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DNA Interaction and Photonicking Properties of DNA-Targeted Acridine (2,2′-Bipyridine)Platinum(II) Complexes

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Abstract—Synthesis of two (2,2′-bipyridine)platinum(II) complexes tethered to one or two acridine chromophores is reported. These acridine complexes efficiently unwind and photocleave supercoiled plasmid DNA under physiological conditions of temperature and pH.
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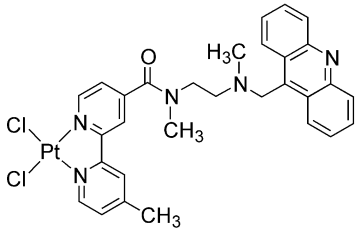
The biological and clinical effectiveness of cisplatin [*cis*-diamminedichloroplatinum(II)] and its analogues as anti-tumour drugs is due to the formation of coordination complexes with DNA which result in intra-strand crosslinks and a smaller proportion of inter-strand crosslinks.[1,2] These covalent adducts induce conformational changes within the DNA double helix that inhibit DNA replication and RNA transcription. Although adduct formation at the N1 and N3 atoms of purine bases has been reported,[3] the primary site is at N7 of guanine, with a preference for binding to GG sequences.[4–7]

Attachment of intercalating agents to platinum complexes[8–29] has proven to enhance rates of DNA platination[23,25–28] while minimising exposure of platinum to inactivating cellular agents such as thiols. These complexes bind covalently and intercalatively to DNA to form novel adducts endowed with the capability of evading cellular DNA repair mechanisms.



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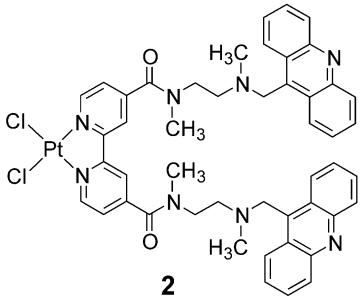

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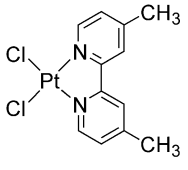

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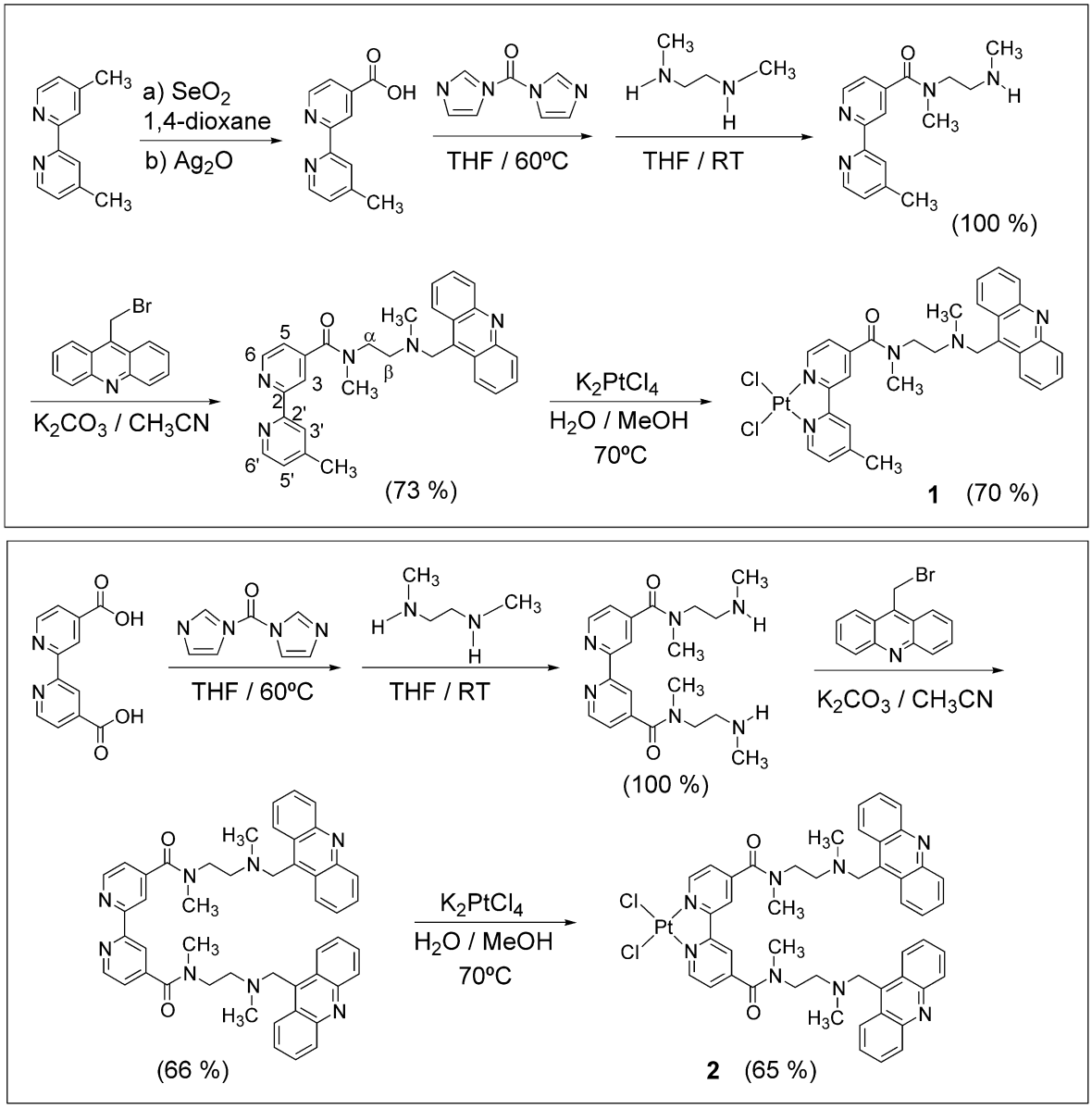
In previous papers,[30,31] we have described the synthesis of water soluble bis- and cyclo-bisintercalants which effect photocleavage of duplex DNA. With the aim of developing more selective and efficient photocleavers, we report herein the synthesis of (2,2′-bipyridine)platinum(II) complexes **1** and **2**, covalently tethered to one and two acridine units, respectively. The conjugation of bipyridine and an intercalator in the design of DNA-targeted platinum(II) complexes is, to the best of our knowledge, unprecedented. The 2,2′-bipyridine moiety constitutes a suitable rigid linker which allows the acridine units to be located at enough distance for a potential bisintercalation taking place in accordance with the neighbour exclusion principle. Thus, the strategy employed was to link the platinum and acridine functionalities in such a way that each could interact freely with DNA. As the acridine subunits are photoactive, it was envisaged that the strong and selective binding between platinum and DNA would lead to effective and selective DNA photocleavage. To this end, we describe experiments in which the platinum(II) complexes **1** and **2** are shown to interact with DNA by cooperative binding and effect complete DNA photocleavage at concentrations $\geq$2.5–3.8 μM.

Synthesis of platinum(II) complexes **1** and **2** was carried out as shown in Scheme 1. Reaction of 4′-methyl-2,2′-bipyridine-4-carboxylic acid and 2,2′-bipyridine-4,4′-dicarboxylic acid with Staab's reagent readily afforded the corresponding amides directly via the imidazolides in a facile, one-pot type process. The ensuing reaction of the amides with 9-bromomethylacridine gave rise to the desired ligands as a mixture of *Z*–*E* isomers. The platinum complexes **1** and **2** were then synthesised in good yields by reaction of the corresponding ligands with K_2PtCl_4 in a solution of water and methanol 1:1 (v/v) at 70 °C. Complexes **1** and **2** and their corresponding ligands were fully characterised by NMR, IR and MS.[32]



Scheme 1.

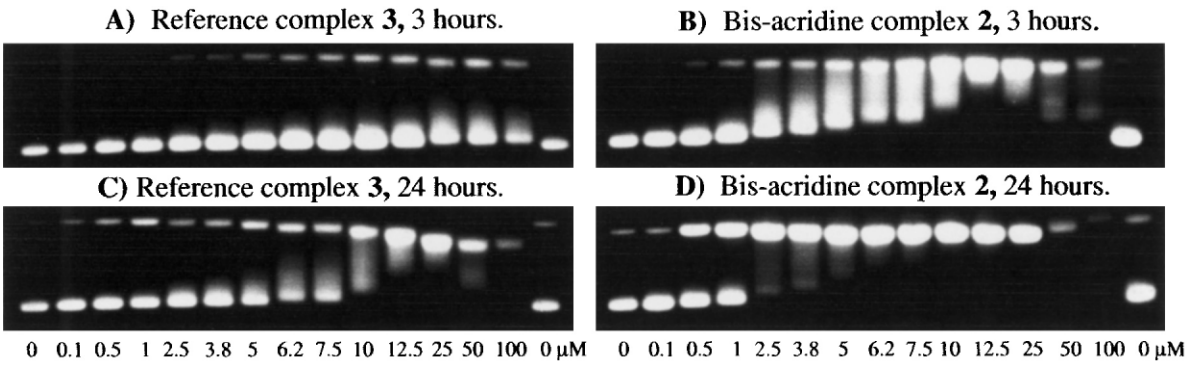


Figure 1. Changes in the electrophoretic mobility of form I (botton bands) and form II (upper bands) of pUC19 plasmid. DNA was equilibrated with increasing concentrations of complex for 3 and 24 h in the dark (39.5 μM bp pUC19, 10 mM sodium phosphate buffer pH 7.4, 37 °C). The plasmid was resolved on 1% agarose gels which were run in the dark for 16 h at 1 V/cm, stained with ethidium bromide (0.5 μg/mL), and visualised with a Molecular Dynamics FluorImager SI Gel Imaging System. In the control lanes, complex was substituted by an equivalent volume of reaction buffer.

The interaction of complexes **1** and **2** with DNA was studied by testing their ability to alter the electrophoretic mobilities of the supercoiled and nicked forms of pUC19 plasmid DNA. It has been previously established that binding of cisplatin and analogues to DNA retards the mobility of form I (supercoiled) and increases the mobility of form II (nicked).[33] Shown in Figure 1 is a representative experiment in which bis-acridine platinum(II) complex **2** and reference complex 4,4′-dimethyl-2,2′-bipyridinedichloroplatinum(II) (**3**) were allowed to react with DNA in the dark for 3 and 24 h at 37 °C, pH 7.4. It is evident from examination of the gel that, after 3 h, complex **2** unwinds DNA at very low concentrations, while no significant unwinding is observed for platinum reference **3**. In additional experiments conducted with the corresponding bis-acridine ligand, the effect of complex **2** on DNA tertiary structure was indicative of cooperative binding, as neither the platinum reference nor the ligand alone exhibited unwinding in the concentration range studied (data not shown). All in all, acridine has been proven to act as an effective DNA targeting molecule, increasing the rate of DNA platination. Even after 24 h, unwinding induced by reference **3**, although significant, does not equal that produced by complex **2** after the 3-h equilibration (Fig. 1).

Mono–acridine complex **1** was also capable of unwinding cooperatively supercoiled plasmid DNA, but the concentrations required to exhibit this interaction were higher. Indeed, it was found that 12.5 μM of complex **1** was needed to produce total unwinding after a 24-h equilibration, while only 5 μM of bis-acridine complex **2** was required.

The ability of complexes **1** and **2** to effect DNA photocleavage was studied next. In these experiments, it was also bis-acridine complex **2** that induced the highest levels of DNA modification, although the difference in reactivity with respect to compound **1** was less pronounced. In the gel shown in Figure 2, pUC19 plasmid DNA was equilibrated with increasing concentrations of bis-acridine complex **2** for 3 h (37 °C, pH 7.4), after which the reactions were irradiated at 350 nm for 50 min under aerobic conditions in a ventilated Rayonet Photochemical Reactor. It is apparent that practically all of the DNA has been converted from the supercoiled to nicked form at 2.5 μM concentrations of the complex. For mono-acridine complex **1**, similar concentrations of $\geq$3.8 μM were required for complete photocleavage. Additional control experiments conducted with ligand molecules revealed their capability to yield nicked DNA upon irradiation, although higher concentrations of compound relative to complexes were needed (25 and 5 μM for mono and bis-ligand, respectively).

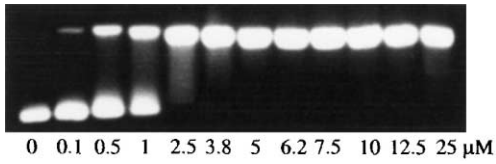


Figure 2. DNA was equilibrated with complex **2** for 3 h in the dark (39.5 μM bp pUC19, 10 mM sodium phosphate buffer pH 7.4, 37 °C), after which the samples were irradiated for 50 min with 10 350 nm 24 W Rayonet lamps. The cleaved plasmid was resolved on a 1% agarose gel which was run for 16 h at 1 V/cm, and stained with ethidium bromide (0.5 μg/mL). In the control lane, complex 2 was substituted by an equivalent volume of reaction buffer.

In conclusion, the above experiments indicate that acridine platinum(II) complexes **1** and **2** are capable of completely unwinding supercoiled plasmid DNA at 5–12.5 μM concentrations (37 °C, pH 7.4). Moreover, complexes **1** and **2** are efficient DNA photocleavers. Upon irradiation at 350 nm, even lower concentrations of each complex produce significant DNA damage. Our current work is focused on the determination of binding modes to provide insights into operating mechanisms underlying the interactions of these acridine complexes with DNA.

Acknowledgements

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References and Notes

1. Bruhn, S. L.; Toney, J. H.; Lippard, S. J. *Prog. Inorg. Chem.: Bioinorg. Chem.* **1991**, *38*, 477.
2. Dabrowiak, J. C.; Bradner, W. T. *Prog. Med. Chem.* **1987**, *24*, 129.
3. Raudaschl-Sieber, G.; Schollhorn, H.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1985**, *107*, 3591.
4. Royer Pokora, B.; Gordon, L. K.; Haseltime, W. A. *Nucleic Acids Res.* **1981**, *9*, 4595.
5. Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616.
6. Ponti, M.; Forrow, S. M.; Souhami, R. L.; D'Incalci, M.; Hartley, J. A. *Nucleic Acids Res.* **1991**, *19*, 2929.
7. Murray, V.; Motyka, H.; England, P. R.; Wickham, G.; Lee, H. H.; Denny, W. A.; McFayden, W. D. *J. Biol. Chem.* **1992**, *267*, 18805.
8. Bowler, B. E.; Hollis, L. S.; Lippard, S. E. *J. Am. Chem. Soc.* **1984**, *106*, 6102.
9. Bowler, B. E.; Lippard, S. E. *Biochemistry* **1986**, *25*, 3031.
10. Pasini, A. *Gazz. Chim. Ital.* **1987**, *117*, 763.
11. Bowler, B. E.; Ahmed, K. J.; Sundquist, W. I.; Hollis, L. S.; Whang, E. E.; Lippard, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 1299.
12. Sundquist, W. I.; Bancroft, D. P.; Lippard, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 1590.
13. Palmer, B. D.; Lee, H. H.; Johnson, P.; Baguley, B. C.; Wickham, G.; Wakelin, L. P. G.; McFayden, W. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 3008.
14. Gibson, D.; Gean, K. F.; Ben-Shoshan, R.; Ramu, A.; Ringel, I.; Katzhendler, J. *J. Med. Chem.* **1991**, *34*, 414.
15. Lee, H. H.; Palmer, B. D.; Baguley, B. C.; Chin, M.; McFayden, W. D.; Wickham, G.; Thorsbourne-Palmer, D.; Wakelin, L. P. G.; Denny, W. A. *J. Med. Chem.* **1992**, *35*, 2983.
16. Murray, V.; Motyka, H.; England, P. R.; Wickham, G.; Lee, H. H.; Denny, W. A.; McFayden, W. D. *J. Biol. Chem.* **1992**, *267*, 18805.
17. Kech, M. V.; Lippard, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 3386.
18. Murray, V.; Motyka, H.; England, P. R.; Wickham, G.; Lee, H. H.; Denny, W. A.; McFayden, W. D. *Biochemistry* **1992**, *31*, 11812.
19. McKeage, M. J.; Kelland, L. R., In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S., Waring, M., eds.; CRC: Boca Raton, FL, 1993; Vol. 1, p 169.
20. Culliname, C.; Wickham, G.; McFayden, W. D.; Denny, W. A.; Palmer, B. D.; Phillips, D. R. *Nucleic Acids Res.* **1993**, *21*, 393.
21. Mikata, Y.; Mogami, K.; Kato, M.; Okura, I.; Yano, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1083.
22. Gibson, D.; Binyamin, I.; Haj, M.; Ringel, I.; Ramu, A.; Katzhendler, J. *Eur. J. Med. Chem. Chim. Ther.* **1997**, *32*, 823.
23. Whittaker, J.; McFayden, W. D.; Wickham, G.; Wakelin, L. P. G.; Murray, V. *Nucleic Acids Res.* **1998**, *26*, 3933.
24. Mikata, Y.; Yokoyama, M; Mogami, K.; Kato, M.; Okura, I; Chikira, M.; Yano, S. *Inorg. Chim. Acta* **1998**, *279*, 51.
25. Pérez, J. M.; López-Solera, I.; Montero, E. I.; Braña, M. F.; Alonso, C.; Robinson, S. P.; Navarro-Ranninger, C. *J. Med. Chem.* **1999**, *42*, 5482.
26. Perrin, L. C.; Culliname, C.; McFayden, W. D.; Phillips, D. R. *Anti-Cancer Drug Des.* **1999**, *14*, 243.
27. Perrin, L. C.; Prenzler, P. D.; Culliname, C.; Phillips, D. R.; Denny, W. A.; McFayden, W. D. *J. Inorg. Biochem.* **2000**, *81*, 111.
28. Temple, M. D.; McFayden, W. D.; Holmes, R. J.; Denny, W. A.; Murray, V. *Biochemistry* **2000**, *39*, 5593.
29. Holmes, R. J.; McKeage, M. J.; Murray, V.; Denny, W. A.; McFayden, W. D. *J. Inorg. Biochem.* **2001**, *85*, 209.
30. Lorente, A.; Fernández-Saiz, M.; Herraiz, F.; Lehn, J.-M.; Vigneron, J.-P. *Tetrahedron Lett.* **1999**, *40*, 5901.
31. Fernández, M.-J.; Grant, K. B.; Herraiz, F.; Yang, X.; Lorente, A. *Tetrahedron Lett.* **2001**, *42*, 5701.
32. Characterization of compounds: **Mono-acridine ligand:** mp 48–51 °C; IR (KBr) υ 3048, 2942, 2852, 1735, 1698, 1638, 1595, 1456, 752 cm^{-1}; EI–MS *m*/*z*: 475 (M^+, 33), 283 (67), 235 (69), 192 (100); *Z* isomer/*E* isomer = 59/41; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.66 (d, *J* = 4.9 Hz, H-6 *Z*), 8.55 (d, *J* = 4.9 Hz, H-6′ *Z*), 8.51 (d, *J* = 4.9 Hz, H-6 *E*), 8.51 (d, *J* = 8.8 Hz, H-1,8 Acr *Z*), 8.42 (d, *J* = 4.9 Hz, H-6′ *E*), 8.38 (d, *J* = 8.8 Hz, H-1,8 Acr *E*), 8.33 (br s, H-3), 8.24 (two overlapped d, H-4,5 Acr), 8.23 (s, H-3′ *Z*), 8.16 (s, H-3′ *E*), 7.76 (m, H-3,6 Acr), 7.58 (m, H-2,7 Acr), 7.16 (d, *J* = 4.9 Hz, H-5′ *Z*), 7.10 (d, *J* = 4.9 Hz, H-5′ *E*), 6.98 (dd, *J* = 4.9, 1.6 Hz, H-5 *E*), 6.93 (dd, *J* = 4.9, 1.6 Hz, H-5 *Z*), 4.55 (s, CH_2–Acr *Z*), 4.43 (s, CH_2–Acr *E*), 3.67 (t, *J* = 6.2 Hz, $CH_2\alpha$ *Z*), 3.32 (t, *J* = 6.2 Hz, $CH_2\alpha$ *E*), 2.82 (t, *J* = 6.2 Hz, $CH_2\beta$ *Z*), 2.67 (t, *J* = 6.2 Hz, $CH_2\beta$ *E*), 2.80 (s, CH_3NCO *E*), 2.60 (s, CH_3NCO *Z*), 2.47 and 2.46 (two s, CH_3–Ar), 2.41 (s, CH_3N *Z*), 1.99 (s, CH_3N *E*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.43 and 168.92 (C=O), 156.66 and 156.42 (C-2), 155.11 and 154.92 (C-2′), 149.35 (C-6), 148.98 (C-6′), 148.57 (C4° Acr), 148.16 and 148.02 (C-4′), 145.01 (C-4), 141.24 (C4° Acr), 140.52 (C4° Acr), 130.15 (C-4,5 Acr), 129.84 and 129.76 (C-3,6 Acr), 126.05 and 125.91 (C-2,7 Acr), 125.01 and 124.93 (C-5′), 124.80 and 124.53 (C-1,8 Acr), 121.97 and 121.79 (C-3′), 121.01 and 120.91 (C-5), 118.55 and 118.39 (C-3), 55.04 ($CH_2\beta$ *E*), 54.50 ($CH_2\beta$ *Z*), 53.85 (CH_2–Acr), 48.95 ($CH_2\alpha$ *E*), 44.67 ($CH_2\alpha$ *Z*), 42.19 (CH_3N *Z*), 41.92 (CH_3N *E*), 37.43 (CH_3NCO *Z*), 32.78 (CH_3NCO *E*), 21.15 and 21.10 (CH_3–Ar).

Complex 1: mp 255 °C dec.; IR (KBr) υ 3060, 2931, 1627, 1546, 1522, 1438, 1417, 756 cm^{-1}, (Nujol, CsI) υ 336, 324 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 9.52–9.24 (m, H-6,6′), 8.62–8.45 (m, H-1,8 Acr), 8.30–8.07 (m, H-3,3′, H-4,5 Acr), 7.88–7.75 (m, H-3,6 Acr), 7.70–7.56 (m, H-2,7 Acr, H-5′), 7.48 (d, *J* = 5.9 Hz, H-5), 7.16 (d, *J* = 5.9 Hz, H-5), 4.56 (s, CH_2–Acr), 4.47 (s, CH_2–Acr), 3.59 (t, *J* = 6 Hz, $CH_2\alpha$), 3.35 (br, $CH_2\alpha$), 2.80–2.60 (m, CH_3NCO, $CH_2\beta$), 2.35 (s, CH_3–Ar, CH_3N), 1.99 (s, CH_3N); ^{195}Pt NMR (DMF-d_7, 64.2 MHz) δ −2325 (^{195}Pt NMR chemical shifts are referenced externally to K_2PtCl_4 in D_2O at −1624 ppm); FAB-MS (m-NBA) *m*/*z* 742.00 ($M^+ + 2$), calcd for $C_{30}H_{29}Cl_2N_5OPt$ (740.1397).

Bis-acridine ligand: mp 79–82 °C; IR (KBr) υ 3055, 2941, 2850, 1734, 1636, 1547, 1519, 1458, 1403, 752 cm^{-1}; FAB-MS (m-NBA) *m*/*z* 767.25 ($M^+ + 1$), calcd for $C_{48}H_{46}N_8O_2$ (766.3744); *Z,Z* isomer/*ZE* isomer/*EE* isomer = 30/44/26%; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.67 (d, *J* = 4.8 Hz, H-6 *ZZ*), 8.52–8.48 (m, H-1,8 Acr *ZZ* + *ZE*, H-6 *EE*, H-6 *ZE*), 8.39–8.31 (m, H-1,8 Acr *EE* + *ZE*, H-3 *ZZ* + *EE* + *ZE*, H-6 *ZE*), 8.27–8.21 (m, H-3 *ZE*, H-4,5 Acr), 7.79–7.70 (m, H-3,6 Acr), 7.61–7.52 (m, H-2,7 Acr), 6.99 (dd, *J* = 4.8, 1.6 Hz, H-5 *EE*), 6.93 (dd, *J* = 4.8, 1.6 Hz, H-5 *ZE* + *ZZ*), 6.84 (dd, *J* = 4.8, 1.6 Hz, H-5 *ZE*), 4.54, 4.53, 4.44 and 4.39 (s, CH_2–Acr), 3.67 and 3.64 (two t, *J* = 5.9 Hz $CH_2\alpha$ *ZZ* + *ZE*), 3.30 (t, *J* = 5.9 Hz, $CH_2\alpha$ *EE*), 3.24 (t, *J* = 5.9 Hz, $CH_2\alpha$ *ZE*), 2.82 (t, *J* = 5.9 Hz, $CH_2\beta$ *ZZ* + *ZE*), 2.80 (s, CH_3NCO *EE*), 2.76 (s, CH_3NCO *ZE*), 2.67 (t, *J* = 5.9 Hz, $CH_2\beta$ *EE* + *ZE*), 2.59 (s, CH_3NCO *ZZ*), 2.54 (s, CH_3NCO *ZE*), 2.48 (s, CH_3N *ZZ*), 2.46 (s, CH_3N *ZE*), 1.97 (s, CH_3N *EE*), 1.90 (s, CH_3N *ZE*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.38 and 168.83 (C=O), 155.77, 155.58, 155.52 and 155.34 (C-2), 149.51 and 149.44 (C-6), 148.59 (C4°

Acr), 145.12 and 144.99 (C-4), 141.31 (C4° Acr), 140.56 (C4° Acr), 130.18 (C-4,5 Acr), 129.93 and 129.82 (C-3,6 Acr), 126.13, 126.06 and 125.94 (C-2,7 Acr), 124.82 and 124.54 (C-1,8 Acr), 121.40 and 121.25 (C-5), 118.69, 118.49, 118.43 and 118.22 (C-3), 54.99 and 54.47 ($CH_2\beta$), 53.91 (CH_2–Acr), 48.98 and 44.71 ($CH_2\alpha$), 42.28, 41.97 and 41.90 (CH_3N), 37.47 and 32.79 (CH_3NCO)

Complex 2: mp 260 °C dec.; IR (KBr) υ 3064, 2932, 2853, 1687, 1636, 1547, 1520, 1459, 1412, 1069, 753 cm^{-1}, (Nujol, CsI) υ 328, 324 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 9.60–9.37 (m, H-6), 8.63–8.57 (m, H-1,8 Acr), 8.52–8.40 (m, H-1,8 Acr), 8.26–8.05 (m, H-3, H-4,5 Acr), 7.83–7.78 (m, H-3,6 Acr), 7.64–7.56 (m, H-2,7 Acr), 7.50–7.46 (m, H-5), 7.26–7.22 (m, H-5), 4.58–4.43 (m, CH_2-Acr), 3.60 (m, $CH_2\alpha$), 3.40 (m, $CH_2\alpha$), 2.80–2.60 (m, CH_3NCO, $CH_2\beta$), 2.35 (br, CH_3N), 1.99 (s, CH_3N), 1.93 (s, CH_3N); ^{195}Pt NMR (DMF-d_7, 64.2 MHz) δ −2329; FAB-MS (m-NBA) m/z 1033.26 ($M^+ + 2$), calcd for $C_{48}H_{46}Cl_2N_8O_2Pt$ (1031.2768).

33. Cohen, G. L.; Bauer, W. R.; Barton, J. K.; Lippard, S. J. *Science* **1979**, *203*, 1014.