Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 3195–3199

TETRAHEDRON

Synthesis and recognition of amino acids by binaphthyl-crown receptors

Kazunori Tsubaki,* Hiroyuki Tanaka, Hiroshi Morikawa and Kaoru Fuji*

Institute for Chemical Research, Kyoto University, Gokasho, Uji, Kyoto 6110011, Japan

Received 17 February 2003; revised 24 March 2003; accepted 24 March 2003

This paper is dedicated to Emeritus Professor Soichi Misumi for his 77th birthday

Abstract—Two binaphthyl crown receptors containing phenylboric acid **2** and 2,4-dinitrophenylurea **3** as lariat parts were prepared from the optically active binaphthyl crown alcohol **1** in two and four steps, respectively. Host **2** showed a 30% extraction efficiency for GABA by a solid–liquid extraction method in DMSO. Chromogenic Host **3** discriminated the guest linear amino acid by molecular length and the information was revealed through color changes.

1. Introduction

In the field of host–guest chemistry, attention has gradually shifted from metallic cationic and organic cationic guests[1] to anionic guests[2] and guests with more complicated properties such as amino acids,[3] sugars,[4] and oligo peptides.[5] Recently, we proposed a new binaphthyl crown ether skeleton **1**, which possessing a scaffolding hydroxy group in front of binaphthyl to effectively discriminate the chirality of the guest molecules.[6] Furthermore, tailor-made host molecules can be synthesized using short steps based on complementary properties of the guest molecules. Here, this approach is used to introduce components on the hydroxy group of **1** to form the two host molecules, which contain phenylboric acid **2** and 2,4-dinitrophenylurea **3** and recognize amino acids (Fig. 1).

2. Results and discussion

Scheme 1 shows the steps required to synthesize Hosts **2** and **3** from the key intermediate **1**. Compound **1** was reacted with 2-bromobenzyl bromide to yield 90% of bromide **6**, which was then lithiated, converted to the boric ester, and hydrolyzed to form Host **2** in an overall yield of 60%.[7] On the other hand, tosylate **7** was prepared from **1** in a 76% yield. **7** was subjected to azidation of tosyl groups in a 94% yield and then reduced to form **9** in a 98% yield. Amine **9** was reacted with a large excess of activated carbamate, which was generated in situ between 2,4-dinitroaniline and

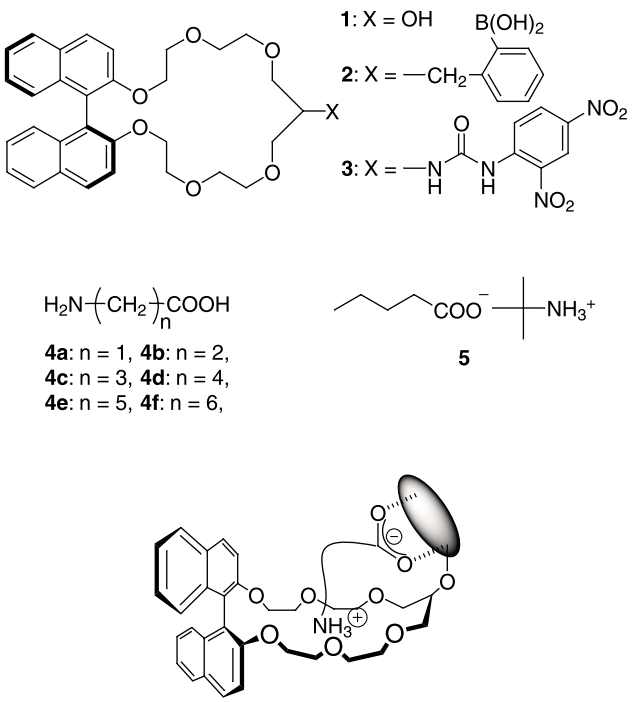


Figure 1. Key compounds **1**–**5** and proposed complex structure.

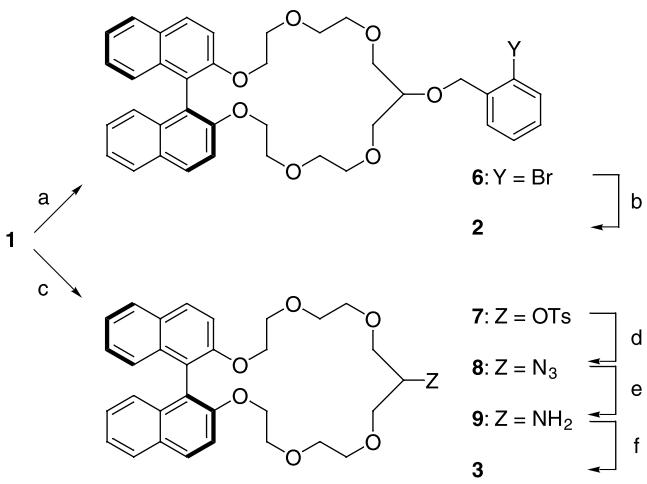
phenyl chloroformate, to yield Host **3** in a 75% yield and an overall yield of 53%.

When Host **2** was designed so that the crown ether part would capture the ammonium group and the boric acid would bind the carboxylate group. In fact, Reetz reported a crystal structure of a three component complex of phenylalanine, 18-crown-6 ether, and 3,5-bis(trifluoromethyl)phenylboric acid.[8] The interaction between Host **2**

Keywords: crown ethers; molecular recognition; amino acids; solid–liquid extraction.

* Corresponding authors. Tel.: +81-774-38-3193; fax: +81-774-38-3197; e-mail: tsubaki@fos.kuicr.kyoto-u.ac.jp

0040–4020/03/$ - see front matter
doi:10.1016/S0040-4020(03)00478-2



Scheme 1. (a) 2-Bromobenzyl bromide, NaH, 90%; (b) (i) *n*-BuLi, (ii) $B(OMe)_3$, (iii) H^+, 60% (three steps); (c) TsCl, pyridine, 76%, (d) NaN_3, 94%; (e) Pd/C, HCO_2NH_4, 98%; (f) 2,4-dinitroaniline, phenyl chloroformate, 75%.

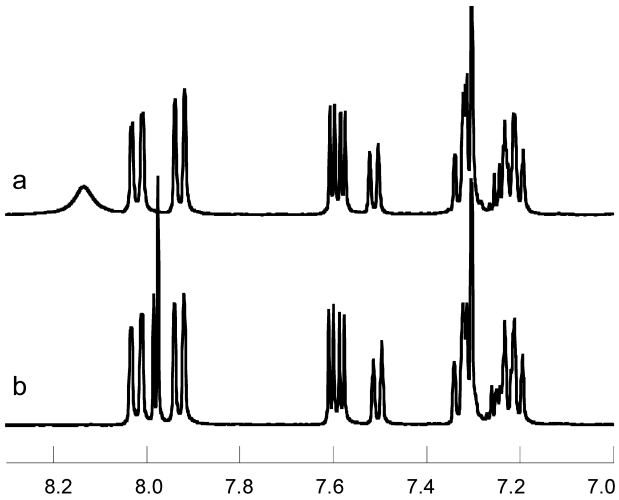


Figure 3. Partial ^{1}H NMR (400 MHz) spectra of (a) Host **2** after extraction of Guest **4c**, (b) Host **2** in DMSO at 22°C. [Host **2**]=1.56×10^{-2} M.

and various amino acids was investigated by a solid–liquid extraction method and estimated using an NMR method.[9] Thus, our NMR studies were conducted under the identical conditions, the same concentration of Host **2** and lot number of $DMSO_{d\text{-}6}$ (99.9% atom) as the solvent. Using the DMSO signal (2.5 ppm) as an internal standard, the integral values of the corresponding signals determined the degree of extraction. In spite of adding solid α-amino acids to the $DMSO_{d\text{-}6}$ solution of Host **2**, the signals ascribed from α-amino acids were barely discernible after filtering. Contrary to α-amino acids, Host **2** obviously extracted GABA[10] (γ-aminobutylic acid, **4c**). Figures 2 and 3 show partial ^{1}H NMR spectra.

In Figure 2, a small amount of GABA (**4c**) oozed into DMSO even in the absence of Host **2**. In the presence of Host **2**, the signal intensities assigned to Guest **4c** obviously increased and shifted downfield. Those lower magnetic field shifts may result from two conflicting effects. One is the increased zwitter ionic character, which would shift the **4c** to a lower magnetic field due to the decrease in electron density of the nitrogen atom. The other is the shielding field of the binaphthyl group since the ammonium group of **4c** is inside the crown ring of Host **2**. In Figure 3, the $B(OH)_2$ signal shifted from 7.98 to 8.13 ppm, which indicates hydrogen bonding between $B(OH)_2$ of Host **2** and the carboxylate of Guest **4c**. Based on the ^{1}H NMR integrated intensities of Host **2** and **4c**, the estimated amount of **4c** extracted was 30% against Host **2**.[11] Applying solid–liquid extraction methods to chiral recognition showed small selectivities as follows; Boc-D-Lys (18.5%), Boc-L-Lys (14.1%) and H-D-Asp-NH_2 (8.2%), H-L-Asp-NH_2 (4.3%).

Host **3** was also designed to bind amino acids. It contained a pendant 2,4-dinitrophenylurea part that was expected to bind guest carboxylates and change color.[12] First, the mode of complexation using *tert*-butylammonium valerate (**5**), which is a sufficiently soluble guest molecule in DMSO, was investigated. Figures 4 and 5 show the partial ^{1}H NMR spectrum and NOESY spectrum between Host **3** and Guest **5**.

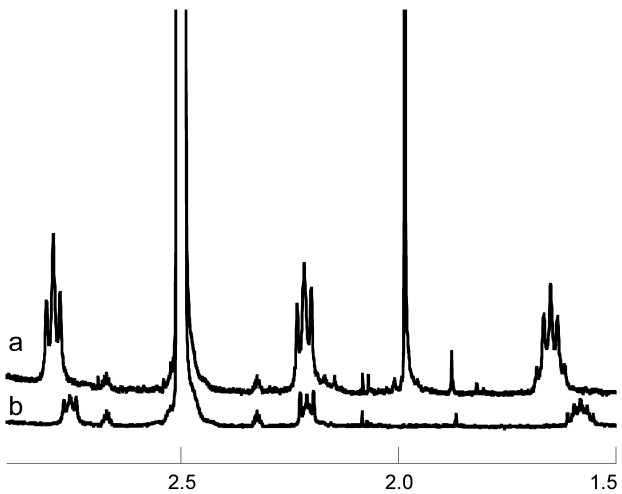


Figure 2. Partial ^{1}H NMR (400 MHz) spectra of (a) GABA (**4c**) in the presence of Host **2**, (b) GABA (**4c**) in the absence of Host **2** after extraction in DMSO at 22°C. [Host **2**]=1.56×10^{-2} M.

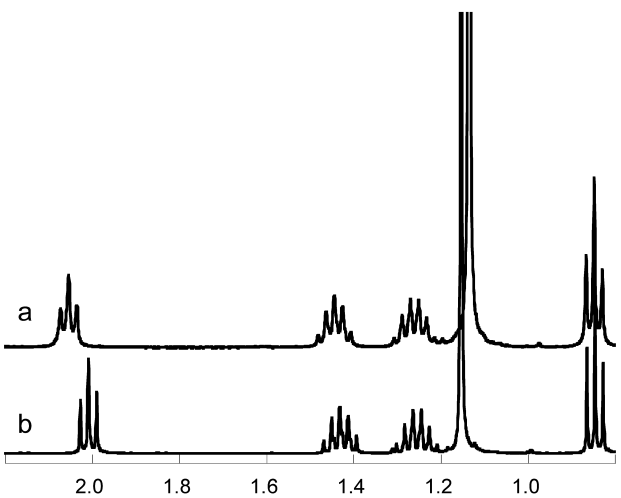


Figure 4. Partial ^{1}H NMR (400 MHz) spectra of (a) 1:1 mixture of Host **3** (1.4×10^{-2} M) and Guest **5** (b) Guest **5** in DMSO at 21°C.

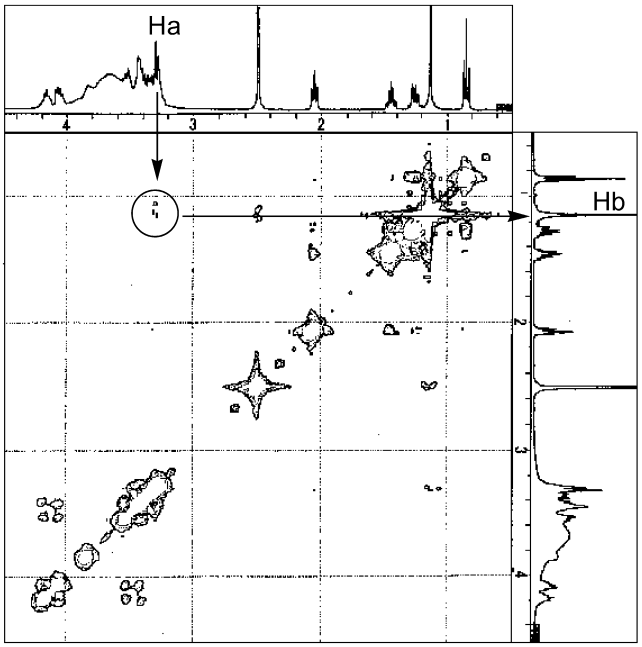


Figure 5. NOESY (400 MHz) spectrum of a 1:1 mixture of Host **3** (1.4×10^{-2} M) and guest **5** in DMSO at 21°C. Ha means the protons of crown ether part of Host **3**, Hb means the *tert*-butyl protons of Guest **5**, respectively.

In Figure 4 a signal ascribed to the α-methylene of carbonyl group of Guest **5** in a 1:1 mixture was shifted downfield, which indicates that hydrogen bonds are formed between urea of Host **3** and carboxylate of Guest **5**. A singlet signal, derived from *tert*-butyl amine, shifted to an upfield due to the shielding field of the naphthyl rings in Host **3**. Furthermore, cross-peaks between the crown methylene protons of **3** and the methyl protons of *tert*-butyl amine were observed in the NOESY spectrum (Fig. 5). These findings indicate that the ammonium ion of Guest **5** is incorporated into the crown ring of Host **3** and the carboxylate of Guest **5** is captured by the acidic urea group of Host **3**. Next, a series of amino acids with various methylene chains lengths (**4a**–**f**) were investigated by the solid–liquid extraction method and monitored by UV–vis spectroscopy (Fig. 6).

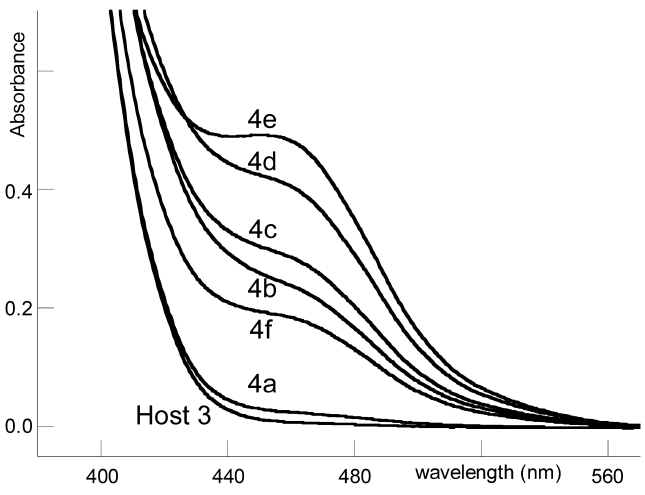


Figure 6. Absorption spectra of after extraction of Guest **4** with Host **3** in DMSO at 20°C. [Host **3**]=5.0×10^{-4} M.

After extracting, the solvent changed from colorless to yellow due to the increased absorbance around 460 nm. The solubility of the guest amino acids **4** and the binding of Host **3** with Guests **4** should influence the degree of color development. Guest **4e** caused the most significant color change. Thus, the extent of the color change indicated the length of the guest amino acid by Host **3**. Although the color change can be detected by the naked eye, small amount of **4e** (~3%) was observed by solid–liquid extraction method and analyzed by 400 MHz NMR.[13]

3. Experimental

3.1. General

Nuclear magnetic resonance (NMR) spectra were taken at 200 or 400 MHz in $CDCl_3$ or $DMSO_{d\text{-}6}$ with chemical shifts being reported as δ ppm from tetramethylsilane as an internal standard and couplings are expressed in Hertz. FT-IR and UV spectra were obtained on a JASCO FT/IR-300 and Shimadzu-2200, respectively.

3.1.1. Solid–liquid extraction method.[9] A solid amino acid (50 mg) was added to a Host **3** solution (5.0×10^{-4} M, DMSO, 2 mL) and the suspension was stirred for 1 min at 20°C. The suspension was centrifuged (3500 rpm, 2 min) and filtered (sartorius, Minisart RC15). The filtrate was measured by UV–vis spectroscopy.

3.1.2. Bromide (*S*)-6. To a solution of the key intermediate (*S*)-**1** (900 mg, 1.74 mmol) in DMF (10 ml), sodium hydride (60% mineral oil suspension, 104 mg, 2.64 mmol) was portionwise added at 0°C and stirred for 10 min. A solution of 2-bromobenzyl bromide (522 mg, 2.10 mmol) in DMF (10 ml) was added dropwise to the mixture and stirred at room temperature for over night. The reaction mixture was poured into the mixed solvent of ethyl acetate and water. The organic layer was separated, washed successively with water (twice) and brine. After dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate=1/1) to afford (*S*)-**6** as a colorless viscous oil (1.07 g, 90% yield). $[\alpha]_D^{20}=-87$ (c=2.14, $CHCl_3$); IR (film) 3057, 2871, 1621, 1592, 1507 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.15–3.75 (m, 17H), 3.90–4.05 (m, 2H), 4.10–4.20 (m, 2H), 4.70 (s, 2H), 7.05–7.50 (m, 8H), 7.50–7.60 (m, 4H), 7.85 (d, J=8.0 Hz, 2H), 7.92 (dd, J=9.0, 2.0 Hz, 2H); HRMS calcd for $C_{38}H_{39}Br^{79}O_7$: 686.1879. Found: 686.1895, calcd for $C_{38}H_{39}Br^{81}O_7$: 688.1859. Found: 688.1872; Anal. calcd for $C_{38}H_{39}BrO_7$: C, 66.38; H, 5.72. Found: C, 66.15; H, 5.72.

3.1.3. Host (*S*)-2. To a solution of the bromide (*S*)-**6** (1.20 g, 1.75 mmol) in dry THF (15 ml), *n*-BuLi (1.75 mol/l in hexane, 1.7 ml, 2.97 mmol) was added dropwise at −78°C under nitrogen atmosphere and the mixture was stirred for 2.5 h. Trimethyl borate (1.7 ml, 17.45 mmol) was added to the solution at −78°C. The reaction mixture was stirred for over night at room temperature. The reaction mixture was poured into the mixed solvent of ethyl acetate and 1N hydrochloric acid solution. The organic layer was separated, washed successively with hydrochloric acid, water (twice)

and brine. After dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate=1/1) to afford (*S*)-**2** as a colorless viscous oil (687 mg, 60% yield). $[\alpha]_D^{20}=-109$ (c=1.30, $CHCl_3$); IR (film) 3368, 2871, 1620, 1593 cm^{-1}; ^1H NMR (400 MHz, $DMSO_{d\text{-}6}$) δ 3.20–3.70 (m, 17H), 4.00–4.10 (m, 2H), 4.10–4.20 (m, 2H), 4.67 (s, 2H), 6.91 (dd, J=8.6, 2.2 Hz, 2H), 7.15–7.35 (m, 7H), 7.51 (d, J=7.1 Hz, 1H), 7.59 (d, J=7.6 Hz, 1H), 7.60 (d, J=7.6 Hz, 1H), 7.93 (d, J=8.1 Hz, 2H), 7.98 (s, 2H), 8.02 (d, J=9.0 Hz, 2H); FAB-MS 501, 313, 268. The molecular ion peak was not detected by EI and FAB mass spectrometer. Anal. calcd for $C_{38}H_{41}BO_9$: C, 69.94; H, 6.33. Found: C, 69.89; H, 6.40.

3.1.4. Tosylate (*S*)-7. Known.[6]

3.1.5. Azide (*S*)-8. A mixture of the tosylate (*S*)-**7** (5.50 g, 8.2 mmol) and sodium azide (2.66 g, 40.8 mmol) in DMF (50 ml) was stirred at 60°C for 3 days. The reaction mixture was poured into the mixed solvent of ethyl acetate and water. The organic layer was separated, washed successively with water (three times) and brine. After dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate=1/1) to afford (*S*)-**8** as a pale yellow viscous oil (4.19 g, 94% yield). $[\alpha]_D^{20}=-141$ (c=0.88, $CHCl_3$); IR (film) 2870, 2102, 1592, 1507 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.10–3.80 (m, 17H), 3.90–4.10 (m, 2H), 4.10–4.30 (m, 2H), 7.10–7.40 (m, 6H), 7.47 (dd, J=8.9, 3.0 Hz, 2H), 7.86 (d, J=8.0 Hz, 2H), 7.94 (d, J=9.2 Hz, 2H); HRMS calcd for $C_{31}H_{33}N_3O_6$: 543.2370. Found: 543.2385; Anal. calcd for $C_{31}H_{33}N_3O_6$: C, 68.49; H, 6.12; N, 7.73. Found: C, 68.67; H, 6.13; N, 7.72.

3.1.6. Amine (*S*)-9. A mixture of the azide (*S*)-**8** (3.8 g, 7.0 mmol), 10% palladium on carbon (0.3 g) and ammonium formate (2.2 g, 35.0 mmol) in ethanol (80 ml) was refluxed for 1 h. After filtration, the filtrate was evaporated in vacuo. The residue was dissolved in ethyl acetate and washed successively with sodium hydrogencarbonate solution, water and brine and dried over sodium sulfate. Evaporation to dryness in vacuo gave (*S*)-**9** (3.56 g, 98% yield), which was directly used for the next step without further purification. A small amount of (*S*)-**9** was subjected to further purification by PTLC to give analytical sample as a colorless viscous oil. $[\alpha]_D^{20}=-111$ (c=0.73, $CHCl_3$); IR (film) 3007, 2870, 1621, 1592 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.80–3.60 (m, 19H), 3.80–4.00 (m, 2H), 4.00–4.30 (m, 2H), 7.00–7.30 (m, 6H), 7.30–7.50 (m, 2H), 7.70–8.00 (m, 4H); HRMS calcd for $C_{31}H_{35}NO_6$: 517.5465. Found: 517.2468.

3.1.7. Host (*S*)-3. To a solution of 2,4-dinitroaniline (6.2 g, 33.8 mmol) in pyridine (100 ml), chlorophenylcarbonate (17.0 ml, 135.2 mmol) was added dropwise at 0°C. The mixture was stirred at room temperature for 1 day. The reaction mixture was poured into the mixed solvent of ethyl acetate and water. The organic layer was separated, washed successively with water, 1 M hydrochloric acid, water, 0.5 M sodium hydroxide solution, water, 1 M hydrochloric acid, water and brine (twice). After dried over sodium sulfate, the solvent was evaporated in vacuo. The amine (*S*)-**9** (3.5 g, 6.8 mmol) in DMSO (50 ml) was added to the residue and the mixture was stirred at room temperature for 3 days. The reaction mixture was poured into the mixed solvent of ethyl acetate and water. The organic layer was separated, washed successively with water, 0.5 M sodium hydroxide solution, water, 1 M hydrochloric acid, water (twice) and brine (twice). After dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate=1/1) to afford (*S*)-**3** as a pale yellow foam (3.67 g, 75% yield). $[\alpha]_D^{20}=-143$ (c=1.05, $CHCl_3$); IR (film) 3325, 3011, 2871, 1711, 1618, 1596 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.90–4.50 (m, 21H), 6.60–6.80 (m, 1H), 7.05–7.75 (m, 10H), 7.86 (d, J=8.0 Hz, 1H), 7.97 (d, J=9.1 Hz, 1H), 8.39 (dd, J=9.8, 2.6 Hz, 1H), 9.02 (d, J=9.8 Hz, 1H), 9.10 (d, J=2.6 Hz, 1H), 9.72 (brs, 1H); HRMS calcd for $C_{38}H_{39}N_4O_{11}$: 727.2616. Found: 727.2625; Anal. calcd for $C_{38}H_{39}N_4O_{11}$: C, 62.80; H, 5.27; N, 7.71. Found: C, 63.20; H, 5.34; N, 7.37.

References

1. Review on related enantiomeric recognition of amine compounds; Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997**, *97*, 3313–3361.
2. For review of artificial organic host molecules for anions; (a) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609–1646. (b) Bucher, C.; Zimmerman, R. S.; Lynch, V.; Král, V.; Sessler, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 2099–2100.
3. (a) Barboiu, M. D.; Hovnanian, N. D.; Luca, C.; Cot, L. *Tetrahedron* **1999**, *55*, 9221–9232. (b) Wehner, M.; Schrader, T.; Finocchiaro, P.; Failla, S.; Consiglio, G. *Org. Lett.* **2000**, *2*, 605–608. (c) Aït-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296–11297. (d) Sasaki, S.; Hashizume, A.; Citterio, D.; Fujii, E.; Suzuki, K. *Angew. Chem. Int. Ed.* **2002**, *41*, 3005–3007.
4. Review on related carbohydrate recognition; (a) Davis, A. P.; Wareham, R. S. *Angew. Chem. Int. Ed.* **1999**, *38*, 2978–2996. (b) Mazik, M.; Sicking, W. *Chem. Eur. J.* **2001**, *7*, 664–670. (c) Kim, Y. H.; Hong, J. I. *Angew. Chem. Int. Ed.* **2002**, *41*, 2947–2950.
5. For recent review; (a) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155–163. (b) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479–2494. (c) Dowden, J.; Edwards, P. D.; Flack, S. S.; Kilburn, J. D. *Chem. Eur. J.* **1999**, *5*, 79–89. (d) Tsubaki, K.; Kusumoto, T.; Hayashi, N.; Nuruzzaman, M.; Fuji, K. *Org. Lett.* **2002**, *4*, 2313–2316.
6. Tsubaki, K.; Tanaka, H.; Kinoshita, T.; Fuji, K. *Tetrahedron* **2002**, *58*, 1679–1684.
7. (a) Paugam, M.-F.; Bien, J. T.; Smith, B. D.; Chrisstoffels, L. A. J.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1996**, *118*, 9820–9825. (b) Finch, H.; Reece, D. H.; Sharp, J. T. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1193–1203.
8. Reetz, M. T.; Huff, J.; Rudolph, J.; Töllner, K.; Deege, A.; Goddard, R. *J. Am. Chem. Soc.* **1994**, *116*, 11588–11589.
9. (a) Diederich, F.; Dick, K. *J. Am. Chem. Soc.* **1984**, *106*, 8024–8036. (b) Peterson, B. R.; Mordasini-Denti, T.; Diederich, F. *Chem. Biol.* **1995**, *2*, 139–146.
10. For recent review of an important inhibitory neurotransmitter

GABA; Endo, S. L.; Wolff, J. R. *J. Neurochem.* **1990**, *54*, 363–372.
11. In the case of compound **1** and **4c**, the estimated amount of **4c** extracted was 7% against compound **1**.
12. For recent review of receptors for carboxylates. (a) Fitzmaurice, R. J.; Kyne, G. M.; Douheret, D.; Kilburn, J. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 841–864. (b) Kato, R.; Nishizawa, S.; Hayashita, T.; Teramae, N. *Tetrahedron Lett.* **2001**, *42*, 5053–5056.
13. A binding constant between Host **3** and Guest **4e** based on the solid–liquid extraction method[9a] could not be estimated, due to the small extractability.