Towards new multivalent supramolecular helical structures based on phthalocyanines for PDT applications

Miguel García-Iglesias,*[a,b] Elisa Huerta,[a]

[a] Institute of Complex Molecular Systems, Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB, Eindhoven, The Netherlands.

[b] Department of Organic Chemistry, Universidad Autónoma de Madrid (UAM), Calle Francisco Tomás y Valiente, 7, 28049 Madrid, ES.

ABSTRACT:. The self-assembly of α-D-Glucose octafunctionalized Zn(II) phtalocyanine (ZnPc) in aqueous media has been investigated using different techniques such as UV, CD and diffusion-ordered ^{1}H-NMR spectroscopy (DOSY). The formation of supramolecular columnar helical aggregates with a preferred handedness due to the presence of the sugar moiety has been confirmed by these techniques. Moreover, the stability of the supramolecular polymers formed has been assessed by using pyridine as a zinc ligand, disrupting the aggregates or inducing their formation by dilution of the system.

INTRODUCTION

The study of nanometer-sized structures formed by self-organization of functional dyes in order to form molecular materials is an important goal nowadays in chemistry.[1-4] Phthalocyanines (Pcs) are flat aromatic macrocycles known as one of the best synthetic porphyrine analogues.[5-8] Due to their excellent electronic and optical properties, Pcs are one the most commonly used chromophores, finding applications ranging from photovoltaic devices [9-11] to non-linear optics [12-13]. The extended flat hydrophobic aromatic surface of Pcs can interact with each other by attractive $\pi-\pi$ stacking interactions (or solvophobic effect) leading to aggregation into columnar structures in solution.[14-18] Mimicking biological functions using organized synthetic dyes into supramolecular polymers in aqueous solution is a challenging area for biomimetic and biomedical chemistry.[19-20] Phthalocyanines can behave as photosensitizers, generating the highly reactive singlet oxygen leading to cell death, thus they are suitable to be used on photodynamic therapy (PDT), when properly substituted to improve their solubility in water.[21]

Saccharides are also involved in several biological processes, including selective recognition processes triggered by interaction with receptors. [22-23] However, the interaction of only one or a few saccharides with their corresponding receptors is usually not strong enough. In this context, several covalent architectures endowed with multiple saccharides have been synthetized in order to create a multivalency effect, enhancing dramatically the interaction between the drug and the receptor. [24-26] The presence of saccharides at the periphery of the phthalocyanines enhances their solubility in water and it could as well increase the interaction with the membranes and improve tumor selectivity. [27] Unfortunaltely, the design of such multivalent

systems is still complicated due to the lack of information about the location of the saccharides with respect to the receptors at the cell surface, and the absence of flexibility of the covalent multivalent systems to adapt their structure to the receptors localization. [24] Recently, supramolecular polymers have demostrated to have dynamic and adaptative nature in multivalent systems in water. [28-29] Therefore, the possibility of endowing a dynamic, well-defined scaffold, such as a supramolecular polymer based on phthalocyanines, with multiple carbohydrates opens the door to a new type of drugs. Moreover, recently it has been observed that the cell adhesion in the supramolecular hydrogels depends on the handedness of the self-assembled nanofibers. [30-31]

The synthesis and tendency to aggregate in water of an α-D-glucose functionalized Pc was described in the literature by Hanack and coworkers. [32-33] Moreover, excellent triplet quantum yield (0.68) and singlet oxygen quantum yield (0.67) were found for this phthalocyanine in DMSO as a first step for its use in PDT.[34] However, the dynamic nature, the size, and the chirality of those aggregates have been never studied before.

Herein, we study the self-assembly of α-D-glucose functionalized phthalocyanines into supramolecular chiral polymers in water by UV-vis, circular dichroism (CD) and diffusion-ordered ^{1}H-NMR spectroscopy (DOSY) spectroscopies, in order to determine the structural features and the stability of the agreggates.

EXPERIMENTAL

Materials: Chemicals were obtained from either Acros or Aldrich at the highest purity available and used without further purification. All solvents were of AR quality and purchased from Biosolve. Synthesis of 2,3,9,10,16,17,23,24-Octa(2,3,4,6-tetra-*O*-acetyl-a-D-glucopyranosyl) phthalocyaninatozinc(II) Pc, was carried out following the procedure described in the literature by M. Hanack, T. Ziegler and coworkers. [32]

Methods: CD measurements were performed on a Jasco J-815 spectropolarimeter where the sensitivity, time constant and scan rate were chosen appropriately. Corresponding temperature dependent measurements were performed with a Jasco PTC-348WI Peltier-type temperature controller, with a temperature range of 263-383 K and adjustable temperature slope. Ultraviolet-visible (UV-vis) absorbance spectra were recorded on and a Jasco V-650 UV-vis spectrometer with a Jasco ETCT-762 temperature controller. Solutions were prepared by weighing in the necessary amount of compound for a given concentration, where after this amount was transferred to a volumetric flask (flasks of 10, 25 and 50 mL were employed). Then the flask was filled for 3/4 with the spectroscopic grade solvent and put in an oscillation bath at 40 °C for 45 min, where after the flask was allowed to cool down and filled up to its meniscus. The $\Delta\varepsilon$ value was calculated from $\Delta\varepsilon =$ CD effect/(32980·c·l) in which c is the concentration in mol/L and l is the optical path length in cm. UV-Vis and CD measurements were performed using quartz cuvettes (1 mm). ^{1}H-NMR Diffusion measurements (DOSY) were carried out at 298 K on Varian Mercury Vx 500 MHz. The ledbpgp2s pulse sequence was selected for the DOSY NMR by using gradients varied linearly from 5% up to 95% in 32 steps, with 16 scans per step. Chemical shifts are given in ppm (δ) values relative to residual solvent or tetramethylsilane (TMS). DOSY experiments with compound 1 in 5 were carried out at 5mM concentration.

The model used to calculate the hydrodynamic radii of the aggregates is the Stokes–Einstein relation [35] for the diffusion of a spherical particle:

$$Dt = \frac{kT}{6\pi\eta RH}$$

k = Boltzmann constant = 1.38065 10^{23} m²·kg·s⁻²·K⁻¹

T = absolute temperature / K

η = dynamic viscosity of D₂O and DMSO-d^6 / Pa·s

$\eta_{(D2O)}$ (298 K) = 1.99 x 10⁻³ Pa·s and $\eta_{(DMSO-d6)}$ (298 K) = 1.00 x 10⁻³ Pa·s)

RH = hydrodynamic radius of the aggregate / m

RESULTS AND DISCUSSION

The absorption spectrum of the α-D-glucose Zn(II) phthalocyanine (Figure 1a) in water reveals the presence of a hypochromic shift of the Q band from 686 nm to 645 nm with respect to that in DMSO (Figure 1b). This feature confirms the presence of H-aggregates in water. Furthermore, the presence of stacks can be seen by "nacked eye" going from bright green, when molecularly dissolved (in DMSO), to dark blue when stacked (in water).
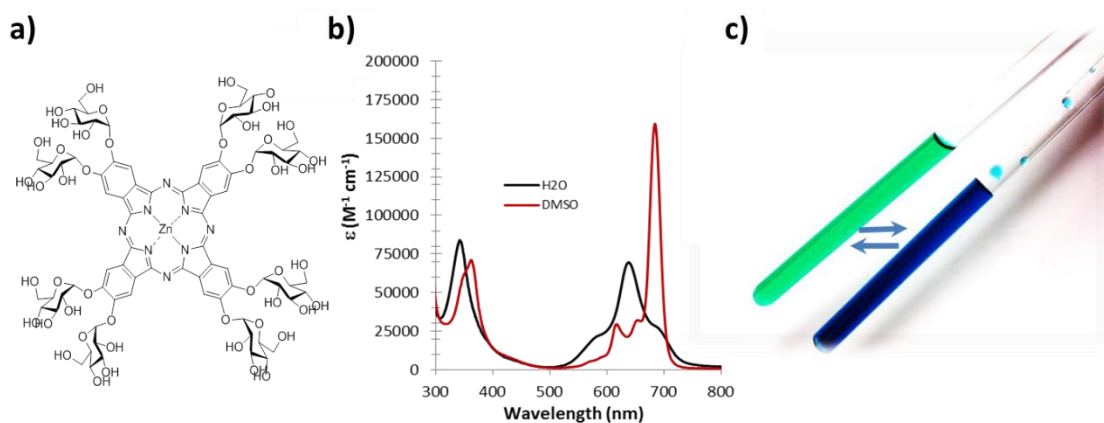

Fig 1. a) Structure of the phthalocyanine Pc b) UV trace of Pc in water and DMSO c) color changes upon changing the solvent DMSO (bright green) and water (dark blue).

In order to determine the stability of the stacks, temperature- and concentration-dependent experiments have been carried out by UV spectroscopy. When the system was diluted 10 fold or 100 fold, no spectroscopic changes were observed (Figure 2b). Besides, cooling curves and variable temperature experiments (from 90 to 20℃) performed at different concentrations revealed very subtle changes, which account for the high stability presented by the Pc aggregates in water (Figure 2a).
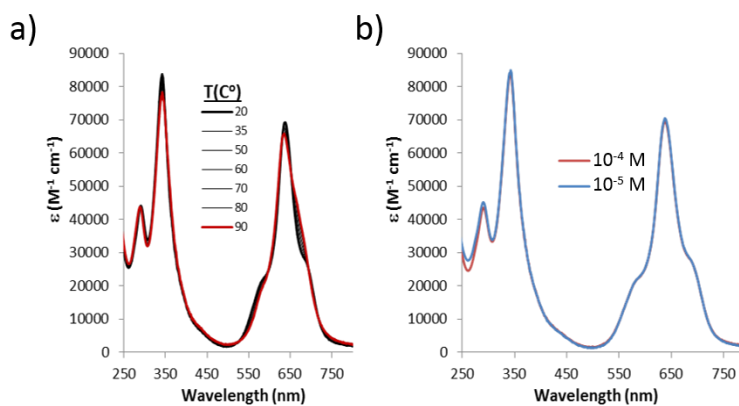
Fig. 2. UV trace of Pc in water a) at different temperatures and b) at different concentrations.

Furthermore, CD experiments were carried out in water in order to study the possibility of the sugars biasing the helical sense of the stacks. Aqueous solution of Pc showed a remarkable Cotton effect, confirming the formation of helical columnar aggregates with a preferred handedness. In contrast, when the Pc was dissolved in DMSO no Cotton effect was observed, which is indicative of a molecularly dissolved state. Moreover, concentration-dependent experiments were again carried out observing, as by UV spectroscopy, no changes in the aggregation degree (Figure 3).
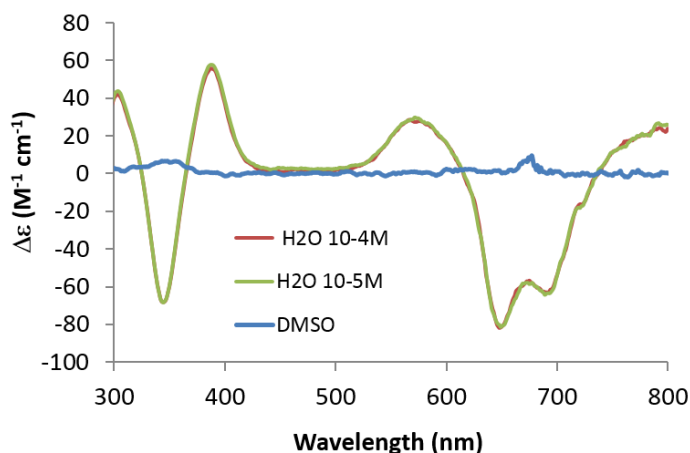

Fig. 3. CD trace of Pc in water at different concentrations and in DMSO.

DOSY was employed to confirm the formation of Pc self-assembled objects and their average nanometer-size. Two different measurements were carried out at the same concentration (5 mM) using as solvents DMSO-d^6 and D_2O. The diffusion coefficients of Pc in DMSO-d^6 and D_2O were determined to be 1.98 x 10^{-10} and 1.61 x 10^{-10} m^2 s^{-1}, corroborating the formation of aggregates of Pc in water media. By using the Stokes–Einstein equation, these diffusion coefficients were converted to an approximate hydrodynamic radius of the monomers in DMSO (0.56 nm) and H_2O (1.36 nm) for the discrete objects of spherical size (see ESI, Fig. S1 and S2). These results are in good agreement with previous the UV and CD experiments showing molecularly dissolved state of Pc in DMSO and the formation of small one dimensional supramolecular helical aggregates in water media, where, interestingly an increment in the viscosty of the samples was observed.

In order to have a better understanding of the stability of these Pc aggregates, specific interactions like metal-ligand coordination were used to shift the equilibrium to the formation of the monomer using pyridine (Py) as a zinc ligand. A clear shift of the absorption Q-band from 645 nm (aggregated) to 686 nm (molecularly dissolved) was only observed by UV-vis after a very large excess of Py was added (Figure 3a),

whereas in the CD spectra a completely loss of Cotton effect was observed, confirming the disruption of the helical columnar aggregates. A sharp transition was observed when adding Py to the aggregated Pc, indicating the probable cooperative polymerization mechanism [36] of the Pc monomers in water (Figure 3b), which points to the formation of aggregates larger than dimers.
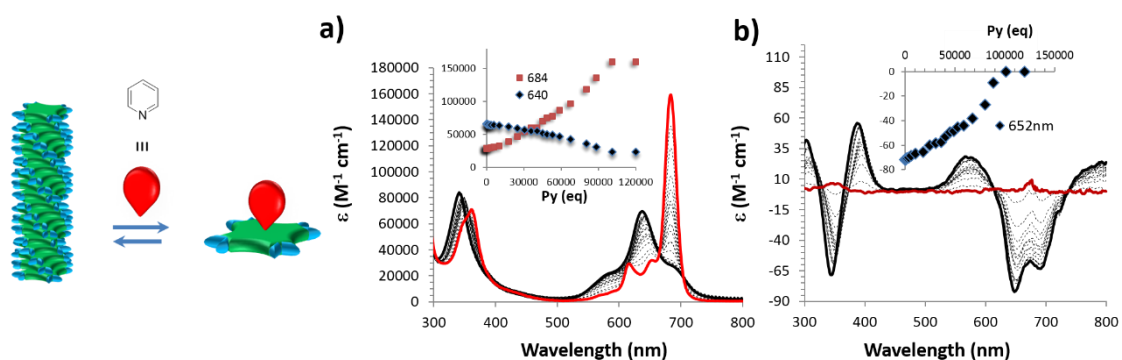
Fig 4. Cartoon of disassembly process of Pcs in water upon addition of Py a) UV and b) CD traces of a titration of Pc ([Pc] = 10^{-4} M) with Py. Insets: a) absorption changes at 684 and 640 nm and b) CD change at 652 nm.

It has been shown that Zn-porphyrins derivatives bearing a Py molecule coordinated to the axial position cannot assemble into hydrogen-bonded stacks in methylcyclohexane. Since the binding constant of the Py with the porphyrin is smaller than the porphyrin with itself, the formation of the stacks can be induced by dilution of the system. [37] A similar behavior could be foressen for this system in water: the axial coordination of a nitrogenated ligand to the zinc should promote the disassembly of the stacks which should reassemble by dilution of the system. As expected, a lower concentration of Pc in water requires a higher excess of Py to completely shift the equilibrium, the excess of Py used for a 10^{-5} M solution of Pc being of 69% v/v. To ensure that the shifting of the equilibrium is due to axial coordination and not to the change in polarity of the solvent mixture, a 10^{-5} M solution of Pc was prepared in a mixture of water and 69% v/v of EtOH, a solvent that has the same polarity than Py. The UV traces recorded for this solution are superimposable with those taken in pure water, which is indicative of a fully aggregated Pc system (see ESI, S2).

Finally, the equilibrium was shifted back to the formation of the stacks by dilution of the system. A 10^{-4} M solution of molecularly dissolved Pc in water and 81% of Py was diluted with water and monitored by UV-vis and CD (Figure 3a, b). The fully aggregated state was reached after diluting the system only ten times as a consequence of the high stability of these aggregates in water.
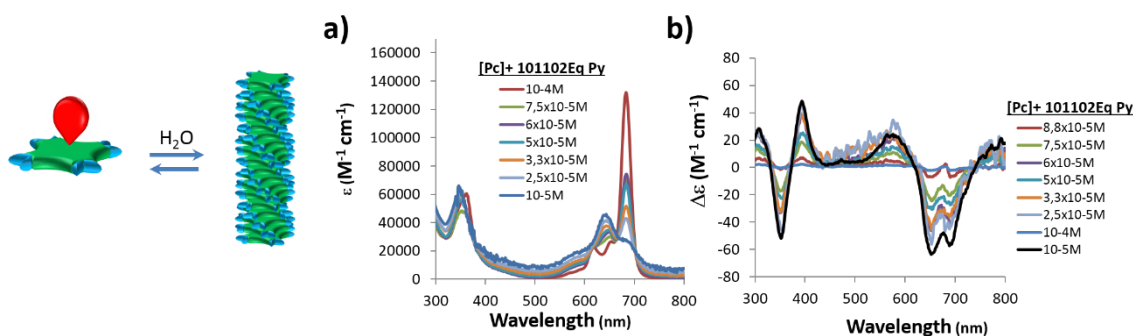

Fig. 5. Cartoon of dilution-induced self-assembly of Pcs in the presence of Py. a) UV-vis and b) CD traces of a dilution experiment in which a 10^{-4} M solution of molecularly dissolved Pc was diluted with water.

CONCLUSIONS

The self-assembly in water of α-D-Glucose octafunctionalized phtalocyanine (Pc) has been meticulously investigated. The nature of the aggregates has been assessed by different techniques, observing the formation of stable one-dimensional helical columnar aggregates by π-π stacking of the hydrophobic cores, whose helicity is biased by the chirality of glucose substituents. The formation of dynamic helical supramolecular polymers in water decorated with saccharides at the periphery together with the excellent singlet oxygen quantum yields shown by Pc [34] can potencially result in a new, and specific multivalent system for PDI treatment.

TOC
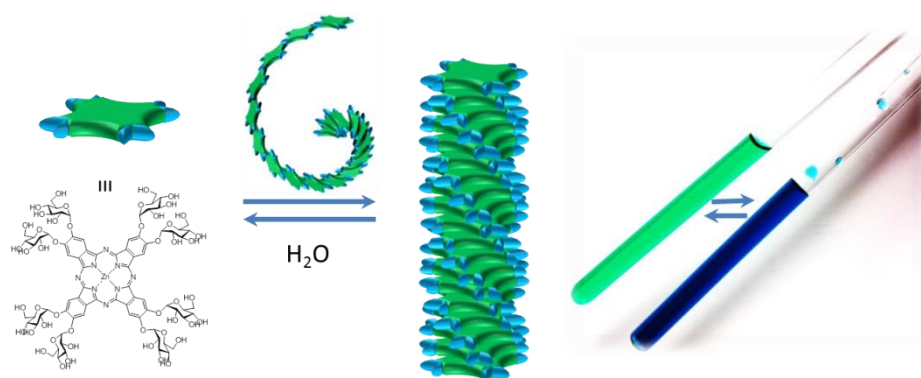
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REFERENCES

1. Van Nostrum CF, Nolte RJM. *Chem. Commun.* 1996, 2385-2392.
2. Elemans JAAW, van Hameren R., Nolte RJM, Rowan AE. *Adv. Mater.* 2006, **18**, 1251-1266.
3. González-Rodríguez D, Schennin APHJ. *Chem. Mater.* 2011, **23**, 310-325.
4. Yagai S, Goto Y, Lin X, Karatsu T, Kitamura A, Kuzuhara D, Yamada H, Kikkawa Y, Saeki A, Seki S. *Angew. Chemie Int. Ed.* 2012, **51**, 6643-6647.
5. Martínez-Díaz MV, Torres T. In Handbook of Porphyrin Science; Kadish KM, Smith KM, Guilard R, Eds.; World Scientific: Singapore, 2010; Vol. 10, Chapter 45.
6. Fukuzumi S. In Handbook of Porphyrin Science; Kadish KM, Smith KM, Guilard R.Eds.; World Scientific: Singapore, 2010; Vol. 10, Chapter 46.
7. Martínez-Díaz MV. de la Torre G, Torres T. *Chem. Commun.* 2010, **46**, 7090−7108.
8. Bottari G, Trukhina O, Ince M, Torres T. *Coord. Chem. Rev.* 2012, **256**, 2453−2477.

9. (a) García-Iglesias M, Cid J.-J, Yum J–H, Forneli A, Vázquez P, Nazeeruddin MK, Palomares E, Grätzel M, Torres T. *Energy Environ. Sci.* 2011, **4**,189-194.

10. Ragoussi ME, Cid JJ, Yum JH, de la Torre G, Di Censo D, Grätzel M, Nazeeruddin MK, Torres T. *Angew. Chem. Int. Ed.* 2012, **51**, 4375-4378.

11. Ragoussi ME, Yum JH, Chandiran AK, Ince M, de la Torre G, Grätzel M, Nazeeruddin MK, Torres T. *ChemPhysChem* 2014, **15**, 1033-1036.

12. De la Torre G, Vazquez P, Agullo-Lopez F, Torres T. *J. Mater. Chem.* 1998, **8**, 1671-1683.

13. Chen Y, Hanack M, Blau W, Dini D, Liu Y, Lin Y, Bai J. *J. Mater. Sci.* 2006, **41**, 2169-2185.

14. Engelkamp H, Middelbeek S, Nolte RJM. *Science* 1999, **284**, 785-788.

15. Kimura M, Ueki H, Ohta K, Shirai H, Kobayashi N. *Langmuir* 2006, **22**, 5051-5056.

16. Sun R, Wangv L, Tian J, Zhang X, Jiang J. *Nanoscale* 2012, **4**, 6990-6996.

17. Adachi K. Chayama K. Watarai H. *Chirality* 2014, **26**, 553-562.

18. García-Iglesias M, Torres T, González-Rodríguez D. *Chem. Commun.* 2016, **52**, 9446-9449.

19. Brinkmann J, Cavatorta E, Sankaran S, Schmidt B, van Weerd J, Jonkheijm P. *Chem. Soc. Rev.* 2014, **43**, 4449-4469.

20. Mikkilä J, Anaya-Plaza E, Liljeström V, Caston JR, Torres T, de la Escosura A, Kostiainen MA. *ACS Nano* 2016, **10**, 1565-1571.

21. Moreira LM.; Vieira dos Santos F, Lyon JP, Maftoum-Costa M, Pacheco-Soares C, Soares da Silva N. Australian Journal of Chemistry, 2008, **61** , 741–754.

22. Guo Y, Feinberg H, Conroy E, Mitchell DA, Alvarez R, Blixt O, Taylor ME, Weis WI, Drickamer K. *Nat Struct Mol Biol* 2004, **11**, 591-598.

23. Imperiali B. *J. Am. Chem. Soc.* 2012, **134**, 17835-17839.

24. Fasting C, Schalley CA, Weber M, Seitz O, Hecht S, Koksch B, Dernedde J, Graf C, Knapp EW, Haag R. *Angew. Chemie Int. Ed.* 2012, **51**, 10472-10498.

25. Cecioni S, Imberty A, Vidal S. *Chem. Rev.* 2015, **115**, 525-561.

26. Muñoz A, Sigwalt D, Illescas BM, Luczkowiak J, Rodríguez-Pérez L, Nierengarten I, Holler M, Remy JS, Buffet K, Vincent SP, Rojo J, Delgado R, Nierengarten JF, Martín N. *Nat Chem* 2016, **8**, 50-57.

27. Singh S, Aggarwal A, Bhupathiraju NVSDK, Arianna G, Tiwari K, Drain CM. *Chem. Rev.* 2015, **115**, 10261-10306.

28. Albertazzi L, Martinez-Veracoechea FJ, Leenders CMA, Voets IK, Frenkel D, Meijer EW. *Proc. Natl. Acad. Sci.* 2013, **110**, 12203-12208.

29. Krieg E, Bastings MMC, Besenius P, Rybtchinski B, *Chem. Rev.* 2016, **116** , 2414 - 2477.

30. Liu GF, Zhang D, Feng CL, *Angew. Chemie Int. Ed.* 2014, **53**, 7789-7793.

31. Marchesan S, Easton CD, Styan KE, Waddington LJ, Kushkaki F, Goodall L, McLean KM, Forsythe JS, Hartley PG. *Nanoscale* 2014, **6**, 5172-5180.

32. Iqbal Z, Lyubimtsev A, Herrmann T, Hanack M, Ziegler T. *Synthesis* 2010,**18**, 3097-3104.

33. Lyubimtsev A, Iqbal Z, Crucius G, Syrbu S, Taraymovich ES, Ziegler T, Hanack M. *J. Porphyr. Phthalocyanines* 2011, **15**, 39-46.

34. Iqbal Z, Masilela N, Nyokong T, Lyubimtsev A, Hanack M, Ziegler T. *Photochem. Photobiol. Sci.* 2012, **11**, 679-686.

35. Li D, Kagan G, Hopson R, Williard PG. *J. Am. Chem. Soc.* 2009, **131**: 5627-5634.

36. Korevaar PA, Schaefer C, de Greef TFA, Meijer EW. *J. Am. Chem. Soc.* 2012, **134**, 13482-13491.

37. Helmich F, Lee CC, Nieuwenhuizen MML, Gielen JC, Christianen PCM, Larsen A, Fytas G, Leclère PELG,Schenning APHJ, Meijer EW. *Angew. Chemie Int. Ed.* 2010, **49**, 3939-3942.