Circularly Polarized-Electrochemiluminescence from a Chiral Bispyrene Organic Macrocycle

Francesco Zinna,[a],[b] Silvia Voci,[c] Lorenzo Arrico,[a] Elodie Brun,[b] Alexandre Homberg,[b] Laurent Bouffier,[c] Tiziana Funaioli,[a] Jérôme Lacour,*[b] Neso Sojic,*[c] Lorenzo Di Bari*[a]

Abstract: The first observation of circular polarization of electrochemiluminescence (ECL) from a purely organic derivative is reported. A bis-pyrene scaffold mounted on a constrained polyether macrocycle displaying intense excimer fluorescence and highly circularly polarized (CP) photoluminescence has been selected for this purpose. Such compound displays an ECL dissymmetry factor of ca. |8×10⁻³|, in good agreement with the corresponding photoluminescence value. This observation is the first step towards the molecular engineering of bespoke suitable dyes acting both as ECL and CP-ECL reporters for (bio)analysis by bringing a new level of information when dealing with chiral environments. In addition, it provides an extra-dimension to the ECL phenomenon and opens the way to chiral detection and discrimination.
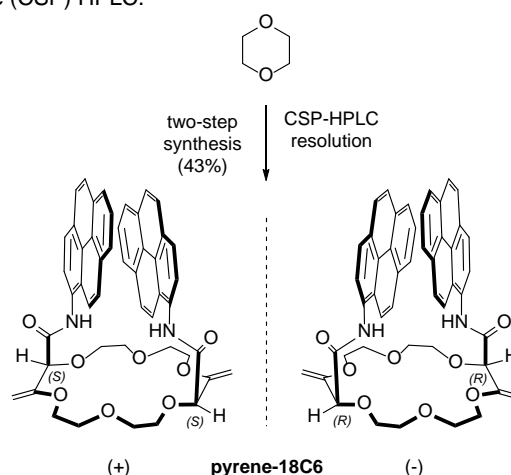
Electrochemiluminescence (ECL) is the production of light as a consequence of very exergonic electron transfer reactions between electrogenerated species.[1] Since the mid 1960's, a thriving scientific research has brought ECL to become one of the most highly sensitive techniques for the detection of bioanalytes, and nowadays commercial ECL-based immunoassays are extensively used in clinical diagnosis and in food or environment investigations.[2] Besides these well-established applications, thanks to the exceptional spatial and temporal resolutions as well as intensity control of the emitted light, ECL systems have been employed in the development of new imaging methods, ranging from the quantification of relevant cancer biomarkers[3] to single cell and cell membrane proteins visualization.[4]

Being able to detect the circular polarization of the emission would add further advantage in (bio)assays in terms of selectivity, enhanced contrast and specific response in a chiral environment.[5] It would provide original valuable information on the interaction between the molecular probe and the targeted analyte. In this context, circularly polarized photoluminescence

[a] Dr F. Zinna, L. Arrico. Dr T. Funaioli, Prof. L. Di Bari
Dipartimento di Chimica e Chimica Industriale
University of Pisa
via G. Moruzzi 13, 56124, Pisa, Italy
E-mail: lorenzo.dibari@unipi.it

[b] Dr F. Zinna, Dr E. Brun, A. Homberg, Prof. J. Lacour
Department of Organic Chemistry
University of Geneva
Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland.
E-mail: Jerome.lacour@unige.ch

[c] S. Voci, Dr. L. Bouffier, Prof N. Sojic
Univ. Bordeaux, Bordeaux INP, CNRS, Institut des Sciences
Moléculaires, UMR 5255
16 avenue Pey-Berland, 33607 Pessac, France
E-mail: neso.sojic@enscbp.fr

Supporting information for this article is given via a link at the end of the document

(CP-PL), which consists in exciting the sample using unpolarized light, has been strongly evidenced.[6] For example, lanthanide complexes able to modulate their CP-PL profile upon interaction with proteins or smaller chiral molecules were developed.[5b, 5c, 7] Organic molecules, even achiral or racemic, can also signal their interaction with chiral (bio)analytes through CP-PL.

To the best of our knowledge, circularly polarized electrochemiluminescence (CP-ECL) was only evidenced once in a pioneering work by Blok et al. in 1987 with dextrorotatory [Ru(phen)₃][ClO₄]₂ complex.[8] So far, the question as to whether CP-ECL could be generalized and observed for organic systems has remained unaddressed, probably also due to the limited number of single molecules capable to give intense enough polarized response. Herein, we report the first observation of CP-ECL on a purely organic dye. An ECL dissymmetry factor of ca. |8×10⁻³| is obtained using a bis-pyrene scaffold mounted on a constrained polyether macrocycle; the result is self-consistent with the corresponding photoluminescence value.

Recently, synthetic protocols have been developed for the synthesis of unsaturated macrocycles via (i) [3+6+3+6] condensations of cyclic ethers and α-diazo-β-ketoesters and (ii) tandem amidation – olefin transpositions.[9] These macrocycles comprise a constrained polyether ring and two aromatic subunits positioned rigidly. A compound like **pyrene-18C6** can be obtained in combined 43% yield from 1,4-dioxane as a racemate (Scheme 1).[10] The two enantiomers can be conveniently and effectively separated via semi-preparative chiral stationary phase (CSP) HPLC.[11]

Scheme 1. Molecular structure of pyrene-18C6.

Pyrene-18C6 shows a photoluminescence profile dominated by a strong excimer band around 490 nm as well as good

photoluminescence quantum yield (24% in CH_3CN, relative to anthracene).[10-12] Such strong excimer is formed intramolecularly thanks to the peculiar molecular geometry, which brings the two pyrene moieties in close spatial proximity. Moreover, the chiral elements of the macrocyclic ring induce a defined configuration of the skewed arrangement of the two pyrene units, resulting in highly CP-PL (Figure S1).[11]

To determine the appropriate conditions for ECL generation, the electrochemical properties of **pyrene-18C6** were first investigated in acetonitrile with a glassy carbon (GC) working electrode. Indeed, the stability of the electrogenerated luminophore radicals is of fundamental importance to produce strong ECL intensity. Due to the known instability of the cation radical of pyrene resulting from the electrochemical oxidation,[13] we focused our study in the cathodic domain. As shown in Figure 1 (top), the voltammogram exhibits two stepwise reversible reduction waves, at $E_{1,red}$ = -1.92 V, and $E_{2,red}$ = -2.24 V $vs.$ Ag pseudo-reference electrode. The peak current of the first reduction wave is equal to the second one. By contrast, the voltammetric response of pyrene shows a single 1-electron reduction wave at -2.1 V $vs.$ Ag in the same potential window. The shift of $E_{1,red}$ to less cathodic potential in comparison to pyrene means that the first pyrene in the complex is easier to reduce than the pyrene alone. The two successive cathodic waves of **pyrene-18C6** can be attributed to the 1-electron reduction of each pyrene in the complex. After the first reduction step, the second pyrene is more difficult to reduce ($E_{1,red} > E_{2,red}$) and it indicates that there is an electronic communication between both pyrene moieties. In any case, it shows that both the mono-anion and the di-anion of **pyrene-18C6** produced during the cathodic processes, are stable in solution during at least the timescale of the voltammetric experiments. This reversible reduction behavior should therefore enable the ECL generation following the reductive-oxidation pathway with an appropriate co-reactant.

In the present work, the good stability of the reduced forms of **pyrene-18C6** was exploited and ECL emission was thus tested using benzoyl peroxide (BPO) as a sacrificial cathodic co-reactant. In a BPO solution, the potential of the GC working electrode was swept from +0.5 V to -2.9 V $vs.$ Ag (Figure 1, bottom). Reduction of BPO (black curve) is visible with a wave peaking at -0.7 V but ECL emission (blue curve) occurs only at the potentials where the pyrene moieties of the complex are reduced. In addition, it is noteworthy that ECL signal is composed of two overlapping peaks with a maximum at -2.6 V $vs.$ Ag. Afterwards, it decays very rapidly. These two peaks are most probably related to both reduction waves of the pyrene moieties, even if a small potential shift is observed. The reduction of BPO leads to its dissociation, which produces a strong oxidant radical Ph-CO_2•. This latter oxidizes the reduced forms of the **pyrene-18C6** and generates the excited state. Finally, radiative relaxation of the excimer to the ground state produces the ECL emission. In brief, **pyrene-18C6** produces a

strong and stable ECL emission by applying a potential of -2.45 V $vs.$ Ag in presence of BPO.

The ECL spectrum (Figure 2, middle) was self-consistent with the PL spectrum collected under 366 nm irradiation ($vide$ $infra$). As expected, the main spectral feature was the excimer emission, as already observed in photoluminescence, while the weak monomer contribution remained hidden under the broad excimer band. As already reported, monomeric pyrene gives only a very weak ECL signal.[13a, 13b]
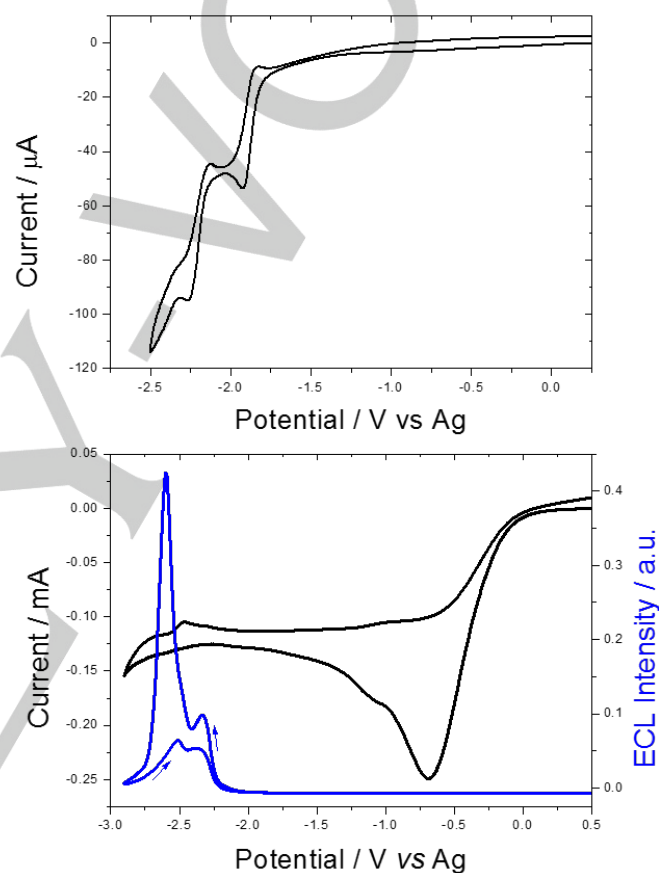

Figure 1. (Top) Voltammetric characterization of 1 mM **pyrene-18C6** in degassed CH_3CN solution with 0.1 M $TBAPF_6$ as supporting electrolyte. (Bottom) Cyclic voltammogram (black curve) and corresponding ECL signal (blue curve) recorded in degassed CH_3CN solution containing 0.1 mM **pyrene-18C6**, 10 mM BPO and 0.1 M $TBAPF_6$. A GC disk and a Ag wire were used as working electrode and pseudo-reference electrode, respectively. Scan rate: 0.1 V/s.

With these experimental conditions in hand, we verified that the ECL emission was stable at least for the time of acquisition of the spectra (~240 s, Figure S2, bottom).Then we proceeded to measure the circular polarization of ECL of the two enantiomers of **pyrene-18C6** by using our home-made spectrofluoropolarimeter.[14] The spectroelectrochemical cell was accommodated into the sample holder of the instrument, and the instrumental parameters (slit width, photomultiplier tube voltage, integration time, see ESI) were optimized for the measurement.

A clear positive CP-ECL signal was unambiguously observed for (−)-**pyrene-18C6**, allied with the electrogenerated excimer chemiluminescence, while a negative band was observed for (+)-**pyrene-18C6**. At least 6 measurements for each enantiomer were carried out to check the reproducibility. The persistence of CP-ECL signal (Figure S2, top) indicated the chemical stability of the compounds during the measurement. Both enantiomers gave indeed mirror image spectra, as expected. The corresponding averaged CP-ECL spectra are reported in Figure 2, (top). Moreover, as a control experiment, the CP-ECL of racemic **pyrene-18C6** was measured in the same conditions and no significant polarization was observed. All these results excluded the presence of artefacts due to linearly polarized components of light in the CP-ECL measurements.

Analogously to photoluminescence, CP-ECL can be quantified by an ECL dissymmetry factor (g_{ECL}), defined as $2(I_L-I_R)/(I_L+I_R)$, where I_L and I_R are the intensities of the left and right circularly polarized components of the electrogenerated emission.[6] The g_{ECL} values observed around 490 nm, on the maximum of excimer emission, were $+8.4\times10^{-3} \pm 1.4\times10^{-3}$ and $-7.3\times10^{-3} \pm 2.0\times10^{-3}$ for (−) and (+)-**pyrene-18C6** respectively (Figure 2, bottom). As expected, the g_{ECL} associated with the racemic ECL mixture is erratic and becomes vanishingly small when averaged throughout the spectrum, with a maximum g_{ECL} values around $0.3\times10^{-3} \pm 1.4\times10^{-3}$. This defines a lower limit of quantitation for CP-ECL in the conditions employed and ensures that the signals measured for the enantiopure compounds are significant (~30 times higher than the racemic signal).

The CP-ECL profile closely retraces the one measured for CP-PL (see Figure S3), moreover g_{ECL} and the photoluminescence dissymmetry factor g_{PL} (measured under 366 nm irradiation) are well-comparable (g_{PL} = +9.0 and -8.8×10^{-3} for (−) and (+) enantiomers, respectively). These observations confirm that the same electronic state is involved in both PL and ECL. Finally, the aforementioned g_{ECL} values are almost one order of magnitude higher than the one measured by Blok et al. for (+)-[Ru(Phen)₃][ClO₄]₂ in the only report so far on CP-ECL.[8, 15]
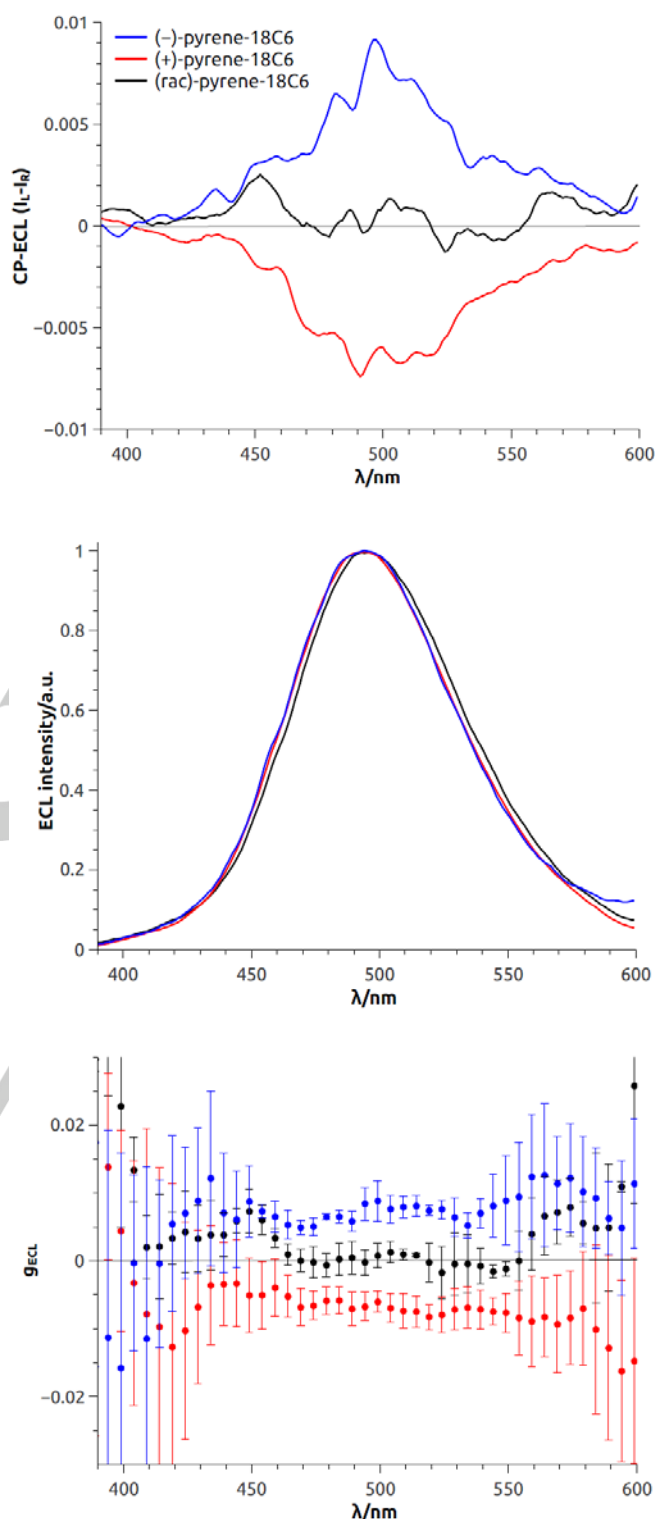

Figure 2. CP-ECL (top), ECL (middle) and g_{ECL} (bottom) of **pyrene-18C6** (both enantiomers and racemic mixture). In g_{ECL} plot, the error bars represent ± one standard deviation and are generated over 6 replicates for each enantiomer and 3 for the racemic mixture. Note that errors on g_{ECL} diverge on the edges of the spectra, where total emission intensity becomes vanishing small.

In conclusion, we have shown that it is possible to obtain and measure highly circularly polarized ECL from purely organic molecules. We disclosed a new nature of the ECL signal, showing for the first time that this analytical technique can discriminate enantiomers. These findings can be generalized to other appropriate molecules and tailored structures can be developed to be used as selective CP-ECL reporters for (bio)analytes and other chiral systems of interest.

Acknowledgements

Keywords: Circularly polarized electrochemiluminescence • Circularly polarized luminescence • CPL • excimer fluorescence • pyrene

[1] M. M. Richter, *Chem. Rev.* **2004**, *104*, 3003-3036.

[2] a) Z. Liu, W. Qi, G. Xu, *Chem. Soc. Rev.* **2015**, *44*, 3117-3142; b) W. Miao, *Chem. Rev.* **2008**, *108*, 2506-2553; c) G. Valenti, E. Rampazzo, S. Kesarkar, D. Genovese, A. Fiorani, A. Zanut, F. Palomba, M. Marcaccio, F. Paolucci, L. Prodi, *Coord. Chem. Rev.* **2018**, *367*, 65-81; d) E. M. Gross, S. S. Maddipati, S. M. Snyder, *Bioanalysis* **2016**, *8*, 2071-2089; e) L. Hu, G. Xu, *Chem. Soc. Rev.* **2010**, *39*, 3275-3304.

[3] M.-S. Wu, Z. Liu, H.-W. Shi, H.-Y. Chen, J.-J. Xu, *Anal. Chem.* **2014**, *87*, 530-537.

[4] a) G. Valenti, S. Scarabino, B. Goudeau, A. Lesch, M. Jovic, E. Villani, M. Sentic, S. Rapino, S. Arbault, F. Paolucci, *J. Am. Chem. Soc.* **2017**, *139*, 16830-16837; b) S. Voci, B. Goudeau, G. Valenti, A. Lesch, M. Jovic, S. Rapino, F. Paolucci, S. Arbault, N. Sojic, *J. Am. Chem. Soc.* **2018**, *140*, 14753-14760.

[5] a) C. P. Montgomery, B. S. Murray, E. J. New, R. Pal, D. Parker, *Acc. Chem. Res.* **2009**, *42*, 925-937; b) R. Carr, N. H. Evans, D. Parker, *Chem. Soc. Rev.* **2012**, *41*, 7673-7686; c) F. Zinna, L. Di Bari, *Chirality* **2015**, *27*, 1-13.

[6] a) E. M. Sánchez-Carnerero, A. R. Agarrabeitia, F. Moreno, B. L. Maroto, G. Muller, M. J. Ortiz, S. de la Moya, *Chem. –Eur. J.* **2015**, *21*, 13488-13500; b) G. Longhi, E. Castiglioni, J. Koshoubu, G. Mazzeo, S. Abbate, *Chirality* **2016**, *28*, 696-707.

[7] a) B. T. Nguyen, A. J. Ingram, G. Muller, *Chirality* **2016**, *28*, 325-331; b) E. Brichtová, J. Hudecová, N. Vršková, J. Šebestík, P. Bouř, T. Wu, *Chem.–Eur. J.* **2018**, *24*, 8664-8669; c) T. Wu, J. Kessler, J. Kaminský, P. Bouř, *Chem.–Asian J.* **2018**, *13*, 3865-3870; d) T. Wu, P. Bouř, *Chem. Commun.* **2018**, *54*, 1790-1792; e) J. Yuasa, T. Ohno, H. Tsumatori, R. Shiba, H. Kamikubo, M. Kataoka, Y. Hasegawa, T. Kawai, *Chem. Commun.* **2013**, *49*, 4604-4606; f) M. Górecki, F. Zinna, T. Biver, L. Di Bari, *J. Pharmaceut. Biomed.* **2017**, *144*, 6-11.

[8] P. Blok, P. Cartwright, H. Dekkers, R. Gillard, *J. Chem. Soc. Chem. Commun.* **1987**, 1232-1233.

[9] a) D. Poggiali, A. Homberg, T. e. Lathion, C. Piguet, J. Lacour, *ACS Catalysis* **2016**, *6*, 4877-4881; b) W. Zeghida, C. Besnard, J. Lacour, *Angew. Chem. Int. Ed.* **2010**, *49*, 7253-7256; c) M. Vishe, R. Hrdina, A. I. Poblador-Bahamonde, C. Besnard, L. Guénée, T. Bürgi, J. Lacour, *Chem. Sci.* **2015**, *6*, 4923-4928.

[10] Z. Jarolímová, M. Vishe, J. Lacour, E. Bakker, *Chem. Sci.* **2016**, *7*, 525-533.

[11] A. Homberg, E. Brun, F. Zinna, S. Pascal, M. Górecki, L. Monnier, C. Besnard, G. Pescitelli, L. Di Bari, J. Lacour, *Chem. Sci.* **2018**, *9*, 7043-7052.

[12] M. Vishe, T. Lathion, S. Pascal, O. Yushchenko, A. Homberg, E. Brun, E. Vauthey, C. Piguet, J. Lacour, *Helv. Chim. Acta* **2018**, *101*, e1700265.

[13] a) B. Fleet, G. Kirkbright, C. Pickford, *J. Electroanal. Chem. Interfacial Electrochem.* **1971**, *30*, 115-121; b) R. Y. Lai, J. J. Fleming, B. L. Merner, R. J. Vermeij, G. J. Bodwell, A. J. Bard, *J. Phys. Chem. A* **2004**, *108*, 376-383; c) J. W. Oh, Y. O. Lee, T. H. Kim, K. C. Ko, J. Y. Lee, H. Kim, J. S. Kim, *Angew. Chem. Int. Ed.* **2009**, *48*, 2522-2524.

[14] F. Zinna, T. Bruhn, C. A. Guido, J. Ahrens, M. Bröring, L. Di Bari, G. Pescitelli, *Chem.–Eur. J.* **2016**, *22*, 16089-16098.

[15] Attempts to measure CP-ECL in CH_3CN of the enantiomers of $[Ru(bpy)_3][PF_6]_2$ (prepared following ref. [16]), were performed in similar conditions. A signal could not be detected despite an intense and stable ECL signal. In the report by Blok et al. (see ref. [8]), neither the exact ECL experimental conditions nor the corresponding ECL data were clearly displayed; only the ECL dissymmetry factor value was given. In addition, only the dextrorotatory enantiomer of $[Ru(phen)_3][ClO_4]_2$ was tested.

[16] M. Oppermann, B. Bauer, T. Rossi, F. Zinna, J. Helbing, J. Lacour, M. Chergui, *Optica* **2019**, *6*, 56-59.

Entry for the Table of Contents

Layout 1:

COMMUNICATION

Circularly polarized electrochemiluminescence is observed from a chiral organic molecule. The chiral macrocyclic scaffold leads to efficient excimer formation between two pyrene moieties mounted on it. Thus, highly polarized emission is obtained upon electrochemical excitation.
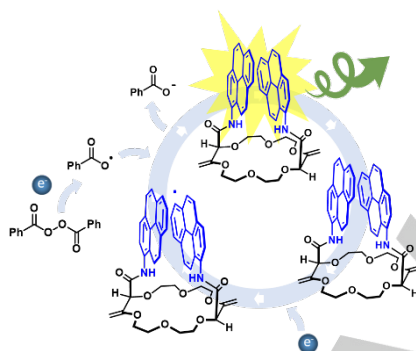
Author(s), Corresponding Author(s)*

Page No. – Page No.

Title