SYNTHESIS OF A NOVEL MULTI-RECEPTOR CONTAINING HYDROGEN BONDING SITES AND ION BINDING SITES

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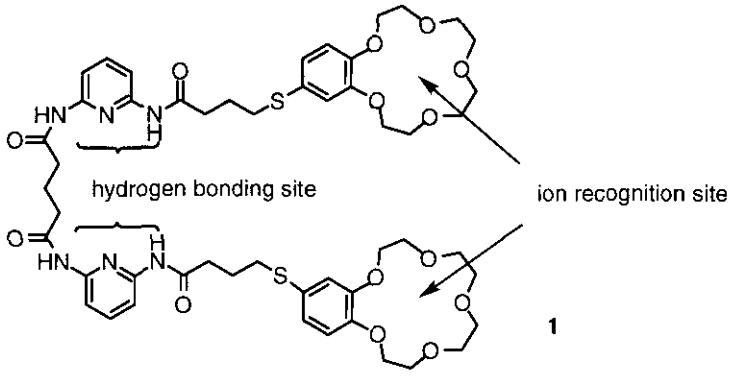
Abstract- A new linear ditopic receptor (**1**) which contains two 2,6-diaminopyridine moieties and two crown ether rings at the termini has been synthesized. The host (**1**) binds a thymine derivative and behaves as a ditopic receptor in a transport experiment.

Artificial receptors bearing several binding sites for different guests have been used for allosteric systems[1-3] and multi-recognition ones.[4] In several these allosteric receptors one guest as an effector causes conformational changes of the host to produce a new binding site for another guest. Thereby, the binding selectivity and strength are changed. Organization of components of recognition sites is also utilized to modulate host-guest interactions.[5,6] We here report new host (**1**) which has two 2,6-bis(acylamino)pyridine moieties as a hydrogen bonding site and two 15-crown-5 ether rings. Thus, **1** may be used as a multi-recognition receptor for guests containing a hydrogen bonding site and an ionic moiety. In addition, a negative allostery on diionic guest binding would be accomplished using **1** if the two crown rings are forced to be separated each other by binding of each acylamino moiety with one guest such as a thymine derivative *via* hydrogen bonding.

Novel Host (**1**) with Several Binding Sites



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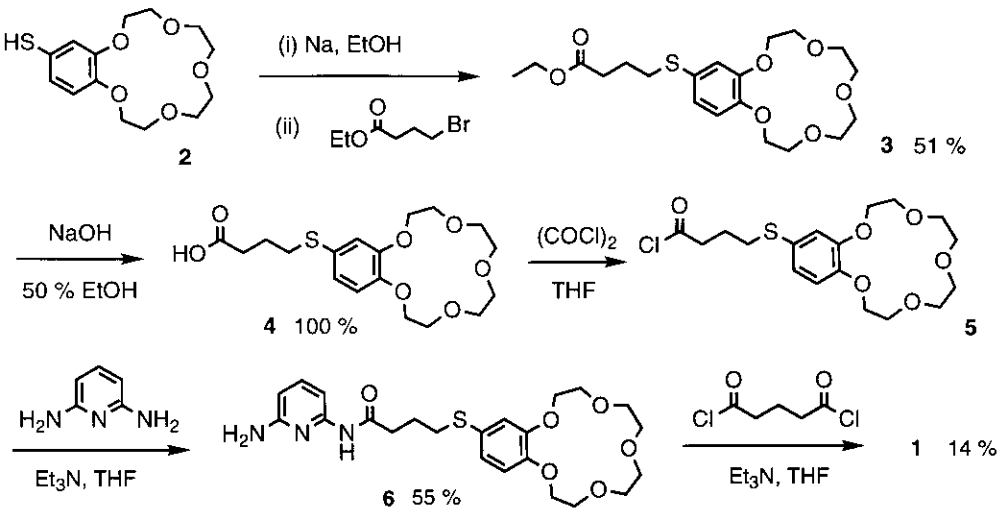
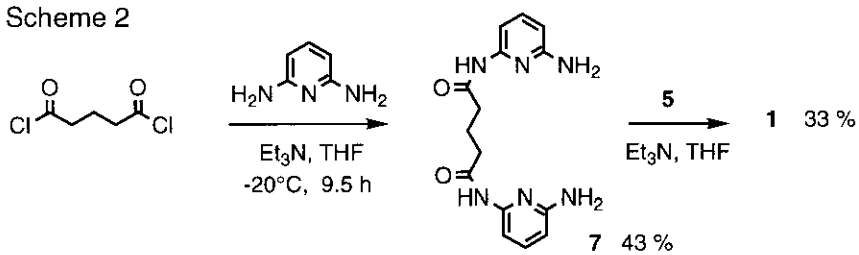
The host (**1**) was prepared according to three routes (Scheme 1~3). In Scheme 1, the mercaptobenzocrown (**2**) was treated with ethyl 4-bromobutyrate to give the ester (**3**). After hydrolysis of **3** followed by reaction with oxalyl chloride, **5** was obtained. 2,6-Diaminopyridine reacted with **5** to give monoamide (**6**) in 55%. **1** was synthesized in 14% yield by the treatment of **6** with glutaryl dichloride in the presence of Et_3N. The

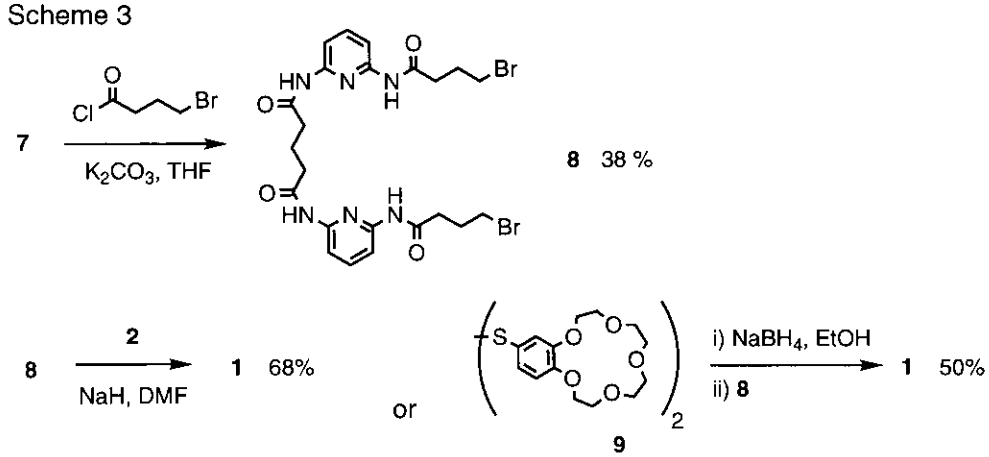
Scheme 1



Scheme 2



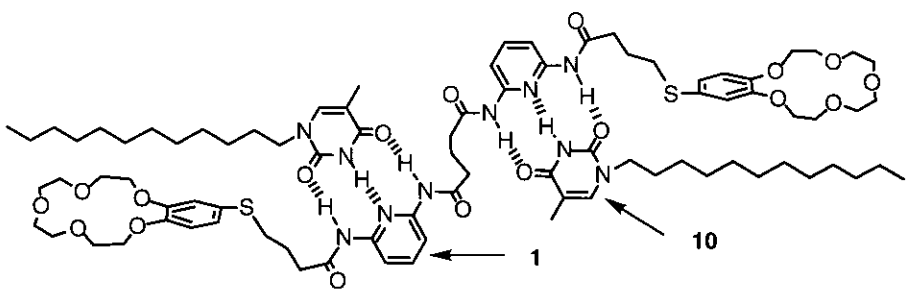
Scheme 3



structure of **1** was ascertained by ^1H, ^{13}C NMR, FAB and ESI mass spectroscopic data and elemental analysis. However, this route consists of many steps and the yield of the last step is quite low (14%). According to Scheme 2, the host (**1**) was also obtained from diamine (**7**) and the acyl chloride (**5**). The yield of the final step is still low (33%). In the third route shown in Scheme 3, the diamine (**7**) was also employed as an intermediate, which was treated with 4-bromobutyryl chloride and K_2CO_3 in THF to give dibromide (**8**) in 38% yield. **8** was used for the next step immediately after purification, because **8** is not stable and decomposed in a few days. **1** was obtained in 68% yield from **2** and **8**. Instead of using the pure **2**, the thiol (**2**) produced *in situ* by the reduction of the corresponding disulfide (**9**) with $NaBH_4$ can be used. Among the three routes described above, the last route shown in Scheme 3 gave the higest overall yield. The procedures are much less laborious than those in Schemes 1 and 2.

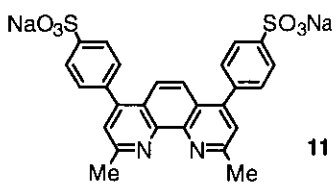
In ^1H NMR spectra in $CDCl_3$, all the amide protons of **1** were shifted downfield on the addition of the thymine derivative (**10**). Similar downfield shifts of **10** were also observed when **1** was added to a solution of **10**. These shifts are ascribed to hydrogen bonds between **1** and **10**, as seen in other hosts containing 2,6-bis(acylamino)pyridine groups.[6,7] ^1H NMR titrations using these chemical shift changes suggest that 1 : 2 complexation between **1** and **10** takes place in cases where either the concentration of **1** or **10** is kept constant (Figure 1). In the Job plots by ^1H NMR spectroscopy, the maximum complex concentration is observed at a mole fraction of 0.4. In general, the maximum value is at a mole fraction of 0.33 if the 1 : 2 binding is predominant. Consequently, this result indicates that 1 : 2 complexation is slightly more favorable than 1 : 1. However, exclusive 1 : 2 complexation can be achieved when a large excess amount of **10** is used. These results show that the two 2,6-bis(acylamino)pyridine moieties can work independently as a hydrogen bonding site.

Figure 1 Plausible Structure of **1** and **10** Complex



Preliminary transport experiments[3] across a 1,2-dichloroethane layer as a liquid membrane indicate that **1** is a ditopic carrier for bathocuproinedisulfonic acid sodium salt (**11**). The guest (**11**) was probably captured as a counter anion of two sodium ions bound in the two crown rings. The transport efficiency of **1** was almost the same as that of benzo-15-crown-5, even when 2-fold concentration of **10** was employed. Thus, in this system allostery was not achieved by **10**.

We are now investigating regulation of molecular recognition by the use of other guests as an effector, and examining the multi-binding ability of **1** toward guests bearing several recognizable sites, such as amino acids and nucleotides.



EXPERIMENTAL

All the reactions were carried under Ar atmosphere. All commercially available reagents were used without further purification.

Ester (**3**). The thiol (**2**) (2.721 g, 9.07 mmol)[8] was added to a solution of Na (0.299 g, 13.0 mmol) in 20 mL of EtOH. To the thiolate solution was added during 5 min a solution of ethyl 4-bromobutyrate (1.862 g, 9.55 mmol) in 5 mL of EtOH. After stirred for 19 h, the reaction mixture was neutralized with 2 N HCl and mixed with 100 mL of AcOEt. The mixture was washed with 50 mL of water and dried over anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo*. Further purification was achieved by silica gel column chromatography eluting with $CHCl_3$/MeOH (40 : 1) to give **3** (1.929 g, 51%) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.96 (1H, dd, J = 8.1, 2.1 Hz), 6.93 (1H, d, J = 2.1 Hz), 6.79 (1H, d, J = 8.1 Hz), 4.15-4.11 (6H, m), 3.93-3.90 (4H, m), 3.8-3.73 (8H, m), 2.86 (2H, t, J = 7.2 Hz), 2.43 (2H, t, J = 7.2 Hz), 1.89 (2H, quint, J = 7.2 Hz), 1.25 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.0, 149.2, 148.5, 127.0, 124.6, 117.3, 114.3, 71.4, 71.1, 70.6, 70.5, 69.53, 69.50, 69.1, 69.0, 60.4, 34.8, 32.8, 24.5, 14.2; IR (neat) cm^{-1} 2928, 1731 (C=O), 1586, 1504, 1454, 1402, 1377, 1257, 1137, 1056; EIMS 414 (M^+). Anal. Calcd for $C_{20}H_{30}O_7S$•0.5H_2O: C, 56.72; H, 7.38; S, 7.57. Found: C, 56.81; H, 7.37; S, 7.77.

Carboxylic acid (**4**). A mixture of **3** (1.199 g, 2.89 mmol) and NaOH (0.456 g, 11.4 mmol) in 20 mL of 50% EtOH was refluxed for 20 h. To the mixture was added 40 mL of 2 N HCl and the product was extracted with $CHCl_3$ (3 x 40 mL). The organic layers were combined and washed with water (3 x 40 mL). The solvent was removed *in vacuo* to give **4** (1.127 g, 100%) as a pale yellow viscous oil. The product was used for the next step without purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.96 (1H, dd, J = 8.2, 2.0 Hz), 6.93 (1H, d, J = 2.0 Hz), 6.87 (1H, d, J = 8.2 Hz), 4.15-4.09 (4H, m), 3.92-3.89 (4H, m), 3.78-3.73 (8H, m), 3.06 (2H, t, J = 7.0 Hz), 2.86 (2H, t, J = 6.9 Hz), 1.94 (2H, quint, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 177.7, 149.1, 148.4, 126.9, 124.8, 117.2, 114.1, 70.94, 70.92, 70.3, 69.47, 69.46, 68.9, 68.7, 34.6, 32.2, 24.4; IR (neat) cm^{-1} 3400-2500 (OH), 2880, 1725 (C=O), 1586, 1504, 1456, 1404, 1359, 1257, 1135, 1054; EIMS 386(M^+).

Acyl chloride (**5**). Oxalyl chloride (0.785 g, 2.03 mmol) in 20 mL of THF was mixed with **4** (0.898 g, 7.07 mmol) and the reaction mixture was refluxed for 2.5 h, and then concentrated *in vacuo*. The product was used for the next step without purification, because ^{1}H NMR suggested that **4** completely disappeared and **5** was produced as a single product: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.97-6.92 (2H, m), 6.79 (1H, d, J = 8.2 Hz), 4.15-4.11 (4H, m), 3.93-3.89 (4H, m), 3.78-3.74 (8H, m), 3.06 (2H, t, J = 7.0 Hz), 2.86 (2H, t, J = 7.0 Hz), 1.94 (2H, quint, J = 7.0 Hz); IR (neat) cm^{-1} 2874, 1796(C=O), 1586, 1504, 1456, 1404, 1361, 1257, 1139, 1058.

Amine (**6**). A solution of **5** which was prepared from 1.033 g (2.67 mmol) of **4** in 15 mL of THF was added to a mixture of 2,6-diaminopyridine (0.863 g, 7.91 mmol) and Et_3N (0.342 g, 3.38 mmol) in 20 mL of THF. After stirred for 11 h, the mixture was concentrated *in vacuo*. The residue thus obtained was mixed with 30 mL of $CHCl_3$, washed with water (4 x 30 mL), and concentrated *in vacuo*. Purification by silica gel column chromatography eluting with AcOEt/MeOH (3 : 1) gave **6** (0.704 g, 55%) as a brown oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.60-7.50 (2H, m) 7.42 (1H, t, J = 8 Hz), 6.98-6.95 (2H, m), 6.77

(1H, d, J = 8 Hz), 6.23 (1H, d, J = 8 Hz), 4.14-4.09 (4H, m), 3.92-3.89 (4H, m), 3.76-3.74 (8H, m), 2.92 (2H, t, J = 6.9 Hz), 2.49 (2H, t, J = 6.9 Hz), 2.00 (2H, quint, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.7, 157.0, 149.3, 149.1, 148.3, 140.2, 127.0, 124.6, 117.1, 114.1, 104.4, 102.8, 70.9, 70.3, 69.44, 69.39, 68.85, 68.79, 35.8, 34.8, 24.9; IR (neat) cm^{-1} 3352 (NH), 2924, 2872, 1688 (C=O), 1622 (NH), 1578, 1504, 1458, 1296, 1255, 1222, 1137; FABMS 478 ($[M+1]^+$).

Diamine (**7**). A solution of glutaryl chloride (2.378 g, 14.1 mmol) in 10 mL of THF was added during 10 min to a solution of 2,6-diaminopyridine (8.138 g, 74.6 mmol) and Et_3N (7.567 g, 74.9 mmol) in 100 mL of THF at -20°C. The reaction mixture was stirred for 4.5 h at -20°C, filtered, and concentrated *in vacuo*. The residue thus obtained was purified by column chromatography over silica gel eluting with AcOEt-acetone (3 : 1) to give **7** (1.923 g, 43%) as a pale brown solid: mp 77.5-79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.91 (2H, br s), 7.53 (2H, d, J = 8.0 Hz), 7.44 (2H, t, J = 8.0 Hz), 6.24 (2H, d, J = 8.0 Hz), 4.32 (4H, br s), 2.48 (4H, t, J = 7.0 Hz), 2.12 (2H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.7, 157.0, 149.6, 140.2, 104.3, 103.3, 36.3, 21.0; IR (KBr) cm^{-1} 3352 (NH), 2944, 1682 (C=O), 1576 (NH), 1539, 1458; EIMS 314 (M^+). Anal. Calcd for $C_{15}H_{18}N_6O_2$: C, 57.31; H, 5.83; N, 26.74. Found: C, 57.24; H, 5.83; N, 26.74.

Host (**1**) (Scheme 1). Glutaryl dichloride (0.11 mL, 0.959 mmol) was added to the mixture of **6** (1.049 g, 2.20 mmol) and Et_3N (0.65 mL, 4.82 mmol) in 10 mL of THF. After stirred for 13 h, a 20 mL of acetone was added, and the reaction mixture was filtered to remove Et_3N•HCl. The filtrate was concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography eluting with AcOEt/MeOH (1 : 1) and by HPLC (Merck, Lobar column RP-18) eluting with MeOH-H_2O (4 : 1) to give **1** (0.114 g) in 14% yield as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.14 (2H, br s), 7.91-7.79 (4H, m), 7.68 (2H, t, J = 8 Hz), 7.59 (2H, br s), 6.99-6.90 (4H, m), 6.78 (2H, d, J = 8 Hz), 4.13-4.05 (8H, m), 3.91-3.85 (8H, m), 3.79-3.70 (16H, br s), 2.93 (4H, t, J = 7 Hz), 2.55 (4H, t, J = 7 Hz), 2.50 (4H, t, J = 7 Hz), 2.15 (2H, quint, J = 7 Hz); ^{13}C NMR (100 MHz) δ (ppm) 171.4, 171.0, 149.5, 149.1, 148.3, 140.6, 126.9, 124.6, 117.0, 114.0, 109.3, 70.8, 70.5, 70.2, 69.3, 69.0, 68.7, 68.3, 36.1, 35.6, 34.7, 25.1, 21.0; IR (KBr) cm^{-1} 3314 (NH), 2924, 1702 (C=O), 1589 (NH), 1510, 1450, 1257, 1224, 1141; FABMS 1051 ($[M+1]^+$). Anal. Calcd for $C_{51}H_{66}N_6O_{14}S_2$•$2H_2O$: C, 56.34; H, 6.49; N, 7.73; S, 5.90. Found: C, 56.26; H, 6.27; N, 7.64; S, 5.69.

Host (**1**) (Scheme 2). A solution of **5** which was prepared from 0.758 g (2.03 mmol) of **4** in 20 mL of THF was added during 10 min to a solution of **7** (0.228 g, 0.725 mmol) and Et_3N (0.272 g, 2.7 mmol) in 12 mL of THF. After stirred for 4.3 h, the reaction mixture was filtered to remove Et_3N•HCl. The filtrate was concentrated *in vacuo*. The resultant residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (10:1) and by HPLC (Merck, Lobar column RP-18) eluting with MeOH-H_2O (3 : 1) to give **1** (0.250 g, 33%) as a colorless solid.

Host **1** (Scheme 3). A solution of 4-bromobutyryl chloride (0.917 g, 4.9 mmol) in 5 mL of THF was added to a suspension of **7** (0.807 g, 2.6 mmol) and K_2CO_3 (0.869 g, 63 mmol) in 10 mL of THF and the mixture was stirred for 75 min at rt to give a gel mixture. MeOH was added to the mixture to dissolve the gel, and the resulting suspension was filtered to remove insoluble materials. The filtrate was concentrated *in vacuo*. The crude product thus obtained was purified by recrystallization from MeOH to give **8** (0.605

g, 38%) as a pale yellow powder: mp 148-152 °C (decomp); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.89-7.85 (6H, m), 7.73-7.68 (4H, m), 3.53 (4H, t, *J* = 7 Hz), 2.60 (4H, t, *J* = 7 Hz), 2.55 (4H, t, *J* = 7 Hz), 2.28 (4H, quint, *J* = 7 Hz), 2.16 (4H, quint, *J* = 7 Hz); IR (KBr) cm^{-1} 3292 (NH), 2922, 1671 (C=O), 1584 (NH), 1518, 1450, 685; FABMS 613 ([M+1]$^+$).

For the purpose of host preparation, this compound was used without chromatographic purification.

The thiol (**2**) (98 mg, 0.33 mmol) was added to a suspension of 60% NaH (28 mg, 0.70 mmol) in DMF (2 mL). The mixture was stirred for 10 min and then **8** was added. After stirring for 17 h, 2 N HCl was added to the reaction mixture to acidify (pH 5). The mixture was mixed with 30 mL of $CHCl_3$ and washed with water (4 x 30 mL). The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed *in vacuo*. The resultant residue was purified by HPLC (Merck, Lobar column RP-18) eluting with MeOH-H_2O (4 : 1) to give **1** (0.108 g, 68 %) as a colorless solid.

Instead of **2**, the disulfide was also used as a starting material. $NaBH_4$ (65 mg, 1.7 mmol) was added to a solution of the disulfide (**9**) (0.395 g, 0.66 mmol) in 5 mL of EtOH. The reaction mixture was stirred for 15 min and then **8** (0.399 g, 6.5 mmol) was added. After stirred for 15 h, the mixture was mixed with 50 mL of $CHCl_3$ and 50 mL of H_2O, and neutralized with 2 N HCl. The organic layer was separated, and the aqueous layer was extracted with 50 mL of $CHCl_3$. The organic layers were combined, washed with H_2O (50 mL x 3), and concentrated *in vacuo*. The resultant residue was purified by HPLC (Merck, Lobar column RP-18) eluting with MeOH-H_2O (3 : 1) to give **1** (0.339 g, 50%) as a colorless solid.

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