Pergamon

Tetrahedron Letters 43 (2002) 4723–4727

TETRAHEDRON LETTERS

Anthracene and naphthalene (2,2′-bipyridine)platinum(II) conjugates: synthesis and DNA photocleavage

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Accepted 15 May 2002

Abstract—A series of (2,2′-bipyridine)platinum(II) complexes covalently tethered to one or two anthracene or naphthalene chromophores was synthesized in high yields. The compounds were equilibrated with pUC19 plasmid and then irradiated at 350 nm. DNA cleaving reactions showed that the complex attached to two anthracene units exhibited the highest photocleavage efficiency, leading to complete conversion of supercoiled to linear DNA at 0.25 μM concentrations of the complex (37°C, pH 7.4).

The effectiveness of platinum(II) complexes as antitumor agents can be improved by linking the chemically reactive platinum functionality to a DNA binding agent such as acridine,[1–13] anthraquinone[14–16] and other intercalators.[17–20] This multifunctional binding induces a significant modification in DNA structure, unwinding the double helix to a greater extent than that induced by the binding of the individual components. Moreover, the degree of unwinding by platinum complexes has been correlated in some cases to corresponding rates of repair of these adducts. Since DNA intercalation is a rapid process, the effect of attachment of the intercalator to a platinum complex is to localize the platinum in the vicinity of the target DNA. This action increases the rate of platination,[12,17,19] circumventing the first chloride hydrolysis step and reducing side reactions of platinum with cellular thiols such as glutathione. DNA-targeted platinum(II) complexes can furthermore alter sequence specificity,[12] producing lesions that may escape the DNA repair process.

In this communication, we report the synthesis of (2,2′-bipyridine)platinum(II) complexes linked to one or two anthracene or naphthalene chromophores. Platinum(II) complexes covalently linked to photoactive intercalators such as anthracene and naphthalene are potential therapeutic agents that can be activated in vivo by a light source. To this end, we describe the DNA photonicking properties of individual anthracene and naphthalene ligands and their corresponding platinum(II) complexes.

Platinum(II) complexes attached to naphthalene (**2** and **6**) and anthracene (**4** and **8**) chromophores were synthesized according to the procedures depicted in Scheme 1. Ligands **1** and **3** were obtained through a Williamson reaction from 4′-methyl-2,2′-bipyridine-4-methanol and a suitable halide compound. In the same fashion, ligands **5** and **7**[21] were prepared from 2,2′-bipyridine-4,4′-dimethanol and halide. Platinum(II) complexes were then obtained in excellent yields by means of reaction of the corresponding ligand with K_2PtCl_4 in a solution of acidic water–methanol 1:1 at ~80°C. Compounds **1** through **8** were fully characterized by NMR, IR and MS.[22]

DNA photocleavage efficiency of synthesized compounds **1**–**8** was tested and then compared to the reference complex 4,4′-dimethyl-2,2′-bipyridinedichloroplatinum(II) by monitoring the conversion of supercoiled plasmid DNA (type I) to the nicked (type II) and linear (type III) forms. After 3 and 24 h equilibrations (37°C, pH 7.4), reactions were irradiated at 350 nm under aerobic conditions in a ventilated Rayonet Photochemical Reactor. Electrophoretic gels were run for 16 h at 1 V/cm and densitometric analysis was performed after UV imaging.

Keywords: DNA; photocleavage; (2,2′-bipyridine)platinum(II) complexes; naphthalene; anthracene.
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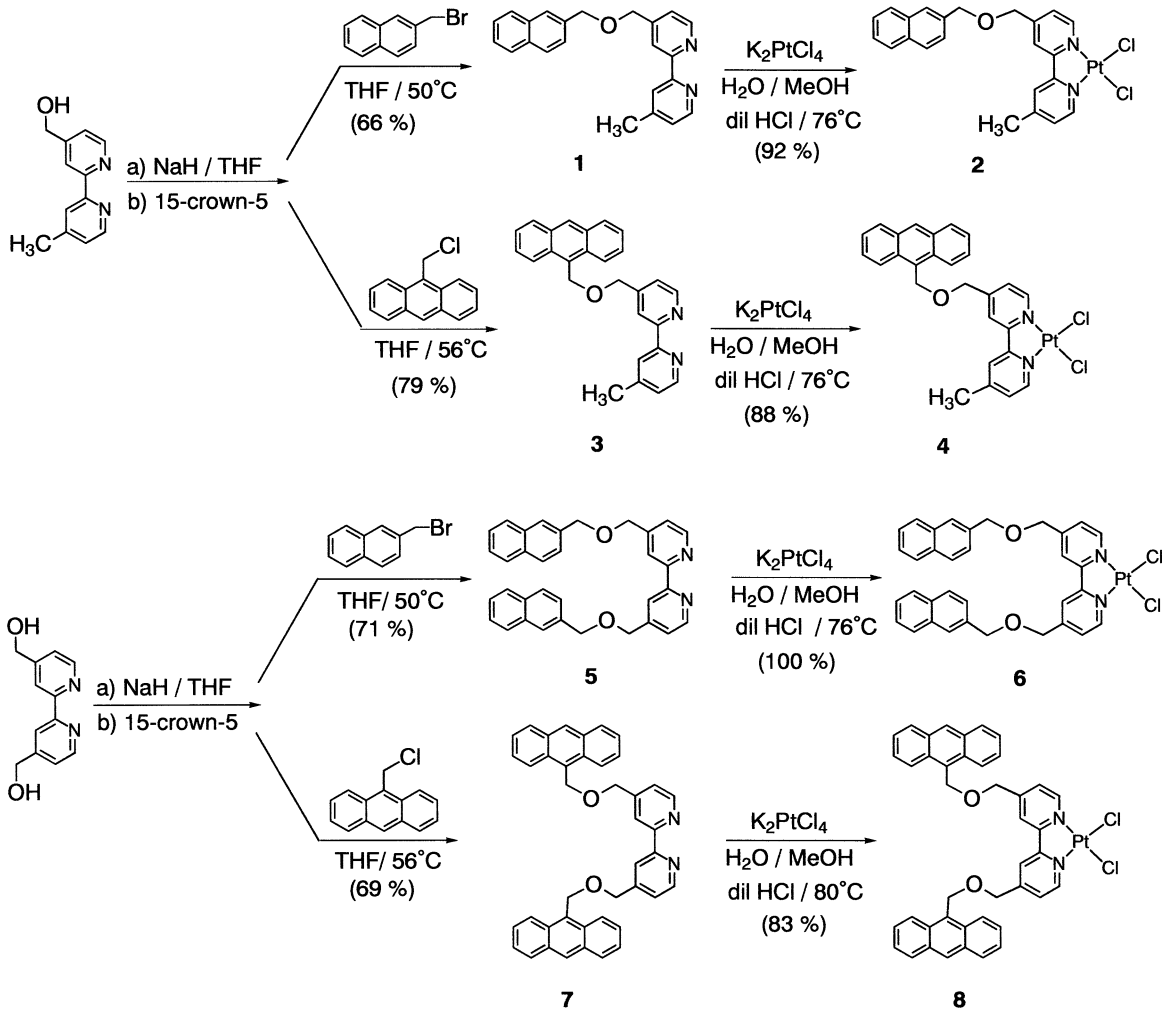
PII: S0040-4039(02)00937-1



Scheme 1.

Our initial experiments demonstrated that the anthracene compounds were superior DNA photocleavers. After a 24 h equilibration, photocleavage was exhibited at concentrations as low as 50 nM for compounds **3**, **4**, and **7** and 25 nM for bis-anthracene complex **8**. In the representative experiment shown in Fig. 1A, irradiation of pUC19 plasmid DNA in the presence of 10 nM to 500 nM concentrations of bis-anthracene complex **8** produced complete conversion of supercoiled to nicked DNA at 100 nM concentrations. Although quantitation of cleavage yields was difficult due to the slight gel shift produced by platination at adjacent guanine bases and subsequent DNA compression, it is evident that linear DNA was obtained at 250 nM of complex **8**. When the experiment was conducted with the reference complex 4,4′-dimethyl-2,2′-bipyridinedichloroplatinum(II), no significant cleaving activity was observed in the 10–500 nM concentration range. In fact, the reference complex induced DNA photocleavage only at concentrations notably higher (Fig. 1B).

A summary of photocleavage yields produced by 0.1 μM concentrations of anthracene containing compounds **3**, **4**, **7** and **8** is reported in Table 1. The relative efficiency in pUC19 DNA cleavage for the series of compounds investigated was found to be: **8**>**7**>**3**≥**4**. (In all cases, no DNA cleavage was produced in parallel control reactions run in the dark; data not shown.)

In comparison to the anthracene series, the naphthalene compounds were unable to effect photocleavage at such low concentrations. Indeed, the photocleavage efficiency of ligands **1** and **5** could not be assessed due to the proximity of their λ_{max} values (ca. 285 nm) to the absorption maximum of DNA ($\lambda_{max}=260$ nm) (Irradiation with higher energy light sources would lead to non-specific DNA photodegradation.) Within the complexes, only compound **2** was able to photocleave DNA efficiently, producing complete conversion of the supercoiled to linear form at 12.5 μM after 3 h equilibration. This result points to the importance of destabilizing steric interactions, since complex **6** with two units of naphthalene did not produce photocleavage at this concentration. Furthermore, unwinding assays conducted in the dark with complex **2** showed significant DNA interaction relative to complex **6**. Mono-naphthalene complex was found to unwind DNA in the 2.5–12.5 μM range with the introduction of positive supercoils at 25–50 μM concentrations (Fig. 2).

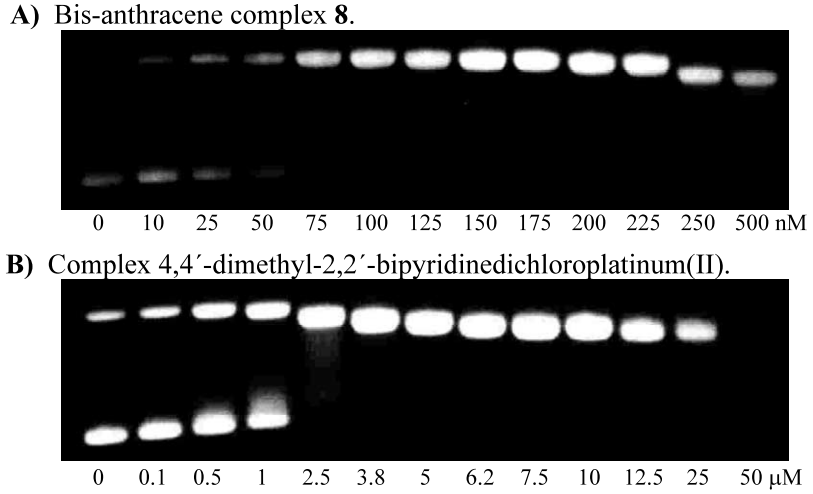


Figure 1. DNA was equilibrated with increasing concentrations of complex for 24 h in the dark (39.5 μM bp pUC19, 10 mM sodium phosphate buffer pH 7.4, 37°C), after which the samples were irradiated for 50 min with ten 350 nm 24W Rayonet lamps. The cleaved plasmid was then resolved on 1% agarose gels which were run for 16 h at 1 V/cm, stained with ethidium bromide (0.5 μg/ml), and visualized with a Molecular Dynamics FluorImager SI Gel Imaging System. In the control lanes, complex was substituted by equivalent volumes of reaction buffer. (A) Photocleavage reactions with complex **8**. (B) Photocleavage reactions with reference complex 4,4′-dimethyl-2,2′-bipyridinedichloroplatinum(II). (Concentration range is extended in order to appreciate cleaving activity.)

Table 1. Approximate % yields of DNA photocleavage

Compound	–[a]	Ref. [b]	**3**[b]	**4**[b]	**7**[b]	**8**[b]
hv (350 nm, 50 min):						
3 h equilibration	18	18	32	33	57	91
24 h equilibration	23	34	60	50	93	100

[a] DNA cleavage in the absence of compound.
[b] Percent nicked+linear plasmid obtained in the presence of 0.1 μM of compound.

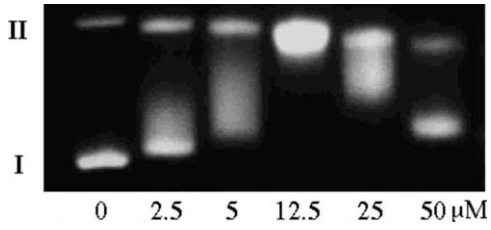


Figure 2. Changes in electrophoretic mobility of type I and type II forms of pUC19 modified by complex **2**. DNA was equilibrated with increasing concentrations of complex for 24 h in the dark (39.5 μM bp pUC19, 10 mM sodium phosphate buffer pH 7.4, 37°C). The plasmid was resolved on a 1% agarose gel which was run in the dark for 16 h at 1V/cm and stained with ethidium bromide (0.5 μg/ml). In the control lane, compound **2** was substituted by an equivalent volume of reaction buffer.

In summary, we have prepared several anthracene and naphthalene ligands and their corresponding (2,2′-bipyridine)platinum(II) complexes. We have found that very low concentrations of each anthracene compound, especially the bis-anthracene complex **8**, were endowed with the capability of photocleaving DNA under physiological conditions of temperature and pH. Due to the existence of differences in reactivity and to the potential applications of these compounds in phototherapy, we are now investigating DNA binding modes in order to establish key interactions and to envisage the design of new molecules with enhanced photocleavage activities.

Acknowledgements

Support of this research by the CICYT (Project SAF96-1704; A.L.) is gratefully acknowledged. We also thank the National Science Foundation for funding under NSF CAREER Award (CHE-9984772; K.B.G.) and the American Chemical Society Petroleum Research Fund for a Type G Grant (32897-G3; K.B.G.). L.G. thanks Consejería de Educación de la Comunidad de Madrid for a postgraduate fellowship.

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22. Compound **1**: mp 98–100°C; IR (KBr) ν 3056, 2854, 1595, 1508, 1461 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) 8.67 (d, $J=5.0$ Hz, 1H, H-6), 8.54 (d, $J=5.0$ Hz, 1H, H-6′), 8.37 (br s, 1H, H-3), 8.24 (br s, 1H, H-3′), 7.87-7.83 (m, 4H, H-1,4,5,8 Naph.), 7.52 (dd, $J=8.5$, 1.6 Hz, 1H, H-3 Naph.), 7.48 (m, 2H, H-6,7 Naph.), 7.40 (dd, $J=5.0$, 1.6 Hz, 1H, H-5), 7.14 (d, $J=5.0$ Hz, 1H, H-5′), 4.80 (s, 2H, CH_2-Naph.), 4.70 (s, 2H, CH_2-Py), 2.44 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.67 (C-2), 155.23 (C-2′), 149.34 (C-6), 149.15 (C-6′), 148.8 (C-4), 148.03 (C-4′), 135.80 (C-2 Naph.), 133.01, 132.71 (C-4a,8a Naph.), 128.11, 127.90, 127.70 (C-4,5,8 Naph.), 126.34, 126.09, 126.01 (C-1,3,6,7 Naph.), 125.07 (C-5′), 122.20 (C-5), 121.42 (C-3′), 118.68 (C-3), 72.30 (CH_2-Naph.), 70.29 (CH_2-Py), 20.85 (CH_3); EI-MS m/z: 340 (M^+, 5%), 199 (7), 184 (100), 157 (6), 142 (22), 141 (23).
Compound **2**: mp 248–251°C dec.; IR (KBr) ν 3055, 2857, 1623, 1509, 1484, 1433 cm^{-1}, (Nujol, CsI) ν 348, 335 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.37 (d, 1H, $J=6.0$ Hz), 9.23 (d, 1H, $J=6.0$ Hz), 8.44 (s, 1H), 8.42 (s, 1H), 7.92 (m, 4H), 7.81 (d, 1H, $J=6.0$ Hz), 7.63 (d, 1H, $J=6.0$ Hz), 7.49–7.57 (m, 3H), 4.84 (s, 2H), 4.73 (s, 2H), 2.45 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 156.71, 156.19, 152.76, 148.28, 147.65, 135.40, 132.96, 132.75, 128.19, 127.94, 127.73, 126.65, 126.42, 126.22, 126.11, 125.28, 124.96, 121.89, 72.53, 69.53, 21.22; ^{195}Pt NMR (DMF-d_7, 64.2 MHz) δ −2327, (^{195}Pt NMR chemical shifts are referenced externally to K_2PtCl_4 in D_2O at −1624 ppm); FAB-MS (*m*-NBA) *m*/z 606 (M^++1), calcd for $C_{23}H_{20}Cl_2N_2OPt$ (605.06).
Compound **3**: mp 140–142°C; IR (KBr) ν 3050, 2900, 2855, 1595, 1524, 1460 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.63 (d, $J=4.9$ Hz, 1H, H-6), 8.53 (d, $J=4.9$ Hz, 1H, H-6′), 8.48 (s, 1H, H-10 Anthr.), 8.35 (d, $J=8.1$ Hz, 2H, H-1,8 Anthr.), 8.35 (s, 1H, H-3), 8.22 (s, 1H, H-3′), 8.02 (d, 2H, $J=8.1$ Hz H-4,5 Anthr.), 7.54 (m, 2H, H-2,7 Anthr.), 7.47 (m, 2H, H-3,6 Anthr.), 7.35 (dd, $J=4.9$, 1.6 Hz, 1H, H-5), 7.13 (dd, $J=4.9$, 1.6 Hz, 1H, H-5′), 5.60 (s, 2H, CH_2-Anthr.), 4.78 (s, 2H, CH_2-Py) 2.44 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.27 (C-2), 155.74 (C-2′), 149.32 (C-6), 148.87 (C-6′), 148.69 (C-4), 148.16 (C-4′), 131.39, 131.08 (C-4a,8a,9a,10a Anthr.), 129.07 (C-4,5 Anthr.), 128.68 (C-10 Anthr.), 128.03 (C-9 Anthr.), 126.37 (C-2,7 Anthr.), 125.00 (C-3,6 Anthr.), 124.76 (C-5′), 124.15 (C-1,8 Anthr.), 122.08 (C-5), 121.99 (C-3′), 119.60 (C-3), 70.77 (CH_2-Py), 64.78 (CH_2-Anthr.), 21.19 (CH_3); EI-MS m/z: 390 (M^+, 17%), 206 (11), 192 (38), 191 (100), 184 (61), 179 (47), 178 (29).
Compound **4**: mp 252–254°C dec.; IR (KBr) ν 3054, 2915, 2854, 1622, 1524, 1481, 1431 cm^{-1}, (Nujol, CsI) ν 343, 336 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.23 (d, $J=6.0$ Hz. 1H), 9.20 (d, $J=6.0$ Hz, 1H), 8.62 (s, 1H), 8.47 (d, $J=8.0$ Hz, 2H), 8.25 (s, 1H), 8.16 (s, 1H), 8.08 (d, $J=8.0$ Hz, 2H); 7.49–7.61 (m, 6H); 5.67 (s, 2H), 4.83 (s, 2H), 2.42 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 156.80, 156.56, 153.30, 153.12, 148.57, 148.08, 131.56, 131.25, 129.57, 129.08, 128.75, 127.18, 125.87, 125.50, 125.29, 125.11, 122.20, 70.09, 65.17, 21.73; ^{195}Pt NMR (DMF-d_7, 64.2 MHz) δ −2328; FAB-MS (*m*-NBA) *m*/*z* 656.1 (M^++1), calcd for $C_{27}H_{22}Cl_2N_2OPt$ (655.08).
Compound **5**: mp 113–115°C; IR (KBr) ν 3061, 2844, 1597, 1508, 1413 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) 8.67 (d, $J=4.9$ Hz, 2H, H-6,6′), 8.40 (br s, 2H, H-3,3′), 7.88–7.82 (m, 8H, H-1,4,5,8 Naph.), 7.53 (dd, $J=8.4$, 1.8 Hz, 2H, H-3 Naph.), 7.49 (m, 4H, H-6,7 Naph.), 7.40 (dd, $J=4.9$, 1.7, 2H, H-5,5′), 4.80 (s, 4H, CH_2-Naph.), 4.70 (s, 4H, CH_2-Py); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.20 (C-2,2′), 149.37 (C-6,6′), 148.54 (C-4,4′), 135.20 (C-2 Naph.), 133.28, 133.09 (C-4a,8a Naph.), 128.30 (C-4 Naph.), 127.87, 127.68 (C-5,8 Naph.), 126.61 (C-1 Naph.), 126.15, 125.96, (C-6,7 Naph.), 125.70 (C-3 Naph.), 121.99 (C-5,5′), 119.46 (C-3), 72.93 (CH_2-Naph.), 70.66 (CH_2-Py); EI-MS m/z: 496 (M^+, 6%), 340 (59), 198 (100), 141 (95).
Compound **6**: mp 212–215°C dec.; IR (KBr) ν 3050, 2852, 1625, 1508, 1467, 1429 cm^{-1}, (Nujol, CsI) ν 344, 336 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) 9.38 (d, $J=5.9$ Hz, 2H), 8.44 (s, 2H), 7.93–7.89 (m, 8H), 7.82 (d, $J=5.9$ Hz, 2H), 7.51 (m, 6H), 4.83 (s, 4H), 4.73 (s, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 156.56, 152.93, 148.37, 135.41, 132.99, 132.75, 128.24, 127.97, 127.77, 126.68, 126.46, 126.26, 126.15, 125.39, 122.04, 72.54, 69.49; ^{195}Pt NMR (DMF-d_7, 64.2 MHz) δ −2327; FAB-MS (*m*-NBA) *m*/*z* 762 (M^++1), calcd for $C_{34}H_{28}Cl_2N_2O_2Pt$ (761.12).
Compound **7**: mp 195–197°C; IR (KBr) ν 3049, 2857, 1596, 1504, 1446 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.63 (d, $J=4.9$ Hz, 2H, H-6,6′), 8.48 (s, 2H, H-10 Anthr.), 8.36 (d, $J=8.5$ Hz, 4H, H-1,8 Anthr.), 8.35 (s, 2H, H-3,3′), 8.02 (d, $J=8.5$ Hz, 4H, H-4,5 Anthr.), 7.54 (m, 4H, H-2,7 Anthr.), 7.47 (m, 4H, H-3,6 Anthr.), 7.35

(dd, $J=4.9$, 1.5 Hz, 2H, H-5,5′), 5.60 (s, 4H, CH_2-Anthr.), 4.78 (s, 4H, CH_2-Py); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.02 (C-2,2′), 149.38 (C-6), 148.65 (C-4), 131.38, 131.08 (C-4a,8a,9a,10a Anthr.), 129.06 (C-4,5 Anthr.), 128.68 (C-10 Anthr.), 128.01 (C-9 Anthr.), 126.37 (C-2,7 Anthr.), 124.99 (C-3,6 Anthr.), 124.14 (C-1,8 Anthr.), 122.18 (C-5,5′), 119.60 (C-3,3′), 70.75 (CH_2-Py), 64.78 (CH_2-Anthr.); MS (EI) m/z 596 (M^+, 1%), 404 (6), 192 (39, 191 (100).

Compound **8**: mp 233–235°C dec.; IR (KBr) ν 3053, 2925, 2855, 1623, 1475, 1428 cm^{-1}, (Nujol, CsI) ν 339, 335 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 9.27 (d, $J=6.2$ Hz, 2H, H-6,6′), 8.61 (s, 2H, H-10 Anthr.), 8.46 (d, $J=9.0$ Hz, 4H, H-1,8 Anthr.), 8.07 (d, $J=8.3$ Hz, 4H, H-4,5 Anthr.); 7.99 (s, 2H, H-3,3′), 7.65 (d, $J=6.2$ Hz, 2H, H-5,5′), 7.55 (m, 4H, H-2,7 Anthr.), 7.48 (m, 4H, H-3,6 Anthr.), 5.68 (s, 4H CH_2-Anthr.), 4.81 (s, 4H, CH_2-Py); ^{195}Pt NMR (DMF-d_7, 64.2 MHz) δ −2329; FAB-MS (*m*-NBA) m/z 862 (M^++1), calcd for $C_{42}H_{32}Cl_2N_2O_2Pt$ (861.15).