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Azobenzene coupled chromogenic receptors for the selective detection of copper(II) and its application as a chemosensor kit

Soo Jin Lee,[a] Shim Sung Lee,[a] Il Yun Jeong,[b] Jin Yong Lee[c] and Jong Hwa Jung[a,*]

[a]*Department of Chemistry and Research Institute for Natural Science, Gyeongsang National University, Chinju 660-701, South Korea*
[b]*Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, South Korea*
[c]*Department of Chemistry and Institute of Basic Science, Sungkyunkwan University, Suwon 440-746, South Korea*

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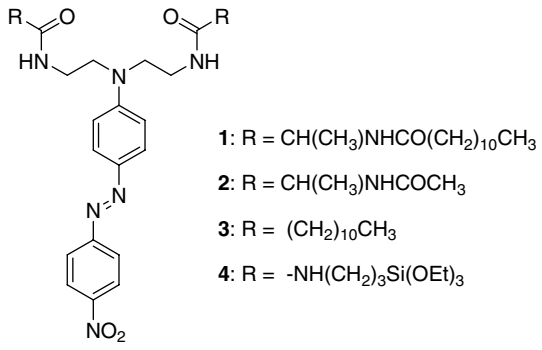
Abstract—Azobenzene-based receptors **1**–**4** as colorimetric sensing materials were synthesized and their sensing properties were examined. In solution, the proposed sensing materials give rise to a large cation-induced hypochromic shift for Cu^{2+} resulting in a change from red to pale-yellow, whereas no significant color change was observed upon addition of other selected metal ions. The use of the silica gel plate modified with immobilization of receptor **4** to detect Cu^{2+} was also reported.

The development of artificial receptors for the sensing and recognition of environmentally and biologically important ionic species, especially transition-metal ions, is currently of great interest.[1–4] Due to its industrial usage as a pollutant and an essential trace element in biological systems, the chemosensors for copper(II) based on the chromogenic probes which are expected quickly, nondestructively, and sensitively to detect copper has drawn much attention.[5–8] Recently, we have focused our efforts on the development of novel colorimetric sensors for the ionic species,[9–11] with the aim of preparing the simple-to-use and naked-eye diagnostic tools for the recognition of essential electrolytes and molecules in serum for critical care analysis. We herein report the synthesis and the spectroscopic evaluation of a series of chromogenic receptors **1**–**4** (Schemes S1–S3), which show selective metal-induced color changes upon the addition of Cu^{2+}.

Each chemosensor receptor (**1**–**4**) proposed contains azobenzene moiety, in which one of the aromatic rings also may act as an integrated part of the ion recognition. Phenyl iminoethylenediamide and nitrobenzene moieties were employed as electron donor and acceptor, respectively. In case of **1**, D-alanine moiety was introduced to provide an extra chelating site especially for Cu^{2+}.



As expected, **1** showed a high selectivity and sensitivity for Cu^{2+}. To the best of our knowledge, **1** is a rare example of azobenzene-based chemosensor for Cu^{2+}.

Synthesis of the receptors began with tosylation of *N*-phenyldiethylene diamine to produce the *N*-phenyldiethylene ditosylate. Treatment with NaN_3 to remove the tosyl group gave *N*-phenyldiethylene diazide. Reduction with Pd/C yielded *N*-phenyldiethylene diamine as a key precursor.[12] The treatment with alanine-appended fatty acid in ethyl acetate successfully produced the *N*-phenylated alanine–fatty acid amide. Subsequently, the diazonium salt generated in situ from the reaction of *p*-nitroaniline with $NaNO_2$ was added to a solution of the corresponding *N*-phenylated fatty acid amide, affording the desired product **1** as a red powder (see Scheme S1). Receptors **2** and **3** were synthesized

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* Corresponding author. Tel.: +82 55 751 6027; fax: +82 55 758 6027; e-mail: jonghwa@gnu.ac.kr

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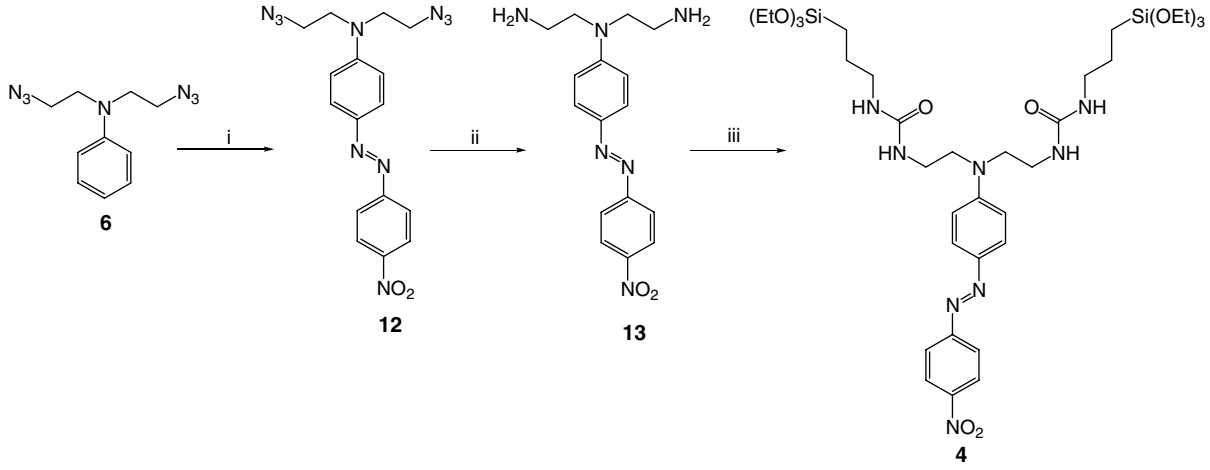
similarly (Schemes S1 and S2). Compound **4** was synthesized by Scheme 1. Treatment with **13** and 3-isocyanatopropyl-triethoxysilane afforded the desired product **4** as a red powder. The red products were analyzed by conventional methods.[13]

First, the metal-binding properties of **1**–**4** were examined by UV–vis spectrophotometry with respect to color changes (all as nitrates in acetonitrile, Fig. 1a). Receptor **1** exhibits an intense absorption at 480 nm (red). The addition of Cu^{2+} resulted in the largest hypochromic shift for **1** to 320 nm ($\Delta\lambda = 160$ nm), changing its solution color from red to pale-yellow in only acetonitrile (Fig. 2). However, no significant color change was observed upon addition of excess amounts of Li^+, Na^+, K^+, NH_4^+, Co^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}, and Ag^+, indicating that only Cu^{2+} forms a strong coordination through the donor atoms of **1**. Also, the color change of receptor **1** was observed by the addition of Cu^{2+} ion in multi-component system in the presence of Li^+, Na^+, K^+, NH_4^+, Co^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}, and Ag^+. However, the color of receptor **1** was changed from red to yellow by the addition of Cu^{2+} ion in the presence of other metal ions (Fig. S2). The results also support that these receptors could be useful as selective chemosensors for Cu^{2+} in medical diagnostics such as serum studies.[7]

The titration of **1** with $Cu(NO_3)_2$ resulted in the 480 nm absorption gradually decreasing, whereas the 320 nm absorption gradually increased to give an isosbestic point at 379 nm (Fig. 1b). Job's plot for binding between **1** and Cu^{2+} shows a 1:1 stoichiometry (inset of Fig. 1b). Furthermore, the existence of **1**–Cu^{2+} complex species (m/z 449) was confirmed in the FAB mass spectrum (Fig. S1). The $\log K$ values for the 1:1 complex formation calculated through linear least squares analysis of the titration data profiles by the Rose–Drago method[14] were 4.41 for $[Cu(\mathbf{1})]^{2+}$, 4.39 for $[Cu(\mathbf{2})]^{2+}$, and 3.38 for $[Cu(\mathbf{3})]^{2+}$. The results indicate that **1**–**3** form relatively stable complexes with Cu^{2+} in acetonitrile. Among receptors **1**–**3**, the $\log K$ value for $[Cu(\mathbf{3})]^{2+}$ is smaller than those of $[Cu(\mathbf{1})]^{2+}$ and $[Cu(\mathbf{2})]^{2+}$, implying that donor atoms of alanine moieties play an important role for complex formation. The $\log K$ values for Li^+, Na^+, K^+, NH_4^+, Co^{2+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, and Zn^{2+} (all as nitrates) were too small to be determined by this method.

The metal-induced color changes of **2**, which possesses methyl groups instead of long alkyl chains were similar



Scheme 1. Synthetic method. Reaction conditions: (i) diazonium salt, DMF, 0 °C; (ii) Ph_3P, THF/H_2O; (iii) (3-isocyanatopropyl)triethoxy silane, CH_2Cl_2. DMF = *N*,*N*′-dimethyl formamide, THF = tetrahydrofuran.

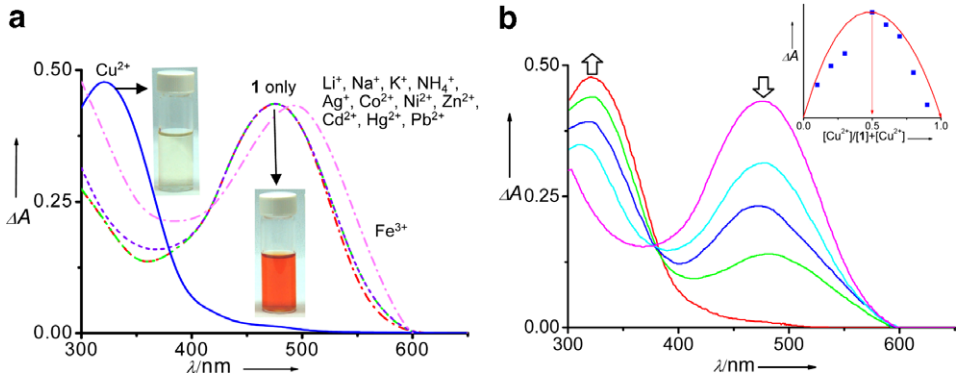


Figure 1. (a) UV–vis spectra of **1** (0.025 mM) in the presence of metal nitrates (5.0 equiv) in acetonitrile and (b) UV–vis titrations of **1** (0.025 mM) with $Cu(NO_3)_2$ (0–5.0 equiv) in acetonitrile; (inset) Job's plot.

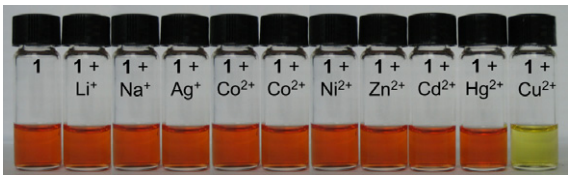


Figure 2. Pictures of **1** (0.025 mM) upon addition of various metal ions (5.0 equiv) in acetonitrile.

to those observed for **1** (Fig. S3), suggesting that the lengths of alkyl chains are not important for selective coloration. Receptor **3** with no alanine moiety produced a smaller hyphochromic shift to 360 nm upon addition of Cu^{2+}, resulting in a color change to yellow (Fig. S4). Once again, this result suggests that the alanine units play an important role in forming the complex with Cu^{2+}.

Our repeated efforts to obtain crystal structures to elucidate the coordination behavior between **1** or **2** and Cu^{2+} were not successful. Also, NMR study for complexation was not available due to the paramagnetic property of Cu^{2+}. Instead we measured IR spectra of **1** and its complex to examine the binding site. The characteristic peaks due to –C=O and –N–C groups in **1** at 1639 and 1338 cm^{-1} were observed to shift to 1628 and 1327 cm^{-1}, respectively, in its Cu^{2+} complex (Fig. S5). The resulting shifts to shorter wavelengths are attributed to the coordination of the Cu^{2+} to the carbonyl amides, and to the amino moiety (Fig. S6).[15,16]

Based on the successful color changes for the receptors obtained in solution state, we also prepared the portable chemosensor kit by immobilizing receptor **4** onto the simple silica gel plate. Immobilization of **4** onto the silica gel plate was conducted in toluene under reflux condition for 24 h. In this process,[17] the triethoxylsilyl group in **4** undergoes hydrolysis and was attached covalently to the surface of the silica gel. In the final step, the red colored silica gel plate was washed with THF and toluene to remove physically adsorbed receptor **4**, and then the plate was dried. As shown in Figure 3, the color of the silica gel plate was changed from white to red after immobilization of **4**, indicating that **4** was covalently attached by the sol–gel reaction.

The content of incorporated chromogenic receptor **4** was determined by TGA and elemental analysis, while UV–vis spectroscopy was used for chromophore determination. The silica gel plate contains approximately 8.2 wt % of the receptor **1** (Fig. S7). The solid UV–vis spectrum of silica gel plate immobilized with **4** exhibits max absorption at 480 nm (Fig. S8). Figure 3c clearly shows that the red color of the modified silica gel plate with **4** was changed to yellow after immersion in Cu^{2+} solution, whereas no significant color change was observed by other selected metal ions (Fig. 3d). When the modified silica gel plate with **4** was employed, it was possible to detect Cu^{2+} content up to 0.01 mM with the naked eye. Hence, the result implies that the proposed receptor-modified plate can be applicable as a

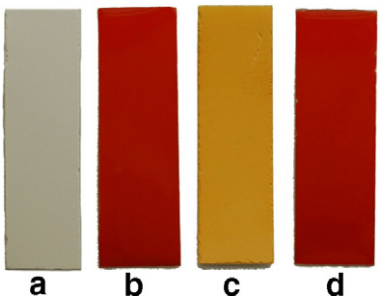


Figure 3. Pictures of silica gel plates: (a) unmodified, (b) modified with **4**, (c) plate b after immersion in Cu^{2+} solution, and (d) plate b after immersion in Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Fe^{3+} or Zn^{2+} solution.

portable sensor kit for the detection of Cu^{2+} in the environmental field.

In conclusion, we have developed highly selective azobenzene-based colorimetric chemosensors **1**–**4** for the detection of Cu^{2+} in solution. The recognition of Cu^{2+} gave rise to major color changes from red to pale-yellow that was clearly visible to the naked eye. The receptor immobilized silica gel plate was also developed for Cu^{2+} detection in a low level. Such Cu^{2+} colorimetric chemosensors could be of great importance in medical diagnostics such as serum studies. In particular, the anchoring of molecular receptors onto suitable solid substrates can be a promising tuning tool for selectivity enhancement which may, in principle, be applied to the design of new chemosensors for a broad range of target species.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.11.066.

References and notes

1. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
2. Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3.
3. Rurack, K. *Spectrochim. Acta* **2001**, *57A*, 2161.
4. Amendola, V.; Fabbrizzi, L.; Forti, F.; Licchelli, M.; Mangano, C.; Pallavicini, P.; Poggi, A.; Sacchi, D.; Taglieti, A. *Coord. Chem. Rev.* **2006**, *250*, 273.
5. Zheng, Y.; Cao, X.; Orbulescu, J.; Konka, V.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M. *Anal. Chem.* **2003**, *75*, 1706.
6. Gunnlaugsson, T.; Leonard, J. P.; Murray, N. S. *Org. Lett.* **2004**, *6*, 1557.

7. Dallali, N.; Darabi, A.; Agrawal, Y. K. *Rev. Anal. Chem.* **2005**, *24*, 263.
8. Kumar, S.; Kaur, N. *Supramol. Chem.* **2006**, *18*, 137.
9. Kim, J. S.; Shon, O. J.; Lee, J. K.; Lee, S. H.; Kim, J. Y.; Park, K.-M.; Lee, S. S. *J. Org. Chem.* **2002**, *67*, 1372.
10. Lee, S. J.; Jung, J. H.; Seo, J.; Yoon, I.; Park, K.-M.; Lindoy, L. F.; Lee, S. S. *Org. Lett.* **2006**, *8*, 1641.
11. Kim, S. K.; Lee, J. K.; Lim, J. M.; Kim, J. W.; Kim, J. S. *Bull. Korean Chem. Soc.* **2004**, *25*, 1247.
12. Lee, S. J.; Lee, S. S.; Kim, J. S.; Lee, J. Y.; Jung, J. H. *Chem. Mater.* **2005**, *17*, 6517–6520.
13. Compound **1**. Mp 240–242 °C. Yield = 30% ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ 8.33 (d, 2H, $J = 7.50$ Hz), 7.92 (m, 2H), 7.83 (m, 2H), 7.02 (d, 2H, $J = 7.80$ Hz), 4.28 (m, 2H), 3.88 (t, 4H, $J = 7.68$ Hz), 3.38 (t, 4H, $J = 7.68$ Hz), 2.13 (t, 4H, $J = 7.14$ Hz), 1.53 (m, 8H), 1.28 (m, 36H), 0.89 (t, 6H, $J = 2.76$ Hz); MS(FAB) m/z 835 $(M+H)^+$. Anal. Calcd for $C_{46}H_{74}N_8O_6$: C, 66.16; H, 8.93; N, 13.42. Found: C, 66.50; H, 8.50; N, 13.11. IR (KBr, pellet): $\tilde{\nu} = 3284$, 2955, 2920, 285, 1644, 1602, 1553, 1516, 1337, 1248, 688 cm^{-1}.
Compound **2**. Mp 221–223 °C. Yield = 40% ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ 8.31 (d, 2H, $J = 7.80$ Hz), 7.88 (m, 2H), 7.84 (m, 2H), 7.01 (d, 2H, $J = 7.50$ Hz), 4.28 (m, 2H), 3.88 (t, 4H, $J = 7.68$ Hz), 3.38 (t, 4H, $J = 7.68$ Hz), 2.14 (s, 6H), 1.53 (s, 6H); MS(FAB) m/z 835 $(M+H)^+$. Anal. Calcd for $C_{46}H_{74}N_8O_6$: C, 66.16; H, 8.93; N, 13.42. Found: C, 66.50; H, 8.50; N, 13.11.
Compound **3**. Mp 198–200 °C. Yield = 43% ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.34 (d, 2H, $J = 7.2$ Hz), 7.97 (m, 4H), 6.84 (d, 2H, $J = 9.3$ Hz), 5.99 (s, 2H), 3.64 (t, 4H, $J = 6.00$ Hz), 3.53 (d, 4H, $J = 6.6$ Hz), 2.17 (d, 4H, $J = 7.8$ Hz), 1.61 (m, 4H), 1.26 (m, 32H), 0.90 (t, 6H, $J = 6.90$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ 174, 157, 152, 144, 129, 126, 123, 113, 112, 62, 38, 37, 29, 26, 23, 20, 14; MS(FAB) m/z 694 $(M+H)^+$. Anal. Calcd for $C_{40}H_{64}N_6O_4$: C, 69.33; H, 9.31; N, 12.13. Found: C, 68.89; H, 9.20; N, 12.02.
Compound **12**. A diazonium salt of 4-nitroaniline (155 g, 1.12 mmol) was prepared by adding an aqueous solution of $NaNO_2$ (78 mg, 1.12 mmol) dropwise into a homogeneous mixture of 1.6 mL of sulfuric acid and 4.2 mL of glacial acetic acid. The mixture was stirred at 0 °C for 5 min. The diazonium salt solution was added dropwise into a solution of compound **6** (200 mg, 0.86 mmol) in DMF(10 mL) at 0 °C. The reaction mixture was stirred for an additional 12 h at 0 °C, treated with H_2O (150 mL) and extracted with $CHCl_3$ (200 mL). The organic layer was dried over $MgSO_4$. Removal of the organic solvent in vacuo was afforded a reddish oil. Column chromatography on silica gel with CH_2Cl_2/MeOH/TEA, 90/10/0.1 as the eluent provided **12** as a reddish crystalline solid in 75% yield. Mp 93–94 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ 8.35 (d, 2H, $J = 8.7$ Hz), 7.96 (d, 4H, $J = 9$ Hz), 6.84 (d, 2H, $J = 9$ Hz), 3.74 (t, 4H, $J = 6$ Hz), 3.62 (t, 4H, $J = 5.7$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ 154, 150, 148, 147, 141, 122, 114, 59, 55, 47, 40, 25, 18, 7. HRMS calcd for $C_{16}H_{16}N_{10}O_2$ $[M]^+$ 380.1458. Found: 380.1455. IR (KBr, pellet): $\tilde{\nu} = 3333$, 2973, 2927, 2886, 1646, 1627, 1596, 1514, 1440, 1389, 1338, 1254, 1138, 1103, 1077, 955, 856, 780 cm^{-1}.
Compound **13**. Compound **12** (500 mg, 1.31 mmol) was dissolved in THF (10 mL) and triphenylphosphane (758 mg, 2.9 mmol) in THF (5 mL) was added to the stirred, ice-cooled solution under an N_2 atmosphere. Water (2 mL) was added and the mixture was stirred at room temperature for 12 h and for a further 6 h at 50 °C. The solution was cooled and then neutralized by addition of concd HCl and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (40 mL) and 0.5 M HCl (40 mL), and the aqueous phase was extracted twice with CH_2Cl_2 (30 mL each). The aqueous phase was made alkaline (pH = 9–10) with NaOH and the product re-extracted with CH_2Cl_2 (4×30 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford compound **13** (211 mg, 0.66 mmol) as a red solid, and was used directly in the next step.
Compound **4**. (3-Isocyanatopropyl)triethoxysilane (100 mg, 0.30 mmol) was slowly added to the solution of compound **13** (165 mg, 0.67 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. After the mixture was stirred for overnight, the solvent was removed in vacuo to yield an off-red solid. Unreacted (3-isocyanatopropyl)triethoxysilane was removed by washing with dry *n*-pentane. After drying under vacuum, compound **4** was obtained as a reddish solid. Yield: 5.3 g, 80%. Mp 202–204 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, 2H, $J = 9$ Hz), 7.96 (d, 4H, $J = 9.3$ Hz), 6.91 (d, 2H, $J = 9$ Hz), 3.81 (q, 12H), 3.75 (t, 4H, $J = 6$ Hz), 3.64 (t, 4H, $J = 5.7$ Hz), 3.34 (t, 4H, $J = 5.1$ Hz), 1.62 (m, 4H),1.26 (m, 18H), 0.66 (m, 4H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.84, 58.37, 42.85, 40.33, 30.27, 29.29, 29.20, 29.14, 26.76, 23.69, 18.26, 7.62 ppm. HRMS calcd for $C_{36}H_{62}N_8O_{10}Si_2$ $[M]^+$ 822.4127. Found: 822.4120; IR (KBr, pellet): $\tilde{\nu} = 3332$, 2973, 2927, 2885, 1627, 1597, 1514, 1138, 1103, 1077 cm^{-1}.
14. (a) Rose, N. J.; Drago, R. S. *J. Am. Chem. Soc.* **1959**, *81*, 6138; (b) Hirose, K. *J. Incl. Phenom. Macro.* **2001**, *39*, 193.
15. (a) Banerjee, I. A.; Yu, L.; Matsui, H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14678; (b) Yoon, J.; Ohler, N. E.; Vance, D. H.; Aumiller, W. D.; Czarnik, A. W. *Tetrahedron Lett.* **1997**, *38*, 3845; (c) Pappalardo, G.; Impellizzeri, G.; Bonomo, R. P.; Camagna, T.; Grasso, G.; Saita, M. G. *New J. Chem.* **2002**, *26*, 593.
16. Krämer, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 772.
17. Immobilization of **4** onto the Silica Gel Plate. Compound **4** (50 mg) was dissolved in toluene (10 mL). Silica gel plate (15 mm × 50 mm) was added to the solution of compound **4**. The solution was refluxed for 24 h in toluene. After cooling to room temperature, silica gel plate was taken out from toluene. The red color silica gel plate was washed with THF and toluene to remove physically adsorbed **4** onto the silica gel plate, and then dried. IR (KBr, pellet): $\tilde{\nu} = 3406$, 2959, 2926, 2854, 1635, 1522, 1382, 796, 576, 457 cm^{-1}; UV–vis (solid): $\lambda_{max} = 480$ nm. Anal. Calcd for $C_{24}H_{114}N_8O_{190}Si_{93}$: C, 4.67; H, 1.86; N, 1.82. Found: C, 4.58; H, 1.48; N, 1.79.