Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 4275–4279

Calix[4]pyrrole–TCBQ assembly: a signal magnifier of TCBQ for colorimetric determining amino acids and amines

Kai Liu,[a,b] Lijun He,[c] Xiaoming He,[a,b] Yong Guo,[a] Shijun Shao[a,*] and Shengxiang Jiang[a]

[a]*Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*
[c]*School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450052, China*

Received 12 March 2007; accepted 9 April 2007
Available online 14 April 2007

Abstract—The interaction and colorimetric sensing properties of the calix[4]pyrrole–TCBQ charge-transfer complex with amino acids and amines in $CHCl_3/EtOH/H_2O$ were investigated using UV/vis spectroscopic techniques. The obvious spectral and visual changes of the complex solution in the presence of basic amino acids (namely, lysine, arginine, and histidine) and aliphatic amines were observed, and the calix[4]pyrrole–TCBQ supramolecular assembly, like a 'signal magnifier', markedly improves sensing sensitivity and selectivity of TCBQ for amino acids and amines.

Proteic primary amino acids are a class of the most important biomolecules and play an important role in biological processes. The selective detection and recognition for amino acids has been a challenging subject.[1] Over the past few years, only a few amino acids can be detected by recognizing a certain functional donor group, which includes the amino group of lysine,[2a] imidazole of histidine,[2b] thiol of cysteine,[2c,d] carboxylate of aspartic acid,[2e] and glutamic acid.[2f] As early as 1963, Birks and Slifkin[3] first reported the interaction between amino acids and *p*-chloranil (TCBQ) by forming n–π charge-transfer (CT) complex. Later, the interactions of TCBQ with amino acids, proteins, and amines have been studied extensively.[4] However, these approaches, which were mostly carried out in an alkaline buffer solution because the n–π CT interactions depend on the presence of an unprotonated amino group, only serve to determine the total amino group and are inefficient to detect specific amino acids by spectral and visual changes. Furthermore, the formation and the spectra of the complexes were affected by solvent, pH, and reaction time, and the sensing both in color and in spectrum of TCBQ for amino acids were very slow at room temperature.[4b,c] The development of a simpler and more sensitive spectrophotometric detection with enhanced selectivity is of great benefit.

Calix[4]pyrroles and their homologues, used as receptors for recognition of anions and neutral substrates mainly through multiple hydrogen bonding interactions, have been explored extensively in anion binding, sensing, and separation technologies.[5] At the same time, calix[4]pyrrole can also act as electron donor to interact with electrondeficient substrate in both solution and solid phase.[6] Herein we reported that calix[4]pyrrole-based supramolecular assemblies, formed by the π–π CT interaction between calix[4]pyrroles and TCBQ in chloroform,[6e] could be used for selective sensing of amino acids and amines by visual color changes in an unbuffered water-containing media at room temperature.

Calix[4]pyrrole **1** can rapidly form the π–π CT complex with TCBQ in $CHCl_3$ at room temperature. Experiments also showed that the formation of complex **1·2** was very slow in $CHCl_3$/EtOH mixture, moreover, no significant color and absorption spectra responses of the formation of the CT complex were observed when more polar solvents, namely, MeCN and DMSO, were used. It was found that once complex **1·2** had been formed in $CHCl_3$, the complex could stand in $CHCl_3$/EtOH or $CHCl_3/EtOH/H_2O$ mixture. The effect of the mixed solvent on the absorption spectra of complex

Keywords: Colorimetric sensors; Charge-transfer complex; Amino-acids; Calix[4]pyrrole; TCBQ.
* Corresponding author. Tel.: +86 931 4968209; fax: +86 931 8277088; e-mail: shaoguo@lzb.ac.cn

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.04.042

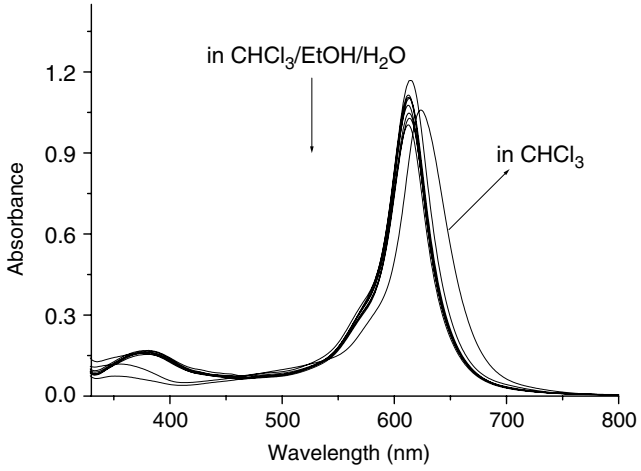
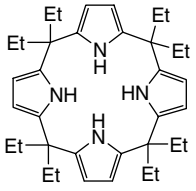
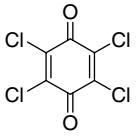


Figure 1. Absorption spectra of complex **1·2** (2.0×10^{-3} M **1** and 2.0×10^{-4} M **2**) in various volume ratios of $CHCl_3/EtOH/H_2O$ mixture. From up to low, the volume ratio is 1:8:0, 1:8:1, 1:7:2, 2:7:1, 1:6:3, 2:6:2, 2:5:3, and 1:5:4.

1·2 was studied. As shown in Figure 1, in the presence of abundant EtOH or $EtOH/H_2O$, the CT absorption peak at 622 nm of complex **1·2** shifted to 612 nm along with the less intensity changes, due to the solvent effect. Actually, CT complex **1·2** exhibited negligible perturbation, and the miscible ensemble solution still maintained sky-blue color. UV/vis spectroscopy titrations were employed to determine the stability constant of complex **1·2** in $CHCl_3/EtOH/H_2O$ system, the binding constants were estimated at $K_a = 5.0\times10^2\ M^{-1}$ in 1:6:3 (v/v/v) $CHCl_3/EtOH/H_2O$ and $K_a = 4.8\times10^2\ M^{-1}$ in 1:8:1 (v/v/v) $CHCl_3/EtOH/H_2O$ by the nonlinear least-squares analysis the absorbance changes of the peaks at 612 nm (see Fig. S1). The mixed solvent ratios have less effect on the stability of complex **1·2**.



1



2

The acid and alkali stabilities of the calix[4]pyrrole–TCBQ assembly in $CHCl_3/EtOH/H_2O$ mixture were preliminary tested. Maybe due to the hydrolysis of TCBQ subunit in alkaline mediums,[7] the calix[4]pyrrole–TCBQ complex was unstable and easily disaggregated under the alkaline condition. It was also observed that the process of complex **1·2** disaggregation induced by OH^- can be rapidly reversed by acidification, and the ensemble solution reexhibits sky-blue color. Generally, the stability of complex **1·2** solution can remain adequate in pH < 8.

The colorimetric properties of complex **1·2** in $CHCl_3/EtOH/H_2O$ (1:6:3, v/v/v) for amino acids were investigated by UV/vis spectroscopy. Figure 2 shows the spectral changes of the ensemble solution of complex **1·2** and

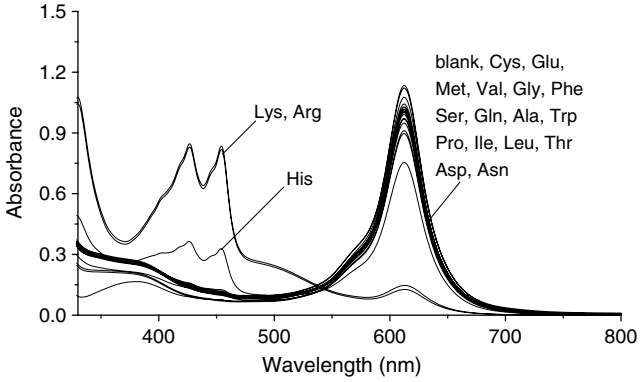


Figure 2. Absorption spectra of complex **1·2** (2.0×10^{-3} M **1** and 2.0×10^{-4} M **2**) in $CHCl_3/C_2H_5OH/H_2O$ (1/6/3, v/v/v) in the presence of various amino acids (7.0×10^{-4} M), at room temperature.

various amino acids, which were prepared by addition of 6-fold EtOH, 2-fold H_2O, and 1-fold aqueous solution of amino acid to the $CHCl_3$ stock solution of complex **1·2**. Upon the addition of basic amino acids, namely, lysine and arginine, the blue complex solution instantly turned to orange-yellow (Fig. 3). The CT absorption band at 612 nm of complex **1·2** nearly vanished, while a new absorption band appeared in the region of 400–500 nm ($\lambda_{max} = 427$ and 453 nm). A similar, but less dramatic color change from blue to yellow-green was observed for histidine, in this case, the peak at 612 nm undergoes decreasing to a certain extent, and the new absorption peak at 427 and 455 nm was also observed. On the other hand, no remarkable changes in color and absorption spectra in long wave region were observed when the solution of complex **1·2** was exposed to various neutral and acidic amino acids under the same condition (even at much higher amino acids concentration), which means that complex **1·2** exhibits negligible perturbation. The above results indicated that complex **1·2** could be used for selective colorimetric sensing of basic amino acids.

The selectivity trends of the colorimetric recognition amino acids assays are accordant with the selectivity in binding affinities of TCBQ for amino acids, which were supported by control studies carried out using individual TCBQ and calix[4]pyrrole **1**, respectively, in $CHCl_3/C_2H_5OH/H_2O$ (1/6/3, v/v/v) mixture. The addition of various amino acids (7.0×10^{-4} M) to the solution of calix[4]pyrrole **1** (2.0×10^{-3} M) caused no changes in color and absorption spectra. Upon the addition of amino acids to the solution of TCBQ (2.0×10^{-4} M), a less

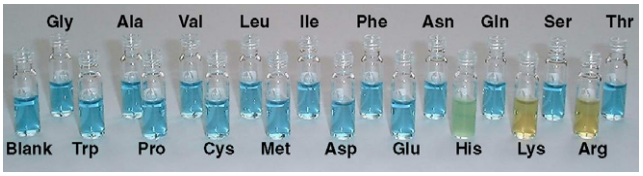


Figure 3. Color changes of complex **1·2** (containing 2.0×10^{-3} M **1** and 2.0×10^{-4} M **2**) in $CHCl_3/EtOH/H_2O$ (1/6/3, v/v/v) before and after the addition of 7.0×10^{-4} M of various amino acids.

dramatic color change from pale yellow to bright yellow could be observed only in the presence of lysine or arginine, in this case, a very weak absorption band appeared in the region of 400–500 nm ($\lambda_{max} = 427$ and 453 nm, see Fig. S2), but the absorption intensity is by far lower than the case of complex **1·2**.

The interactions of complex **1·2** with various amines were also studied. The spectral changes of the solution of complex **1·2** in the presence of aliphatic amines are similar to those of basic amino acids (Fig. 4). However, the addition of aromatic amine and pyridine caused less significant changes in color and the CT absorption band at 612 nm of complex **1·2**. The results, which can be briefly understood on the basis of the amine basicity (aliphatic amines > pyridine > aromatic amine), mean that complex **1·2** enables color discrimination between aliphatic amines and aromatic amines.

It was found that the color and absorption spectra changes of complex **1·2**, induced by basic amino acids and aliphatic amines, could be reversed by acidification. Upon the addition of hydrochloric acid to the mixture system of complex **1·2** and basic amino acids or aliphatic amines, the ensemble solution instantly changed to sky-blue color and the absorption peak at 612 nm reappeared, which means the renewal of the CT interaction between calix[4]pyrrole and TCBQ.

The interaction of TCBQ with amino acids and amines has been reported before.[4] Some of these studies indicated that the formation and the spectra of the n–π CT complexes were affected by solvent, pH, temperature, and reaction time. Our experiments showed that the interaction of TCBQ with amino acids was very weak in the unbuffered $CHCl_3/C_2H_5OH/H_2O$ (1/6/3, v/v/v) solution at room temperature. Although the basic amino acids-induced changes could be observed, TCBQ itself, unlike the calix[4]pyrrole–TCBQ complex, is unfit to be used for practical colorimetric assay for amino acids under present experiment conditions, due to the weak responses in color and absorption spectra. On the other hand, compared with the interaction systems of TCBQ with various amines (see Fig. S3), the calix[4]pyrrole–TCBQ complex displayed straightforward selectivity for amines.

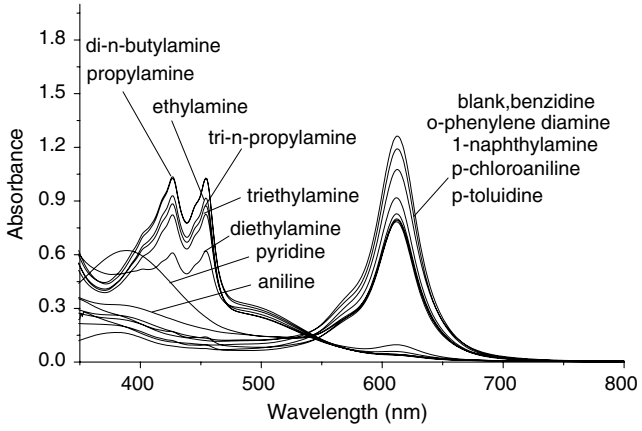


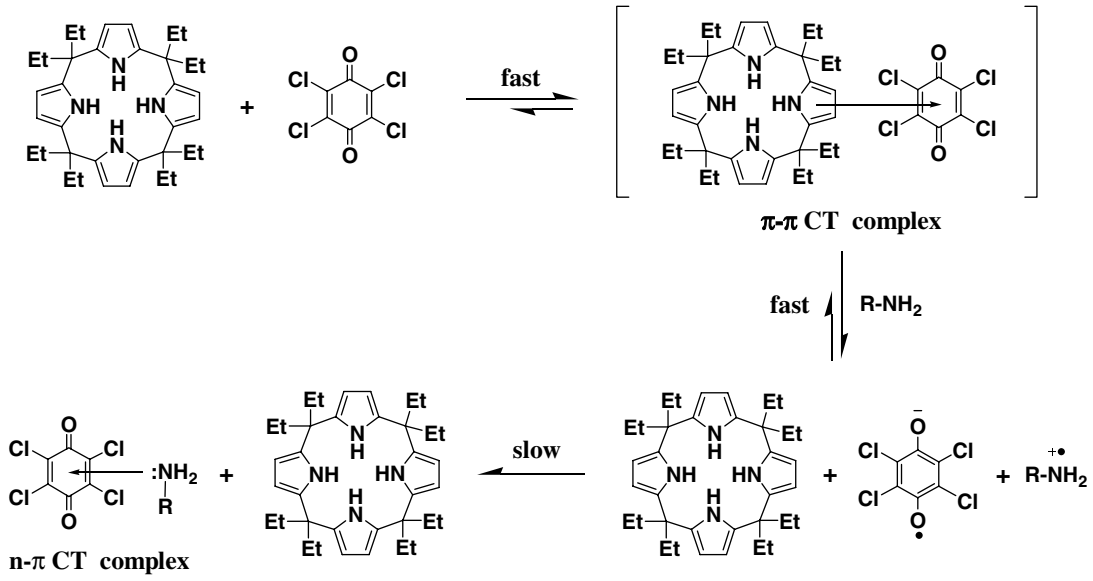
Figure 4. Absorption spectral changes of the complex **1·2** (2.0×10^{-3} M **1** and 2.0×10^{-4} M **2**) in $CHCl_3/EtOH/H_2O$ (1:6:3, v/v/v) in the presences of various amines (3.5×10^{-3} M), at room temperature.

TCBQ subunit of complex **1·2** plays a critical role in colorimetric assay. The new absorption peaks at 427 and 453 nm, which have not occurred during the formation of π–π CT complex **1·2** in $CHCl_3$ or $CHCl_3/C_2H_5OH/H_2O$, should be assigned to tetrachlorosemiquinone anion radical ($TCBQ^{\cdot -}$)[7] as a result of the interaction between TCBQ subunit and the basic amino acids/aliphatic amines with the free amino group. $TCBQ^{\cdot -}$ is difficult to form in the presence of neutral and acidic amino acids. These may be explained by the fact that these amino acids, existing as dipolar ions in the unbuffered solution, lack the activated free amino groups. Furthermore, compared with aliphatic amines, the conjugative effect decreases the electron density on the nitrogen atoms of aromatic amines, thereby decreases their n-electron-donating abilities for TCBQ subunit. The formation of $TCBQ^{\cdot -}$ resulted in the disaggregation of the π–π CT complex **1·2**. As expected, in pH 7.0 $CHCl_3/C_2H_5OH/H_2O$ (1/6/3, v/v/v) buffer solution, the basic amino acids and aliphatic amines fail to induce the changes in color and absorption spectra of complex **1·2**, because the free amino groups of basic amino acids and aliphatic amines are protonated in pH 7.0 buffer solutions, which decrease their n-electron-donating abilities, that is, to say, restrain their basicity.

Further experiments showed that the $TCBQ^{\cdot -}$ absorption band induced by basic amino acids, should completely disappear after one day, and a strong peak appeared at about 350 nm, which indicated the formation of the n–π CT complex of TCBQ with basic amino acids (see Fig. S4). In this case, the change process could not be reversed by acidification, due to the stability of amino acids–TCBQ complex on acidification.[4d]

From the above results, the remarkable changes in color and absorption spectra of complex **1·2** in the presence of basic amino acids and aliphatic amines resulted from (i) the disaggregation of the calix[4]pyrrole–TCBQ complex as a result of analytes compete for TCBQ subunit with calix[4]pyrrole subunit; and (ii) the formation of tetrachlorosemiquinone anion radical ($TCBQ^{\cdot -}$). The interaction mode is shown in Scheme 1. The approach is incompletely similar to the common colorimetric displacement assays.[5d] The intensity of the $TCBQ^{\cdot -}$ absorption band in the system of the calix[4]pyrrole–TCBQ complex is more stronger than that in the system of alone TCBQ. The calix[4]pyrrole subunit seems to act as 'auxochrome' for TCBQ, and TCBQ subunit existing as π-electron acceptor would be easier to be dissociated into free radical ions in the presence of n-electron donor. The calix[4]pyrrole–TCBQ assembly, like a 'signal magnifier', markedly improves sensing sensitivity and selectivity of TCBQ for amino acids and amines.

Flow-injection analysis (FIA) combined with electrochemistry, chemiluminescene, or spectrophotometry, as a means of rapid quantitative analysis, has been em-



Scheme 1.

Table 1. Results of determination of lysine and arginine using the proposed method

	Equation of calibration curves	Linear range ($\times 10^{-6}$ M)	LOD[a] ($\times 10^{-6}$ M)	RSD[b] (%)	Sampling rate (samples/h)
Lysine	$H = 530{,}001\times - 133.74$ ($r^2 = 0.997$, $n = 8$)	2.0–500	2.0	1.1	125
Arginine	$H = 516{,}522\times - 8939.1$ ($r^2 = 0.977$, $n = 8$)	5.0–500	5.0	2.0	125

[a] Limit of detection ($S/N = 3$).
[b] Relative standard deviation at 1.0×10^{-4} M using 10 replicate injections.

ployed widely for the determination of amino acids. Generally, the direct spectrophotometric detection of amino acids by FIA was not selective because the absorption spectra of the colored products formed by derivative reaction are completely overlapped or all amino acids reacted with chromogenic reagent at the same sensitivity, thus the method was hampered by the lack of specificity. Based on complex **1·2**, a more quantitative determination for lysine and arginine was preliminary performed by FIA coupled with spectrophotometry (see Supplementary data). Six amino acids, that is, lysine, arginine, alanine, serine, glutamic acid, and aspartic acid, were selected as compounds to cover a range of properties and elucidate the intragroup selectivity of amino acids. Under the established flow-injection conditions, the magnitude of the response of these amino acids (1.0×10^{-4} M) is in order of: lysine and arginine ($\geqslant$48,885 mv) $\gg$ serine, glutamic acid and aspartic acid ($\leqslant$3041 mv). The determination results obtained for lysine and arginine (shown in Table 1) indicate that the method is rapid and sensitive and can provide a high sampling rate and a wide linear range. A control experiment, carried out using solution of TCBQ as the reagent stream for determination of lysine, showed that no suitable linear ranges could be obtained, and the LOD is 40 times higher than that using the complex **1·2** solution. However, the new methodology of the determination of basic amino acids was only preliminarily evaluated, further studies, relating to interference of other co-existing amino acids, are currently in progress.

In summary, a new colorimetric assay based on calix[4]pyrrole–TCBQ supramolecular assembly has been developed. Compared with conventional TCBQ methods for the spectrophotometric detection of amino acids and amines, the approach, which carried out in an unbuffered water-containing media at room temperature, improves detection sensitivity of TCBQ for amino acids and amines, and allows for selective colorimetric sensing of basic amino acids and aliphatic amines.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No. 20372067).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.04.042.

References and notes

1. Fabbrizzi, L.; Licchelli, M.; Rabaioli, G.; Taglietti, A. *Coord. Chem. Rev.* **2000**, *205*, 85–108, and references cited therein.
2. (a) Sasaki, S.; Hashizume, A.; Citterio, D.; Fujii, E.; Suzuki, K. *Tetrahedron Lett.* **2002**, *43*, 7243–7245; (b) Hortala, M. A.; Fabbrizzi, L.; Marcotte, N.; Stomeo, F.;

Taglietti, A. *J. Am. Chem. Soc.* **2003**, *125*, 20–21; (c) Min-Su, H. D.; Kim, H. *Tetrahedron* **2004**, *60*, 11251–11257; (d) Guo, Y.; Shao, S.; Xu, J.; Shi, Y.; Jiang, S. *Tetrahedron Lett.* **2004**, *45*, 6447–6480; (e) Ait-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296–11297; (f) Bonizzoni, M.; Fabbrizzi, L.; Piovani, G.; Taglietti, A. *Tetrahedron* **2004**, *60*, 11159–11162.

3. Birks, J. B.; Slifkin, M. A. *Nature* **1963**, *197*, 42–45.
4. (a) Slifkin, M. A. *Spectrochim. Acta* **1964**, *20*, 1543–1554; (b) Al-Sulimany, F.; Townshend, A. *Anal. Chim. Acta* **1973**, *66*, 195–199; (c) Al-Ghabsha, T. S.; Rahim, S. A. *Anal. Chim. Acta* **1976**, *85*, 189–194; (d) Lin, B. Y.; Cheng, K. L. *Anal. Chim. Acta* **1980**, *120*, 335–345.
5. (a) Gale, P. A.; Sessler, J. L.; Král, V.; Lynch, V. *J. Am. Chem. Soc.* **1996**, *118*, 5140–5141; (b) Gale, P. A.; Sessler, J. L.; Král, V. *Chem. Commun.* **1998**, 1–8, and references cited therein; (c) Gale, P. A.; Anzenbacher, P., Jr.; Sessler, J. L. *Coord. Chem. Rev.* **2001**, *222*, 57–102, and references cited therein; (d) Gale, P. A.; Twyman, L. J.; Handlin, C. I.; Sessler, J. L. *Chem. Commun.* **1999**, 1851–1852; (e) Miyaji, H.; Sato, W.; Sessler, J. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 1777–1780; (f) Sessler, J. L.; Gale, P. A.; Genge, J. W. *Chem. Eur. J.* **1998**, *4*, 1095–1099.
6. (a) Bates, G. W.; Kostermans, M.; Dehaen, W.; Gale, P. A.; Light, M. E. *CrystEngCommun.* **2006**, *8*, 444–447; (b) Bates, G. W.; Gale, P. A.; Light, M. E. *CrystEngCommun.* **2006**, *8*, 300–302; (c) Custelcean, R.; Delmau, L. H.; Moyer, B. A.; Sessler, J. L.; Cho, W.-S.; Gross, D.; Bates, G. W.; Brooks, S. J.; Light, M. E.; Gale, P. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 2537–2542; (d) Sessler, J. L.; Gross, D. E.; Cho, W.-S.; Lynch, V. M.; Schmidtchen, F. P.; Bates, G. W.; Light, M. E.; Gale, P. A. *J. Am. Chem. Soc.* **2006**, *128*, 12281–12288; (e) Shao, S.; Guo, Y.; He, L.; Jiang, S.; Yu, X. *Tetrahedron Lett.* **2003**, *44*, 2175–2178.
7. (a) Osakai, T.; Akagi, N.; Hotta, H.; Ding, J.; Sawada, S. *J. Electroanal. Chem.* **2000**, *90*, 85–92; (b) Oyama, M.; Park, H. *J. Electroanal. Chem.* **2002**, *523*, 79–85; (c) Andre, J. J.; Weill, G. *Mol. Phys.* **1968**, *15*, 97–99; (d) Kawai, K.; Shirota, Y.; Tsubomura, H.; Mikawa, H. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 77–81.