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RESEARCH ARTICLE

Cooperative recognition of Cu^{2+} based on amino acids tethered benzothiadiazoyl-bistriazoles

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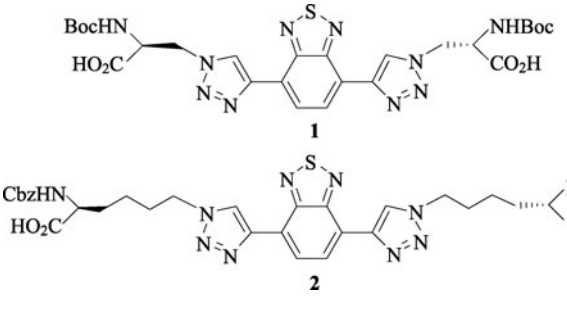
Fluoroionophoric properties of benzothiadiazoyl-bistriazoyl amino acids derivatives toward metal ions were investigated by UV-Vis and fluorescence spectroscopy. Our results show that the water soluble lysine derivative 2 exhibited a significant selectivity toward Cu^{2+} in "on-off" type response in buffer solution at pH 7.4.

Keywords benzothiadiazoyl-bistriazoles, fluoroionophores, Cu^{2+}, fluorescence quenching, cooperative recognition

1 Introduction

Benzothiadiazoles (BTD) with good optical properties and strong electron-withdrawing characters have attracted much attention due to their potential applications in organic functional materials [1–10]. For instance, when coupled with *N,N*-diaryl N terminus via various π-conjugate spacers, the corresponding BTD derivatives exhibited large two-photon absorption in orange-red color [8]. While being incorporated into conjugate polymer, BTD could modulate the photophysical properties of polymer which had great importance in organic optical-electrical materials [11–13]. Combining with other non-covalent interactions, such as hydrogen bonding, van der Waals, and different kinds of functionalities like liquid crystal displays, light-harvesting antennas have also been constructed [14]. Furthermore, Wang et al. demonstrated that charged water-soluble BTD-containing polyfluorenes used for fluorescence assays of phosphatase and peptidase induced intramolecular energy transfer from the fluorene units to BTD sites [15]. Similar approach has been also realized in naked-eye detection of heparin by Liu's group; they made use of a multicolor cationic conjugated polymer with BTD as energy acceptor [16]. For continuous work, they further applied BTD-containing conjugated polymer to fluorescent sensing of concanavalin A [17]. However, to the best of our knowledge, BTD is rarely applied in metal ions sensing which has been a research field of interest in recent years. Our group has recently reported a benzothiadiazoyl-triazoyl modified cyclodextrin as a selective fluoroionophore for Ni^{2+}. We have found that a multi-chromophoric system displayed an enhanced sensitivity toward Ni^{2+}compared to monomeric BTD linked to methyl glucoside [18]. This could be explained by a cooperative interaction of fluorophore and multiple binding sites in the cyclodextrin molecule. However, this molecule suffered from low water solubility and from interference with Cu^{2+} and Hg^{2+}. As a continuing program on the development of novel fluoroionophores and special coordination properties of 1,2,3-triazoles in metal ion sensing [19,20], we attempt to improve the water solubility of the BTD-based ligands by incorporation of amino acids as the terminal group through Cu(I)-catalyzed Huisgen 1,3-dipolaire cycloaddition reaction [21], in order to realize selective metal ions sensing in aqueous solution. The molecules under investigation (**1** and **2**) have been previously synthesized by our group and their structures are shown in Scheme 1 [22]. Compound **1** can be considered as an alanine derivative of BTD, while compound **2** as a lysine derivative. Compared with our previous work on Ni^{2+} sensor, ditopics of the ligands might facilitate cooperative multi binding recognition to improve the sensitivity, meanwhile endow superior photophysical properties since triazole is an electron donating group [23]. The amino acid functions are also favorable for metal complexation [24].



Scheme 1 Structure of compounds **1** and **2** under investigation.

2 Experimental section

Absorption spectra were recorded on a Uvikon-940 KONTRON or a Varian Cary 5E spectrophotometer and corrected emission spectra were taken on a Jobin-Yvon Spex Fluorolog 1681 spectrofluorometer (1 cm quartz cell was used). Stock

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solutions of compounds **1** and **2** were prepared in DMSO. Stock solutions of metal perchlorate salts were prepared in MeCN. The fluorescence quantum yield (Φ_F) was determined by standard method using coumarin 153 in ethanol as reference. The refractive index of the solvent was taken into account in the measurement. IR spectra were collected on a Thermo-Optek Nexus FTIR instrument.

3 Results and discussion

3.1 Photophysical properties

Due to its strong electron-withdrawing ability [8], the spectral properties of BTD could be easily modulated by chemical environment. We then first investigated the absorption spectra of **1** and **2** in HEPES buffer solution at pH 7.4. As shown in Table 1, compounds **1** and **2** have almost the same molar extinction coefficient around 9000 $L \cdot mol^{-1} \cdot cm^{-1}$ and exhibit absorption band respectively centered at 384 and 391 nm in HEPES buffer solution. The slight blue shift in **1** may come from existence of the intramolecular hydrogen bonding between triazole and carboxylic acid group which reduced the electron donating ability of triazole. Solvatochromism of **2** was investigated in different solvents. The absorption band showed a blue shift in protic solvents like CH_3OH and H_2O as compared with non-protic solvents like CH_3CN and dichloromethane (DCM) (Figure 1). This result is consistent with our hypothesis that hydrogen bonding interaction induced blue shift in the absorption spectra. However, no dependence between polarity of solvents and absorption wavelength was observed. Despite the difference in absorption spectra of both ligands, these two compounds have almost the same emission band at ca. 542 nm, with large Stokes shift around 155 nm and quantum yields more than 20%. Compared with our previous work on the monotopic BTD [18], compounds **1** and **2** showed more red shift in absorption spectra and could be excited in the visible region. Meanwhile, larger Stokes shift could be another advantage for practical analysis.

3.2 Metal ion binding properties

Our previous results have shown that triazole and BTD both contributed to the coordination interaction with metal ions [18]. Since the potential binding sites of nitrogen-containing compounds could be protonated under acid condition and hence result in changes of the photophysical properties and binding ability with metal ions, first we have investigated the pH effect. As shown in Figure 2, with increasing pH values from 3.0 to 6.2, the absorption of **2** shifted from 404 to 392 nm, with a concomitant enhancement and red shift of fluorescence from 529 to 552 nm. When the pH value was higher than 6.2, the fluorescence intensity stayed at a plateau.

Table 1 Spectroscopic data of **1** and **2**

compound	solvent	λ_{max}^{A} /nm	λ_{max}^{F} /nm	$\Delta\lambda_{F\text{-}A}$ /nm	ε_{00} /($L \cdot mol^{-1} \cdot cm^{-1}$)	Φ_F
1	H_2O	388	542	154	9046	0.21
	H7W	384	542	158	nd	nd
2	H_2O	391	541	150	8893	0.29
	H7W	391	542	151	nd	nd

λ_{max}^{A}: absorbance wavelength; λ_{max}^{F}: emission wavelength; $\Delta\lambda_{F\text{-}A}$: Stokes shift; ε_{00}: molar absorbance coefficient; Φ_F: fluorescence quantum yield; H7W: HEPES: water buffer at pH 7.4; nd: not determined.

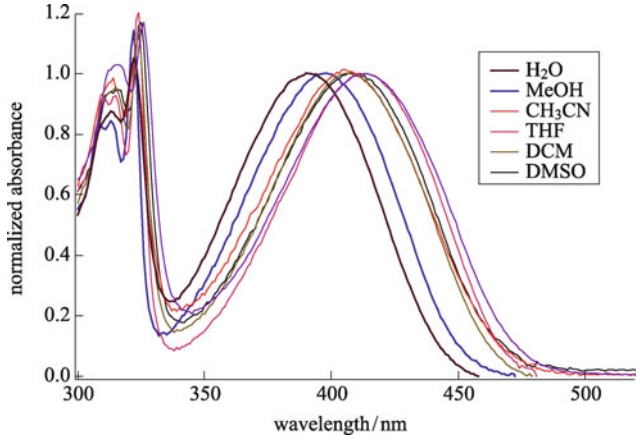

Figure 1 Normalized absorption spectra of **2** in different solvents.

However, no significant pH effect was observed on ligand **1** (the absorption spectra of **1** had almost no change within the pH range from 3.0 to 11.5). The insensibility of ligand **1** toward pH variation might be explained by intramolecular hydrogen bonding between amino acid moiety and the chromophore.

Preliminary selectivity assay in water showed that Cu^{2+} induced the fluorescence quenching of compounds **1** and **2**. We then decided to study the response of **1** and **2** to Cu^{2+} under different pH conditions. The results shown in Figure 3 demonstrated that under neutral condition, fluorescence spectra of **2** showed the most change before and after the addition of 20 equiv. of Cu^{2+}. This result might be explained that under acidic condition, nitrogen atoms could be prontonated and then prevented the binding between ligand and Cu^{2+}; while at higher pH, compound **2** is in carboxylate form and OH^- might compete with ligand. According to our results on pH effect, we decided to perform our experiments using HEPES buffer solution at pH 7.4.

To get insight into the binding properties of benzothiadiazoyl-bistriazoles **1** and **2** toward metal ions, we first investigated fluorescence changes upon the addition of 20 equiv. of selected cations to the solution of **1** and **2** in HEPES buffer solution. As presented in Figure 4(b), the fluorescence emission was not affected by Hg^{2+}, Mn^{2+}, Mg^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+} and Pb^{2+}, but slightly quenched by

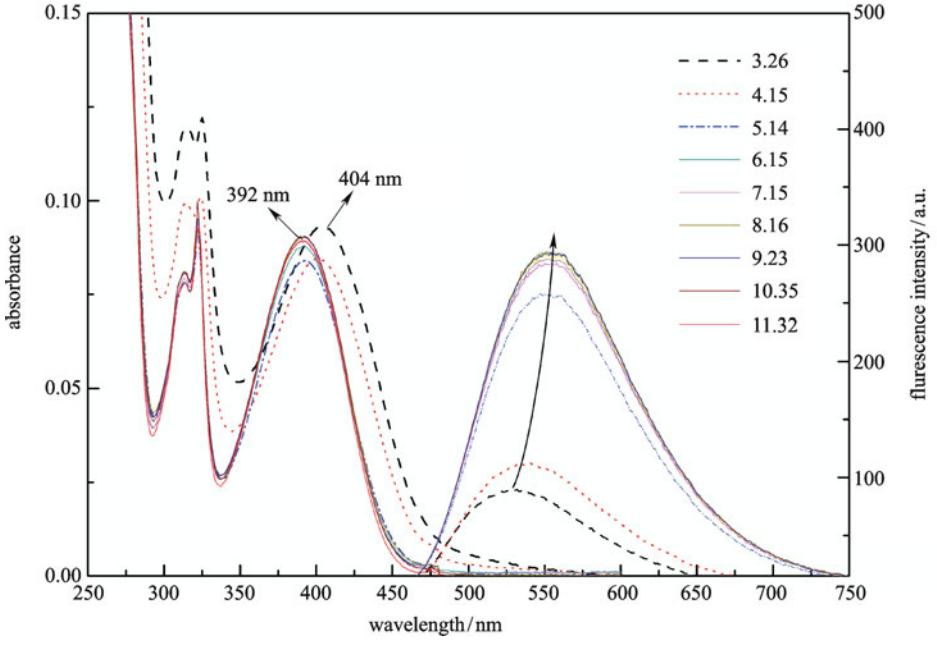

Figure 2 The absorption and fluorescence spectra of **2** in HEPES solution at different pH. [**2**] = 10 μmol·L^{-1}, [HEPES] = 10 mmol·L^{-1}, fluorescence spectra were excited at the maximum absorption wavelength.

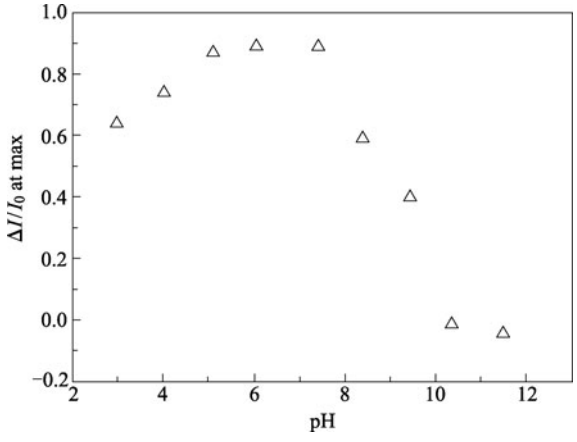

Figure 3 pH Effect on the fluorescence response of **2** to Cu^{2+} at maximum emission wavelength. [**2**] = 10 μmol·L^{-1}, [Cu^{2+}] = 0.2 mmol·L^{-1}.

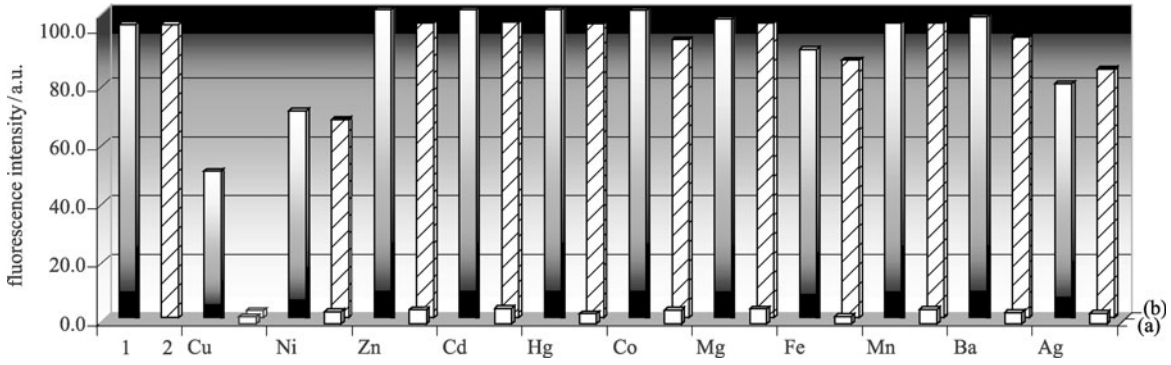

Figure 4 Fluorescence intensity change profiles of ligands **1** and **2** in HEPES buffer solution at pH 7.4 with selected cations in the presence and absence of Cu^{2+}. [**1**] = [**2**] = 10 μmol·L^{-1}, [M^{n+}] = 0.2 mmol·L^{-1}, λ_{ex} = 391 nm. (a) Competitivity of ligand **2** with Cu^{2+} as competitive ion. (b) Comparison of selectivity of ligand **1** and **2** with different metal ions.

Fe^{2+}, Ag^{+} and Ni^{2+} for both ligands. However, a significant quenching toward Cu^{2+} was observed. Moreover, ligand **2** appeared to be more sensitive than compound **1**, which might be due to the participation of more flexible amino acid moiety in **2** for Cu^{2+} complexation. The selectivity of compound **2** toward Cu^{2+} was further ascertained by the competition experiment by adding 20 equivalents of Cu^{2+} ion to the competing metal ion-ligand mixtures, where the emission was quenched as in the presence of Cu^{2+} alone (Figure 4(a)). The different selectivity observed between BDT-functionalized multichromophoric cyclodextrin for Ni^{2+} [18] and compounds **1** and **2** for Cu^{2+} indicates that the selectivity of BDT series is dependent on the nature of linked functional groups and their cooperativity. The presence of amino acid function might favor the selectivity for Cu^{2+} [24].

Finally, we carried out the titration of compound **2** in HEPES buffer solution at pH 7.4. As shown in Figure 5, with increasing concentration of Cu^{2+}, the absorption band at 391 nm gradually decreased and underwent a red shift to 418 nm. Simultaneously, a clear isosbestic point at 418 nm was observed and indicated the formation of complex between Cu^{2+} and **2**. Fluorescence titration showed that at the beginning of titration ($\leqslant$ 2 equiv.), fluorescence intensity at 542 nm decreased slowly. However, after the addition of 2 equiv. of Cu^{2+}, the quenching efficiency was enhanced and reached a plateau at 10 equiv. (Figure 6). The binding curve showed typical sigmoidal behavior, which is characteristic of cooperativity. The changes in fluorescence could be analyzed by Hill equation to yield a Hill coefficient of 2.6 and an apparent binding constant up to 5.0×10^{11} (Figure 7). Similar

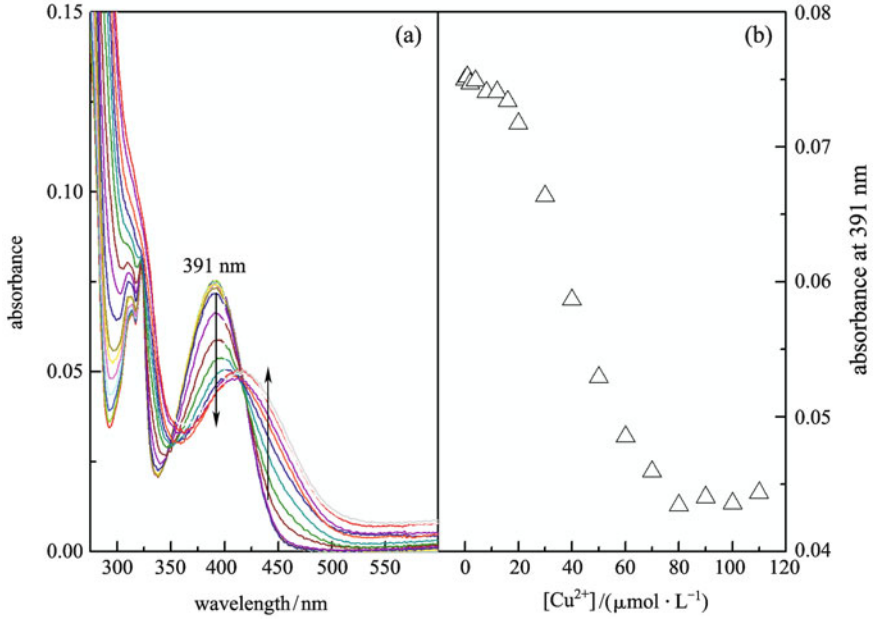

Figure 5 (a) Absorption spectra of **2** with increasing concentration of Cu^{2+} in HEPES buffer at pH 7.4; (b) plot of absorbance at 391 nm vs Cu^{2+} concentration. [**2**] = 10 μmol·L^{-1}.

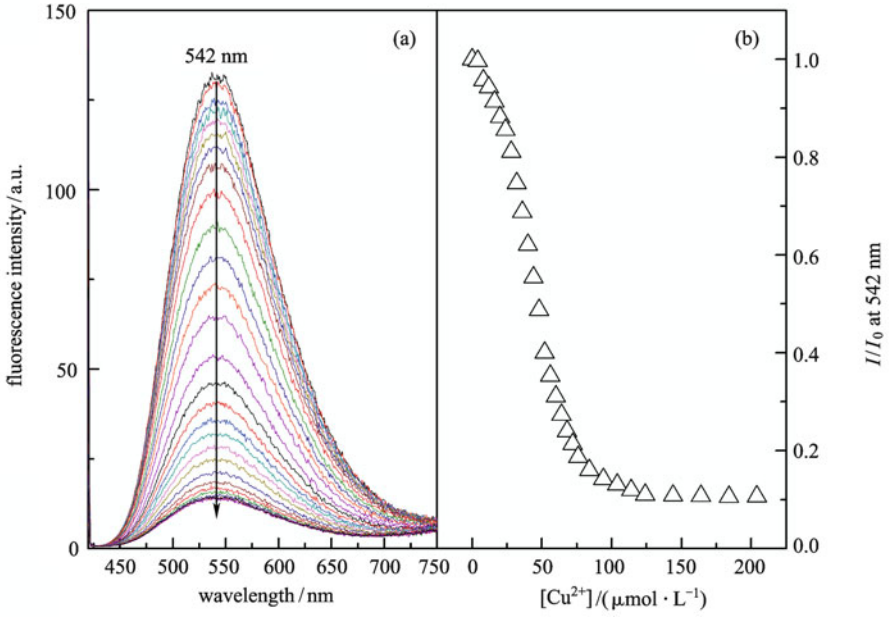

Figure 6 (a) Fluorescence spectra of **2** with increasing concentration of Cu^{2+} in HEPES buffer at pH 7.4; (b) plot of fluorescence intensity at 542 nm vs Cu^{2+} concentration. [**2**] = 10 μmol·L^{-1}, λ_{ex} = 418 nm.

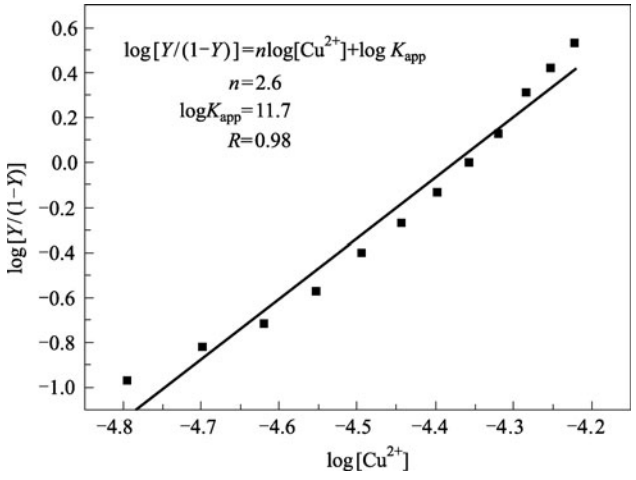

Figure 7 Hill plot for the binding of Cu^{2+} to **2** based on the fluorescence spectral changes in low concentration range. Hill equation: $\log[Y/(1-Y)] = n\log[Cu^{2+}] + \log K_{app}$, where Y is the fractional occupancy of the host, n the Hill coefficient, and K_{app} the apparent binding constant [26].

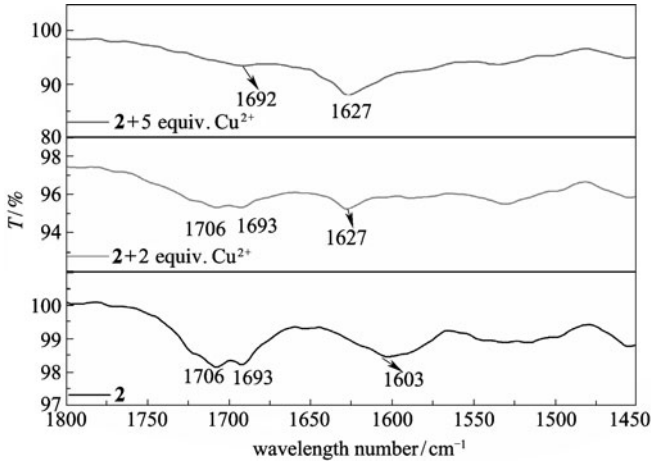

Figure 8 Comparison of expanded IR spectra of **2** in the absence and presence of 2 and 5 equiv. $Cu(ClO_4)_2$.

cooperative effect has been observed on 8-hydroxyquinoline-based chemosensor [25].

To rationalize the change of fluorescence, infrared spectra of **2** were collected. As displayed in Figure 8, the stretching vibration of C = O bond at 1722, 1706, 1693 cm^{-1} and of C = N bond at 1603 cm^{-1} disappeared after the addition of 5 equiv. Cu^{2+}, while a new band at 1627 cm^{-1} emerged. The result demonstrated that both amino acid group and BTD group participated in the coordination interaction with Cu^{2+}.

4 Conclusion

In conclusion, we have demonstrated that triazole conjugation of benzothiadiazole with amino acids led to a new class of water-soluble fluoroionophores. Compound **2** with a longer linker between benzothiadiazole and amino acid part (Lys) was a better ligand to Cu^{2+} than its homolog **1** with a shorter linker (Ala), probably due to the participation of the more flexible amino acid group in **2**. These results would be interesting for the further development of new benzothiadiazoles-based fluoroionophores.

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Reference:

1. Justin Thomas, K. R.; Lin, J. T.; Velusamy, M.; Tao, Y. T.; Chuen, C. H., *Adv. Funct. Mater*. **2004**, *14*, 83–90
2. Niu, Y. H.; Huang, J.; Gao, Y., *Adv. Mater*. **2003**, *15*, 807–811
3. Raimundo, J. M.; Blanchard, P.; Brisset, H.; Akoudad, S.; Roncali, J., *Chem. Commun*. **2000**, 939–940
4. Kitamura, C.; Tanaka, S.; Yamashita, Y., *Chem. Mater*. **1996**, *8*, 570–578
5. van Mullekom, H. A. M.; Vekemans, J. A. J. M.; Meijer, E. W., *Chem. Eur. J*. **1998**, *4*, 1235–1243
6. Bangcuyo, C. G.; Evans, U.; Myrick, M. L.; Bunz, U. H. F., *Macromolecules* **2001**, *34*, 7592–7594
7. Velusamy, M.; Justin Thomas, K. R.; Lin, J. T.; Hsu, Y. C.; Ho, K. C., *Org. Lett*. **2005**, *7*, 1899–1902
8. Kato, S. I.; Matsumoto, T.; Ishi-i, T.; Thiemann, T.; Shigeiwa, M.; Gorohmaru, H.; Maeda, S.; Yamashita, Y.; et al., *Chem. Commun*. **2004**, 2342–2343
9. Zhu, W.; Meng, X.; Yang, Y.; Zhang, Q.; Xie, Y.; Tian, H., *Chem. Eur. J*. **2010**, *16*, 899–906
10. Ma, X.; Hua, J.; Wu, W.; Jin, Y.; Meng, F.; Zhan, W.; Tian, H., *Tetrahedron* **2008**, *64*, 345–350
11. Bouffard, J.; Swager, T. M., *Macromolecules* **2008**, *41*, 5559–5562
12. Pu, K. Y.; Cai, L.; Liu, B., *Macromolecules* **2009**, *42*, 5933–5940
13. Neto, B. A. D.; Lopes, A. S. A.; Ebeling, G.; Gonçalves, R. S.; Costa, V. E. U.; Quina, F. H.; Dupont, J., *Tetrahedron* **2005**, *61*, 10975–10982
14. Ishi-i, T.; Nakamura, N.; Esaki, N.; Amemori, S., *Chem. Lett*. **2008**, *37*, 1166–1167
15. An, L.; Tang, Y.; Feng, F.; He, F.; Wang, S., *J. Mater. Chem*. **2007**, *17*, 4147
16. Pu, K. Y.; Liu, B., *Macromolecules* **2008**, *41*, 6636–6640
17. Shi, J.; Cai, L.; Pu, K. Y.; Liu, B., *Chem. Asian J*. **2010**, *5*, 301–308
18. Maisonneuve, S.; Fang, Q.; Xie, J., *Tetrahedron* **2008**, *64*, 8716–8720
19. David, O.; Maisonneuve, S.; Xie, J., *Tetrahedron Lett*. **2007**, *48*, 6527–6530
20. Xie, J.; Ménand, M.; Maisonneuve, S.; Métivier, R., *J. Org. Chem*. **2007**, *72*, 5980–5985
21. Kolb, H. C.; Finn, M. G.; Sharpless, K. B., *Angew. Chem. Int. Ed*. **2001**, *40*, 2004–2021
22. Li, C.; Tang, J.; Xie, J., *Tetrahedron* **2009**, *65*, 7935–7941
23. Zhou, Z.; Fahrni, C. J., *J. Am. Chem. Soc*. **2004**, *126*, 8862–8863
24. Zheng, Y.; Huo, Q.; Kele, P.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M., *Org. Lett*. **2001**, *3*, 3277–3280
25. Tang, X. L.; Peng, X. H.; Dou, W.; Mao, J.; Zheng, J. R.; Qin, W. W.; Liu, W. S.; Chang, J.; et al., *Org. Lett*. **2008**, *10*, 3653–3656
26. Shinkai, S.; Ikeda, M.; Sugasaki, A.; Takeuchi, M., *Acc. Chem. Res*. **2001**, *34*, 494–503