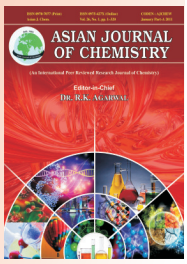
Asian Journal of Chemistry; Vol. 26, No. 14 (2014), 4457-4461

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16964



Biological Interaction Between Copper(II) Complex and Nucleosides

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Received: 30 December 2013; *Accepted*: 23 March 2014; *Published online*: 5 July 2014; AJC-15496

A copper-coordinated complex based on 7-membered amide cycle has been designed and synthesized. Its binding properties for various nucleosides, guanosine-5'-diphosphate (GDP), uridine-5'-diphosphate (UDP), inosine-5'- diphosphate (IDP) and guanosine-5'-triphosphate (GTP) has been studied by UV-visible spectra and crystal structure. Results indicated that the compound showed the highest binding ability with guanosine-5'-triphosphate in water solution among the studied nucleosides. The interaction of host-guest derived from hydrogen bonding and π-π staking and the host-guest binding ability depended on the chain length of nucleosides. In addition, the compound can be used to label nucleoside.

Keywords: Biological interaction, Nucleoside, Copper(II) complex.

INTRODUCTION

In recent years, increasing attention in the field of host-guest chemistry has been devoted to the fast development of anion recognition systems[1-7]. Detection of nucleosides has paramount importance as they form the fundamental units of all the life forms[8-12]. Most known molecular receptors for the nucleosides use complementary hydrogen bonding, but such recognition in the aqueous medium would be limited due to the interference from hydroxyl groups of the sugar moiety and competitive hydrogen bonding of the solvent[13-15]. Moreover, the sugar moiety of the nucleosides can interfere in such recognition and hence masking of the hydroxyl groups prior to the recognition event is essential[16]. In previous reported literature, the recognition of nucleosides was focused on supramolecular catalysis area at certain pH condition[17,18]. In addition, the literature about the recognition of ATP has been reported due to the important role that ATP transports chemical energy within cells for metabolism[16,19-21]. Nowadays, the methods detecting nucleotides are usually liquid chromatography (LC), high performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI-MS)[22-24]. However, studies on detecting nucleotide with new compounds are less reported.

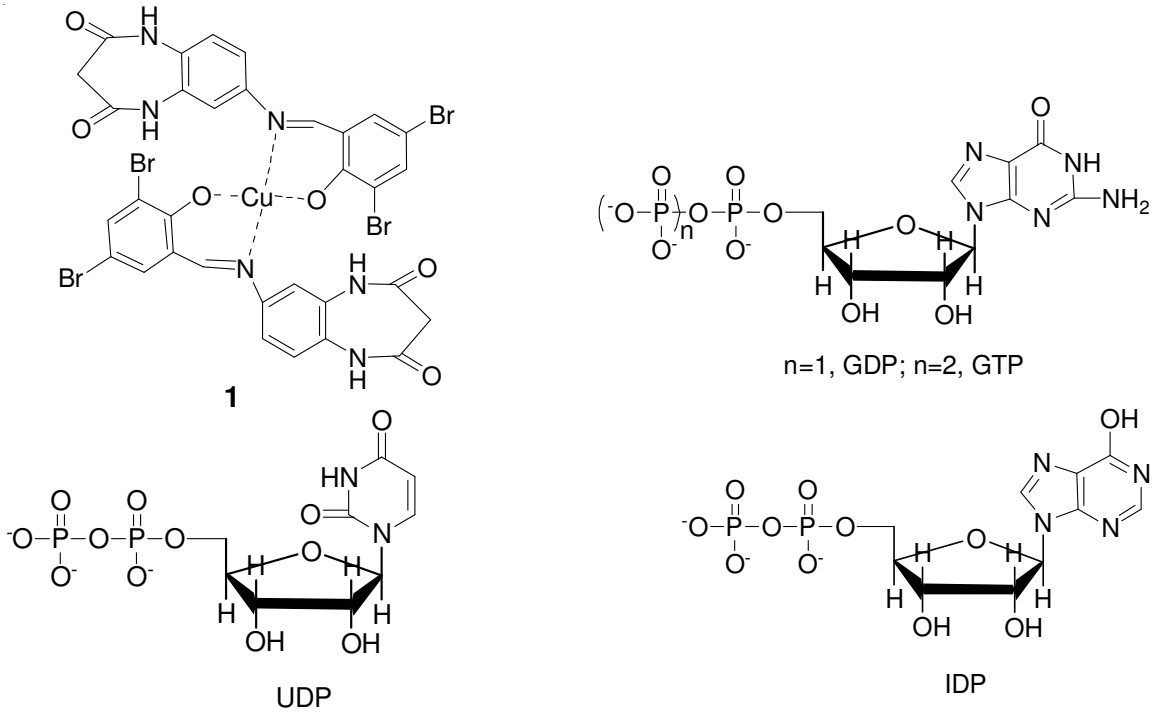
According to the above information, we synthesized a new compound **1** (**Scheme-I**) and fortunately obtained its crystal. The nucleotide binding ability of compound **1** with guanosine-5'-triphosphate (GDP), uridine-5'-diphosphate (UDP), inosine-5'- diphosphate (IDP) and guanosine-5'-triphosphate (GTP) which selectively complexes with GTP in neutral water solution and signals the event through changes in absorption spectroscopy.

EXPERIMENTAL

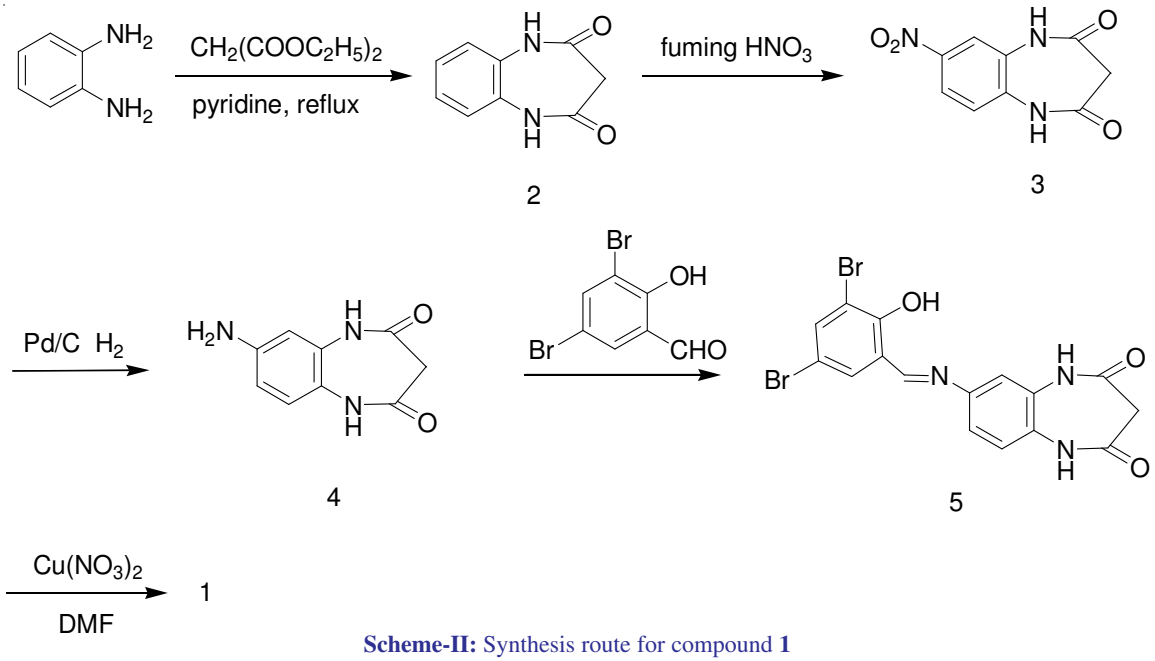
Most of the starting materials were obtained commercially and all reagents and solvents employed were of analytical grade. All nucleosides (GDP, UDP, IDP and GTP) were purchased from Sigma-Aldrich Chemical Co., stored in a desiccator under vacuum containing self-indicating silica and used without any further purification. Dimethyl sulfoxide (DMSO) was distilled *in vacuo* after dried with CaH_2. C, H, N elemental analysis was made on Vanio-EL. ^{1}H NMR spectrum was recorded on a Varian UNITY Plus-400 MHz Spectrometer. FAB-MS was made on VG ZAB-HS. ESI-MS was performed with a MARINER apparatus. UV-visible Spectroscopy titrations were made on a Shimadzu UV2550 Spectrophotometer at 298 K. The stability constants K_s were obtained by the non-linear least square calculation method through data fitting.

Compound **1** was synthesized according to the route shown in **Scheme-II**.

Benzo-1,4-diazacycloheptane[2,3-d]-5,7-dione (2)[25]: 1,2-Phenylenediamine (10.8 g, 0.1 mol), diethyl malonate (16 mL, 0.1 mol) and pyridine (200 mL) were put in a 250 mL three-neck flask. The mixture was refluxed with N_2 for 72 h. After cooling, the mixture was filtrated and the colourless solid obtained. The solid was washed with ethanol and ether



Scheme-I: Chemical structures of compound **1** and nucleosides



Scheme-II: Synthesis route for compound **1**

sequentially and dried in vacuum. Yield: 72 %. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ 10.38 (s, 2H), 7.11-7.18 (m, 4H), 3.17 (s, 2H). Elemental analysis: Calc. for $C_9H_8N_2O_2$: C, 61.36; H, 4.58; N, 15.90; Found: C, 61.69; H 4.59; N, 15.96. FAB-MS (*m/z*): 177.0 $(M + H)^+$.

(4'-Nitrobenzo)[1', 2'-d]-1,4-diazacycloheptane[2,3-d]-5,7-dione (3): Benzo-1,4- diazacycloheptane[2,3-d]-5,7-dione (10 mmol, 1.7 g) was dissolved in concentrated H_2SO_4 (43 mL). Fuming HNO_3 (1.1 mL) was added dropwise with stirring at 273 K. After the addition was completed, the mixture was stirred for 2 h and then poured into 200 mL ice-water. The solution was filtered to give a yellow solid, which was washed with distilled water, recrystallized from methanol and dried in vacuum. Yield: 85 %. ^{1}H NMR(400 MHz, DMSO-d_6, 298 K) δ 10.96 (s, 1H), 10.74 (s, 1H), 8.04, 7.3 (3H), 3.3 (s, 2H), Elemental analysis: Calc. for $C_9H_7N_3O_4$: C, 48.88; H, 3.19; N, 19.00; Found: C, 48.76; H 3.58; N, 18.61. ESI-MS (*m/z*): 220.9 $(M-H)^-$.

(4'-Aminobenzo)[1',2'-d]-1,4-diazacycloheptane[2,3-d]-5,7-dione (4): A slurry of compound (4'-nitrobenzo)[1',2'-d]-1,4-diazacycloheptane[2,3-d]-5,7- dione (221 mg) and Pd/C (10 %, 70 mg) in dry ethanol (200 mL) was maintained under hydrogen with stirring for 12 h. The mixture was filtered through a bed of Celite and then washed twice with ethanol (2 × 20 mL).

The solvents were removed under reduced pressure and the yellowish solid dried in vacuum. Yield: 92 %. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ 10.15 (s, 1H), 9.91 (s, 1H), 6.76 (d, 1H), 6.38 (m, 1H), 6.27 (d, 2H), 5.16 (s, 2H) 3.08 (s, 2H). Elemental analysis: Calc. for $C_9H_9N_3O_2$: C, 56.54; H, 4.74; N, 21.98; Found: C, 56.41; H 4.96; N, 21.87. ESI-MS (*m/z*): 190.3 $(M-H)^-$.

N-(2''-Hydroxyl-3'',5''-dibromophenyl-methylene-yl)-4'-imino-benzo[1',2'-d]-1,4-diazacycloheptane[2,3-d]-5,7-dione [HODBrphC=NphDNHexDO](5): (4'-amino-benzo)-[1',2'-d]-1,4-diazacycloheptane[2,3-d]-5,7-dione (1 mmol, 191 mg) and 3,5-dibromo-salicylaldehyde (1 mmol, 278 mg) were suspended in dry ethanol (100 mL). The mixture was heated under reflux for 8 h and the orange-yellow precipitate was separated by filtration. The solid was washed with diethyl ether and dried under vacuum. Yield: 89 %. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ 14.41 (s, 1H), 10.54 (s, 2H), 8.97 (s, 1H), 7.9 (d, 2H), 7.3 (d, 2H), 7.2 (m, 1H) 3.24 (s, 2H). Elemental analysis: Calc. for $C_{16}H_{11}N_3O_3Br_2$: C, 42.41; H, 2.45; N, 9.27; Found: C, 42.35; H 2.88; N, 9.45. ESI-MS (*m/z*): 449.8 $(M-H)^-$.

Cu(II)[HODBrphC=NphDNHexDO]2 (1): 5 (0.1 mmol) and $Cu(NO_3)_2$ (0.05 mmol) were stirred for 1 h in DMF (20 mL), then stood at room temperature. After 24 h, the green expected compound appeared. Elemental analysis: Calc. for $C_{32}H_{20}N_6O_6Br_4Cu{\cdot}5H_2O$: C, 39.72; H, 2.08; N, 8.68; Found: C, 39.22; H 3.55; N, 8.91.

X-Ray crystallography: A green crystal of 1 with dimensions of 0.20 × 0.14 × 0.06 mm was mounted on a glass fiber. X-ray single-crystal diffraction data were collected on a Rigaku Saturn CCD area detector at 294(2) K with MoK_α radiation (λ = 0.71073 Å) . The structure was solved by direct methods and refined on F^2 by full-matrix least squares methods with SHELXL-97[26].

RESULTS AND DISCUSSION

Compound **1** was obtained by the reaction of **5** and $Cu(NO_3)_2$ in N,N-dimethylformamide (DMF). The crystals of compound **1** suitable for X-ray crystal analysis has been obtained and the structure has been confirmed (Fig. 1). The overall coordination environment of the Cu(II) atom involved two compound **1** and two DMF molecules. Four of the Cu1-O1, Cu1-O1A, Cu1-N1, Cu1-N1A bonds were relatively longer (bond lengths 1.913 Å, 1.914 Å, 2.033 Å and 2.033 Å) and they constituted a plane quadrangular geometry around the Cu(II) atom (O1-Cu1-O1A, 180°, O1-Cu1-N1, 90.7°, O1-Cu1-N1A, 89.3°, O1A-Cu1-N1, 89.3°, O1A-Cu1-N1A, 90.7°, N1-Cu1-N1A, 180°); the other two Cu-O4, Cu-O4A contacts were significantly shorter (bond lengths 1.653 Å and 1.653 Å), completing a distorted octahedron as the overall geometry around the Cu(II) atom. The NH of amide formed hydrogen bonds with oxygen atoms of DMF molecules that were derived from two resources: one was coordinated DMF (O4), the other was uncoordinated DMF (O5) (Table-1). The overall crystal structure featured chain type with DMF molecules by hydrogen bonds along the b axis.

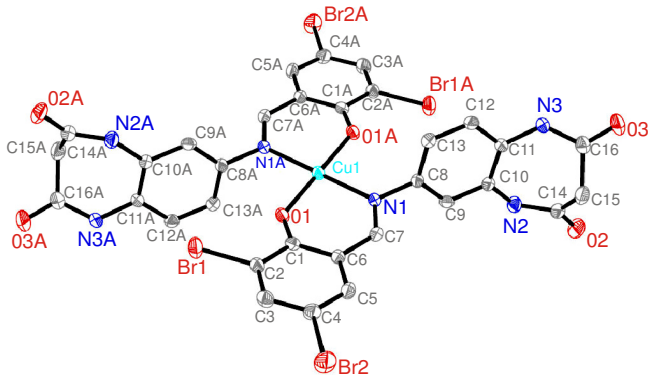


Fig. 1. Crystal structure of 1 and the hydrogen atoms are shown as small circles with arbitrary radii (ellipsoids at 50 % probability)

The nucleotide binding ability of **1** with GDP, UDP, IDP and GTP were investigated through UV-visible spectral titrations in DMSO/H_2O (90:10) by the addition of GDP, UDP, IDP and GTP to the solution of **1**. The interacted absorption spectra of compound 1 with nucleosides was shown in Fig. 2. With the addition of GDP, the absorbance band at about 323 nm decreased gradually and a new absorbance band developed at about 425 nm. In addition, one clear isosbestic point appeared at about 373 nm, which showed a stable concentration of the complex was formed in the solution with a certain stoichiometric ratio between **1** and GDP. The addition of UDP, IDP and GTP also induced similar spectral changes of compound **1**, compared with GDP. Differently, the concentration of nucleosides was different when the equilibrium developed. The above results showed compound **1** interacted with GDP, UDP, IDP and GTP. Job-plot of compound **1** with GDP showed compound **1** bound GDP at 1:1.

Stability constants of compound **1** for nucleosides were calculated according to the equation (1), 1:1 host-guest complexation[27-30].

$$X = X_0 + 0.5\Delta\varepsilon\{c_H + c_G + 1/K_s - [(c_H + c_G + 1/K_s)^2 - 4c_H c_G]^{1/2}\} \quad (1)$$

where c_G and c_H are the concentration of guest and host, respectively. X is the intensity of absorbance at certain concentration of host and guest. X_0 is the intensity of absorbance of host when the anion isn't added. K_s is the affinity constant of host-guest complexation. Δε is the change in molar extinction coefficient.

Curve fitting (about 425 nm) of the interaction between the compound and GDP, UDP, IDP, GTP according to equation (1) was shown in Fig. 3. The high correlation also indicated compound **1** bound nucleosides at 1:1.

According to UV-visible titration data, the stability constants of compound **1** with various nucleosides were determined by

TABLE-1
INTERMOLECULAR HYDROGEN BONDS IN COMPOUND **1**

Donor---H… Acceptor	[ARU]	D-H (Å)	H…A(Å)	D…A(Å)	D-H … A(°)
N2---H2 … O5	x, 1+y, z	0.872	1.991	2.855	170.10
N3A---H3A … O4	x, -1+y, z	0.943	0.943	2.890	166.98

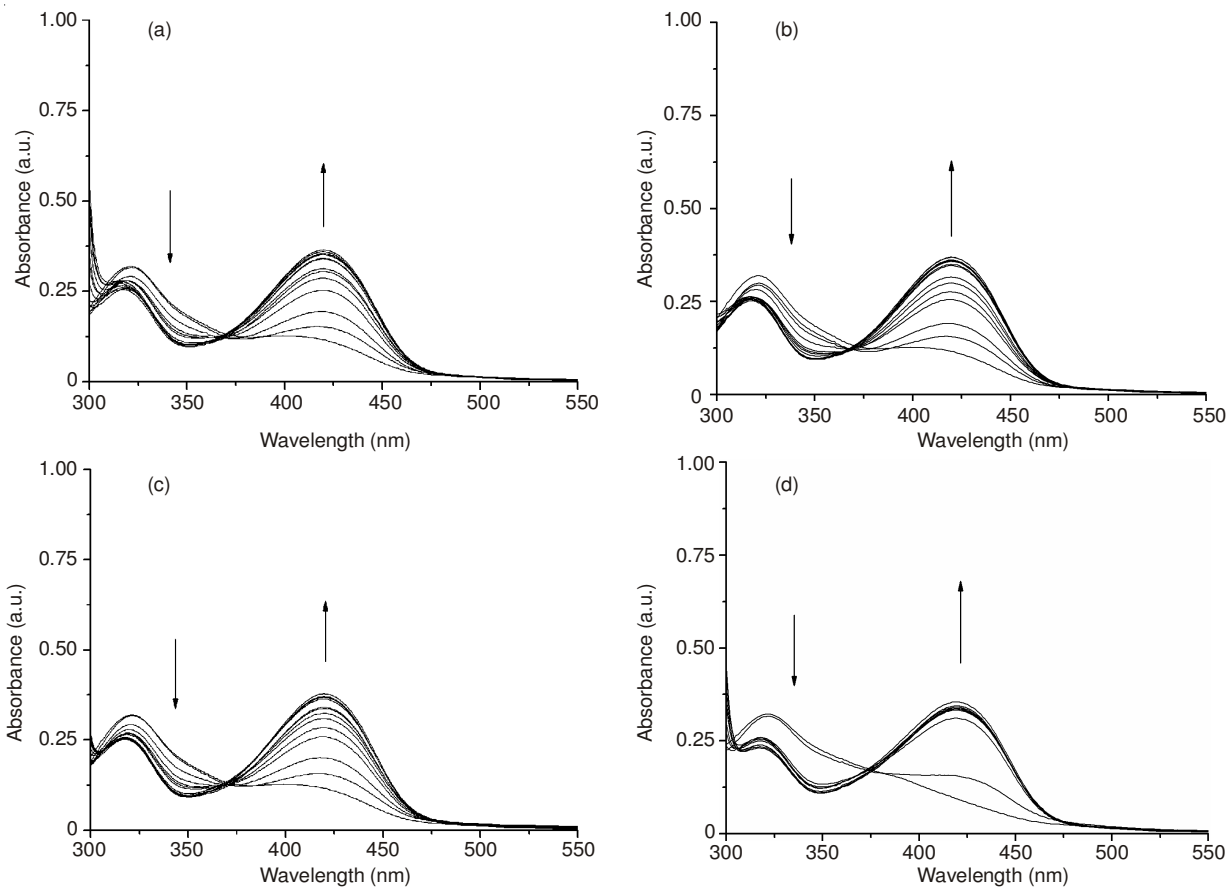


Fig. 2. UV-visible spectral changes of compound **1** (2×10^{-5} mol L^{-1}) upon the addition of GDP(a), UDP(b), IDP(c), GTP(d), the concentration of nucleosides is from 0 to 160×10^{-5} mol L^{-1}. Arrows indicate the direction of increasing nucleosides concentration

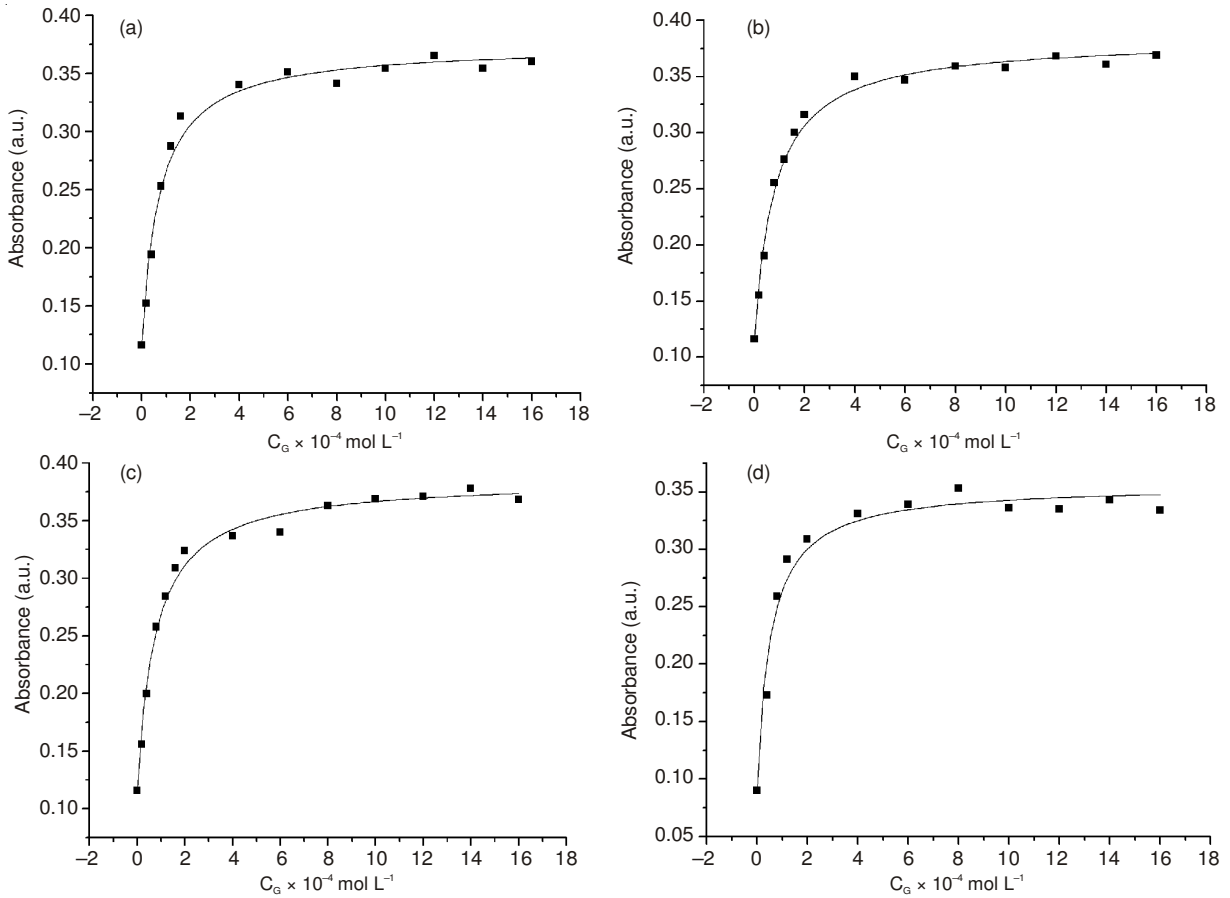


Fig. 3. Curve fitting (about 425 nm) of the interaction between the compound **1** and GDP(a), UDP(b), IDP(c), GTP(d)

non-linear least square method and listed in Table-2. Obviously, the binding ability of nucleosides with compound **1** was in the order: GTP > GDP-IDP-UDP (in the range of error). The binding ability of GTP with compound **1** was the strongest among the studied nucleosides. As for the same length of phosphate chain, the value of stability constant between GDP, UDP, IDP and compound **1** was almost equal in the range of error. According to the crystal structure of compound **1**, nucleosides may interact with compound **1** by electrostatic interaction (Cu^{2+}---O^{-}), which Cu^{2+} was from the receptor and O^{-} was from phosphate of nucleosides. In addition, nucleosides formed π-π staking with 7-membered amide cycle of compound **1**. Therefore, the binding ability of nucleosides with compound **1** was influenced by the chain length of nucleosides. According to the stability constant, GTP, the long chain among studied nucleosides, stacked well with compound **1**.

TABLE-2
STABILITY CONSTANT OF COMPOUND **1**
WITH NUCLEOSIDES

Nucleosides	K_s (mol^{-1} L)
GDP	13800 ± 1539
UDP	12343 ± 967
IDP	13086 ± 1102
GTP	18780 ± 2941

Conclusion

In conclusion, we demonstrated a sensitive absorption assay for GTP through beneficial properties of compound **1** and the UV-visible indicator. Compound **1** showed the strongest binding ability for GTP among studied nucleosides through the visual change in absorption intensity and can be used a supramolecular optic sensor for GTP. Studies are in progress to evaluate the binding ability of compound **1** with nucleosides in cell or living body.

ACKNOWLEDGEMENTS

This project was supported by the National Natural Science Foundation of China (81301269).

REFERENCES

1. J.L. Sessler and J.M. Davis, *Acc. Chem. Res*., **34**, 989 (2001).
2. S.V. Shevchuk, V.M. Lynch and J.L. Sessler, *Tetrahedron*, **60**, 11283 (2004).
3. F.P. Schmidtchen and M. Berger, *Chem. Rev*., **97**, 1609 (1997).
4. E. Quinlan, S.E. Matthews and T. Gunnlaugsson, *Tetrahedron Lett*., **47**, 9333 (2006).
5. J. Shao, X.D. Yu, X.F. Xu, H. Lin, Z.S. Cai and H.K. Lin, *Talanta*, **79**, 547 (2009).
6. Z. Yang, K. Zhang, F. Gong, S. Li, J. Chen, J.S. Ma, L.N. Sobenina, A.I. Mikhaleva, B.A. Trofimov and G. Yang, *J. Photochem. Photobiol. A*., **217**, 29 (2011).
7. R. Velu, V.T. Ramakrishnan and P. Ramamurthy, *J. Photochem. Photobiol. A*., **217**, 313 (2011).
8. R. Martinez-Manez and F. Sancenon, *Chem. Rev*., **103**, 4419 (2003).
9. A.P. De Silva, H.Q.N. Gunaratne, T. Gunnlaugsson, A.J.M. Huxley , C.P. McCoy , J.T. Rademacher and T.E. Rice, *Chem. Rev.*, **97**, 1515 (1997).
10. C.V. Kumar and A. Buranaprapuk, *Angew. Chem. Int. Ed. Engl*., **36**, 2085 (1997).
11. M.W. Hosseini, A.J. Blacker and J.-M. Lehn, *J. Am. Chem. Soc*., **112**, 3896 (1990).
12. C. Marquez, U. Pischel and W.M. Nau, *Org. Lett*., **5**, 3911 (2003).
13. N. Marcotte and A. Taglietti, *Supramol. Chem*., **15**, 617 (2003).
14. A. Ojida, S. Park, Y. Mito-oka and I. Hamachi, *Tetrahedron Lett*., **43**, 6193 (2002).
15. J. Rebek Jr, *Science*, **235**, 1478 (1987).
16. P.P. Neelakandan, M. Hariharan and D. Ramaiah, *Org. Lett*., **7**, 5765 (2005).
17. Y. Guo, Q. Ge, H. Lin, H.K. Lin, S. Zhu and C. Zhou, *J. Mol. Recognit.*, **16**, 102 (2003).
18. Y. Guo, Q. Ge, H. Lin, H.K. Lin, S. Zhu and C. Zhou, *Biophys. Chem*., **105**, 119 (2003).
19. W.N. Lipscomb and N. Strater, *Chem. Rev*., **96**, 2375 (1996).
20. J.M. Berg, J.L. Tymoczko and L. Stryer, Biochemistry, W.H. Freeman, New York, edn 5 (2002).
21. D.A. Jose, S. Mishra, A. Ghosh, A. Shrivastav, S.K. Mishra and A. Das, *Org. Lett*., **9**, 1979 (2007).
22. K. Albert, M. Krucker, T. Glaser, A. Schefer, A. Lienau and D. Zeeb, *Anal. Bioanal. Chem*., **372**, 25 (2002).
23. A.C. Pinto, D.H.S. Silva, V.S. Bolzani, N.P. Lopes and R.A. Epifanio, *Quim. Nova*, **25**, 45 (2002).
24. A.E.M. Crotti, R.L. Vessecchi, J.L.C. Lopes and N.P. Lopes, *Quim. Nova*, **29**, 287 (2006).
25. W.B. Lu, *Guangzhou Chem*., **27**, 26 (2002).
26. G.M. Sheldrick, SHELX97, Programs for Crystal Structure Analysis (Release 97-2). University of Göttingen, Germany (1997).
27. C.M. Fouqué-Brouard and J.M. Fournier, *Talanta*, **43**, 1793 (1996).
28. Y. Liu, B.H. Han and H.Y. Zhang, *Curr. Org. Chem*., **8**, 35 (2004).
29. Y. Liu, C.C. You and H.Y. Zhang, Supramolecular Chemistry Nankai University Publication, Tianjin, China (2001).
30. J. Bourson, J. Pouget and B. Valeur, *J. Phys. Chem*., **97**, 4552 (1993).