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The synthesis of novel fluorescent purine analogues modified by azacrown ether at C6

Hai-Ming Guo [a,*], Jing Wu [a], Hong-Ying Niu [b], Dong-Chao Wang [a], Feng Zhang [a], Gui-Rong Qu [a,*]

[a] College of Chemistry and Environmental Science, Henan Normal University, Xinxiang, 453007 Henan, China
[b] School of Chemistry and Chemical Engineering, Henan Institute of Science and Technology, Xinxiang 453003, China

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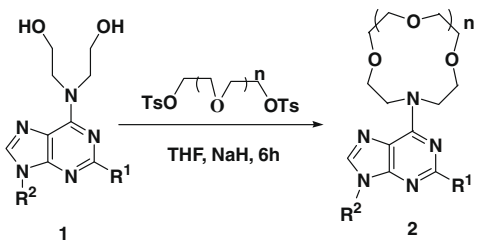
ABSTRACT

The synthesis and fluorescence properties of novel purine analogues linked azacrown ether at C6 position were investigated. These new purine analogues could be prepared from a series of 6-chloropurines and showed selective and efficient signaling behaviors toward micromolar concentration of Ag^+ ion over other common metal ions in an aqueous environment.

Purines are one of the most ubiquitous heterocyclic ring systems found in nature.[1] They are components in numerous biologically significant molecules and thus present an excellent scaffold for the construction of bioprobes.[2] Purine derivatives with various substituents at C6 have received considerable attention due to their broad spectrum of biological activities.[3] This prompts a number of studies on the modification at C6 position of the purine ring.

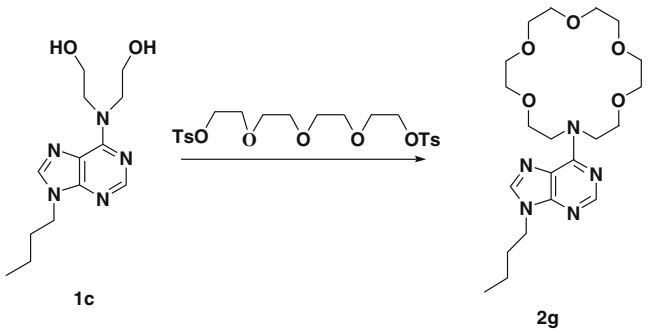
Crown ethers, first introduced in 1967 by Pedersen, are very intriguing compounds in the field of supramolecular chemistry.[4] In order to develop metal ion-selective and sensitive material, there is a growing interest in synthesizing crown ether derivatives with fluorescence properties which will show marked changes upon metal complexation.[5]



Scheme 1. Reaction of (poly)ethylene glycol ditosylates with 6-[*N*,*N*-bis(2-hydroxyethyl)amino]purines.

Currently, considerable attention has been focused on fluorescent chemosensors for the selective and rapid determination of

Table 1
Effect of solvent and the optimization of reaction conditions[a]



Entry	Base	Solvent	*T* (°C)	Time (h)	Yield[b] (%)
1	NaH	THF	Reflux	6	43
2	NaH	THF	25	24	40
3	NaH	DMF	50	6	35
4	KOH	THF	Reflux	6	30
5	NaOH	THF	Reflux	6	35
6	LiOH	THF	Reflux	24	Trace
7	Cs_2CO_3	THF	Reflux	24	Trace
8	Cs_2CO_3	DMF	50	24	Trace
9	Cs_2CO_3	CH_3CN	Reflux	24	Trace
10	Na_2CO_3	THF	Reflux	24	NR

[a] Reagents and conditions: 1 mmol **1c**, 1 mmol (poly)ethylene glycol ditosylate and 2 mmol bases in 30 mL solvent for the indicated time.
[b] Isolated yields based on nucleobases.

* Corresponding authors. Fax: +86 373 3329276 (H.-M.G,).
E-mail address: guohm518@hotmail.com (H.-M. Guo).

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the toxic heavy metal ions, such as Pb^{2+}, Cd^{2+}, Hg^{2+} and Ag^{+} ion.[6] Ag^{+} ion is received more attention because of its bioaccumulation and toxicity.[7] Furthermore, the mechanism of antimicrobial activities of Ag^{+} has not been well established because of the lack of suitable detection and imaging methodologies.[8]

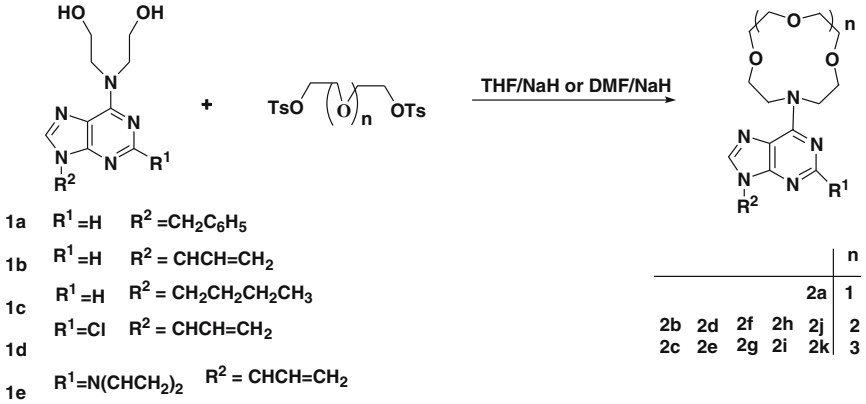
The focus of attention on crown ethers bearing purine base has traditionally been directed toward the relationship between the structure of the host molecule and its ability to bind and transport guest species, especially alkali metal cations.[9] Crown ether and nucleotide base systems have also been designed to determine the extent of association as well as what interactions occur between the nucleotide bases.[10] To our knowledge, there has been no report of purine analogues modified by azacrown ether at C6 position. Based on our preliminary study on various modification of purine analogues,[11] we designed and prepared novel C6-modified purine analogues by azacrown ether which displayed highly selective and efficient signaling behaviors toward micromolar concentration of Ag^{+} ion over other common metal ions in an aqueous environment.

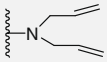
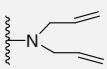
The key starting material, 6-[*N*,*N*-bis(2-hydroxyethyl)amino]-purines **1**, were prepared according to literature.[12] Crown ethers bearing purines **2** were synthesized via the cyclization of **1** with (poly)ethylene glycol ditosylates in THF in the presence of sodium hydride (Scheme 1).[13] They were purified by column chromatography in acceptable yields of 12–43% and their structures were confirmed by NMR spectra and high-resolution mass spectrometry.

Significant differences in the outcome of the reaction were observed depending on the solvents, bases and reaction conditions used. As shown in Table 1, when Na_2CO_3 was used as the base (entry 10), no reaction was observed. When Cs_2CO_3 (entries 7–9) or LiOH (entry 6) were used, the product **2g** was obtained with trace yield. While higher yields were obtained in the present of KOH (entry 4) or NaOH (entry 5) in refluxing THF. When NaH was used as the base (entries 1–3), the isolated yields were further improved. At room temperature, the reaction completed within 24 h in 40% yield (entry 2). Increasing the temperature to reflux, the reaction completed within 6 h in 43% yield (entry 1).

To investigate the versatility of the reaction, a series of C6-crowned purines (**2a–2k**) were synthesized (Table 2). For the substrates substituted by *n*-butyl at N9, the yields are higher than others. For example, the yield of product **2g** (entry 9) was 43%, higher than that of **2e** (entry 8) and **2c** (entry 7). Aza-15-crown-5

Table 2
Reaction of (poly)ethylene glycol ditosylates with various 6-[*N*,*N*-bis(2-hydroxyethyl)amino]purines[a]



Entry	*n*	Product	R^1	R^2	Yield[b] (%)
1[c]	**1**	**2a**	H		26
2[c]	**2**	**2b**	H		23
3	**2**	**2d**	H		19
4	**2**	**2f**	H		40
5	**2**	**2h**	Cl		12
6	**2**	**2j**			24
7[c]	**3**	**2c**	H		33
8	**3**	**2e**	H		40
9	**3**	**2g**	H		43
10	**3**	**2i**	Cl		37
11	**3**	**2k**			30

[a] Reagents and conditions: NaH (2 mmol), **1b** (**1c**, **1d**, **1e**) (1 mmol), and (poly)ethylene glycol ditosylates (1 mmol) in 30 mL THF refluxed for 6 h.
[b] Isolated yields based on nucleobases.
[c] NaH (2 mmol), **1a** (1 mmol), and (poly)ethylene glycol ditosylates (1 mmol) in 30 mL DMF stirred at 50 °C for 6 h.

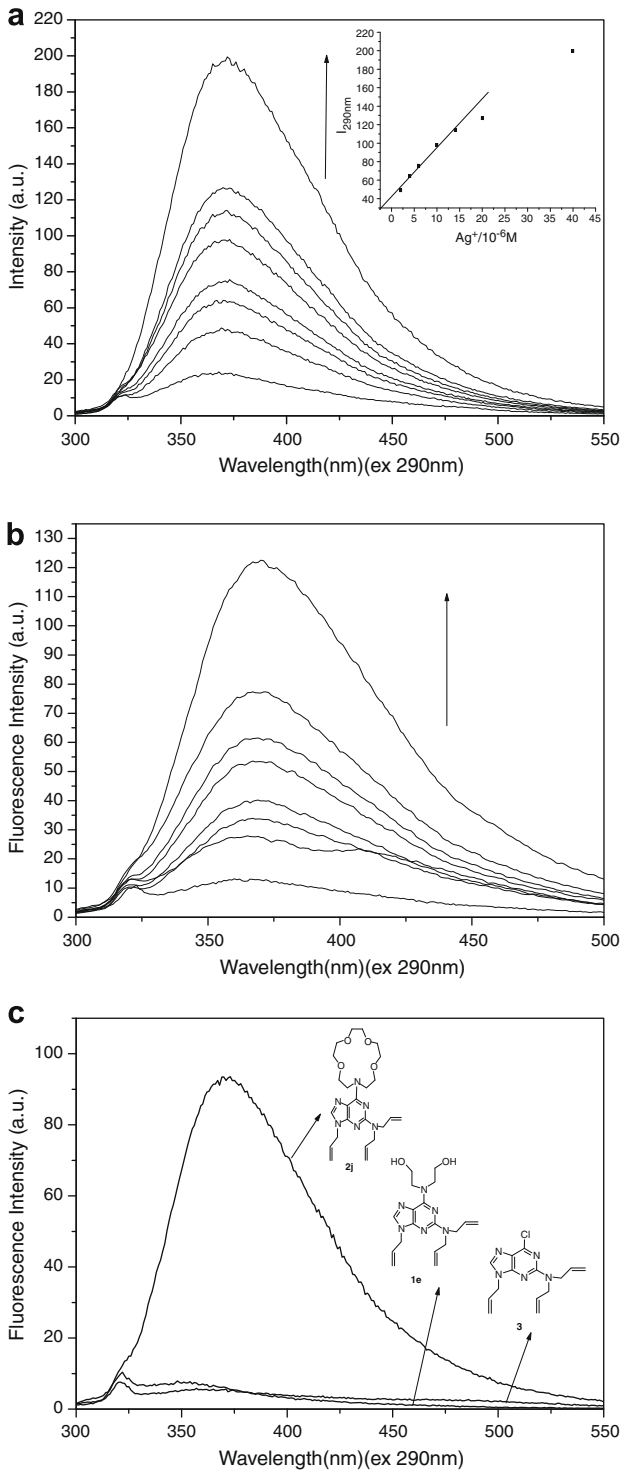


Figure 1. (a) Fluorescence spectra of **2j** at different concentrations (1×10^{-6} M–4×10^{-5} M) in an ethanol–water solution (2:100, v/v) at 375 nm, λ_{ex} = 290 nm. (b) Fluorescence spectra of **2k** at different concentrations (1×10^{-6} M–4×10^{-5} M) in an ethanol–water solution (2:100, v/v) at 375 nm, λ_{ex} = 290 nm. (c) Fluorescence spectra of **2j** (1×10^{-5} M), **1e** (1×10^{-5} M) and **3** (1×10^{-5} M) in an ethanol–water solution (2:100, v/v).

2f (entry 4) was obtained in 40% yield which was higher than that of crown ethers **2d** (entry 3) and **2b** (entry 2). The influence of substituent groups at C2 was also studied. When H at C2 was replaced by Cl, **2h** was afforded with 12% yield (entry 5) and **2i** 37% yield (entry 10), lower than that of the corresponding crown ethers **2d** (entry 3) and **2e** (entry 8). By contrast, replacement of H by the nitrogen-containing substituent, 2-(*N*,*N*-diallyl)-6-azacrowned purines gave slightly better yield for **2j** (entry 6), but the lower yield for **2k** (entry 11). Moreover, the yields of the azacrown ethers were improved by extending the scope of this cyclization, **2a** (entry 1) was obtained from compound **1a** in 16% yield, **2b** (entry 2) in 23% yield and **2c** (entry 7) in about 33% yield.

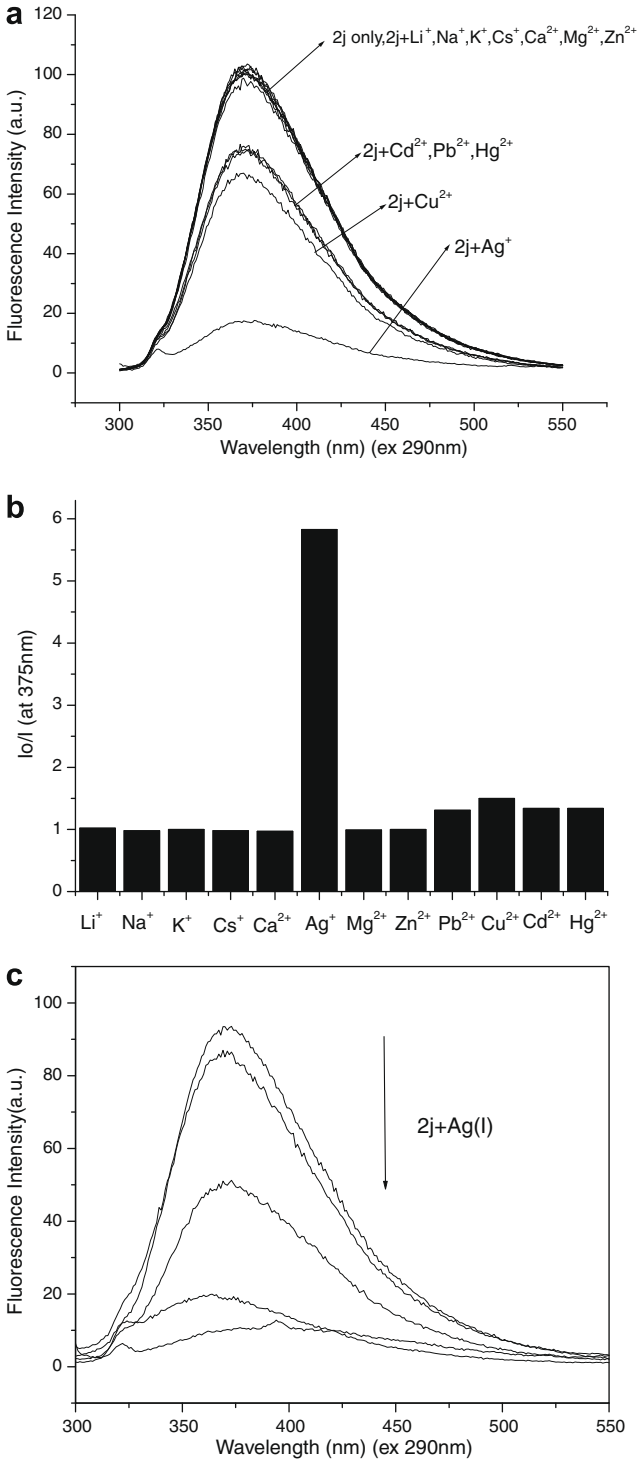
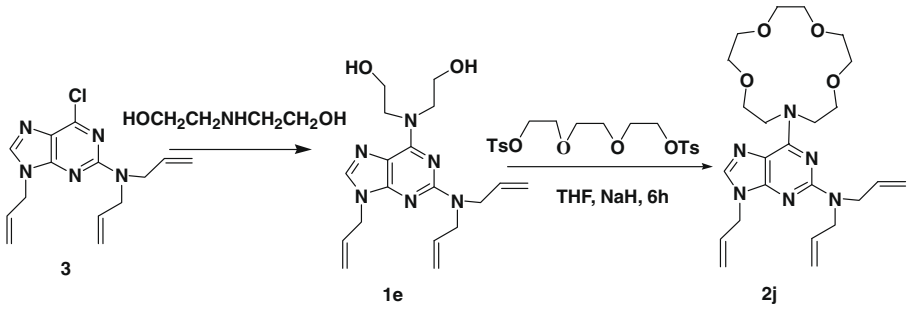


Figure 2. (a) Fluorescence spectra of **2j** in the presence of various metal ions in an ethanol–water solution (2:100, v/v). [**2j**] = 1×10^{-5} M, [M^{n+}] = 1×10^{-4} M. λ_{ex} = 290 nm. (b) The quenching efficiency of **2j** expressed by the ratio of I_o/I (I_o and I represent fluorescence intensity of **2j** in the absence and presence of metal ions). [**2j**] = 1×10^{-5} M, [M^{n+}] = 1×10^{-4} M. (c) Fluorescence spectra of **2j** with Ag^+. [**2j**] = 1×10^{-5} M, [Ag^+] = 1×10^{-5} M, 2×10^{-5} M, 5×10^{-5} M, 1×10^{-4} M.



Scheme 2. Synthetic pathway for *N*-[9-allyl-2-(*N*,*N*-diallyl)purin-6-yl]aza-15-crown-5 **2j**.

The fluorescence behavior of the purine analogues were investigated in 2% ethanol solution. As shown in Figure 1(a), compound **2j** displayed characteristic emission band around 375 nm when excited at 290 nm. The fluorescence intensity increased linearly with the concentration of **2j** in the range of 10^{-6}–10^{-5} M. Under the same condition, crown ether **2k** also exhibited emission band around 375 nm (Fig. 1(b)). Compared with **2j**, **2k** displayed weak emission at the same concentration.

The fluorescent properties of **2j** as well as the corresponding starting materials **3** and **1e** were also examined (Scheme 2, Fig. 2(c)). At the same concentration, compound **3** showed almost no fluorescence in the range of 300–550 nm, and compound **1e** (formed from **3**) did not show any significant change in fluorescence intensities. While the azacrown ether bearing purines **2j** exhibited significant fluorescence. This result implied that the crown ether group at C6 may contribute to the fluorescence enhancement of the purine analogues.

The fluorescence response of crown ether **2j** toward various metal ions (Li^+, Na^+, K^+, Cs^+, Ca^+, Mg^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}) was also examined (Fig. 2(a)). The characteristic fluorescence spectrum of **2j** was effectively quenched upon treatment with Ag^+ ions. The quenching efficiency can be expressed by the ratio of I_o/I at 375 nm (I_o and I represent the fluorescence intensity of **2j** in the absence and in the presence of metal ions, respectively). Figure 2(b) demonstrates that metal ions other than Ag^+ did not induce noticeable changes in the fluorescence intensity. Fluorescence intensity changes of **2j** with different concentrations of Ag^+ were shown in Figure 2(c), the intensity decreased when $AgNO_3$ had been added from 1 equiv to 10 equiv. These results indicated that compound **2j** had good selectivity toward Ag^+ and that other competitive metal ions would induce a rather low interfering effect on this fluorescence assay for Ag^+.

In conclusion, the first synthesis of purine analogues containing a crown ether group at C6 was developed, thus widening the scope of crown ether chemistry and opening a new rout for modification at C6 of purine analogues. The resulting crowned purine analogues showed selective and efficient signaling behaviors toward micromolar concentration of Ag^+ ion over other common metal ions in an aqueous environment. The further extension of the synthesis of the crown ether bearing purine base and their fluorogenic behavior are undergoing in our laboratories.

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.bmcl.2010.03.102.

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13. Typical experimental procedure for the synthesis of *N*-(9-butyl purin-6-yl)aza-18-crown-6 (**2g**)
To a suspension of NaH (0.08 g, 2 mmol, 60% in mineral oil) in 10 mL dry THF at room temperature was added a solution of **1c** (0.279 g, 1 mmol) in 10 mL of THF. The reaction mixture was refluxed for 1 h. After cooling to room temperature, a solution of tetraethylene glycol ditosylate (0.502 g, 1 mmol) in 10 mL of THF was slowly added to the reaction mixture. The suspension was refluxed for 6 h. The solvent was evaporated and the crude product was purified by flash column chromatography on silica gel (eluent: ethyl acetate–petroleum ether 60–90 = 1:3) to give **2g** 0.188 g (43%). Yellow oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (s, 1H), 7.67 (s, 1H), 4.60–4.53 (m, 2H) 4.16–4.12 (m, 4H), 3.83 (d, J = 4.8 Hz, 4H), 3.67 (d, J = 8 Hz, 16H), 1.85–1.81 (m, 2H), 1.37–1.30 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.2, 152.1, 150.6, 138.4, 119.8, 70.8, 70.5, 70.4, 70.0, 43.3, 32.0 19.8, 13.5. HRMS: calcd for $C_{21}H_{35}N_5NaO_5$ [M+Na$^+$] 460.2536, found 460.2534.