A dizinc complex for selective fluorescence sensing of uridine and uridine-containing dinucleotides†

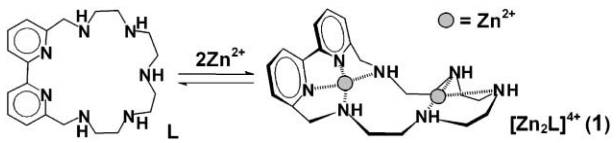
Carla Bazzicalupi,[a] Andrea Bencini,*[a] Laura Bussotti,[b] Emanuela Berni,[a] Silvia Biagini,[a] Enrico Faggi,[a] Paolo Foggi,*[bc] Claudia Giorgi,[a] Andrea Lapini,[a] Agnese Marcelli[b] and Barbara Valtancoli[a]

Received (in Cambridge, UK) 29th November 2006, Accepted 31st January 2007
First published as an Advance Article on the web 12th February 2007
DOI: 10.1039/b617453a

A dizinc complex with a polyamine macrocycle is able to selectively bind and sense uridine (U) as well as the uridine-containing ribodinucleotides U(3′–5′)pU and U(3′–5′)pA, thanks to an exciplex emission arising from a π-stacked complex involving the dipyridine unit and Zn(II)-bound uridine moieties.

There is a current interest in the design of luminescent molecular chemosensors for metabolites in aqueous solutions, due to their potential application in medicinal as well as in analytical chemistry.[1] As one of the approaches to develop luminescent chemosensors, synthetic metal complexes containing fluorogenic units have been successfully used for signaling of a variety of substrates, such as amino acids or carboxylic acids.[2–8] Early work by Kimura has shown that Zn(II) complexes with cyclen-based polyamine ligands can selectively bind thymine- or uracil-containing nucleosides or oligonucleotides, such as TpT, over the corresponding substrates with different nucleobases.[9] This selectivity was attributed to the imide function –CO–NH–CO– of thymine or uracil which can easily deprotonate upon coordination to Zn(II).[9,10] On the other hand, fluorogenic metal complexes able to sense specific nucleosides or dinucleotides are still unknown.

Recently, we reported that ligand L, which contains a pentaamine chain linking the 6,6′ positions of a 2,2′-dipyridine moiety, can form a stable dinuclear Zn(II) complex in aqueous solution (**1** in Scheme 1).[11] In this complex the two metals may behave as separated docking sites for substrates. At the same time, the dinuclear complex displays an emission band at 330 nm, due to the Zn(II)-bound dipyridine fluorophore. Therefore, complex **1** is a promising chemosensor for substrates containing two separated binding units, such as dinucleotides.



Scheme 1

Actually, addition of increasing amounts of uridinyl(3′–5′) uridine (UpU) to an aqueous solution of complex **1** at slightly alkaline pH values leads to a decrease of the fluorescence emission at 330 nm and to a new intense, non-structured and red-shifted emission band with a maximum at 445 nm (Fig. 1a). The fluorescence emission intensity at 445 nm increases linearly up to 0.9 : 1 UpU to **1** molar ratio, to achieve a constant value for molar ratios greater than 1.2 (Fig. 1b), indicating the formation of a stable 1 : 1 adduct between UpU and the dimetal complex.

This exciplex type emission is likely to be due to a π-stacking complex in the excited state, involving dipyridine and Zn(II)-bound UpU. On the other hand, while the excitation spectrum at pH 8.6 recorded at 330 nm is coincident with the absorption spectrum, the excitation spectrum recorded at 445 nm is slightly red shifted, as expected from a ground state association between Zn(II)-bound dipyridine and deprotonated uracil. Time resolved fluorescence measurements with λ_{exc} = 290 nm at pH 8.6 recorded around

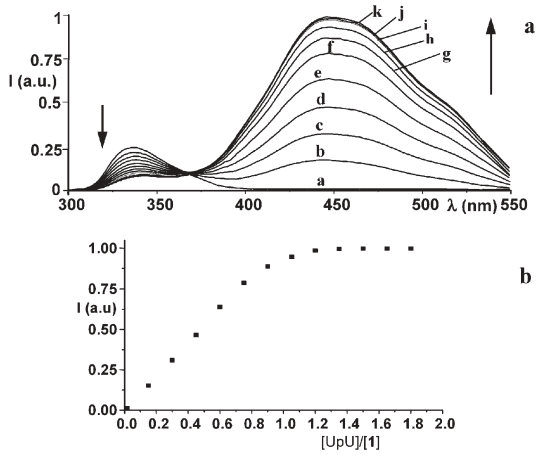


Fig. 1 (a) Fluorescence emission spectra of **1** in the presence of increasing amounts of UpU at pH 8.6 (UpU/**1** molar ratio: 0 (a), 0.15 (b), 0.30 (c), 0.45 (d), 0.60 (e), 0.75 (f), 0.90 (g), 1.05 (h), 1.2 (i), 1.35 (j), 1.5 (k). (b) Fluorescence emission intensity at 445 nm recorded on solutions containing **1** and increasing amounts of UpU at pH 8.6 ([**1**] = [UpU] = 2.5.10–5 M, λ_{exc} = 290 nm, 298.1 K).

[a]*Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019-Sesto Fiorentino, Firenze, Italy. E-mail: andrea.bencini@unifi.it; Fax: +39-055-5773364; Tel: +39-055-5773371*
[b]*European Laboratory for non-Linear Spectroscopy (LENS), University of Florence, Via N. Carrara 1, 50019-Sesto Fiorentino, Firenze, Italy. E-mail: foggi@lens.unifi.it; Fax: +39-055-4572451; Tel: +39 055 4572494*
[c]*Department of Chemistry, University of Perugia, Via Elce di Sotto 8, 06100 Perugia, Italy*

† Electronic supplementary information (ESI) available: Stability constants of the Zn(II) complexes; experimental details for potentiometric and fluorimetric experiments; fluorescence emission spectra of UpU, UpA and U in the presence of complex **1** at different pH values; fluorescence emission spectra of **1** in the presence of increasing amounts of UpA; fluorescence emission spectra of **1** in the presence of increasing amounts of 5′-AAUUAA-3′; ^{1}H NMR spectra of U in the presence of complex **1** at different pH values. See DOI: 10.1039/b617453a

445 nm as well as around 540 nm (central wavelengths) show a monoexponential decay (lifetime 1.4 ns), suggesting the presence of a unique π-stacked complex in the excited state.‡

Addition of the 3′–5′ ribodinucleotides ApA, GpG or CpC to solutions of **1**, instead, does not give any effect on its fluorescence emission, even in the presence of large excess (1 : 30) of these substrates.

The exciplex emission is strongly pH-dependent. As shown in Fig. 2a, the intensity of the emission at 445 nm increases from neutral pH to pH 8.8 and then decreases at more alkaline pH values, giving rise to a bell-shaped profile.

Actually, a potentiometric study on UpU coordination to **1** shows that UpU binding occurs at slightly acidic pH values and is accompanied by release of an acidic proton to give a [**1**·UpU(H_{-1})] adduct, as expected considering deprotonation of the imine group of one uracil moiety upon metal coordination.§ The release of a further acidic proton is then observed at slightly alkaline pH values, due to metal-assisted deprotonation of the second uracil unit of UpU to give a [**1**·UpU(H_{-2})] complex (Fig. 2a).

Fig. 2a shows that the exciplex emission is given by the [**1**·UpU(H_{-2})] complex, which contains two Zn(II)-bound deprotonated uracil moieties, while the [**1**·UpU(H_{-1})] complex does not display exciplex emission. This emission is likely to be due to metal-assisted formation of an array of three heteroaromatic units fixed at close distances (Fig. 2b), which leads to π-stacking pairing between uracil and dipyridine. In [**1**·UpU(H_{-1})] either the single Zn(II)-bound uracil is anchored on the metal not coordinated to dipyridine, far from the fluorogenic center, or it assumes a spatial disposition unable to give a π-stacking interaction with dipyridine strong enough to lead to the exciplex emission.

The observed quenching of the exciplex emission above pH 10 is due to binding of hydroxide anions to complex **1**; this leads to the formation of a $[Zn_2L(OH)_2]^{2+}$ complex and consequent detachment of UpU from the dizinc complex.

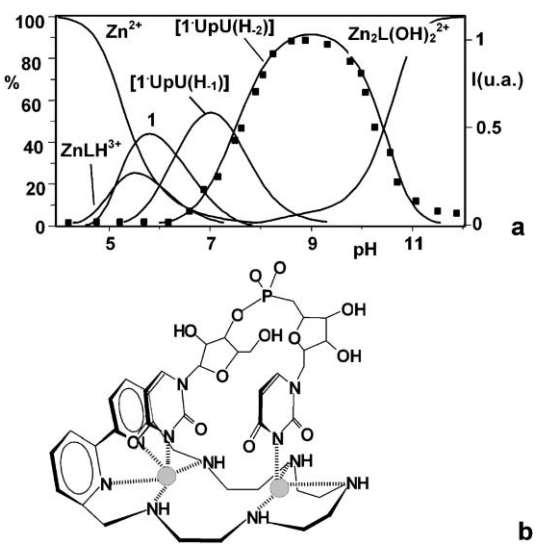


Fig. 2 (a) Emission intensity at 445 nm (■, right *y* axis) at different pH values and distribution curves of the complexes (solid curves, left *y* axis) for a system containing **1** and UpU in equimolecular ratio (both $2.5 \cdot 10^{-5}$ M). (b) Proposed structure for the [**1**·UpU(H_{-2})] complex.

In the case of CpC, ApA and GpG no interaction with **1** is detected by means of potentiometric measurements, in agreement with the fact that no exciplex emission is observed in presence of these substrates.

To shed further light on the structural and photophysical features of the UpU complexes, we analyzed the binding ability of **1** toward nucleosides and the ribodinucleotide uridinyl(3′–5′) adenosine (UpA), which contains a single uracil moiety.

Similarly to UpU, complex **1** is also able to selectively bind uridine (U) over adenosine (A), cytidine (C) and guanidine (G). Potentiometric measurements show that binding of a first U unit takes place at acidic pH values and is accompanied by release of an acidic proton, as expected considering metal-assisted deprotonation of the nucleobase to form a [**1**·U(H_{-1})] species (Fig. 3c). A second deprotonated uridine is then coordinated to give a [**1**·[U(H_{-1})]$_2$] complex.§ U binding was also confirmed by ^{1}H NMR spectra recorded on solutions containing U and **1** at different pH values, which show that the signals of both the aromatic protons of U and dipyridine are remarkably upfield shifted upon formation of the [**1**·[U(H_{-1})]$_2$] adduct at slightly alkaline pH values (Fig. S3, ESI),¶ due to π-stacking interactions involving these units. No interaction was found between **1** and A, G or C either by potentiometry or by ^{1}H NMR measurements.

A similar coordination behavior was also observed in the case of UpA, with the formation of a 1 : 1 [**1**·UpA(H_{-1})] and a 2 : 1 [**1**·[UpA(H_{-1})]$_2$] complex, containing respectively one and two depronated UpA moieties.§ Adeninose does not show any tendency to deprotonate and/or to bind to complex **1** and, therefore, the assembly of these complexes would involve deprotonation and binding to Zn(II) of the uracil moiety of UpA.

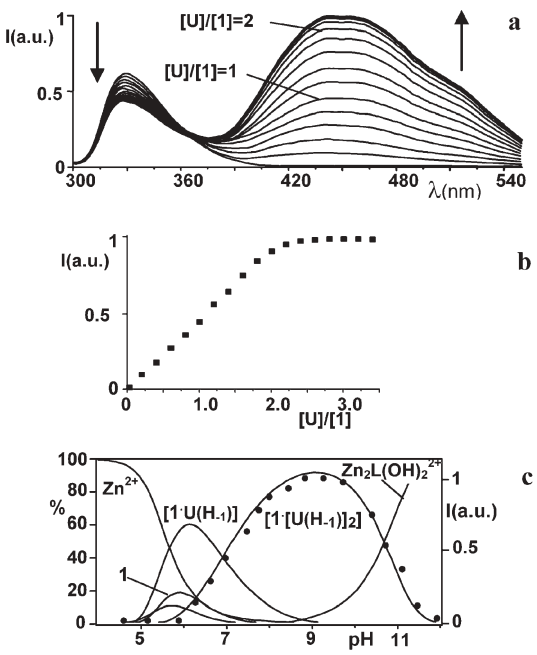


Fig. 3 (a) Fluorescence spectra and (b) emission intensity of **1** at 440 nm in presence of increasing amounts of U at pH 9. (c) Emission intensity at 440 nm (●, right *y* axis) as a function of pH compared to the distribution curves of the complexes (solid curves, left *y* axis) for a system containing **1** and U in 1 : 2 molar ratio ([**1**] = $2.5 \cdot 10^{-5}$ M, λ_{exc} = 290 nm, 298.1 K).

As in the case of UpU, a pH-dependent exciplex emission with a maximum at 440 nm is observed in the presence of uridine or UpA (see Fig. 3 for uridine and ESI, Fig. S4, for UpA).

In the case of uridine, (Fig. 3b), the emission intensity at 440 nm at pH 9 increases linearly with U concentration up to a 1.8 : 1 molar ratio between U and **1** and reaches a constant value for molar ratios greater than 2.4, confirming the formation of a stable 2 : 1 adduct. The only emissive species, however, are the [**1**·[U(H_{-1})]$_2$] (Fig. 3c) and [**1**·[UpA(H_{-1})]$_2$] (see Fig. S6, ESI) complexes; the emission intensity, in fact, increases with the formation of these 2 : 1 complexes from pH 6 to 9 and then decreases above pH 10, where hydroxide binding to **1** leads to disruption of the adducts.

This result confirms that metal-assisted formation of an assembly involving two metal-bound uracil moieties is indeed necessary to give a π-stacked exciplex complex. In contrast, the fluorescence emission of **1** is not affected by the presence of the mononucleosides G, A or C, as expected considering that these substrates do not interact with the complex.

Once again, the decay of the exciplex emission is fitted by a single exponential, in accord with the presence of a unique π-stacking complex; the measured lifetimes around 440 nm of the [**1**·[U(H_{-1})]$_2$] and [**1**·[UpA(H_{-1})]$_2$] complexes (750 and 300 ps, respectively) are smaller than that observed for the [**1**·UpU(H_{-2})] complex (1.4 ns). The observed higher value for the UpU complex is in accord with the presence in [**1**·UpU(H_{-2})] of a more "rigid" and stable π-stacked assembly involving the two uracil units and dipyridine, probably due to the presence of a linker between the two uracil moieties of UpU.‡

Complex **1** represents a potential chemosensor for the UpU sequence in polyribonucleotides. Actually, addition of the UpU-containing hexaribonucleotide 5′-AAUUAA-3′ to a solution of **1** at pH 8.6 gives rise to the formation of a new emission band at *ca.* 440 nm (see ESI, Fig. S7). In contrast, the emission spectrum of **1** is not affected by the presence of the hexaribonucleotides 5′-AUAAUA-3′ or 5′-AAAUAA-3′, which contain respectively two U units separated by two A nucleotides and a single U unit. Therefore, the band at 440 nm observed in the case of 5′-AAUUAA-3′ can be reasonably ascribed to binding of complex **1** to the UpU sequence of this hexanucleotide, in a similar fashion to that proposed for the [**1**·UpU(H_{-2})] complex.

Notes and references

‡ The emission decay was analysed in the spectral intervals 300–375 nm, 410–470 nm and 500–575 nm. We determined a lifetime in the interval 300–375 nm of 650 ps for complex **1**. For the adducts of **1** with the different substrates, the emission decay recorded around 330 nm shows two contributions, ascribable respectively to the exciplex emission and to a fast component due to the dipyridine emission, similar to or shorter than that found for the exciplex emission. The presence of the component corresponding to the exciplex emission introduces a large uncertainty in determining the value of the dipyridine one. Therefore, it is not possible to obtain reliable values for the lifetimes at 330 nm and to make a quantitative comparison between the value of the dipyridine emission decay in free complex **1** and in its adducts.

§ Addition constants (log *K*) of deprotonated UpU, U and UpA to **1**: **1** + UpU(H_{-1}) = [**1**·UpU(H_{-1})], log *K* = 8.0; [**1**·UpU(H_{-1})] = [**1**·UpU(H_{-2})] + H^+, pK_a = 8.1; **1** + U(H_{-1}) = [**1**·U(H_{-1})], log *K* = 8.1; [**1**·U(H_{-1})] + U(H_{-1}) = [**1**·[U(H_{-1})]$_2$], log *K* = 7.2; **1** + UpA(H_{-1}) = [**1**·UpA(H_{-1})], log *K* = 6.1; [**1**·UpA(H_{-1})] + UpA(H_{-1}) = [**1**·[UpA(H_{-1})]$_2$], log *K* = 4.9.

¶ (a) Observed upfield shifts at pH 9: U, 0.38 (H6), 0.43 (H5) ppm; dipyridine, 0.24 (H4), 0.37 (H5), 0.38 (H6) ppm. In the case of the U signals, deprotonation of the nucleobase upon metal binding also contributes to the observed upfield shifts. Deprotonation of unbound U, in fact, leads to a 0.15 and 0.13 ppm upfield shift of the H6 and H5 signals (see ESI). (b) UpU and UpA binding by **1** could not be analyzed by ^{1}H NMR, due to a marked fluxionality of the spectra.

1 Selected reviews: (*a*) L. Fabbrizzi, M. Licchelli, G. Rabaioli and G. A. Taglietti, *Coord. Chem. Rev.*, 2000, **105**, 85–108; (*b*) V. Amendola, M. Bonizzoni, D. Esteban-Gomez, L. Fabbrizzi, M. Licchelli, F. Sancenon and A. Taglietti, *Coord. Chem. Rev.*, 2006, **250**, 273–299; (*c*) R. Martínez-Máñez and F. Sancenón, *Coord. Chem. Rev.*, 2006, **250**, 3081–3093; (*d*) T. Gunnlaugsson, M. Glynn, G. M. Tocci (née Hussey), P. E. Kruger and F. M. Pfeffer, *Coord. Chem. Rev.*, 2006, **250**, 3094–3117; (*e*) B. T. Nguyen and E. V. Anslyn, *Coord. Chem. Rev.*, 2006, **250**, 3118–3127; (*f*) M. D. Best, S. L. Tobey and E. V. Anslyn, *Coord. Chem. Rev.*, 2003, **240**, 3–15; (*g*) R. Martinez-Mañez and F. Sancenon, *Chem. Rev.*, 2003, **103**, 4419–4476; (*h*) P. D. Beer and S. R. Bayly, *Top. Curr. Chem.*, 2005, **255**, 125–162; (*i*) F. Pina, M. A. Bernardo and E. Garcia-España, *Eur. J. Inorg. Chem.*, 2000, 2143–2157; (*j*) 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.*, 1997, **97**, 1515–1566; (*k*) J. L. Sessler and J. M. Davis, *Acc. Chem. Res.*, 2001, **34**, 989–997; (*l*) P. D. Beer and P. A. Gale, *Angew. Chem.*, 2001, **40**, 486–616; (*m*) J. L. Sessler, S. Camiolo and P. A. Gale, *Coord. Chem. Rev.*, 2003, **240**, 17–55.

2 H. Miyaji, H.-K. Kim, E.-K. Sim, C.-K. Lee, W.-S. Cho, J. L. Sessler and C.-H. Lee, *J. Am. Chem. Soc.*, 2005, **127**, 12510–12512.

3 (*a*) L. Fabbrizzi, F. Foti and A. Taglietti, *Org. Lett.*, 2005, **7**, 2603–2606; (*b*) M. Boiocchi, M. Bonizzoni, L. Fabbrizzi, G. Piovani and A. Taglietti, *Angew. Chem., Int. Ed.*, 2004, **43**, 1962–1965; (*c*) M. A. Hortala, L. Fabbrizzi, N. Marcotte, F. Stomeo and A. Taglietti, *J. Am. Chem. Soc.*, 2003, **125**, 20–21.

4 (*a*) B. Garcia-Acosta, X. Albiach-Marti, E. Garcia, L. Gil, R. Martinez-Manez, K. Rurack, F. Sancenon and J. Soto, *Chem. Commun.*, 2004, 774–775; (*b*) F. Sancenon, R. Martinez-Manez and J. Soto, *Angew. Chem., Int. Ed.*, 2002, **41**, 1416–1419.

5 (*a*) M. D. Pratt and P. D. Beer, *Tetrahedron*, 2004, **60**, 11227–11238; (*b*) P. D. Beer, M. R. Sambrook and D. Curiel, *Chem. Commun.*, 2006, 2105–2117.

6 (*a*) M. D. Best and E. V. Anslyn, *Chem.–Eur. J.*, 2003, **9**, 51–57; (*b*) S. L. Tobey and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 14807–14815; (*c*) L. A. Cabell, M. D. Best, J. J. Lavigne, S. E. Schneider, D. M. Perreault, M.-K. Monahan and E. V. Anslyn, *J. Chem. Soc., Perkin Trans. 2*, 2001, 315–323.

7 (*a*) S. Aoki, D. Kagata, M. Shiro, K. Takeda and E. Kimura, *J. Am. Chem. Soc.*, 2004, **126**, 13377–13390; (*b*) S. Aoki, M. Zulkefeli, M. Shiro, K. Takeda and E. Kimura, *J. Am. Chem. Soc.*, 2005, **127**, 9129–9139.

8 S. Yamaguchi, I. Yoshimura, T. Kohira, I. Tamaru and I. Hamachi, *J. Am. Chem. Soc.*, 2005, **127**, 11835–11841.

9 (*a*) S. Aoki and E. Kimura, *Chem. Rev.*, 2004, **104**, 769–787; (*b*) M. Shionoya, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1993, **115**, 6730–6737; (*c*) M. Shionoya, T. Ikeda, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1994, **116**, 3848–3859; (*d*) E. Kimura, M. Kikuchi, H. Kitamura and T. Koike, *Chem.–Eur. J.*, 1999, **5**, 3113–3123.

10 Q. Wang and H. Loennberg, *J. Am. Chem. Soc.*, 2006, **128**, 10716–10728.

11 (*a*) C. Lodeiro, A. J. Parola, F. Pina, C. Bazzicalupi, A. Bencini, A. Bianchi, A. Masotti, C. Giorgi and B. Valtancoli, *Inorg. Chem.*, 2001, **40**, 2968–2975; (*b*) C. Bazzicalupi, A. Bencini, E. Berni, A. Bianchi, P. Fornasari, C. Giorgi and B. Valtancoli, *Inorg. Chem.*, 2004, **43**, 6255–6265.

