Inorg. Chem. **2009**, *48*, 408−410

Metallohelical Triangles for Selective Detection of Adenosine Triphosphate in Aqueous Media

Hongmei Wu, Cheng He,* Zhihua Lin, Yang Liu, and Chunying Duan*

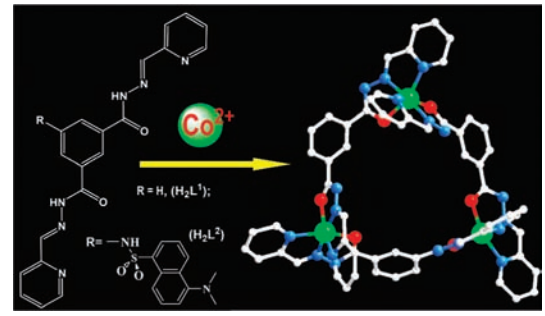
State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China

Received July 19, 2008

Metallohelical triangles consisting of chromophore units and hydrogen-bonding trigger sites were prepared by modulating two tridentate N_2O units containing amide groups within a central benzene ring at the meta sites, for the selective detection of adenosine trisphosphate in aqueous media over other ribonucleotides.

Among biologically important anions, a great deal of interest has been focused on the molecular recognition of the nucleotide polyphosphates; in particular, adenosine triphosphate is one of the basic components in bioenergetic processes of living organisms, with its polyphosphate chain being the center for chemical energy storage and transfer.[1] Although several intriguing strategies have been developed to detect adenosine trisphosphate (ATP), such as synthetic host−guest receptors,[2,3] peptides,[4] and RNA aptamers,[5,6] the complexation selectivity, particularly the selectivity to adenine over all other nucleobases, is still insufficient and remains as a challenging problem.[7]

Scheme 1. Molecular Structures of Ligands H_2L^1 and H_2L^2 as Well as the Cationic Helical Triangle $[Co_3(HL^1)_3]^{3+a}$



[a] The metal, oxygen, nitrogen, and carbon atoms are drawn as green, red, blue, and white, respectively.

On the other hand, self-assembled, metal−ligand coordination molecular hollows are appealing as synthetic hosts for application as a fascinating class of size- or shape-selective dynamic molecular chemosensors with various responses and metal-triggered functions.[8,9] By using carefully functionalized amide groups as the trigger sites for efficient host−guest interactions, these Werner-type capsules and some of the organic capsules that have the potential to recognize the important biological molecules have been realized.[10,11] Herein, we develop a new strategy for preparing helical triangles through modulation of the tridentate N_2O units containing amide groups within a central benzene ring at the meta sites (Scheme 1). We reasoned that these amide groups in the positively charged cages matched the static,

* To whom correspondence should be addressed. E-mail: hecheng@dlut.edu.cn (C.H.); cyduan@dlut.edu.cn (C.D.).

(1) (a) Lipscomb, W. N.; Strater, N. *Chem. Rev.* **1996**, *96*, 2375–2434. (b) Berg, J. M.; Stryer, L.; Tymoczko, J. L. *Biochemistry*, 5th ed.; W. H. Freeman: New York, 2002. (c) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*, 4th ed.; Newton: Tokyo, 2002.

(2) (a) Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920–3925. (b) Yin, C.; Gao, F.; Huo, F.; Yang, P. *Chem. Commun.* **2004**, 934–935. (c) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S.-J.; Yoon, J. *J. Am. Chem. Soc.* **2007**, *129*, 3828–3829.

(3) (a) Wang, H.; Chan, W.-H. *Org. Biomol. Chem.* **2008**, *6*, 162–168. (b) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777–4780. (c) Li, C.; Numata, M.; Takeuchi, M.; Shinkai, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 6371–6374.

(4) (a) McCleskey, S. C.; Griffin, M. J.; Schneider, S. E.; McDevitt, J. T.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 1114–1115. (b) Butterfield, S. M.; Waters, M. L. *J. Am. Chem. Soc.* **2003**, *125*, 9580–9581. (c) Cruz-Aguado, J. A.; Chen, Y.; Zhang, Z.; Elowe, N. H.; Brook, M. A.; Brennan, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 6878–6879.

(5) (a) Sazani, P. L.; Larralde, R.; Szostak, J. W. *J. Am. Chem. Soc.* **2004**, *126*, 8370–8371. (b) Stojanovic, M. N.; Kolpashchikov, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 9266–9270.

(6) (a) Cho, E. J.; Yang, L.; Levy, M.; Ellington, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 2022–2023. (b) Hagihara, M.; Fukuda, M.; Hasegawa, T.; Morii, T. *J. Am. Chem. Soc.* **2006**, *128*, 12932–12940. (c) Zuo, X.; Song, S.; Zhang, J.; Pan, D.; Wang, L.; Fan, C. *J. Am. Chem. Soc.* **2007**, *129*, 1042–1043.

(7) Hisamatsu, Y.; Hasada, K.; Amano, F.; Tsubota, Y.; Wasada-Tsutsui, Y.; Shirai, N.; Ikeda, S.-i.; Odashima, K. *Chem.—Eur. J.* **2006**, *12*, 7733–7741.

(8) (a) Cronin, L. *Angew. Chem., Int. Ed.* **2006**, *45*, 3576–3578. (b) Schmuck, C. *Angew. Chem., Int. Ed.* **2007**, *46*, 5830–5833.

(9) (a) Pluth, M. D.; Bergman, R. G.; Raymond, K. N. *Science* **2007**, *316*, 85–88. (b) Tashiro, S.; Kobayashi, M.; Fujita, M. *J. Am. Chem. Soc.* **2006**, *128*, 9280–9281. (c) Chen, J.; Wang, X.; Shao, Y.; Zhu, J.; Zhu, Y.; Li, Y.; Xu, Q.; Guo, Z. *Inorg. Chem.* **2007**, *46*, 3306–3312.

(10) (a) Hasegawa, S.; Horike, S.; Matsuda, R.; Furukawa, S.; Mochizuki, K.; Kinoshita, Y.; Kitagawa, S. *J. Am. Chem. Soc.* **2007**, *129*, 2607–2614. (b) Mazik, M.; KÖnig, A. *J. Org. Chem.* **2006**, *71*, 7854–7857. (c) Ferrand, Y.; Crump, M. P.; Davis, A. P. *Science* **2007**, *318*, 619–622.

(11) (a) Nativi, C.; Cacciarini, M.; Francesconi, O.; Vacca, A.; Moneti, G.; Ienco, A.; Roelens, S. *J. Am. Chem. Soc.* **2007**, *129*, 4377–4385. (b) Mazik, M.; Cavga, H.; Jones, P. G. *J. Am. Chem. Soc.* **2005**, *127*, 9045–9052. (c) He, C.; Lin, Z.; He, Z.; Duan, C.; Xu, C.; Wang, Z.; Yan, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 877–881.

10.1021/ic801350h CCC: $40.75
Published on Web 12/12/2008

geometric, coordinative, and functional requirements of the bowl-like capsules for recognition of nucleotide anions.[12] Also, the formation of hydrogen bonds of the amide groups is likely to perturb the electronic distribution of the conjugated backbone of the ligand, leading to significant changes in optical properties.[13]

The reaction of 1,3-dicarbohydrazidobenzene with 2-pyridinecarboxaldehyde gave a ligand, H_2L.[1] Adding NH_4PF_6 into the methanol solution of ligand H_2L[1] and $Co(NO_3)_2 \cdot 6H_2O$ led to the formation of the cobalt(II)-based helical triangle **MC-1**.[14] The triangular cation $[Co_3(HL_1)_3]^{3+}$ has an ideal C_3 symmetry with three identical cobalt(II) centers exhibiting the same chiral coordination geometries. The three benzene rings of the ligands lie on the same side of the averaged plane (Scheme 1). The presence of three PF_6^- anions corresponding to each cationic cage suggests that three of the six amide groups lose their protons during coordination to metal ions.

Ligand H_2L[1] exhibits an absorption band at 300 nm in *N*,*N*-dimethylformamide (DMF)/water (8:2) aqueous media, while the Co-based compound **MC-1** shows a band at about 380 nm. The absorbance of **MC-1** was a slight change over a wide concentration range of acid/base (from log [HCl] = −4.2 to log [NaOH] = −2.5; see Figure S9 in the Supporting Information). The addition of ATP to the solution of **MC-1** causes a significant absorbance increase at 300 nm and an obvious absorbance decrease at 380 nm. The presence of a sharp isosbestic point at 330 nm indicates that only two species coexist in the equilibrium. The individual profile of the ratio of the two absorbencies A_{300}/A_{380} demonstrates a 1:1 stoichiometric complexation, with the association constant (log K_{ass}) being calculated as 2.55 ± 0.03.

Upon the addition of adenosine 5'-monophosphate (AMP) and adenosine diphosphate (ADP) instead of ATP, no obvious spectral changes were found under the same experimental conditions, indicating that the affinity of **MC-1** for ATP was stronger than that for the other adenosine-based nucleotides. In the meantime, the addition of other ribonucleotides (XNP, where X = U (uridine), C (cytidine), and G (guanosine), N = mono-, di-, and tri-, and P = phosphate) could not cause any significant spectral changes of the UV−vis spectra under the same experimental conditions. Compared to the selectivity of ATP with respect to the less charged ADP, AMP, or inorganic phosphate anions, the selective recognition and sensing of ATP over the other triphosphate nucleotides is a much more difficult goal. The excellent selectivity of **MC-1** for ATP over the other ribonucleotides thus not only suggests that the receptor molecule **MC-1** exhibits size-selective recognition for nucleotides via electrostatic interactions between the cationic binding sites of the receptor and the negatively charged polyphosphate groups but also indicates that the amide groups in **MC-1** situate in the correct place and are able to conceivably interact with adenosine, from which the chromogenical response is achieved.[11c]

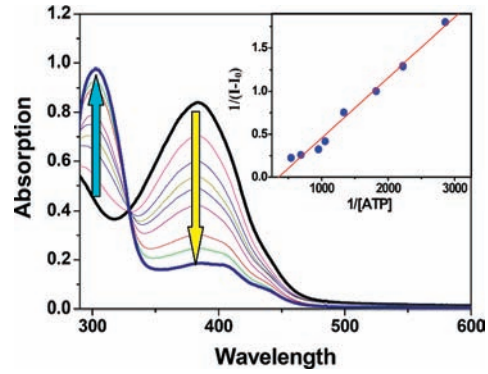


Figure 1. Family of UV−vis spectra of **MC-1** (10 μM) in 8:2 DMF/H_2O aqueous solutions upon the addition of ATP. The inset shows the Benesi−Hildebrand fitting of the titration curve, where I and I_0 are the ratio of the absorbance at 300 and 380 nm (A_{300}/A_{380}) in the presence and absence of ATP, respectively.

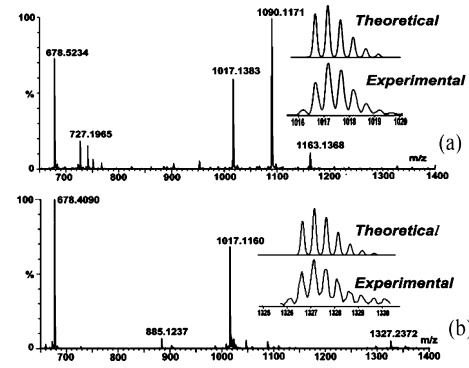


Figure 2. ESI-MS of **MC-2** in a DMF solution (a) and upon the addition of 1 equiv of ATP (b). The insets exhibit the measured and simulated isotopic patterns at m/z = 1017.12 and 1327.24 in parts a and b, respectively.

To further investigate the potential interaction sites, a fluorescent dansyl unit was introduced into another meta position, giving a new ligand, H_2L^2, which has two kinds of optical responding groups and two kinds of guest binding amide groups.[15] The cobalt(II) compound **MC-2** was isolated by a similar synthetic procedure using H_2L^2 instead of H_2L.[1] The electrospray ionization mass spectrometry (ESI-MS) spectrum of **MC-2** in a DMF solution exhibited three intense peaks at m/z = 678.40, 1017.12, and 1090.11, respectively. These signals were assignable to $[Co_3(H_3L^2_3)]^{3+}$, $[Co_3(H_2L^2_3)]^{2+}$, and $[Co_3(H_3L^1_3)(PF_6)]^{2+}$, respectively, reflecting the formation of a [3 + 3] triangle species and the substantial stability of **MC-2** in solution. In the presence of 1 mol equiv of ATP (Figure 2), the peak at m/z = 1090.11 disappeared, indicating that the PF_6^- anion was replaced. In the meantime, two new peaks at m/z = 885.12 and 1327.24 appeared. They were assigned to $(Na[Co_3(H4L^2_3)\supset ATP](DMF)(H_2O))^{3+}$ and $(Na[Co_3(H_3L^2_3)\supset ATP](DMF)(H_2O))^{2+}$,

(12) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896–3904.

(13) (a) Jiang, P.; Guo, Z. *Coord. Chem. Rev.* **2004**, *248*, 205–229. (b) Boiocchi, M.; Boca, L. D.; Gómez, D. E.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *J. Am. Chem. Soc.* **2004**, *126*, 16507–16514.

(14) Crystal data of **MC-1**: $C_{60}H_{60}Co_3F_{18}N_{18}O_{12}P_3$, M = 1834.27, hexagonal, space group $R\bar{3}c$, red block, a = 18.28(1) Å, c = 90.71(5) Å, V = 26250(16) Å^3, Z = 3, D_c = 1.394 g cm^{-3}, μ(Mo Kα) = 0.716 mm^{-1}, T = 293(2) K, 5049 unique reflections [R_{int} = 0.1177]. Final R1 [with $I > 2\sigma(I)$] = 0.0814, wR2 (all data) = 0.2092. CCDC number 678275.

(15) Sessler, J. L.; Kral, V.; Shishkanova, T. V.; Gale, P. A. *Proc. Natl. Acad. Sci U.S.A.* **2002**, *99*, 4848–4853.

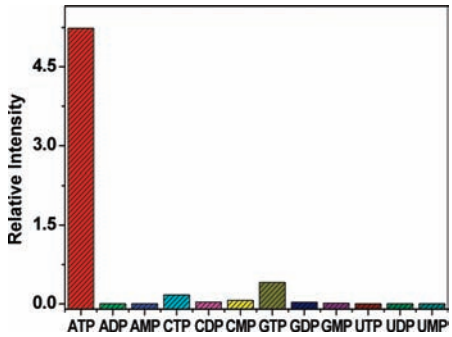


Figure 3. UV−vis responses of **MC-2** (10 μM in a 8:2 DMF/H_2O solution) upon the addition of ribonucleotide polyphosphates (0.4 mM). The bars represent the value of $[(I - I_0)/I_0]$, where I and I_0 are the ratio of the absorbances at 300 and 380 nm (A_{300}/A_{380}) in the presence and absence of nucleotide polyphosphates, respectively.

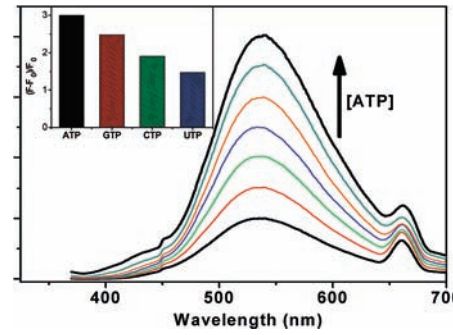


Figure 4. Family of luminescence spectra of **MC-2** (50 μM in a 8:2 DMF/water solution) upon the addition of a standard aqueous solution of ATP. The inset shows the luminescence responses of **MC-2** (50 μM in a 8:2 DMF/water solution) upon the addition of ribonucleotide triphosphates (0.25 mM). Data are recorded at 530 nm; excitation is at 330 nm.

respectively, based on the exact comparison of these peaks with simulations on the basis of natural isotopic abundances, demonstrating the recognition of **MC-2** for ATP and providing evidence for a 1:1 stoichiometric host−guest complexation.

The UV−vis spectral properties of ligand H_2L^2 and **MC-2** were similar to those of H_2L^1 and **MC-1**, respectively. Also, **MC-2** exhibited a similar UV−vis spectral change in a DMF/water (8:2) aqueous solution upon the addition of ATP with a sharp isosbestic point at 330 nm, indicating the reversibility of the sensing of **MC-2** for ATP. The individual profile of the ratio of the absorbance A_{300}/A_{380} also demonstrated the 1:1 stoichiometric host−guest complexation behavior, with an association constant (log K_{ass}) being calculated as 3.86 $\pm$ 0.03. From the structure of **MC-1**, we reasoned that the three dansyl groups in **MC-2** should position in the same direction showing a calix[3]arene fashion, leading to a 10-fold enhancement of the affinity for ATP. Interestingly, the addition of other ribonucleotides (XNP, where X = A, U, C, and G, N = mono-, di-, and tri-, and P = phosphate) could not cause any significant spectral changes of the UV−vis spectra under the same experimental conditions (Figure 3). Because **MC-1** and **MC-2** exhibited almost the same UV−vis spectral changes upon the addition of ATP, it was reasonable to assign the spectral response to the formation of hydrogen bonds between the amide groups and the ATP guests. The response of **MC-1** and **MC-2** toward ATP varied slightly over a wide acid/base concentration range (Figures S10 and S12 in the Supporting Information). Also, the addition of sodium trisphosphate to the two hosts did not cause obvious UV−vis spectral changes (Figure S13 in the Supporting Information).

Furthermore, the presence of dansylsulfonamide groups allows the host−guest property of **MC-2** to be evaluated also by luminescent analysis. Compound **MC-2** exhibits a typical emission of the dansylsulfonamide group at about 530 nm in the DMF/H_2O aqueous solution (50 μM, excitation at 330 nm).[16] Upon the addition of 5 equiv of an ATP aqueous solution, the luminescence intensity exhibits a 4-fold enhancement, with the emission wavelength not being shifted (Figure 4). The 1:1 stoichiometric host−guest complexation was also confirmed by the individual titration profile, with an association constant (log K_{ass}) being calculated as 4.08 $\pm$ 0.03, which is comparable to that calculated by the UV−vis titration data. Clearly, the recognition of **MC-2** to ATP should include the contribution of the hydrogen-bonding interaction about the sulfonamide groups, which block the photoinduced electron transfer process from the amide groups to the dansyl groups, resulting in luminescence enhancement.

Interestingly, the presence of the other ribonucleotide trisphosphates, CTP, GTP, and UTP, could cause similar luminescence enhancement of the dansylsulfonamide groups, but the addition of ribonucleotide monophosphate did not lead to any luminescence enhancements under the same experimental conditions. Thus, the fluorescence response is size-selective for nucleotides via hydrogen bonds between the sulfonamide groups and the negatively charged polyphosphate groups. From a mechanism viewpoint, the amide groups that are fixed at the inflexible backbone of the Werner-type macrocyclic receptors can respond to ATP through hydrogen-bonding patterns between the amide group and the nucleoside. However, the size-selective interactions between the negatively charged polyphosphate groups and the cationic triangle receptor provided the opportunity to distinguish the trisphosphates from other polyphosphates but did not prompt any chromogenical responses. Thus, the UV−vis responses of both **MC-1** and **MC-2** for ATP exhibited high selectivity over all other ribonucleotides. Whereas these sulfonamide groups interacted with the polyphosphate groups, the luminescence enhancements were quite similar for all four ribonucleoside triphosphates.

Acknowledgment. We gratefully acknowledge financial support of the NSFC (Grant 20801008) and the Start-Up Fund of Dalian University of Technology.

Supporting Information Available: CIF file of crystal data of **MC1**, experimental details, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

IC801350H

(16) (a) Guy, J.; Caron, K.; Dufresne, S.; Michnick, S. W.; Skene, W. G.; Keillor, J. W. *J. Am. Chem. Soc.* **2007**, *129*, 11969–11977. (b) Pearson, A. J.; Xiao, W. *J. Org. Chem.* **2003**, *68*, 5361–5368.