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Synthesis, optical absorption and photophysical properties of cone-shaped subnaphthalocyanine †

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The first photophysical studies of a member of the family of subnaphthalocyanines are described. The cone-shaped unsubstituted subnaphthalocyanine, synthesized in 35% yield, provides it with distinctive photophysical properties that are better than those of the related planar phthalo- and naphthalocyanines. SubNc absorbs in the red part of the spectrum, has substantial fluorescence, $\Phi_F = 0.22$, and, significantly for photodynamic therapy applications, triplet and singlet oxygen quantum yields, $\Phi_T = \Phi_\Delta = 0.68$, that are substantially higher than those of the related phthalo- and naphthalocyanines. These results, together with their synthetic availability, high solubility, and low tendency to aggregate, make this new class of sensitisers amenable to being further studied with a view to finding photodynamic therapy applications.

Introduction

Photodynamic therapy is an emerging cancer treatment based on the oxidative action of reactive oxygen species generated by irradiation with visible-light of a drug accumulated in the tumoral tissue.[1] The only drug approved so far for this purpose in North America, Japan and the European Union is Photofrin, a purified mixture of porphyrins derived from haematoporphyrin and possessing seemingly poor chemical and photophysical properties, namely an uncertain composition and low absorption coefficients in the red part of the spectrum. The development of second generation compounds with improved properties is currently the subject of major research efforts. Porphyrin analogues such as phthalocyanines, naphthalocyanines, porphyrazines, chlorins, sapphyrins, texaphyrins, purpurins, and porphycenes, to name just a few, are being considered for this purpose.[2] These compounds share substantial planarity in their basic skeleton, a feature that leads to stacking in biological media with concomitant loss of photosensitising effectiveness. Very recently, a new class of phthalocyanine-related compounds,[3] the so-called subphthalocyanines (SubPcs),[4] have been described. While originally used as intermediate materials for the synthesis of unsymmetrically-substituted phthalocyanines,[5] SubPcs' non-planar, cone-shaped structure[4c,6] provides them with distinctive properties. Thus, SubPcs are far more soluble than their phthalocyanine analogues and aggregate to a much lesser extent. In addition, they have rich, non-linear optical and photophysical properties.[7,8] On the other hand, SubPcs are transparent to light above 600 nm, the region of highest tissue penetration and hence of use for photodynamic therapy applications. Hence, SubPc analogues with absorption spectra extended into the red region could meet both the chemical and photophysical requirements for photodynamic therapy. One group of such analogues, the subnaphthalocyanines (SubNcs, Fig. 1), were first reported in 1995,[6b] though the low yields obtained in their preparation have precluded a systematic exploration of their properties.[4b,9] Two recent papers have reported an improved synthesis of these compounds, comparable in yield to the one described in this work, along with their structural characterisation and some theoretical calculations.[10]

In this work we report the photophysical properties of the unsubstituted SubNc ring and compare them to those of related phthalo- and naphthalocyanines. While the effectiveness of any new drug cannot be assessed from its photophysical properties, its determination is nevertheless required in order to judge its potential and can thus be used as a screening tool before undertaking cytotoxicity studies. In addition, these studies are of fundamental value in increasing our understanding of the excited-state behaviour of non-planar aromatic

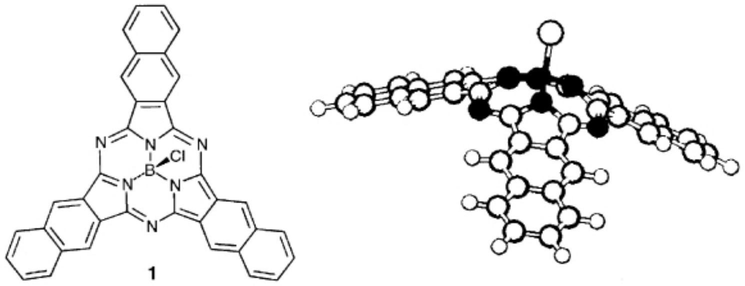

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Fig. 1 Three-dimensional structure of SubNc as modelled by the semiempirical AM1 and PM3 methods.

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† Further data (in graphical format) are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p2/b0/b000633p/

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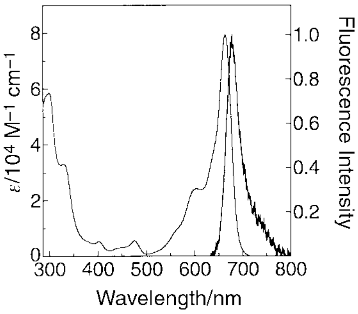

Fig. 2 Ground-state absorption and fluorescence spectra (left: absorption; right: fluorescence).

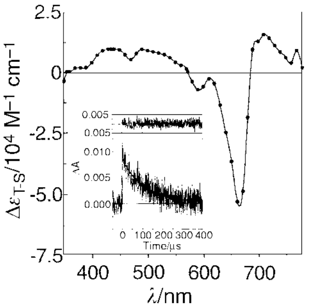

Fig. 3 Triplet-minus-singlet absorption spectrum in argon-saturated toluene ($\lambda_{exc} = 355$ nm). Inset: typical triplet absorption signal ($\lambda_{obs} = 474$ nm) and residuals of the monoexponential fit.

systems, and should facilitate the development of new lead compounds with tailored photophysical properties.

Results and discussion

Subnaphthalocyanine **1**[9] ‡ was obtained in good yield (35%) by reaction of 1,2-dicyanonaphthalene with boron trichloride using a mixture of chlorobenzene and toluene as solvent at 130 °C. While this reaction is not new, the chromatographic purification process described in the Experimental section is critical in order to obtain a pure compound. The product was characterised by the usual spectroscopic techniques (^{1}H-NMR, UV-vis, IR, MS-EI) and the purity checked by elemental analysis. Geometry optimisation at the semiempirical AM1 and PM3 levels predicts that this compound has indeed a cone-shaped geometry (Fig. 1), in agreement with X-ray data.[10a] This is consistent with its high solubility and lack of aggregation in toluene, unprecedented for the planar non-substituted phthalocyanine and naphthalocyanine compounds. The photophysical properties have been characterised following standard protocols described in the Experimental section.[7b] Similar to other pyrrole-based macrocycles, SubNc has an absorption spectrum with bands in the UV (Soret) and visible (Q) regions. Its lowest-energy maximum is at 663 nm ($\varepsilon = 7.94 \times 10^4$ M^{-1} cm^{-1}; *cf.* Fig. 2). The Beer–Lambert plots are linear over at least three orders of magnitude in concentration, thus ruling out the formation of aggregates. Again, this behaviour is strikingly different from that of planar unsubstituted naphthalocyanines and phthalocyanines, which have a high tendency to form aggregates by π–π stacking. Cone-shaped SubNcs are thus advantageous, since aggregation results in the loss of photosensitization ability as new, efficient internal conversion pathways arise.

SubNc fluoresces with quantum yield $\Phi_F = 0.22$, the maximum fluorescence being at 677 nm, and has a singlet lifetime of $\tau_S = 2.5$ ns (Fig. 2). Oxygen had no effect on these values up to 1 atm pressure. Kobayashi *et al.* have reported substantially different results for SubNc, *i.e.*, $\Phi_F = 0.094$ and $\tau_S = 1.93$ ns.[10b] However, their emission spectrum is quite different from ours and their excitation spectrum is also quite different from the absorption spectrum, which suggests the presence of an impurity in their sample. As stated above, chromatographic purification is essential to obtain SubNc with high purity. From the lifetime and the quantum yield, the radiative rate constant is readily calculated as $k_F = \Phi_F/\tau_S = 8.8 \times 10^7$ s^{-1}, a value very similar to that found for aluminium phthalocyanine, $k_F = 8.5 \times 10^7$ s^{-1} ($\Phi_F = 0.58$), and aluminium naphthalocyanine tetrasulfonate, $k_F = 8.0 \times 10^7$ s^{-1} ($\Phi_F = 0.20$).[11]

From fluorescence quenching by 1-iodopropane, with rate constant $k_q^{Ipr} = 1.47 \times 10^8$ M^{-1} s^{-1}, we estimate the singlet-to-lowest-triplet energy gap, $\Delta E_{ST_n} = 42$ kJ mol^{-1}, using the correlation described by Dreeskamp *et al.*[12] This value is significantly lower than those observed for phthalocyanines and naphthalocyanines, which are typically in the range 55–70 kJ mol^{-1},[11,13] and is responsible for the high triplet and singlet oxygen quantum yields observed for SubNc. From ΔE_{ST_n}, the energy of the triplet state reached by intersystem crossing is calculated as $E_{T_n} = 137$ kJ mol^{-1}. This value poses an upper limit for the T$_1$ energy.

Using laser-induced optoacoustic calorimetry, we find an enthalpy change upon triplet formation of $\Phi_T E_T = 88.2$ kJ mol^{-1}, which, combined with the above triplet energy value sets a lower limit for the triplet quantum yield of $\Phi_T \geqslant 0.64$. In addition, $\Phi_T \leqslant 0.78$ as deduced from the fluorescence data ($\Phi_T \leqslant 1 - \Phi_F$). Again, this value is remarkably high compared to those of phthalocyanines and naphthalocyanines, *e.g.*, $\Phi_T = 0.4$ for chloroaluminium phthalocyanine[11a] and $\Phi_T = 0.20$ for bis(tri-*n*-hexylsiloxy)silicon 2,3-naphthalocyanine.[13] Most likely, this reflects the low singlet–triplet energy gap, which is expected to facilitate intersystem crossing according to the energy-gap law. Small singlet–triplet energy gaps in combination with high triplet quantum yields are also encountered in other non-planar aromatic molecules such as the related subphthalocyanines ($\Phi_T = 0.62$ and $\Delta E_{TS} = 34$ kJ mol^{-1})[7b] and the C$_{60}$ buckminsterfullerene ($\Phi_T = 1$ and $\Delta E_{TS} = 36$ kJ mol^{-1}).[14]

The triplet SubNc has a lifetime of $\tau_T = 95$ µs, as determined by laser flash photolysis. The transient absorption decay was strictly monoexponential at low laser fluences though a second component could be increasingly observed at higher fluences, probably reflecting the onset of triplet–triplet annihilation processes. The differential triplet–singlet absorption spectrum is shown in Fig. 3 and has its absolute maximum at 711 nm with differential absorption coefficient $\Delta\varepsilon_{TS} \leqslant 1.62 \times 10^4$ M^{-1} cm^{-1}.

The triplet lifetime is long enough to produce a rich bimolecular chemistry. Significantly for photodynamic therapy applications, energy transfer from the triplet state to molecular oxygen occurs with the rate constant $k_q^{O_2} = 2.5 \times 10^9$ M^{-1} s^{-1} and singlet molecular oxygen, O$_2$($^1\Delta_g$), is produced with quantum yield $\Phi_\Delta = 0.68$. This reduces the uncertainty in the Φ_T value to $0.68 \leqslant \Phi_T \leqslant 0.78$. The consideration that in the related subphthalocyanines the rate constant for fluorescence is very similar to the one we observe for SubNc (7.6×10^7 s^{-1} and 8.8×10^7 s^{-1}, respectively) allows us to suggest that internal conversion may also be similar for the two compounds. We therefore assume for our SubNc the same internal conversion rate constant as for SubPc,[7b] *i.e.*, $k_{ic} = 4.0 \times 10^7$ s^{-1} and calculate that $\Phi_T = 1 - \Phi_F - k_{ic}\tau_S = 0.68$, consistent with the Φ_Δ value. As a further check, we notice that this Φ_T value in turn implies that the efficiency for energy transfer from ^{3}SubNc to oxygen, the so-called S_Δ parameter,[15] is close to 1, as we found for SubPc.[7b] Thus, the rate constant for intersystem crossing is readily calculated as $k_{isc} = \Phi_T/\tau_S = 2.7 \times 10^8$ s^{-1}. For comparison $k_{isc} = 5.9 \times 10^7$ s^{-1} for chloroaluminium phthalocyanine[11a] and $k_{isc} = 7.7 \times 10^7$ s^{-1} for bis(tri-*n*-hexylsiloxy)silicon 2,3-naphthalocyanine,[13] consistent with the larger singlet–triplet energy gaps in these molecules. In combination with the optoacoustic result for $\Phi_T E_T$, the $\Phi_T = 0.68$ value yields the T$_1$ energy as $E_T = 130$ kJ mol^{-1} and $\Delta\varepsilon_{TS} = 1.53 \times 10^4$

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‡ Compound **1** is now commercially available (Aldrich®) but with low purity (~75%).

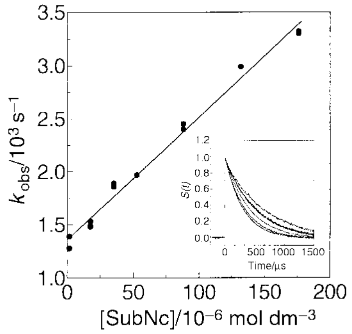

Fig. 4 Stern–Volmer plot for the quenching of $O_2(^1\Delta_g)$ by SubNc in deuterated benzene. The rate constant k_q^{SubNc} was determined from the equation $k_{obs} = k_\Delta(0) + k_q^{SubNc}[SubNc]$, where $k_\Delta(0)$ is the $O_2(^1\Delta_g)$ decay rate constant observed at infinite dilution. Inset: reduction of the $O_2(^1\Delta_g)$ lifetime ($1/k_{obs}$) upon increasing the SubNc concentration.

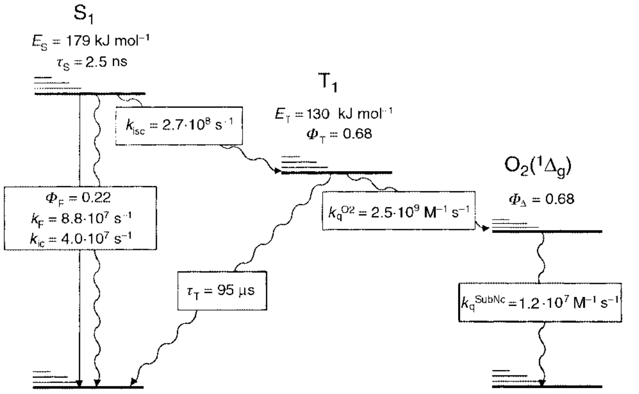

Fig. 5 Summary of the photophysical properties of SubNc.

$M^{-1}\ cm^{-1}$ from the $\Phi_T\Delta\varepsilon_{TS}$ value obtained by laser flash photolysis. Finally, we find that SubNc is a moderate $O_2(^1\Delta_g)$ quencher in perdeuterated benzene, with $k_q = 1.2 \times 10^7\ M^{-1}\ s^{-1}$, the quenching being essentially physical (Fig. 4). No photobleaching could be observed, which leads to an upper photobleaching quantum yield of *ca.* 10^{-4}. The photophysical properties are summarised in Fig. 5.

To summarise, the cone-shape structure of SubNc provides a chromophore with distinctive photophysical properties that compare favourably to those shown by the related planar phthalo- and naphthalocyanines. In particular, SubNcs have higher triplet and singlet oxygen quantum yields, which, combined with their synthetic availability, high solubility and low tendency to aggregate, makes them amenable to cytotoxicity studies to explore their use for photodynamic therapy applications.

Experimental

Chemicals

Toluene and benzene were of spectroscopic grade (SDS). Benzene-d_6 (>99.6%) was also from SDS. Zinc(II) phthalocyanine, benzophenone, 2-hydroxybenzophenone, phenalenone, and 1-iodopropane were purchased from Aldrich and used as received. Argon (>99.9%) and oxygen (>99.9%) were from Abello-Linde.

Preparation of subnaphthalocyanine 1

Dried dicyanonaphthalene (10 mmol, 1.78 g) was suspended in a 1 : 1 mixture of chlorobenzene and toluene (5 ml) under argon. Then BCl_3 (10 mmol, 1.29 g, 0.83 ml) previously condensed at −78 °C was added through a cannula with the help of a slightly positive argon pressure. The mixture was stirred for 10 minutes at rt and then heated at 130 °C for 2 hours. After cooling, the solvent was partially removed and the precipitate was washed with copious amounts of hexane using a soxhlet extractor for 24 hours and the resulting subnaphthalocyanine was purified by column chromatography in silica gel using toluene as solvent to give 670 mg (35%) of compound **1**. Mp = 279–281 °C. ^{1}H-NMR (200 MHz, toluene-d_6) δ 8.1 (m, 12H), 9.42 (s, 6H) ppm; MS–EI *m*/*z* 580; IR (KBr) ν 3399, 3182, 3055, 1720, 1441, 1253, 1141, 1110, 797 cm^{-1}; UV-vis in toluene [λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$)]: 663 (79400), 602 (24700), 475 (5970), 402 (5750), 328 (33500), 299 (58000).

Photophysical measurements[7b]

Unless stated otherwise, all photophysical measurements were carried out in toluene. Absorption spectra were recorded on a Varian Cary 4 spectrophotometer. Absorption coefficients were calculated from the slopes of the Beer–Lambert plots in the concentration range 0.5–50 µM.

Fluorescence spectra were recorded on a Shimadzu RF-540 spectrofluorometer. The fluorescence quantum yield was determined by comparing the areas under the emission curves of a series of optically-matched solutions of SubNc and zinc(II) phthalocyanine, for which a $\Phi_F = 0.30$ was assumed.[11a] The absorbance of these solutions at the excitation wavelength was kept in the range 0.005–0.050. The singlet lifetime was determined by time-correlated single photon counting on an Edinburgh FL900 fluorescence lifetime system. The singlet-to-next-lowest-triplet energy gap, ΔE_{ST_n}, was determined by the method of Dreeskamp *et al.*, from fluorescence quenching by 1-iodopropane.[12] The triplet lifetime, absorption spectrum, and rate constant for quenching by oxygen were determined by nanosecond laser flash photolysis, using a Continuum Surelite Nd-YAG laser for excitation at 355 nm (5 ns pulsewidth, less than 5 mJ per pulse). Photoinduced absorbance changes were monitored at 90 degrees by an analyzing beam produced by a Xe lamp (PTI, 75 W), in combination with a monochromator–photomultiplier. The details of this apparatus have been given elsewhere.[7b] The triplet-minus-singlet absorption coefficients were determined by the comparative method of Bensasson *et al.*[16] using benzophenone as a standard in benzene, for which $\Delta\varepsilon_{TS} = 7220\ M^{-1}\ cm^{-1}$ at 525 nm was assumed.[17] The rate constant for oxygen quenching, $k_q^{O_2}$, was determined from Stern–Volmer analysis of the triplet lifetime in argon-, air-, and oxygen-saturated solutions.

The triplet quantum yield was determined by laser-induced optoacoustic calorimetry (LIOAC) on argon-saturated solutions, using a ceramic piezoelectric transducer to sense the pressure wave induced by the non-radiative relaxation events leading to the production of the triplet state.[18] With the aid of a calorimetric reference that releases all absorbed energy as heat (2-hydroxybenzophenone, 2HBP), the energy stored in the photoproduced triplets, $\Phi_T E_T$, could be readily determined by comparing the maximum amplitude of the optoacoustic waves for optically-matched solutions. The laser pulse energy was kept below 30 µJ in these experiments.

The quantum yield and rate constant for $O_2(^1\Delta_g)$ production and quenching, respectively, by SubNc, were determined by time-resolved near-IR emission spectroscopy based on a germanium detector (North Coast EO-817P).[19] The quantum yield was determined by the comparative method from the zero-time signal intensity using phenalenone as standard with $\Phi_\Delta = 0.92$.[19,20] The quenching rate constant was calculated from the effect of SubNc on the observed decay rate constant for $O_2(^1\Delta_g)$ in benzene-d_6. For the photobleaching studies, a 170 µM solution of SubNc in toluene was irradiated with 100 mJ laser pulses at 355 nm at 10 Hz for several periods of time, after which the absorption spectrum was checked.

Geometry optimizations were performed at AM1 and PM3 semiempirical levels implemented in the Hyperchem 5.1 program.

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References

1 (*a*) *Photodynamic Therapy of Neoplastic Disease*, ed. D. Kessel, CRC Press, Boca Raton, FL, 1990; (*b*) *Photodynamic Therapy. Basic Principles and Clinical Applications*, eds. B. W. Henderson and T. J. Dougherty, Marcel Dekker, New York, 1993.

2 A. Jasat and D. Dolphin, *Chem. Rev.*, 1997, **97**, 2267.

3 (*a*) *Phthalocyanines*: *Properties and Applications*, eds. C. C. Leznoff and A. B. P. Lever, VCH, Weinheim, 1989, 1993, 1996, vols. 1–4; (*b*) M. Hanack, H. Heckmann and R. Polley, *Methods of Organic Chemistry* (*HoebenWeyl*), ed. E. Schaumann, Georg Thieme Verlag, Stuttgart, 1998, vol. E 9d, p. 717.

4 (*a*) A. Meller and A. Ossko, *Monatsh. Chem.*, 1972, **103**, 150; (*b*) M. Geyer, F. Plenzig, J. Rauschnabel, M. Hanack, B. del Rey, A. Sastre and T. Torres, *Synthesis*, 1996, 1139; (*c*) M. K. Engel, J. Yao, H. Maki, H. Yonehara and C. Pac, *Rep. Kawamura Inst. Chem. Res.*, 1998, **9**, 53; (*d*) N. Kobayashi, R. Kondo, S. Nakajima and T. Osa, *J. Am. Chem. Soc.*, 1990, **112**, 9640; (*e*) N. Kobayashi, *J. Chem. Soc.*, *Chem. Commun.*, 1991, 1203.

5 (*a*) A. Sastre, B. del Rey and T. Torres, *J. Org. Chem.*, 1996, **61**, 8591; (*b*) A. Weitemeyer, H. Kliesch and D. Wöhrle, *J. Org. Chem.*, 1995, **60**, 4900; (*c*) A. Sastre, T. Torres and M. Hanack, *Tetrahedron Lett.*, 1995, **36**, 8501; (*d*) S. V. Kudrevich, S. Gilbert and J. E. van Lier, *J. Org. Chem.*, 1996, **61**, 5706.

6 (*a*) H. Kietaibl, *Monatsh. Chem.*, 1974, **105**, 405; (*b*) J. Rauschnabel and M. Hanack, *Tetrahedron Lett.*, 1995, **36**, 1629; (*c*) K. Kasuga, T. Idehara, M. Handa, Y. Ueda, T. Fujiwara and K. Isa, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 2559.

7 (*a*) A. Sastre, T. Torres, M. A. Díaz-García, F. Agullo-López, C. Dhenaut, S. Brasselet, I. Ledoux and J. Zyss, *J. Am. Chem. Soc.*, 1996, **118**, 2746; (*b*) B. del Rey, T. Torres, G. Rojo, F. Agullo-López, S. Brasselet, I. Ledoux, J. Zyss, S. Nonell and C. Martí, *J. Am. Chem. Soc.*, 1998, **120**, 12808; (*c*) G. Rojo, B. del Rey, F. Agulló-López and T. Torres, *Appl. Phys. Lett.*, 1997, 1802; (*d*) M. A. Díaz-García, F. Agulló-López, A. Sastre, T. Torres, W. E. Torruellas and G. I. Stegeman, *J. Phys. Chem.*, 1995, **99**, 14988.

8 R. A. Kipp, J. A. Simon, M. Beggs, H. E. Ensley and R. H. Schmehl, *J. Phys. Chem. A*, 1998, **102**, 5659.

9 Y. Kenichi and S. Yukio, *Kenkyu Hokoku-Kanto Gakuin Daigaku Kogakubu*, 1997, **40**, 215.

10 (*a*) J. R. Stork, R. J. Potucek, W. S. Durfee and B. C. Noll, *Tetrahedron Lett.*, 1999, **40**, 8055; (*b*) N. Kobayashi, T. Ishizaki, K. Ishii and H. Konami, *J. Am. Chem. Soc.*, 1999, **121**, 9096.

11 (*a*) J. R. Darwent, P. Douglas, A. Harriman, G. Porter and M. C. Richoux, *Coord. Chem. Rev.*, 1982, **44**, 83; (*b*) I. McCubbin and D. Phillips, *J. Photochem.*, 1986, **34**, 187.

12 H. Dreeskamp, E. Koch and M. Zander, *Ber. Bunsen-Ges. Phys. Chem.*, 1974, **78**, 1328.

13 P. A. Firey and M. A. J. Rodgers, *Photochem. Photobiol.*, 1987, **45**, 535; P. A. Firey, W. E. Ford, J. R. Sounik, M. E. Kenney and M. A. J. Rodgers, *J. Am. Chem. Soc.*, 1988, **110**, 7626.

14 J. W. Arbogast, A. P. Darmanyan, C. S. Foote, Y. Rubin, F. N. Diederich, M. M. Alvarez, S. J. Anz and R. L. Whetten, *J. Phys. Chem.*, 1991, **95**, 11.

15 A. A. Gorman, I. Hamblett and M. A. J. Rodgers, *J. Am. Chem. Soc.*, 1984, **106**, 4679; F. Wilkinson, W. P. Helman and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1993, **22**, 113.

16 R. V. Bensasson, C. R. Goldsmith, E. J. Land and T. G. Truscott, *Photochem. Photobiol.*, 1978, **28**, 277.

17 I. Carmichael and G. L. Hug, *J. Phys. Chem. Ref. Data*, 1986. **15**, 1.

18 S. E. Braslavsky and G. E. Heibel, *Chem. Rev.*, 1992, **92**, 1381.

19 C. Martí, O. Jürgens, O. Cuenca, M. Casals and S. Nonell, *J. Photochem. Photobiol. A: Chem.*, 1996, **97**, 11.

20 E. Oliveros, P. S. Murasecco, T. A. Saghafi, A. M. Braun and H. J. Hansen, *Helv. Chim. Acta*, 1991, **74**, 79; R. Schmidt, C. Tanielian, R. Dunsbach and C. Wolff, *J. Photochem. Photobiol. A*: *Chem.*, 1994, **79**, 11.