaphthyl Crown Unit on the Upper-Rim

**Hyejae Ihm and Kyungsoo Paek***

*Department of Chemistry, Soong Sil University, Seoul 156-743, Korea*
*Center for Biofunctional Molecules, Pohang P.O. Box 125, Pohang 790-600, Korea*
*Received January 27, 1998*

Chiral recognition is one of the fundamental processes in living systems. Among the various chiral host molecules such as crown ethers,[1] calixarenes,[2] cyclodextrins,[3] porphyrins,[4] etc., calixarenes have attracted considerable interests due to their potential as platforms for the attachment of convergent binding groups at the upper or lower rim.[5] Many chiral recognition properties by calix[4]arene system have been reported,[2,6] but the examples having chiral element on the upper-rim are quite rare.[7] If a chiral substituent having a substantial chiral barrier could be regioselectively incorporated on the upper-rim of cone-structured calix[4] arene, a chiral calix[4]arene host having a hydrophobic cavity into which guest molecules can be bound would be afforded.

Chiral recognition properties of dissymmetric chiral calix [4]crowns having binaphthyl crown unit on the lower-rim have been reported.[6a] Also the cone-structured upper-rim calix[4]crowns which have a hydrophobic cavity as well as a hydrophilic binding site are known to show synergistic binding property for alkylammonium ions.[8] It was therefore thought that the distal incorporation of a chiral binaphthyl crown unit on the upper-rim of a cone-structured calix[4] arene would show interesting chiral recognition properties.

## Experimental Section

**General Details.** All chemicals were reagent grades and used directly unless otherwise specified. All anhydrous reactions were conducted under an argon atmosphere. Alkylammonium picrates were prepared by neutralization of the appropriate amine with picric acid in methanol and purified by recrystallization from methanol.[9] Melting points were measured on an Electrothermal 9100 apparatus and uncorrected. The $^1$H NMR spectra and $^{13}$C NMR spectra were run on a Bruker DPX 300, Gemini-300 (300 MHz) or JEOL lambda-400 (400 MHz) spectrometer. Mass spectrum was run on a VG 70-VSEQ for positive FAB mass. CD spectra were measured at 25 °C with a Jovin-Yvan CD6 CD-ORD spectropolarimeter. UV spectra were obtained using a Shimadzu UV-3101 PC spectrophotometer. The fluorescence spectra were measured in chloroform using Shimadzu RF-5301 PC spectrofluorometer. Gravity column chromatography was performed on E. Merck silica gel 60 (70-230 mesh ASTM). Thin layer chromatography was done on plastic sheets silica gel 60 $F_{254}$ (E. Merck, 0.2 mm).

**(R)-(+)-2,2'-(2-(2-chloroethoxy)ethoxy)-1,1'-binaphthalene (1).** A solution of 2.0 g (7.0 mmol) of (R)-(+)-binaphthol and 11.6 g (83.8 mmol) of potassium carbonate in 200 mL of DMSO was warmed to 55-60 °C and stirred for 30 min. After addition of 19.5 g (69.8 mmol) of 2-(2-chloroethoxy)ethane tosylate, the reaction mixture was stirred for 1.5 d, then cooled to room temperature, and treated with 2 N HCl. The residue was extracted with $CH_2Cl_2$, washed with water, brine and then dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, and then the crude mixture was chromatographed on silica gel gravity column (4×23 cm, hexane : $CH_2Cl_2$=1 : 2) to yield 3.1 g (89%) of the product as a yellow oil: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.97, 7.89, 7.44 (each d, each 2H, Ar*H*), 7.36 (t, 2H, Ar*H*), 7.24 (m, 4H, Ar*H*), 4.13 (m, 4H, $OCH_2$), 3.51 (t, 4H, $CH_2Cl$), 3.13 (m, 8H, $OCH_2$).

**(R)-(+)-2,2'-(2-(2-iodoethoxy)ethoxy)-1,1'-binaphthalene (2).** A solution of 2.1 g (13.9 mmol) of sodium iodide in 60 mL of methyl ethyl ketone was refluxed for 1 h. To a refluxing solution was added 2.0 g (4.0 mmol) of chloro compound **1** in methyl ethyl ketone, and the temperature was controlled to 60-65 °C. The solution was stirred for 2 d, and then cooled to room temperature. After the solvent was removed *in vacuo*, the residue was extracted with $CH_2Cl_2$, washed with water, brine and then dried over anhydrous $MgSO_4$. The concentrated mixture was chromatographed on silica gel gravity column (4.5×17 cm, hexane : $CH_2Cl_2$=1 : 3) to give 1.3 g (80%) of the product as a yellow oil: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.83 (two d, 4H, Ar*H*), 7.43-7.14 (m, 8H, Ar*H*), 4.05 (m, 4H, $OCH_2$), 3.42 (m, 4H, $OCH_2$), 3.08 (m, 4H, $OCH_2$), 2.63 (m, 4H, $CH_2I$).

**Chiral Calix[4]crown (4).** A mixture of 86 mg (3.6 mmol) of NaH and 40 mL of DMF was warmed to 65 °C and to this a solution of 170 mg of diol **3**[10] (0.18 mmol) and 134 mg (0.20 mmol) of iodo compound **2** in 40 mL of DMF was dropwisely added with dropping funnel over 8 h. The reaction mixture was stirred for an additional 24 h, then cooled to room temperature, and treated with methanol. The solvent was removed *in vacuo* and the crude product was extracted with $CH_2Cl_2$. The organic phase was washed with water, brine and then dried over anhydrous $MgSO_4$. The concentrated mixture was chromatographed on silica gel gravity column (2.5×16 cm, hexane : EtOAc=5 : 1) and recrystallized from a mixture of $CH_2Cl_2$ and methanol to give 57 mg (23%) of the product **4** as a white powder: mp 78.6-79.6 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ 7.96, 7.90, 7.49 (each d, each 2H, binaphthalene), 7.33-7.14 (m, 6H, binaphthalene), 7.24 (dd, 4H, ArH), 5.73 (dd, 4H, Ar*H*), 4.36 (dd, *J*=13.2 Hz, 4H, *endo*-ArC*H*), 4.24 (m, 2H, $OCH_2$), 4.06 (t, 4H, $OCH_2$), 3.99 (m, 2H, $OCH_2$), 3.59 (t, 4H, $OCH_2$), 3.56 (m, 2H, $OCH_2$), 3.39 (m, 6H, $OCH_2$), 3.28 (m, 4H, $OCH_2$), 3.05 (dd, *J*=13.2 Hz, 4H, *exo*-ArC*H*), 1.97-1.76 (m, 8H, $OCH_2CH_2$), 1.38-1.26 (m, 24H, $(CH_2)_3CH_3$), 0.91 (t, 12H, $CH_3$); $^{13}$C NMR (100.4 MHz, $CDCl_3$) δ 157.07, 154. 45, 153.60, 149.66 (ArC), 139.07, 138.95 (ArCH), 134.12

(ArC), 133.21, 132.81, 131.39 (ArCH), 131.25 (ArC), 129.55, 129.23 (ArCH), 127.86, 126.24, 125.43, 123.69, 120.93 (ArC), 116.61 (ArCBr), 114.99, 114.25, 113.03 (ArCH), 75.74, 75.14 ($OCH_2$), 70.14, 70.08, 69.59, 67.58 (bridged $OCH_2$), 32.14, 31.91 ($ArCH_2Ar$), 31.16, 31.05, 30.47, 29.23, 25.59, 22.93, 22.69 ($CH_2$), 14.09, 14.01 ($CH_3$); $FAB^+$ MS (3-nitrobenzyl alcohol), m/z 1376 ($M^+$, 100%).

**Liquid-liquid Extraction Experiments.** A two-phase liquid-liquid extraction experiment was carried out between an aqueous solution (1.0 mL, [GPic]=$6.0\times10^{-5}$ M, [GCl]=0.5 M) and a chloroform solution (1.0 mL, [H]=$3.0\times10^{-3}$ M). The two-phase mixture in a tightly-stoppered centrifuge tube was shaken with a Vortex-Genie for 2 min at 25 °C and then centrifuged at 1500 rpm for 1 min. The extractability was determined spectrophotometrically from the decrease in the absorbance of the picrate ion in the aqueous phase ($[GPic]_i$) using the following equation.

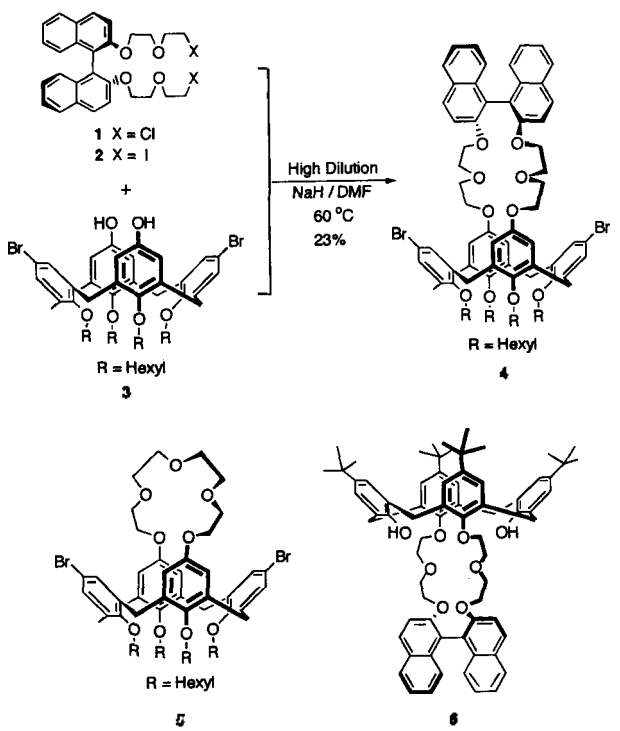
Extractability (%)

$=(6.0\times10^{-5}\text{ M}-[GPic]_i)/6.0\times10^{-5}\text{ M}\times100$

**Spectrofluorometric Titration Experiment.** The amino acid methyl esters were used as HCl salts. The fluorescence spectra were measured in $CHCl_3$ at 25 °C. The slit widths of excitation and emission were 5 nm. Each of the amino esters was titrated in chloroform solution containing the host 4 ($1.0\times10^{-6}$ M). The binding constants ($K_a$) of the host were obtained from the changes in fluorescence intensity of host 4 on addition of amino esters using the following Benesi-Hildebrand equation,[11]

$$1/(F_0-F)=1/K_aC\,(F_0-F_\infty)+1/(F_0-F_\infty)$$

where $C$ is the concentration of amino esters added, $F_0$ and $F$ are the fluorescent intensity in the absence and presence of amino esters, respectively. $F_\infty$ is the intrinsic fluorescence intensity of the complexes.




**Scheme 1.**

## Results and Discussion

(R)-(+)-Binaphthol ($[\alpha]^{21}$+34°, c=1, THF) was treated with 2-(2-chloroethoxy)ethyl tosylate in $K_2CO_3$/DMSO mixture at 55-60 °C to give (R)-2,2'-bis-(2-(2-chloroethoxy)ethoxy)-1,1'-binaphthalene **1** (Scheme 1). Chiral binaphthols are known to be optically stable at 100 °C for 24 h in dioxane-water.[12] Chloro compound **1** was treated with NaI in MEK solution at 60-65 °C to give (R)-2,2'-bis-(2-(2-iodoethoxy) ethoxy)-1,1'-binaphthalene **2** in 80% yield. The optical property of iodo compound **2** was confirmed by its CD spectra shown in Figure 1.

The pinched-cone structured distal diol **3**[10] was coupled with the chiral iodo compound **2** to give the conformationally stable chiral host **4**. Host **4** was obtained in 23% yield by high-dilution reaction between the diol **3** treated with NaH and the chiral iodo compound **2** in DMF solution at 60 °C.

The $^1H$ NMR spectrum of host **4** shows that it is a cone-structured and distal bridged chiral calix[4]crown having $C_2$ symmetry. Aryl protons of calix[4]arene appeared as two doublets of doublet at δ 7.24 and 5.73. Also methylene protons bridging phenyls appeared as two doublets of doublet at δ 4.36 for $H_{endo}$ and at δ 3.05 for $H_{exo}$. The $^{13}C$ NMR and $FAB^+$ MS spectra also supported its anticipated structure. The chirality of host **4** was also confirmed by its CD spectra showing the similar pattern of cotton effect to that of the iodo compound **2** (Figure 1), which means the binaphtyl of host **4** has the same chirality of iodo compound **2**.

The cation recognition property of host **4** was studied by liquid-liquid extraction of aqueous alkali metal, ammonium, or alkylammonium picrates into a $CHCl_3$ solution of the host at 25 °C (Figure 2). The extracted picrate concentration was calculated from the residual picrate concentration in aqueous solution because the typical $\lambda_{max}$ values at around 360 nm of picrate and of binaphthyl unit were overlapped. The extractability of host **4** was compared to that of the host **5**[8a] having a tetraethyleneoxy crown unit on the upper-rim.

The lower-rim cyclized binaphthyl host **6**[6a] which has no

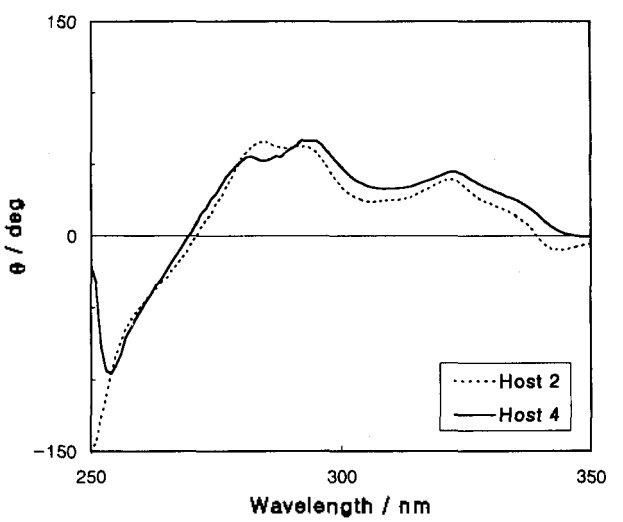



**Figure 1.** CD Spectra of Host **2** and **4** at 25 °C ([**2**]=[**4**]=$1.0\times10^{-4}$ M in $CH_2Cl_2$).

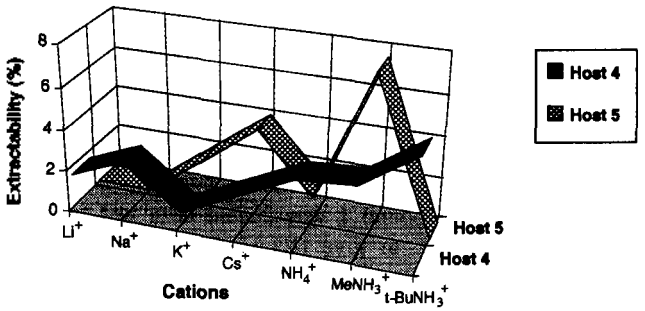



**Figure 2.** Liquid-liquid Extractability Spectra of Calix[4]crown **4** and **5**[8a] of Alkali Metal and Alkylammonium Picrates ([H]=$3.0\times10^{-3}$ M, [GPic]=$6.0\times10^{-5}$ M, [GCl]=0.5 M, Average Values of Two Trials).

**Table 1.** Binding Constants ($K_a$)[a] for the Complexes of Host **4** and **6**[6a] with Hydrochloride Salts of α-Amino Acid Methyl Ester in $CHCl_3$ at 25 °C

| Amino acid ester | $K_a$ ($\times10^{-3}$ $M^{-1}$) | | $K_L/K_D$ | $K_D/K_L$ |
|---|---|---|---|---|
| | **4** | **6** | **4** | **6** |
| L-Phe-OMe | 8.46 | 2.79 | 1.39 | 3.22 |
| D-Phe-OMe | 6.09 | 8.99 | | |
| L-Val-OMe | 5.52 | 6.89 | | |
| L-Leu-OMe | 3.57 | 12.1 | | |
| L-Ile-OMe | 2.51 | 12.8 | | |
| L-Met-OMe | 5.91 | – | 1.33 | – |
| D-Met-OMe | 4.43 | – | | |
| L-Phegly-OMe[b] | 2.45 | – | | |

[a] monitored by spectrofluorometer; $\lambda_{ex}$=328 nm, $\lambda_{em}$=374 nm for host **4**, $\lambda_{ex}$=332 nm, $\lambda_{em}$=380 nm for host **6**; [H]=$1.0\times10^{-5}$ M; determined by Bensei-Hildebrand equation. [b](S)-(+)-2-Phenylglycine methyl ester·HCl.

side arms available as additional ligands showed low affinities for alkali metal, ammonium and *t*-butylammonium ions. The upper-rim cyclized hosts **4** and **5** also showed rather weak binding affinities for these cations (< 7%). Typically spherical cations are bound weakly, because the crown moiety is so large and not well organized for spherical cations. Host **4** showed the highest affinity for *t*-$BuNH_3^+$, whereas host **5** showed the highest affinity for $MeNH_3^+$, which manifests the overall larger binding space of host **4** than that of host **5**. This better affinity of host **4** for larger alkylammonium ion suggests its potential binding ability for amino acid ester·HCl salts having a large alkyl group.

The chiral recognition of amino acid methyl esters by host **4** was studied by spectrofluorometric titration method. The association constants ($K_a$) were calculated using Benesi-Hildebrand equation[11] and compared to those of host **6** having a chiral binaphthyl crown unit on the lower-rim.

As summarized in Table 1, host **4** showed the similar affinities to those of host **6** for amino acid methyl esters. In general host **4** showed larger affinity toward amino ester having a larger alkyl group ($K_a$ of L-Phe-OMe > L-Met-OMe > L-Leu-OMe), which implies that a bulkier alkyl group can be accommodated better in the cavity of the upper-rim. Host **4** showed the higher selectivity for L-PheOMe or L-MetOMe over D-PheOMe or D-MetOMe ($K_L/K_D$=1.39 or 1.33), which contrasts with that of host **6**. But the low selectivity values imply that the designed chiral barrier transfered from binaphtyl to two bromo groups on calix[4]arene unit is inefficient compared to that of 3,3'-substituents on binaphtyl. Host **4** exhibited the lowest affinity for L-PheglyOMe, which suggests the phenyl group of L-PheglyOMe might be inappropriately oriented due to its structural rigidity in point of the proper ammonium binding to crown unit.

In conclusion, chiral calix[4]crown **4** was efficiently synthesized by adopting a chiral binaphthyl unit on the upper-rim. Picrate extraction experiment and spectrofluorometric titration show that host **4** has high binding abilities toward alkylammonium ions and amino acid methyl esters. The low selectivity will be improved when bromo groups are modified to better binding side arms.

**Acknowledgment.** The financial support from the Ministry of Education, Korea (BSRI-97-3437) is gratefully acknowledged.

## References

1. (a) Kyba, E. P.; Timko, J. M.; Kaplan, L. J.; de Jong, F.; Gokel, G. W.; Cram, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 4555. (b) Davidson, R. B.; Bradshaw, J. S.; Jones, B. A.; Dalley, N. K.; Christensen, J. J.; Izatt, R. M.; Morin, F. G.; Grant, D. M. *J. Org. Chem.* **1984**, *49*, 353. (c) Peacock, S. C.; Domeier, L. A.; Gaetu, F. C. A.; Helgeson, R. C.; Timko, J. M.; Cram, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 8190. (d) Sawada, M.; Takai, Y.; Yamada, H.; Hirayama, S.; Kaneda, T.; Tanaka, T.; Kamada, K.; Mizooku, T.; Takeuchi, S.; Ueno, K.; Hirose, K.; Tobe, Y.; Naemura, K. *J. Am. Chem. Soc.* **1995**, *117*, 7726.
2. (a) Morozumi, T.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1219. (b) Morozumi, T.; Shinkai, S. *Chem. Lett.* **1994**, 1515.
3. (a) Lipkowitz, K. B.; Raghothama, S.; Yong, J. *J. Am. Chem. Soc.* **1992**, *114*, 1554. (b) Hamilton, J. A.; Chen, L. *J. Am. Chem. Soc.* **1988**, *110*, 5833.
4. (a) Kuroda, Y.; Kato, Y.; Higashioji, T.; Hasegawa, J.; Kawanami, S.; Takahashi, M.; Shiraishi, N.; Tanabe, K.; Ogoshi, H. *J. Am. Chem. Soc.* **1995**, *117*, 10950. (b) Konishi, K.; Yahara, K.; Toshishige, H.; Aida, T.; Inoue, S. *J. Am. Chem. Soc.* **1994**, *116*, 1337.
5. (a) Gutsche, C. D. *Calixarenes, Monographs in Supramolecular Chemistry*; Stoddart, J. F., Ed.; The Royal Society of Chemistry Press: Cambridge, 1989; Vol. 1. (b) *Calixarenes: A Versatile Class of Macrocyclic Compounds*; Vicens, J.; B hmer, V., Ed.; Kluwer Press: Dordrecht, 1991. (c) Böhmer, H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713.
6. (a) Kim, T.; Ihm, H.; Paek, K. *Bull. Korean Chem. Soc.* **1997**, *18*, 681. (b) Andreetti, G. D.; Böhmer, V.; Jordon, J. G.; Tabatabai, M.; Ugozzoli, F.; Vogt, W.; Wolff, A. *J. Org. Chem.* **1993**, *58*, 4023. (c) Pappalardo, S.; Parisi, M. *Tetrahedron Lett.* **1996**, *37*, 1493.
7. Ikeda, A.; Yoshimura, M.; Lhotak, P.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1945.
8. (a) Ihm, H.; Kim, H.; Paek, K. *J. Chem. Soc., Perkin*

*Trans. 1* **1997**, 1997. (b) Paek, K.; Ihm, H. *Chem. Lett.* **1996**, 311.
9. Almansa, C.; Moyano, A.; Serratosa, F. *Tetrahedron* **1992**, *48*, 1497.
10. Paek, K.; Kim, H.; Chang, S.-K. *Supramol. Chem.* **1995**, *5*, 83.
11. (a) Benesi, H.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703. (b) Shirai, M.; Matoba, Y.; Tsunooka, M. *Eur. Polym. J.* **1995**, *31*, 559.
12. Kyba, E. P.; Gokel, G. W.; de Jong, F.; Koga, K.; Sousa, L. R.; Siegel, M. G.; Kaplan, L.; Sogah, G. D. Y.; Cram, D. J. *J. Org. Chem.* **1997**, *42*, 4173.

# Analyses of Phthalates and Peptides Using a Gradient μLC/MS System

**Won Jo Cheong*, Choong Sik Oh, and Jong-Shin Yoo†**

*Department of Chemistry and Center for Chemical Dynamics, Incheon 402-751, Korea*
*†Mass Spectrometry Analysis Group, Korea Basic Science Institute, Taejon 305-333, Korea*
*Received February 7, 1997*

Liquid Chromatography/Mass Spectrometry (LC/MS) has been rapidly commercialized and extensively used in analytical applications since it first appeared over twenty years ago.[1-3] The incompatibility between the LC and MS systems had retarded the appearance of a useful LC/MS system before. Fundamentally, the LC system was operated in the liquid phase, and the MS system, in the gas phase. The conventional LC system eluted *ca.* 1 mL liquid corresponding to *ca.* 500-1,000 mL gas per minute, but the conventional MS system could endure 10 mL gas or so in those days.

In the last two decades, ultra-high vacuum systems of high capacity have been developed, and physical concepts of separating solutes from the solvent, mechanical designs, and low-temperature ionization techniques have been devised to construct useful LC/MS systems. Such techniques include thermospray,[4-6] particle beam,[7-9] fast atom bombardment,[10-20] atmospheric pressure ionization,[21] and electrospray[22-25] methods. Some of the commercial systems are known to be directly connected to a conventional LC whose flow rate is 1 mL/min or so. However, reducing the LC flow rate as low as possible is strongly recommended to minimize contamination of the MS system for long term maintenance, thus use of a μLC for μLC/MS is rationalized. Commercial packed silica capillary microcolumns are usually employed for such purposes, but handling the silica capillary columns is inconvenient since they are fragile.

We have been studying to make glass-lined stainless steel microcolumns and recently have constructed a gradient μLC system using such columns.[26-31] In this study, we have constructed a gradient μLC/MS system using the glass-lined stainless steel microcolumns. We believe that this is the first report for a gradient μLC/MS system with a glass-lined stainless steel microcolumn. We have found that this system would be a dependable analytical tool after preliminary analyses of a couple of test samples--a phthalate mixture and a peptide mixture.

## Experimental

Two Shimadzu (Tokyo, Japan) 10AD pumps, a Shimadzu DGU-14A membrane degasser, a Tee union with a 1/16 inch I.D. stainless steel frit (as a mixer), a Valco(Houston, USA) CI4W0.05 injector with a 50 nL injection loop, and a 0.5 mm I.D. glass-lined stainless steel C18 microcolumn were combined to construct the micro-flow gradient liquid chromatography part of the system. The Adsorbosphere C18 stationary phase (5 μm) from Alltech (Deerfield, USA) was used as the packing material for the microcolumn. An 1 m × 50 μm I.D. (400 μm O.D.) silica capillary was connected to the column outlet union. A piece of Teflon tubing of 1/16 inch O.D. and 400 μm I.D. was employed to fit the silica capillary in the union. The other end of the silica capillary was introduced into the stainless steel capillary of the electrospray interface of the mass spectrometer. The mass spectrometer used was a VG Biotech (Manchester, UK), Quattro

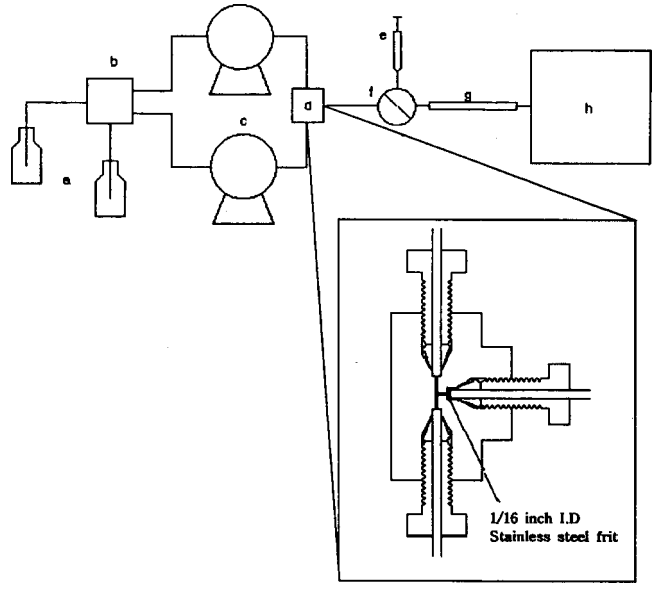



**Figure 1.** The layout of the LC/MS system. a; mobile phase, b; degasser, c; pump, d; Tee, e; sample syringe, f; injector, g; microcolumn, h; mass spectrometer.