Organic LETTERS

Letter

pubs.acs.org/OrgLett

Fluorescent Sensing of Guanine and Guanosine Monophosphate with Conjugated Receptors Incorporating Aniline and Naphthyridine Moieties

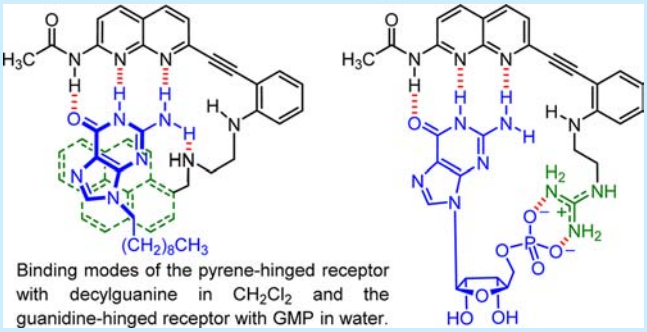
Shao-Hung Lu,[†,§] Riping Phang,[†,§] and Jim-Min Fang*[,†,‡]

[†]Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

[‡]The Genomics Research Center, Academia Sinica, Taipei 115, Taiwan

Supporting Information

ABSTRACT: Ethyne-linked naphthyridine−aniline conjugated molecules are selective sensors of decylguanine in dichloromethane and guanosine monophosphate in water (K_{ass} = 16 000 M^{-1}). The 2-acetamido-1,8-naphthyridine moiety binds with guanine in a DAA−ADD triply hydrogen-bonded motif. The aniline moiety enhances an electron-donating effect, and the substituent is tuned to attain extra hydrogen bonds, $\pi-\pi$ stacking, and electrostatic interactions. The proposed binding modes are supported by a Job plot, ESI-MS, ^{1}H NMR, UV−vis, and fluorescence spectral analyses.



Rational design of new DNA-binding agents provides an effective approach for development of novel chemotherapeutic anticancer drugs.[1] In nature, cytosine (C) binds with guanine (G) to form a C≡G pair through the triply hydrogen-bonded DAA−ADD motif, wherein A represents a hydrogen acceptor and D represents a hydrogen donor. Cytosine analogues have been designed as effective molecular receptors of guanine and guanosine.[2] In addition to triple hydrogen bonds, a cytosine-based ditopic receptor is devised to contain terminal amine groups for enhanced binding with guanosine 5′-monophosphate (GMP) through electrostatic interactions of the basic amine with the phosphoric group.[3]

It is noted that 2-amido-1,8-naphthyridine also possesses a DAA motif with a geometric fit to guanine in the formation of a DAA−ADD triply hydrogen-bonded complex similar to the C≡G pair.[4−6] A single guanine bulge in duplex DNA is found to bind selectively with 2-amido-1,8-naphthyridine derivatives by fully complementary hydrogen bonds.[7−9] Thus, dimeric and tetrameric 2-amido-1,8-naphthyridine compounds are further designed to detect the G−G mismatches in duplex DNA and telomeres.[10−12] Silica gel modified by covalent linkage of 2-amido-1,8-naphthyridine units is also a suitable stationary phase for 2′,3′,5′-tri-*O*-acetylguanosine in HPLC analysis.[13] A Gd^{3+} complex of 7-acetamido naphthyridine−tetraazocyclodecane has been utilized as a magnetic resonance imaging contrast for detection of GMP.[14]

We have previously devised a series of ethyne-linked naphthyridine−aniline receptor molecules, such as compound **1** (Figure 1), to monitor the binding events with guanine derivatives.[5]

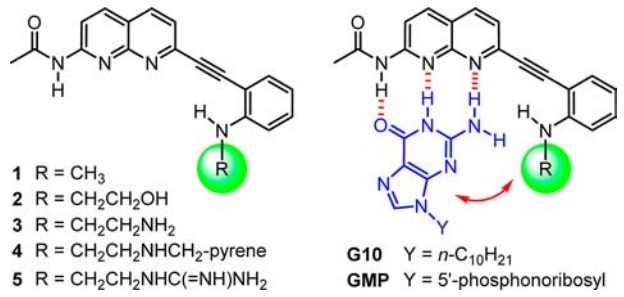


Figure 1. Ethyne-linked naphthyridine−aniline molecules **1**−**5** as fluorescent sensors of 9-decylguanine and GMP. The substituents (R) of aniline provide additional interactions to enhance the DAA−ADD triply hydrogen-bonded motif.

The 2-acetamido-1,8-naphthyridine moiety is an electron-withdrawing group (EWG), and the aniline moiety is an electron-donating group (EDG). The ethynyl linker between naphthyridine and aniline elongates the conjugate system in this sensor molecule to make a better intramolecular charge transfer (ICT) system upon binding with guanine derivatives,[15,16] so that the absorption and fluorescence wavelengths of the host−guest complexes can fall in the visible region for naked-eye detection.

We have shown that the receptor molecule **1** forms a 1:1 complex with 9-decylguanine (G10) in CH_2Cl_2 solution with a binding constant of (9.1 ± 0.2) × 10^4 M^{-1} by fluorescence titration at 535 nm.[5] We conceive that the *N*-methylaniline moiety of **1** can be further modified to attain extra interactions

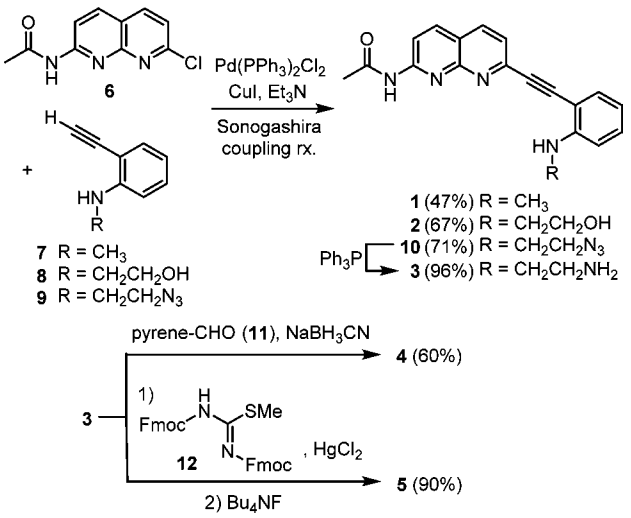
Received: January 30, 2016
Published: March 28, 2016

DOI: 10.1021/acs.orglett.6b00311
Org. Lett. 2016, 18, 1724−1727

with the purine ring of a guanine derivative. Along this line, we designed the new push−pull receptor molecules **2**−**4** (Figure 1) that incorporate appropriate R substituents at the aniline site to increase the binding affinity with a guanine derivative. The hydroxyethyl and aminoethyl substituents in the aniline moieties of compounds **2** and **3** are expected to provide hydrogen bondings with G10, while the pyrene group of **4** may offer hydrophobic and $\pi-\pi$ stacking interactions with the purine ring. To probe GMP in water under physiological conditions, we also designed a receptor molecule **5** (Figure 1) containing a terminal guanidine group to form a stable ion pair with the negatively charged phosphate ion via electrostatic interactions.[17]

Scheme 1 shows the synthetic routes to molecules **1**−**5** using the Sonogashira coupling reaction to construct the naphthyridine−ethyne−aniline scaffold. The azido compound **10** was reduced by Ph_3P in H_2O−THF to give the receptor molecule **3** that bears a terminal amino group for the subsequent elaboration to compounds **4** and **5** by reductive amination with pyrene-1-carbaldehyde and guanidination, respectively. Attempts to remove the Fmoc groups from the guanidination product by piperidine under conventional reaction conditions ($CHCl_3$ solution, rt, 3 h) unexpectedly caused a concomitant deacetylation of the 2-acetamido substituent. Fortunately, the Fmoc protecting groups were selectively removed by treatment with tetrabutylammonium fluoride (0.1 M solution in DMF) at room temperature for 10 min,[18] giving the desired receptor molecule **5** in 90% yield.

Scheme 1. Synthesis of Receptor Molecules 1−5



The photophysical properties of receptor molecules **2**−**5** were studied in various solvents, and the binding events were monitored by UV−vis and fluorescence spectroscopy. Upon titration of **2** with incremental amounts of G10 in CH_2Cl_2 solution (Figure 2), the absorption band at λ_{max} 392 nm gradually shifted to 404 nm. The fluorescence also showed a bathochromic shift from 528 to 539 nm, albeit in lower intensity. The decreased intensity was related to the electron transfer from decylguanine and the energy loss in the relaxation process. A Job plot based on the absorption changes in the titration curves was established to reveal the formation of a **2**−G10 complex in 1:1 stoichiometry (Figure S10 in Supporting Information (SI)). According to the nonlinear least-squares curve-fitting method,[19] the association constant (K_{ass}) of the **2**−G10 complex was determined to be (2.2 ± 0.2) × 10^5 M^{-1} at 298 K in CH_2Cl_2 solution.

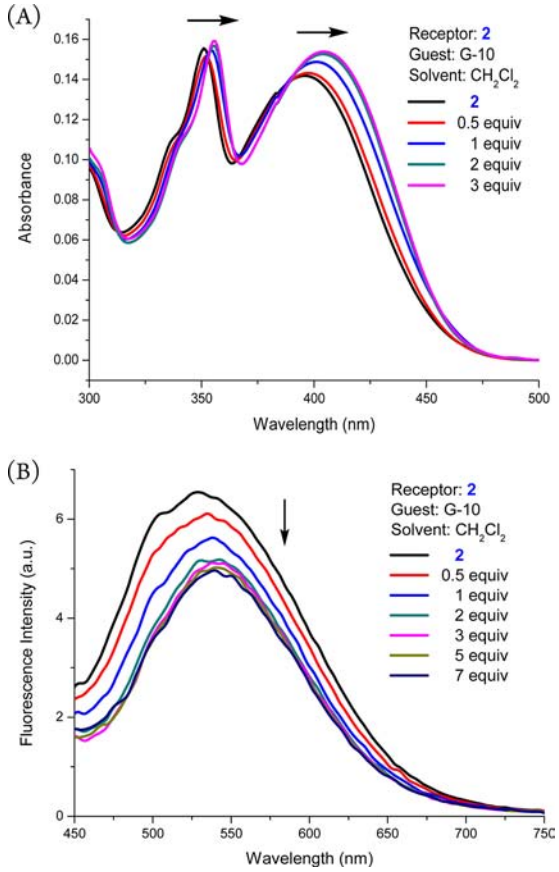


Figure 2. Titration of receptor **2** (1×10^{-5} M) upon incremental additions of 9-decylguanine (1×10^{-2} M) in CH_2Cl_2 solution. (A) UV−vis curves; (B) fluorescence curves (λ_{ex} = 396 nm).

Similar to compound **2**, formation of the **3**−G10 complex in 1:1 stoichiometry was indicated by the Job plot based on the absorption changes in the titration experiment (Figure S13 in SI). The **3**−G10 complex showed a signal at *m*/*z* 637.3727 attributable to the protonated molecular ion $[M + H]^+$ in the ESI-MS spectrum (Figure S14 in SI). Based on the changes of fluorescence intensity at the highest point of each titration curve (528−545 nm), the association constant of the **3**−G10 complex in CH_2Cl_2 solution was determined to be (2.3 ± 0.4) × 10^5 M^{-1}, which was close to the value of the **2**−G10 complex. Thus, receptors **2** and **3** exhibited an affinity ∼2.5-fold stronger than that of **1** toward the G10 molecule, presumably because the terminal OH or NH_2 group in receptors **2** and **3** provided extra hydrogen bondings. In CH_2Cl_2 solution, the affinity of **2** to 9-decyladenine (A10) [K_{ass} = (4.5 ± 0.7) × 10^3 M^{-1}] was 50-fold weaker than that to G10, and the binding of **3** with A10 was too weak to induce any apparent change in the UV−vis and fluorescence titrations. The triply hydrogen-bonded mode of acetamido naphthyridine receptors **1**−**3** with guanine accounted for the selective binding of G10 over A10.

In comparison with the structure of **3**, compound **4** had an additional pyrene group at the terminal end of the aniline site. The UV−vis spectrum of **4** in CH_2Cl_2 solution (Figure S21 in SI) showed the absorption maximum at 397 nm for the naphthyridine−ethyne−aniline chromophore. The fluorescence emitted at 523 nm upon excitation at 400 nm. Such a large Stokes' shift ($\Delta\lambda$ = 126 nm) was attributable to an ICT process that occurred by delocalization of the n,π-electrons from the electron-donating aniline group to the electron-withdrawing

DOI: 10.1021/acs.orglett.6b00311
Org. Lett. 2016, 18, 1724−1727

naphthyridine center along the ethynyl bridge. Upon titration of **4** with incremental amounts of G10 in CH_2Cl_2 solution (Figure S22 in SI, λ_{ex} = 417 nm), the fluorescence maximum gradually shifted to longer wavelengths with decreased intensity. A Job plot analysis of the 550 nm emission indicated that receptor **4** and G10 formed a complex in 1:1 stoichiometry (Figure S23 in SI). A signal at m/z 851.4509 in the ESI-HRMS spectrum was ascribed to the $[M + H]^+$ ion of the **4**−G10 complex (Figure S24 in SI). The association constant of the **4**−G10 complex in CH_2Cl_2 solution was determined to be $(1.4 \pm 0.2) \times 10^6$ M^{-1}, about 6-fold stronger than that in the **3**−G10 complex. In contrast, the binding between **4** and A10 was very weak according to the fluorescence titration experiment (Figure S25 in SI).

The ^{1}H NMR titrations of **2** and **4** were undertaken in $CDCl_3$ solutions to gain more information on their binding modes with G10 (Figure 3). Upon incremental additions of G10, all of the

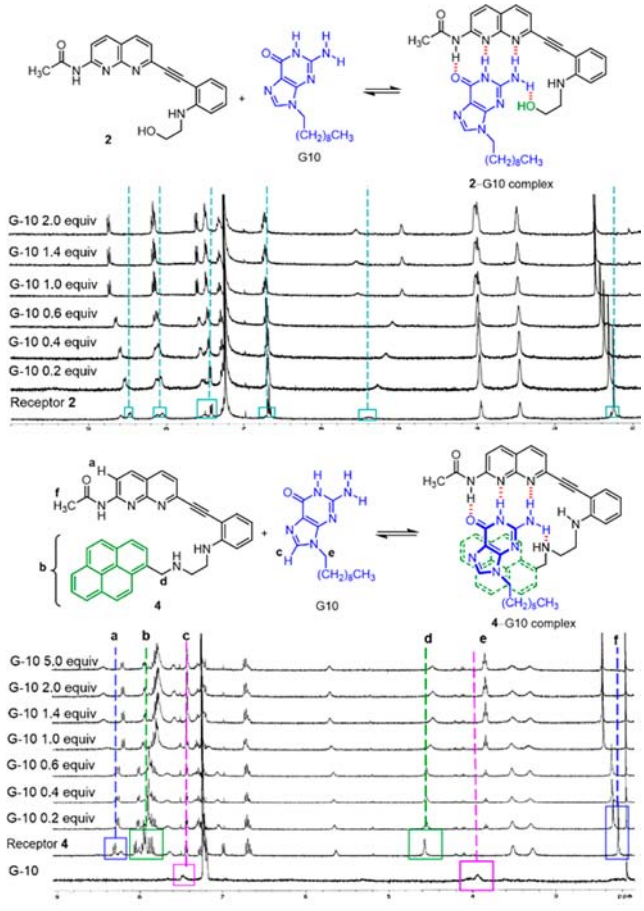


Figure 3. ^{1}H NMR titration of compounds **2** and **4** at 2 mM with incremental additions of 9-decylguanine in $CDCl_3$ solution. The diagnostic protons a−f are marked.

protons on the naphthyridine and aniline rings of compound **2** gradually shifted to lower fields. The downfield shifts were related to the ICT system with multiple hydrogen bonds between **2** and guanine. In contrast, addition of G10 to **4** had a distinct pattern that showed the acetyl protons (H_f at δ 2.24) downfield shifted and the naphthyridine C3 proton (H_a at δ 8.23) upfield shifted. The pyrene protons (H_b) and its adjacent methylene protons (H_d) at δ 4.59 also shifted upfield, indicating the anisotropic effect exerted by the purine ring of G10 through the $\pi-\pi$ stacking interactions. In a reciprocal manner, the anisotropic effect of the pyrene ring also rendered the upfield shifts of the C8 aromatic proton (H_c) and N^9-CH_2 (H_e) in G10. A possible binding mode of the **4**−G10 complex is depicted in Figure 3 to address the multiple hydrogen bonds and $\pi-\pi$ stacking interactions. The greater binding strength of **4**−G10 compared to that of **2**−G10 and **3**−G10 complexes might be attributable to the additional hydrophobic[20] and $\pi-\pi$ stacking[21] interactions between pyrene and the decylpurine moiety. As pyrene is known to be a DNA intercalating agent,[1,22] compound **4** may be explored to serve as a fluorescent probe for detection of a guanosine mutation by simultaneous fluorescence changes from pyrene and the conjugated naphthyridine system.

A guanidine group was introduced to the naphthyridine−aniline molecule **5** to improve its water solubility and acquire binding with the phosphate group in GMP. The fluorescence of **5** appeared at 445 nm in water by excitation at 350 nm. Upon incremental additions of GMP to the aqueous solution of **5**, the intensity of fluorescence decreased in appreciable amounts (Figure S29 in SI). By monitoring the intensity of fluorescence at 450 nm, the Job plot was established to show the formation of a **5**−GMP complex in 1:1 stoichiometry (Figure S30 in SI). According to the fluorescence titration, the binding constant of the **5**−GMP complex in water was calculated to be $(1.6 \pm 0.3) \times 10^4$ M^{-1}. The binding mode of **5**−GMP (Figure 4) is proposed

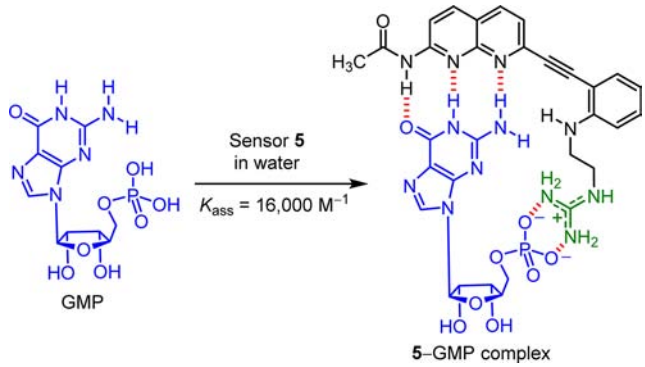


Figure 4. Illustration of the binding mode of the **5**−GMP complex.

to emphasize the strong electrostatic interactions between the guanidinium ion of receptor **5** and the phosphate ion of GMP in an aqueous system. In contrast, adenosine 5′-monophosphate (AMP) and 3′,5′-cyclic GMP (cGMP) did not provide effective complexation with compound **5** in water (Figures S31 and S32 in SI) because AMP could not provide sufficient hydrogen bondings and cGMP lacked the cooperative electrostatic interaction. This study reveals that the guanidine-hinged molecule **5** is highly selective for GMP over cGMP and AMP in aqueous solution. Compound **5** may be useful for monitoring the activity of phosphodiesterase in the hydrolysis of cGMP to GMP because it strongly binds with GMP but is insensitive to cGMP.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00311.

Experimental procedures, NMR, UV−vis, fluorescence, and ESI-MS spectra, and Job plots of synthetic compounds and host−guest complexes (PDF)

DOI: 10.1021/acs.orglett.6b00311
Org. Lett. 2016, 18, 1724−1727

AUTHOR INFORMATION

Corresponding Author

*E-mail: jmfang@ntu.edu.tw.

Author Contributions

§S.-H.L. and R.P. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Ministry of Science and Technology for financial support.

REFERENCES

(1) Jenkins, T. C. *Curr. Med. Chem.* **2000**, *7*, 99−115.
(2) Wilds, C. J.; Maier, M. A.; Manoharan, M.; Egli, M. *Helv. Chim. Acta* **2003**, *86*, 966−978.
(3) Furuta, H.; Magda, D.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 978−985.
(4) Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010−4011.
(5) Lu, S.-H.; Selvi, S.; Fang, J.-M. *J. Org. Chem.* **2007**, *72*, 117−122.
(6) Cywinski, P. J.; Moro, A. J.; Ritschel, T.; Hildebrandt, N.; Löhmannsröben, H.-G. *Anal. Bioanal. Chem.* **2011**, *399*, 1215−1222.
(7) Nakatani, K.; Sando, S.; Yoshida, K.; Saito, I. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 335−337.
(8) Tok, J. B.-H.; Bi, L.; Saenz, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 827−831.
(9) Wang, Y.-X.; Wang, C.-P.; Bo, H.-Y.; Gao, Q.; Qi, H.-L.; Zhang, C.-X. *Sens. Actuators, B* **2013**, *177*, 800−806.
(10) Nakatani, K.; Sando, S.; Kumasawa, H.; Kikuchi, J.; Saito, I. *J. Am. Chem. Soc.* **2001**, *123*, 12650−12657.
(11) Nakatani, K.; Hagihara, S.; Sando, S.; Sakamoto, S.; Yamaguchi, K.; Maesawa, C.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 662−666.
(12) He, H.-P.; Xia, J.-P.; Chang, G.; Peng, X.-Q.; Lou, Z.-W.; Nakatani, K.; Zhou, X.; Wang, S.-F. *Biosens. Bioelectron.* **2013**, *42*, 36−40.
(13) Feibush, B.; Saha, M.; Onan, K.; Karger, B.; Giese, R. *J. Am. Chem. Soc.* **1987**, *109*, 7531−7533.
(14) Kong, J.; Liu, T.; Bao, Y.; Jin, K.; Zhang, X.; Tang, Q.; Duan, C. *Talanta* **2013**, *117*, 412−418.
(15) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: New York, 2006.
(16) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.
(17) Schug, K. A.; Lindner, W. *Chem. Rev.* **2005**, *105*, 67−113.
(18) Ueki, M.; Amemiya, M. *Tetrahedron Lett.* **1987**, *28*, 6617−6620.
(19) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.
(20) Tanaka, K.; Murakami, M.; Jeon, J.-H.; Chujo, Y. *Org. Biomol. Chem.* **2012**, *10*, 90−95.
(21) Sato, Y.; Kudo, M.; Toriyabe, Y.; Kuchitsu, S.; Wang, C.-X.; Nishizawa, S.; Teramae, N. *Chem. Commun.* **2014**, *50*, 515−517.
(22) Wheate, N. J.; Brodie, C. R.; Collins, J. G.; Kemp, S.; Aldrich-Wright, J. R. *Mini-Rev. Med. Chem.* **2007**, *7*, 627−648.

DOI: 10.1021/acs.orglett.6b00311
Org. Lett. 2016, 18, 1724−1727