

CHEMISTRY A European Journal





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Spin-Orbit Charge Transfer Intersystem Crossing (SOCT-ISC) in Perylene-Bodipy Compact Electron Donor-Acceptor Dyads: ISC Mechanism and Application as Novel and Potent Photodynamic Therapy Reagents

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Abstract: Spin-orbit-charge-transfer intersystem crossing (SOCT-ISC) is useful for preparation of heavy atom-free triplet photosensitisers (PSs). Herein we prepared a series of perylene-Bodipy compact electron donor/acceptor dyads showing efficient SOCT-ISC. The photophysical properties of the dyads were studied with steady state and time-resolved spectroscopies. Efficient triplet state formation (quantum yield $\Phi_T = 60\%$) was observed, with triplet state lifetime ($\tau_T = 436 \ \mu s$) much longer than that accessed with the

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conventional heavy atom effect (62 μ s). The SOCT-ISC mechanism was unambiguously confirmed by direct excitation of the charge transfer (CT) absorption band using nanosecond transient absorption spectroscopy and time-resolved electron paramagnetic resonance (TREPR) spectroscopy. The factors affecting the SOCT-ISC efficiency include the geometry, the potential energy surface of the torsion, the spin density for the atoms of linker, solvent polarity, and the energy matching of the ¹CT/³LE states. Remarkably, these heavy atom-free triplet PSs were demonstrated as a new type of efficient photodynamic therapy (PDT) reagents (phototoxicity, EC₅₀ = 75 nM), with a negligible dark toxicity (EC₅₀ = 78.1 μ M) as compared to the conventional heavy atom PS (dark toxicity, EC₅₀ = 6.0 μ M). This study provides in-depth understanding of the SOCT-ISC, unveils the design principles of triplet PSs based on SOCT-ISC, and outlines their applications as a new generation of potent PDT reagents

Introduction

Efficient production of triplet excited states in organic compounds upon visible light photoexcitation is of particular interest, due to its application in photo-redox catalytic preparative organic reactions, $^{[1-5]}$ hydrogen production through photocatalytic water splitting, $^{[6-8]}$ photodynamic therapy, $^{[9-14]}$ and triplet-triplet annihilation upconversion (TTA-UC). $^{[15-17]}$ Due to the electron spin forbidden nature of the $S_1 \rightarrow T_n$ transition, it is tricky to access the triplet state of an organic chromophore directly upon photoexcitation. As the ISC process is a non-radiative process that obeys the Fermi Golden Rule (Eq.1 shows only the electron spin-orbital coupling term; the contribution from the vibrational coupling and vibrational overlap are not included), $^{[18]}$ nearly all the methods for enhancing ISC are based on intensifying the coupling of the S_1/T_n states or decrease their relative energy gap.

$$k_{\rm ISC} \propto \frac{\langle T_{\rm n} | H_{\rm SO} | S_1 \rangle^2}{\langle \Delta E_{\rm S_1-T_n} \rangle^2}$$
 (1)

The mostly used method to enhance ISC is based on the introduction of heavy atoms into the chromophores. However, limitations do exist for this strategy. For instance, the dark toxicity is a great concern for photodynamic therapy (PDT) with these heavy atom-containing molecules;^[10,19] moreover, the triplet

FULL PAPER

lifetime in these systems is generally shortened due to strong heavy atom effect,^[20,21] which is detrimental to the intermolecular electron or energy transfer initiated by the photoexcited PSs. Compared with short-lived photosensitisers, long-lived triplet photosensitisers have been proved to be more efficient in oxygen sensing,^[22] photocatalysis^[23] and PDT.^[24] Hence *heavy atom-free* PSs with long-lived triplet state are highly desired.

The traditional heavy atom-free triplet PSs undergo ISC through different mechanisms, for instance via the exciton coupling effect,[25] radical pair ISC (RP-ISC),[26-29] or with spin converter.^[30-32] In most cases the molecules relying on these mechanisms are synthetically demanding. The ISC can also be facilitated by the presence of low-lying $n\pi^* \leftrightarrow \pi\pi^*$ transitions (El-Sayed's Rule for ISC), as it happens for some aromatic carbonyl compounds,^[31,33,34] of which the molar extinction coefficients are usually small. Energy matched S₁/T_n states is another method to enhance ISC, and the representative example in this sense is anthracene.^[31,34] However, this strategy is elusive and subtle structure derivatization may break the delicate energy matching between S₁/T_n states, making this method unfeasible from a point of view of molecular structure design. As an alternative strategy, singlet fission attracted much attention recently, since one excited singlet can produce two triplet states, with a maximal triplet quantum yield of 200%,^[35,36] but the triplet state energy level obtained with this method, especially with visible light-harvesting chromophores, is low and it is unfavourable for further application based on intermolecular triplet energy transfer or electron transfer. Radical enhanced intersystem crossing (REISC) was also used to prepare heavy atom-free triplet PSs,^[37,38] but the relationship between the molecular structure (electron spin-spin exchange interaction) and the ISC efficiency is not clear, hence it is difficult to predict the efficiency of systems relying on this mechanism.^[39,40]

Another important approach, charge transfer (CT) induced ISC, has been known for decades. Since the formation of a long-lived charge separated state (CSS) is the principal step of natural photosynthesis and it is pivotal in solar energy conversion, it is vital to control the fate of CSS.^[41] The previously reported examples of CT-induced ISC are usually based on the RP-ISC mechanism. A long and rigid linker between the electron donor and acceptor is usually required, to minimize the electron coupling between the electron donor/acceptor, and reduce the electron exchange energy (J), which is the prerequisite of RP-ISC.[27-29] The initial event upon photoexcitation is charge separation (CS), which is a spin selective process, i.e., $S_0 \rightarrow {}^1LE \rightarrow {}^1CT$ transitions, with excited states in the same spin multiplicity, is dominant. Generally, the direct ${}^{1}CT \rightarrow {}^{3}CT$ transition is forbidden, due to the small coupling matrix elements of the two states.[42] However, if the distance between the radical anion and cation is large enough, the electron exchange energy can be extremely small, which results in extremely small ¹CT/³CT energy gap ($\Delta E_{ST} = 2J$. On cm⁻¹ scale).^[28,29] In this case the hyperfine interaction (HFI), i.e., the magnetic coupling between the electron spin and the magnetic nuclear spin, is able to enhance the electron spin flip/rephasing, facilitating the ${}^{1}CT \rightarrow {}^{3}CT$ ISC. Spin selective charge recombination (CR) from ³CT induces the formation of localized triplet states, given the ³LE state is lower in energy than the ³CT state.^[41,43] However, these molecular systems are

synthetically demanding because long and rigid linkers are required for the connection of electron donor and acceptors, to reduce the electron exchange energy.^[26–28,44,45] Moreover, hyperfine coupling enhanced ISC has slow kinetics, which is usually on the nanosecond time scale, hence the CR to the S₀ state will compete strongly with the RP-ISC, reducing the efficiency of triplet state generation.

On the other hand, preparation of compact donor/acceptor dyads, i.e. direct connection of the electron donor/acceptor, may make the synthesis easier. Unfortunately, shortening the linker between the electron donor and acceptor will increase the electronic coupling between the two units, and consequently increase the energy gap between the ¹CT and ³CT states, making the RP-ISC impossible.^[26]

It is clear that a general approach to design heavy atom-free triplet PSs with simple molecular structure but efficient ISC, is highly desired. Concerning this aspect, triplet PSs based on spinorbit charge transfer ISC (SOCT-ISC) have recently attracted much attention. For compact electron donor/acceptor dyads, ISC can be greatly enhanced given the electron donor and acceptor are in orthogonal geometry.^[42,46-49] The mechanism responsible for triplet generation in these systems is similar to the ISC involving $n\pi^* \leftrightarrow \pi\pi^*$ transitions. In this case, the CR process induces a change of molecular orbital angular momentum to compensate the change of electron spin angular momentum during ISC, hence greatly enhancing the formation of triplet states. Since CR is associated with a variation of orbital symmetry, this process is termed as SOCT-ISC.[42,46,47] Inspired by the simple molecular structures and efficient ISC, investigation on SOCT-ISC of compact electron donor/acceptor dyads with orthogonal geometry is booming during the last few years.[42,47-56]

Although a few studies have been recently reported concerning this mechanism, the SOCT-ISC is still in its infancy. Firstly, although a few electron donor-acceptor dyads able to undergo SOCT-ISC have been reported,[47-56] the interplay among different ISC mechanisms (e.g. SOCT-ISC, RP-ISC and others) remains unclear. Pulsed laser excited time-resolved electron paramagnetic resonance (TREPR) spectroscopy, a powerful method to distinguish the different ISC mechanisms by studying the electron spin polarization (ESP) of the triplet state, was rarely applied to study the SOCT-ISC mechanism.[47,53,54] Furthermore, the relationship between the molecular structure and the SOCT-ISC efficiency is still unclear. Recent studies indeed showed that the requirement of orthogonal geometry between electron donor and acceptor is actually non-sufficient for attaining efficient ISC.[48,50,59] However, factors that affect SOCT-ISC efficiency have not been studied in detail yet.[60,61]

One of the most appealing potential applications of the triplet PSs with efficient production of triplet states is PDT, a promising tumour treatment method, due to its non-invasive nature, spatiotemporal selectivity, and negligible drug resistant features.^[13] Traditional triplet PSs used in PDT are usually porphyrin or phthalocyanine compounds, which is easy to aggregate due to the large planar molecular structure.^[57,58] Bodipy, 'porphyrin's little sister', has been used to construct new generation of PDT regents.^[12] However, as Bodipy shows no ISC ability, most Bodipy-derived triplet photosensitisers have to

FULL PAPER



Scheme 1. Preparation of the compact electron donor/acceptor dyads. (i) *N*-Bromosuccinimide (NBS), DMF, stirring at rt for 20 h; (ii) Bis(pinacolato)diboron, potassium acetate, Pd(dppf)Cl₂, heating for 17 h at 70 °C, under N₂, yield: 46%; (iii) *o*-Dichlorobenzene, DMF, POCl₃, 100°C, 4 h, yield: 62%; (iv) For **BDP-Pery-1**: 2,4–dimethylpyrrole, TFA, DDQ, TEA, BF₃·Et₂O, stirring at rt, 4 h, under N₂, yield: 18%; for **BDP-Pery-2**: pyrrole, TFA, DDQ, TEA, BF₃·Et₂O, stirring at rt, 4 h, under N₂, yield: 23%; (v) *N*-lodosuccinimide (NIS), DCM, 30 min, stirring at rt, yield: 50%; (vi) Pd(PPh₃)₄, K₂CO₃, toluene/ethanol/water, heating at 90 °C for 2 h, under N₂, yield: 30%. (vii) ICl, DCM/MeOH, 2 h, stirring at rt, yield: 50%; (viii) Pd(PPh₃)₄, K₂CO₃, toluene/ethanol/water mixed solvent, 90 °C, 1 h, under N₂, yield: 20%.

contain heavy atoms. Concerning the toxicity and sharply reduced triplet lifetime induced by the heavy atom effect, heavy atom-free PDT reagents based on Bodipy are interesting but rarely reported. Previously an orthogonal Bodipy dimer showing efficient singlet oxygen production ability and was used as heavy atom-free PDT regents,^[62] but that ISC strategy doesn't work with larger π -conjugated Bodipy dimers showing red-shifted absorpion.^[63]

To address the above challenges, herein we prepared a series of perylene-Bodipy compact dyads with orthogonal and nonorthogonal geometry between the two units (Scheme 1). The triplet state quantum yields of the dyads were determined to be high ($\Phi_T = 60\%$) and the triplet state is long-lived ($\tau_T = 436 \ \mu$ s) for one dyad. Direct excitation into the CT absorption band gives solid evidence of charge transfer induced ISC. TREPR unambiguously confirms the SOCT-ISC mechanism through the analysis of the ESP of the triplet states. The factors that contribute to the SOCT-ISC efficiency are discussed. Finally, the heavy atom-free Bodipy triplet PSs based on SOCT-ISC, exemplified with BDP-perylene dyads, were used in the PDT study, demonstrating low dark toxicity and potent PDT activity, showing the advantage of this new type of potential PDT reagent over conventional heavy atom containing triplet PSs.

Results and Discussion

Molecular structure design and synthesis of the compact electron donor/acceptor dyads

Recently, a perylene-Bodipy dyad was reported to show charge transfer induced ISC,^[51,53] but the photophysical properties were not studied in detail, and the ISC mechanism was not confirmed with TREPR spectroscopy. These results prompted us to prepare perylene-Bodipy compact dyads with orthogonal and non-

orthogonal geometry to fully study the possibility of SOCT-ISC and the relationship among structure and ISC efficiency in these molecular systems (Scheme 1).

In order to control the molecular geometry, the pervlene moiety was directly connected at the meso-position of the Bodipy π -conjugation core (**BDP-Pery-1**). Due to the rotational steric hindrance exerted by the methyl groups at the 1,7-position of the Bodipy framework, the perylene and Bodipy moieties are expected to adopt an orthogonal geometry in BDP-Pery-1 (Scheme 1). The perylene moiety was also attached at the 2position of Bodipy (BDP-Pery-3), for which the extent of the conformation restriction is lower, thus the different linkage enables us to study the relationship between the molecular structure and the SOCT-ISC efficiency, as well as the molecular geometry-specific spin-selectivity of the ISC process. To tune the mutual orientation of the electron donor/acceptor and the magnitude of the electronic coupling between the Bodipy and the perylene moieties, we also designed the dyads BDP-Pery-2 and BDP-Pery-4 (Scheme 1), in which there are no methyl groups in the 1,3,5,7-position of the Bodipy, allowing for more torsional freedom, thus significant coplanarity and electronic coupling. It should be pointed out that difference between the current compact electron donor/acceptor dyads showing SOCT-ISC, with the purpose to produce localized triplet state, and those electron donor/acceptor dyads showing thermally activated delay fluorescence (TADF), is that the localized triplet state of the TADF molecules should be close to the ¹CT state (e.g. with an energy gap of a few dozens of meV). No such limitation exists for the SOCT-ISC electron donor/acceptor dyads.

The synthesis of the compounds is based on the routine derivatization chemistry of perylene and Bodipy (Scheme 1). The target compounds **BDP-Pery-1** and **BDP-Pery-2** are obtained

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Fable 1. Photophysical parameters of the compounds									
	Solvents ^[a]	$\lambda_{\rm abs}/{\rm nm}^{\rm [b]}$	<i>E</i> ^[C]	$\lambda_{\rm em}/{\rm nm}^{[d]}$	$\varPhi_{\text{F}}^{[e]}$	$\tau_T/\mu S^{[f]}$	$arPhi^{[g]}$	$arPsi_{T}^{[h]}$	
BDP-Pery-1	HEX	505	7.2	518	98.7	_[j]	_[]]	_0	
	TOL	509	6.0	526/555	61.9	215	0.18	0.24	
	THF	505	6.0	524/636	8.2	225	0.21	0.50	
	DCM	506	6.1	522/677	3.7	216	0.42	0.56	
	ACN	502	5.3	524/754	1.7	174	0.11	0.11	
BDP-Pery-2	HEX	502	4.6	596	38.1	54	0.1	0.37	
	TOL	504	4.7	690	6.7	436 ^[k]	0.31	0.60	
	THF	500	4.5	524/774	0.8	308	_0)	0.10	
	DCM	501	4.5	521	0.6	_0	_[]]	_[]]	
BDP-Pery-3	HEX	516	7.7	590	45.9	146	_0	0.09	
	TOL	519	7.0	627	16.7	99	_0)	0.14	
	THF	515	7.7	675	3.5	442	_0	0.09	
	DCM	517	7.2	717	1.0	309	_0	0.07	
BDP-Pery-4	TOL	573	2.2	782	0.3	_[j]	_0	_0	
BDP-1	TOL	503	8.8	515	90.0 ^[i]	_0	_0	_0)	
BDP-2	TOL	503	6.2	522	4.4'	_[i]	_[1]	0)	

[a] $E_{\rm T}(30)$ values of the solvents, HEX (31.0), TOL (33.9), THF (37.4), DCM (40.7) and ACN (45.6) for n-hexane, toluene, tetrahydrofuran, dichloromethane and acetonitrile, respectively. [b] Absorption wavelength. [c] Molar absorption coefficient ($10^4 \, {\rm M}^{-1} {\rm cm}^{-1}$). [d] Fluorescence emission wavelength. [e] Fluorescence quantum yield, with **BDP-1** ($\Phi_{\rm L} = 0.72$ in THF)^[65] and **BDP-2** ($\Phi_{\rm L} = 0.044$ in toluene)^[66] as the standards. [f] Triplet excited state lifetimes, $c = 5.0 \times 10^{-6} \, {\rm M}$. [g] Singlet oxygen quantum yield ($\Phi_{\rm A}$) with **iodo-BDP** as standard ($\Phi_{\rm A} = 0.87$ in DCM).^[67] [h] Triplet quantum yield, with **iodo-BDP** as standard ($\Phi_{\rm T} = 0.88$ in toluene).^[68] [i] Literature values.^[66,68] [j] Not observed. [k] Intrinsic triplet lifetime fitted by Eq. S12.



Figure 1. UV–Vis absorption spectra of (a) **BDP-Pery-1**, **BDP-Pery-2**, **BDP-1** and perylene; (b) **BDP-Pery-3**, **BDP-Pery-4** and **BDP-2**. $c = 1.0 \times 10^{-5}$ M in toluene, 20 °C.

with methods similar to the preparation of pristine Bodipy. Suzuki-Miyaura cross coupling reactions were used for preparation of **BDP-Pery-3** and **BDP-Pery-4**. All the target compounds were verified by ¹H NMR, ¹³C NMR, HR MS and elemental analysis.

UV–Vis absorption and fluorescence spectroscopies: observation of the CT aborption and emission bands

The absorption spectra of the compounds are presented in Figure 1. It is evident that the presence/absence of methyl substituents on the Bodipy influences the electronic coupling magnitude between Bodipy and perylene units, manifested by the CT bands at the red-end of the spectra.

In particular, for **BDP-Pery-1**, where the two moieties are expected to adopt an orthogonal conformation, the UV–Vis absorption is close to the sum of the spectra of pristine perylene and Bodipy. For **BDP-Pery-2**, however, a weak, broad CT absorption band is observed in the 535 nm – 635 nm range (Figure 1a),^[53] indicating the possibility of optical electron transfer (adiabatic electron transfer).^[64] The appearance of this band can be rationalized by the lack of the methyl groups at the 1,7-position of the Bodipy unit, allowing a more coplanar conformation, hence increasing the electronic coupling between the electron donor and acceptor.

For **BDP-Pery-3**, the absorption of the perylene unit is different from that of pristine perylene, and the absorption band of the Bodipy moiety is much broader than that of the pristine Bodipy, indicating an interaction between the two moieties at the ground state. This is reasonable because the conformation restriction ability of the methyl group in 1,3-position of the Bodipy is weaker

FULL PAPER

than that at 1,7-position. In case of **BDP-Pery-4**, the two moieties are expected to have more torsion freedom, the case is similar to the effect of π -conjugation on the absorption.



Figure 2. (a) Fluorescence emission spectra of **BDP-Pery-2** in different solvents: HEX (n-hexane); TOL (toluene); DCM (dichloromethane); THF (tetrahydrofuran); ACN (acetonitrile). Optically matched solutions were used in each panel (each of the solutions gives the same absorbance at the excitation wavelength, $c \approx 1 \times 10^{-5}$ M, variation of the concentration is necessary for preparation of optically matched solution). (b) Normalized fluorescence emission spectra of **BDP-Pery-2** in different solvents. $\lambda_{ex} = 480$ nm. 20 °C.

The solvent polarity-dependency of the fluorescence of the dyads was analysed (for **BDP-Pery-2**, see Figure 2). The common feature for all dyads is that the fluorescence is strongly quenched in polar solvents (Figure S1–S3 in the Supporting Information). This quenching of Bodipy fluorescence is not due to Förster resonance energy transfer (FRET), since the Bodipy unit (with lower energy level) is selectively excited, we assign it to the occurrence of electron transfer upon photoexcitation, which is supported by electrochemical measurements (Figure S5 and Table S2 in the Supporting Information).^[42,53] The photophysical properties of the studied compounds are summarized in Table 1.

Femtosecond transient absorption (fs-TA) spectroscopy: charge separation and charge recombination

In order to analyse the photophysical processes of the dyads in detail, fs-TA spectra of **BDP-Pery-2** were measured in different solvents (Figure 3). These measurements confirm that fast and efficient CS occurs upon photoexcitation, and that both the efficiency and kinetics of the process are influenced by the polarity of the solvent. In order to extract the kinetic information on the excited states evolution, the measured transient absorption data were analysed with global fitting using a sequential model with increasing lifetimes.^[69,70]

The evolution associated difference spectra (EADS) obtained for **BDP-Pery-2** in toluene are shown in Figure 3a. Excitation of the Bodipy moiety induces very fast charge separation, inferred by the first EADS obtained by global analysis (Figure 3a). Several negative bands appear below 450 nm, which are due to the bleaching of the perylene ground state, while the intense negative peak appearing at 500 nm corresponds to the ground state bleaching (GSB) of the Bodipy unit. A positive band at 550 nm is observed, which can be assigned to the absorption of perylene cation and Bodipy anion based on spectroelectrochemical results (Figure S6 and Figure S7 in the Supporting Information), hence confirming the occurrence of charge separation.^[47,71] The appearance of both perylene and Bodipy bleach signals and their respective cation and anion bands implies the occurrence of ultrafast charge separation (sub-picoseconds): the time constant for the evolution between the Bodipy local excited state (initially populated) and the charge separated state cannot be accurately determined, being within the time resolution of our instrument setup.

The lifetime of the CS state in toluene is quite long, as it can be inferred by examination of the kinetic traces presented in Figure 3c, showing that a significant amount of signal is still present at the longest pump-probe delay recorded, 1.5 ns. In comparison, the CS state of **BDP-Pery-2** decays fast in DCM, with the lifetime of the last spectral component of only 367.6 ps (Figure 3b), and the signal going almost to zero on the nanosecond timescale (Figure 3d). These results agree well with the triplet quantum yield of the dyad, which is higher in toluene than that in DCM (Table 1). The fs-TA results show that in medium/high polarity solvents such as DCM, CR to the ground state is very fast and significantly competes with the ISC, hence no triplet state is produced. This is also confirmed by the triplet state production of **BDP-Pery-2** in highly polar solvents using the triplet sensitization method (Figure S11 in the Supporting Information).



Figure 3. Evolution associated difference spectra (EADS) obtained from global analysis of transient absorption data recorded for the dyad **BDP-Pery-2** in (a) toluene and (b) DCM. $\lambda_{ex} = 490$ nm. Kinetic traces at representative wavelengths together with the fit obtained from global analysis are presented in (c) toluene and (d) DCM.

Nanosecond transient absorption (ns-TA) spectroscopy: ISC by excitation into CT absorption band as direct evidence for charge transfer-induced ISC

In order to study the triplet state production in the dyads upon photoexcitation, ns-TA spectra were recorded, exemplified by **BDP-Pery-2** (Figure 4). Upon selective photoexcitation into the

Bodipy unit, an excited state absorption (ESA) profile similar to the triplet state absorption of perylene is observed (Figure 4a). In addition, a negative band at 510 nm is visible, assigned to the GSB of the Bodipy unit. The GSB of the perylene is masked by the ESA bands, due to the smaller molar absorption coefficient of the ground state of perylene.

The triplet state lifetime determined by monitoring the decay trace at 470 nm (perylene ESA band) is 196 µs. The intrinsic triplet lifetime should be much longer than this value considering the contribution of triplet-triplet annihilation (TTA) quenching effect. The intrinsic triplet lifetime of **BDP-Pery-2** was fitted to be 436 μ s (for more details, see Figure S15 in the Supporting Information), which is substantially longer than that accessed with the conventional heavy atom effect (62 µs, intrinsic triplet lifetime, see Figure S12 and Figure S15 in the Supporting Information). Triplet PSs with heavy atoms usually have reduced triplet lifetimes, because the heavy atom effect increases the spin orbit coupling (SOC) of both S₁/T₁ and T₁/S₀ states. In stark contrast, in case of SOCT-ISC, the SOC of ¹CT/³LE is enhanced while the coupling of ³LE/S₀ is decreased, hence both high triplet quantum yield and long triplet lifetime are achieved, showing the advantage of SOCT-ISC over the heavy atom effect. This method may become a general, useful approach to extend the triplet state lifetimes of organic chromophores.



Figure 4. Nanosecond transient absorption spectra of **BDP-Pery-2** upon excitation at (a) $\lambda_{ex} = 512$ nm (S₀ \rightarrow ¹LE transition of Bodipy unit) and (c) $\lambda_{ex} = 555$ nm (S₀ \rightarrow ¹CT band). The corresponding decay curves monitored at 470 nm are presented in (b) and (d), respectively. The spectra were recorded in the co-linear measurement mode of the LP980 ns-TA spectrometer. $c = 5.0 \times 10^{-6}$ M in deaerated toluene, 20 °C.

The dyad was also selectively photo-excited into the CT absorption band (555 nm, Figure 4c). Interestingly, the ns TA spectra are similar to those obtained upon photoexcitation into the Bodipy moiety (Figure 4a). This is a direct evidence that the triplet state of the dyad **BDP-Pery-2** is produced via charge transfer. To the best of our knowledge, this is the first direct confirmation of

the SOCT-ISC mechanism in a compact dyad with excitation into a well-separated CT absorption band.

Time-resolved electron paramagnetic resonance (TREPR) spectroscopy: ISC mechanism, triplet state wave function confinement and geometry-dependency of the spin-selective ISC of the dyads

TREPR is a powerful tool to study the triplet excited states,^[72–78] and radical pairs in organic compounds.^[79] Zero-field splitting (ZFS) parameters *D* and *E* determined by TREPR give crucial information on the localization of the triplet state wavefunction and the rhombicity at triplet state. The magnitude of the electronic coupling of the radical pairs can be directly detected by TREPR.^[79] Moreover, this technique is able to provide information on spin-selectivity of the ISC, i.e. the population ratio of the magnetic sublevels (*T*_X, *T*_Y and *T*_Z) of the triplet state and the ESP pattern, which are specific for the different ISC mechanisms.^[42,46]

Up to now, only a limited number of molecular systems, which are able to form triplet states through SOCT-ISC, have been analysed by TREPR.^[42,46] In particular, for the recently reported Bodipy-derived electron donor/acceptor dyads showing SOCT-ISC, the TREPR spectroscopy was rarely applied to confirm the ISC mechanism.^[47–49,53,54] Herein we performed a detailed TREPR study of the compact donor/acceptor dyads (Figure 5).



Figure 5. TREPR spectra of (a) compounds 1 (3-bromoperylene) and iodo-BDP, (b) dyads BDP-Pery-1, BDP-Pery-2, BDP-Pery-3 and BDP-Pery-4. The spectrum of compound 1 was collected at ~3 μ s after laser flash (355 nm, ~10 mJ per pulse, 10 Hz repetition rate). The spectra of other compounds were collected at ~1 μ s after the laser flash (532 nm, 4 mJ per pulse, 10 Hz repetition rate). The red curves are computer simulations of the spectra of the triplet states with parameters given in Table 2. In frozen toluene, 85 K.

First, the TREPR spectra of the triplet state of the reference compounds were recorded (Figure 5a). For **iodo-BDP**, an (*e*, *e*, *e*, *a*, *a*, *a*) polarization pattern was observed, with the ZFS parameter |D| being 2960 MHz. For 3-bromoperylene (compound 1), an (*e*, *e*, *e*, *a*, *a*, *a*) polarization pattern was observed, with the ZFS parameter |D| = 1620 MHz. The simulated results are listed in Table 2. These results indicate that the *D* parameters of the triplet states of perylene and Bodipy chromophores are drastically different.

Table 2. Zero-Field Splitting parameters (*D* and *E*) and relative populations of the Zero-Field Spin states obtained from simulations of the triplet-state TREPR spectra of the compounds in a frozen toluene matrix at 85 K ^[a]

Compound	D /MHz	E /MHz	$A_X:A_Y:A_Z^{[b]}$	$ A_X - A_Y $: $ A_Z - A_Y $ ^[c]
1	1620	90	[1.0:0.9:0.85]	2.0
iodo-BDP	2960	640	[0.5:0.6:1.0]	0.25
BDP-Pery-1	1650	60	[0.9:0.9:1.0]	0/1
BDP-Pery-2	1650	60	[0.87:0.9:1.0]	0.30
BDP-Pery-3	1660	60	[0:1.0:1.0]	1/0
BDP-Pery-4	1560	80	[0.22:1.0:0.5]	1.56

[a] In all cases g-factors were $g = 2.000\pm0.003$. The approximate accuracies of zero-field splitting parameters are ± 20 MHz for *D*, and ± 10 MHz for *E*. [b] Representative populations of the zero-field spin states normalized to 1. [c] Unique ratios of populations, which determine the ESP.

The TREPR spectra of the triplet states of the compact dyads are shown in Figure 5b. For **BDP-Pery-2**, a polarization pattern (*a*, *a*, *a*, *e*, *e*, *e*) with a |D| value of 1650 MHz was observed. Similar values of |D| were found for all the other perylene-Bodipy dyads (~1600 MHz, Table 2), and all of them are drastically smaller than that for 2-iodoBDP (2960 MHz, Table 2), but similar to the |D| value of 3-bromoperylene (compound 1, 1620 MHz, Table 2). This implies that the triplet state of the dyads is mainly localized on the perylene moiety.

The spin selectivity of the ISC in electron donor-acceptor dyads can be studied through the analysis of the ESP of the triplet state.^[27,28,42,46] The ESP patterns observed for **BDP-Pery-3** (a, a, e, a, e, e) and **BDP-Pery-4** (e, a, e, a, e, a) are different from those for BDP-Pery-1 and BDP-Pery-2 (a, a, a, e, e, e). None of the four perylene-Bodipy dyads shows the footprint of RP-ISC, for which the ESP pattern should be (e, a, a, e, e, a) or (a, e, e, a, a, e), hence RP-ISC mechanism can be excluded for the dyads. Moreover, the ESP patterns of perylene-Bodipy dyads are also different from those of the reference 2-iodoBDP or 3bromoperylene (e, e, e, a, a, a), which indicates that the ISC mechanism is also different from the general spin-orbit ISC (SO-ISC, Figure 5a). As the RP-ISC mechanism is excluded for the current dyads by TREPR, and considering that there is no heavy atoms in perylene-Bodipy dyads and the orthogonal geometry of the electron donor/acceptor, the most probable ISC mechanism is the SOCT-ISC.[42,46]

Moreover, the triplet production by direct excitation into the CT absorption band in nanosecond transient absorption study, the solvent polarity-dependency of fluorescence emission (Figure 2a), the excitation spectrum (Figure S4 in the Supporting Information), the negative ΔG_{CS}° values in polar solvents in electrochemical studies (Table S2) and the observation of the radical cations and radical anions in femtosecond transient absorption study undoubtedly indicate that charge transfer is involved in the ISC of these perylene-Bodipy systems, and should be responsible for the triplet state production.

The solvent polarity dependency of triplet quantum yield (Table 1 and Scheme 2) is also characteristic of SOCT-ISC, but not of RP-ISC (the energy gap between ¹CT state and ³CT state will not be affected by solvent polarity, hence the triplet production

ability based on RP-ISC will not be highly dependent on the polarity of solvent). By combining various experimental evidence as mentioned above, we clearly demonstrate the occurrence of SOCT-ISC in the current dyads.

Photophysics and the factors contributing to SOCT-ISC efficiency

Based on all the aforementioned information collected with different spectroscopies, the photophysical processes occurring in the dyads upon photoexcitation can be summarized in Scheme 2. The factors that contribute to SOCT-ISC efficiency are discussed in the following sections.

Solvent polarity. The polarity of the solvent strongly affects the energy level of the CT state, which plays an important role in the SOCT-ISC efficiency. No triplet state is produced if the energy level of the CT state is too low. This happens to the compact dyad **BDP-Pery-2** in polar solvents, where the decay of the ¹CT to S₀ prevails over ISC, as confirmed by fs-TA (Figure 3d). This postulation was also confirmed by the intermolecular TTET sensitization using 2-iodoBDP (Scheme 1) as triplet PS and **BDP-Pery-2** as triplet acceptor in high polar solvent. In this case the ³LE state of **BDP-Pery-2** was observed in acetonitrile (Figure S11 in the SUPPorting Information). Hence the polarity of solvent is vital for the SOCT-ISC efficiency, as it determines the fate of charge recombination (¹CT→S₀ or ¹CT→³LE), by affecting the energy level of the ¹CT state.



Scheme 2. Photophysical processes involved in **BDP-Pery-2**. The energy levels of ¹CT state in hexane (HEX) and toluene (TOL) was determined according to the crossing point of CT absorption band and CT emission band, the energy level of ¹CT state in tetrahydrofuran (THF) and dichloromethane (DCM) was determined based on the energy difference of different solvents calculated from cyclic voltammogram. The triplet energy levels were obtained from TD-DFT calculation.^[53] For **BDP-Pery-2**, the ISC is most efficient in toluene.

Energy matching of the ¹CT and ³LE states. The triplet state producing ability is solvent dependent. For example, for **BDP-Pery-2**, the best solvent to attain high SOCT-ISC efficiency is toluene ($\Phi_T = 60\%$). As mentioned above, the polarity of solvent strongly affects the energy level of CT state. The energy level of ¹CT state in toluene is close to the ³LE state (Scheme 2). After examination of the other BDP-Perylene dyads, we propose that an energy matching of the ¹CT state and the ³LE state is beneficial for SOCT-ISC.^[80] This can explain the different ISC efficiency of **BDP-Pery-1** and **BDP-Pery-2** in the same solvent (such as in toluene). Due to the different electron withdrawing ability of the

Bodipy moiety of **BDP-Pery-1** and **BDP-Pery-2**, the two dyads have different energy levels of ¹CT state in toluene ($E_{CTS} = 2.0 \text{ eV}$ for **BDP-Pery-2**; $E_{CTS} = 2.4 \text{ eV}$ for **BDP-Pery-1**). The triplet energy levels of the two dyads are similar (~1.57 eV), hence a better ¹CT/³LE states energy matching contribute to a significantly higher ISC efficiency of **BDP-Pery-2** than **BDP-Pery-1** in toluene. Herein we suggest that selection of the electron donor and acceptor with proper redox potentials allows tuning the energy of the ¹CT state to be close to the ³LE state, so that the efficient SOCT-ISC can be attained simply by using proper solvents (E_{CTS} = 2.02 eV for **BDP-Pery-2** in toluene; $E_{CTS} \sim 1.83 \text{ eV}$ for **BDP-Pery-1** in DCM).

Spin density surfaces of the triplet state. BDP-Pery-3 and BDP-Pery-4, with connection at the 2-position of Bodipy, show significantly lower triplet state quantum yield (Table 1), as compared to BDP-Pery-1 and BDP-Pery-2 (in which the electron donor and acceptor are connected via meso-position of Bodipy). Previously, anthracence-Bodipy dyads and Phenoxazine-Bodipy dyads were also found to show more efficient SOCT-ISC if the electron donor was attached at the meso-position of Bodipy than that at the 2-position.^[48,81] The spin density at the 2-position of the Bodipy chromophore (-0.07 e/bohr³) is much lower than that on the meso-C atom (0.29 e/bohr3, Figure 6). As the spin-orbit coupling should be a function of spin density^[46] at the carbon atom in the Bodipy moiety binding the electron donor group, the different spin density on the linker atoms may be another reason for the low SOCT-ISC efficiency for BDP-Pery-3 and BDP-Pery-4, along with the molecular geometry effect.^[46] Based on this observation we propose a design rule for the Bodipy triplet PS based on SOCT-ISC, i.e., that attaching the electron donor to the meso-position will induce more efficient ISC than substitution at the 2-position of Bodipy.



Figure 6. Spin density of the triplet state of Bodipy, calculated at DFT//uM062X//6-31G level with Gaussian 09W, based on the optimized triplet state geometry. The spin density values are in e/bohr³.

Molecular geometry and potential energy surface. Previously it was reported that for SOCT-ISC, the geometry between the electron donor and acceptor should be orthogonal, as such the variation of molecular orbital angular momentum can compensate the change of electron spin angular momentum during ISC process. However, the SOCT-ISC in **BDP-Pery-2** (dihedral angle between perylene and Bodipy, $\phi = 118^{\circ}$, $\Phi_T = 60\%$ in toluene) is as efficient as in **BDP-Pery-1** ($\phi = 90^{\circ}$, $\Phi_T = 56\%$ in DCM). These results indicate that the orthogonal geometry is not strictly required, non-orthogonal geometry can also induce efficient SOCT-ISC.



Figure 7. (a) Potential energy surfaces of the ground states of **BDP-Pery-1**, **BDP-Pery-2**, **BDP-Pery-3** and **BDP-Pery-4**, as a function of the torsion dihedral angle between Bodipy and perylene moieties. Calculated at DFT//M062X//6-31G level with Gaussian 09W, based on the optimized ground singlet state geometry. (b) Magnified potential energy surfaces of (a), RT is the abbreviation of the thermal energy at room temperature ($k_{\rm B}T = 0.026$ eV).

The low triplet state production ability of BDP-Pery-4 can be well explained by its potential energy curves (Figure 7a and 7b). Compared to BDP-Pery-1, BDP-Pery-2 and BDP-Pery-3, the potential energy curve of **BDP-Pery-4** is very flat. Two energy minima on the potential energy curve were observed, for which the dihedral angles between the perylene and the Bodipy moieties are about 40° and 130°, respectively. The energy barrier is 0.07 eV (which is higher than the thermal energy at room temperature, $k_{\rm B}T = 0.026 \text{ eV}$), hence in principle the molecules of **BDP-Pery-4** are thermodynamically trapped at two conformations at room temperature, and deviate greatly from the orthogonal geometry, which is detrimental for SOCT-ISC. For BDP-Pery-1, the geometry is trapped in orthogonal conformation ($70^{\circ} \sim 112^{\circ}$) to large extent, and the potential energy curve is steep. These geometries are beneficial for SOCT-ISC, which is in agreement with the experimental results (Table 1). For BDP-Pery-2 and BDP-Pery-3, two energy minima were observed with the torsion angle of 118° and 60°, respectively.

Application of the compact electron donor/acceptor dyads in PDT

PDT has attracted great attention due to its spatiotemporal selectivity, low side effect and little drug resistance for the treatment of tumours.^[13,14] Compared with traditional heavy atom containing PSs used in PDT, triplet PSs based on SOCT-ISC are free of heavy atoms and therefore lower dark toxicity is expected. Moreover, the current electron donor/acceptor dyads based on SOCT-ISC PSs show long triplet lifetime (τ_T = 436 µs) and hence are suitable to be used in PDT in hypoxic environments (more common for tumour tissue than normal tissue), which is verified by the non-decreased singlet oxygen (${}^{1}O_{2}$) production ability (Φ_{Δ} = 0.34, see Figure S14 in the Supporting Information) even under severe hypoxic environment (0.2% O2). This result shows that the SOCT-ISC PSs have the potential to effectively produce singlet oxygen (¹O₂) in hypoxic tumours, and are expected to overcome the drawbacks of compromised PDT effect associated with hypoxia environment of the tumour tissues. Additionally, the generation of singlet oxygen by SOCT-ISC PSs dyads is highly sensitive to the polarity of the environment (Table 1), which can

FULL PAPER

be exploited to develop external stimuli-activatable PDT reagents and chromophore-assisted light inactivation (CALI), i.e., recognition of the appropriate environment (usually the lowpolarity environment of the target protein) switches on local generation of singlet oxygen.^[82] Hence the SOCT-ISC triplet PSs can be used as new type of promising PDT reagents.

Inspired by the simple molecular structure, efficient ISC, long triplet lifetime and polarity sensitivity of SOCT-ISC triplet photosensitizers, their potential application in PDT towards tumour cells was studied and exemplified with **BDP-Pery-2**. The benchmark Bodipy molecule with heavy atom effect, 2-iodoBDP, was also studied for comparison. The cells were incubated with **BDP-Pery-2** in the darkness for 24 h. Then the cellular imaging was studied by confocal laser scanning microscopy (Figure 8). The green fluorescence from the cell cytoplasm was clearly observed upon excitation at 488 nm, indicating efficient cellular uptake of **BDP-Pery-2** (Figure 8).

The biocompatibility and PDT effect of **BDP-Pery-2** and **iodo-BDP** in cells were evaluated using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) assays (Figure 9). We incubated HeLa cells, a commonly used tumour cell line, with different doses of **BDP-Pery-2** or **iodo-BDP** for 24 h, followed by light irradiation (500 nm, 9.6 J cm⁻²). As shown in Figure 9a, upon photoirradiation, **BDP-Pery-2** induced significant phototoxicity with an EC₅₀ value of 75 nM for HeLa cells. Trypan blue staining of the cells confirmed the cell death due to the increased cellular permeability (Figure 10b). In a control experiment, the cells were not killed in the absence of light irradiation or photosensitiser (Figure 10a and 10c). These results indicate that **BDP-Pery-2** acts as an efficient PDT reagent to cause cell death.

Remarkably, **BDP-Pery-2** exhibited extremely low dark toxicity (EC₅₀ = 78.1 μ M) as compared with the heavy atom PS **iodo-BDP** (EC₅₀ = 6.0 μ M), hence demonstrating the advantage of the heavy atom-free PSs for PDT (Table 3). The phototoxicity index (PI) value (the ratio of dark to light toxicity which reflects the effective PDT range of the PS) of **BDP-Pery-2** is calculated to be 1041, such a high PI value has been rarely reported,^[82] which is attributed to the significantly reduced dark toxicity in the absence of heavy atoms, as well as the high phototoxicity of the PSs.



Figure 8. Confocal laser scanning microscopy images of HeLa cells after incubation with **BDP-Pery-2** ($c = 10 \mu$ M). (a) Green channel (500-600 nm), $\lambda_{ex} = 488$ nm. (b) Bright channel. (c) Merged image. Scale bar: 20 μ m. 20°C.

To further confirm that the cell death is triggered by the in situ generated reactive oxygen species (ROS), the **BDP-Pery-2** pretreated Hela cells were stained with dichlorodihydrofluorescein diacetate (DCF-DA), a ROS probe that can be deacetylated by esterase in cells to form DCFH. The DCFH can rapidly trap the ROS to yield highly fluorescent dichlorofluorescein (DCF), which is commonly used as an indicator of intracellular ROS. After prolonged irradiation of the HeLa cells pre-treated with **BDP-Pery-2** and DCF-DA, obvious enhancement of green fluorescence was observed (Figure S19 in the Supporting Information), indicating the production of a significant amount of ROS. No significant fluorescence has been observed for the reference group in the same conditions without photosensitizer, which rules out any interference of laser.

Table 3. Parameters of the PDT with electron donor/acceptor dyads at triplet $\mbox{PSs}^{[a]}$							
Compound	EC50(light toxicity)	EC50(dark toxicity)	Pl ^[a]				
iodo-BDP	0.004 μM	6.0 μM	1500				
BDP-Perv-2	0.075 uM	78.1 uM	1041				

[a] Phototoxicity index value (the ratio of dark to light toxicity which reflects the effective PDT range of the PS).



Figure 9. Comparison of the cell viability of HeLa cells pre-treated with increasing doses of **BDP-Pery-2** and **iodo-BDP**, (a) with light irradiation (500 nm, 9.6 J cm⁻²); (b) without light irradiation. 20°C.



Figure 10. Microscope images of HeLa cells stained with Trypan blue. (a) Cells incubated with **BDP-Pery-2**, then incubated for another 24 h in the dark in the incubator; (b) Cells incubated with **BDP-Pery-2** and illuminated with 500 nm green light for 40 min, then incubated for another 24 h in the dark in the incubator; (c) Cells illuminated for 24 h without treating with the PS. The dead cells were preferentially stained with Trypan blue because of increased cellular permeability. *c*[**BDP-Pery-2**] = 1 μ M, 37°C.

This work successfully explored a heavy atom-free Bodipy PDT regent based on SOCT-ISC mechanism. In comparison with a benchmark Bodipy PS, we demonstrated the low dark toxicity and high PI value of the heavy atom-free SOCT-ISC PSs. The long

triplet lifetime feature of SOCT-ISC PSs is particularly beneficial for applying these PDT reagents in the hypoxia microenvironment of tumours. Moreover, the sensitivity of the triplet quantum yield to the polarity of the media makes it possible to control the PDT efficiency by the polarity of microenvironment. It can also be used for chromophore-assisted light inactivation.^[77] To the best of our knowledge, SOCT-ISC triplet PSs have been seldom used in PDT and the advantage of applying this kind of triplet PSs in PDT has not been fully recognized and addressed in previous studies.

Conclusions

A series of perylene-Bodipy compact electron donor/acceptor dyads showing efficient spin orbit charge transfer intersystem crossing (SOCT-ISC. Quantum yield Φ_T = 60%, triplet state lifetime $\tau_T = 436 \ \mu s$) were prepared, with the aim of confirming the ISC mechanism and unveiling the molecular structural factors controlling the efficiency of SOCT-ISC, as well as the potential applications of these novel heavy atom-free triplet photosensitizers. The photophysical properties of the compact dyads were studied with steady state and time-resolved optical and time-resolved electron paramagnetic resonance (TREPR) spectroscopies. The SOCT-ISC mechanism was confirmed by combination of various spectroscopies. The nanosecond transient absorption spectroscopy unambiguously confirmed that the production of triplet state is related to the charge transfer, by direct photoexcitation into the CT absorption band. Femtosecond transient absorption spectroscopy demonstrated that the charge separation is extremely fast (<1 ps) and the CR is dependent on the solvent polarity, by which the energy level of ¹CT state is affected. By using the time-resolved EPR (TREPR) spectroscopy, the SOCT-ISC mechanism was confirmed, other ISC mechanisms such as RP-ISC and the SO-ISC were excluded for the dyads. We found that the previously proposed orthogonal geometry is not sufficient for attaining efficient SOCT-ISC, other factors contribute to the SOCT-ISC as well. Examination of the potential energy curves gives a full picture of the relationship between molecular structure and SOCT-ISC efficiency; the spin density on the atoms in the linker between the electron donor and acceptor significantly affects the SOCT-ISC efficiency; a suitable energy level of ¹CT state is crucial and an energy matching between the ¹CT and ³LE states is beneficial for SOCT-ISC. Herein we point out that attachment of the electron donor to the meso-position of Bodipy skeleton will induce more efficient ISC than the attachment to the 2-position of Bodipy. TREPR shows that the geometrical parameters play an important role in the electron spin selectivity, i.e. the population the sublevels of the triplet state. The triplet state lifetime of the pervlene moiety accessed with the SOCT-ISC (436 µs) is much longer than that accessed with the conventional heavy atom effect (62 µs). Remarkably, the heavy atom-free Bodipy PDT regent based on SOCT-ISC mechanism presents a significantly reduced dark toxicity (EC₅₀ = 78.1 μ M for SOCT-ISC PS), vs. the normal triplet PSs based on heavy atom effect (EC₅₀ = 6.0 μ M, 2,6diiodoBodipy).

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Our work provides a detailed investigation and instruction on the molecular design of heavy atom-free triplet PSs, and this kind of PSs based on SOCT-ISC mechanism shows potent photodynamic therapy (PDT) effect. Although efforts to redshift the absorption wavelength to PDT operation window (650 nm – 800 nm) are still undergoing in our laboratory, we believe our current work is the cornerstone of future development of the SOCT-ISC triplet photosensitizers showing NIR absorption, and will pave the way for the design and development of a new generation of triplet PSs as hypoxia suitable, polarity activated phototherapy reagents. These results are also useful for the understanding of the fundamental Photophysics associated with CR-induced ISC and the design of heavy atom-free efficient triplet PSs.

Experimental Section

Synthesis and characterization. Synthesis and characterization of the materials used in this study can be found in the Supporting Information.

Nanosecond Transient Absorption Spectra Measurements: The nanosecond time-resolved transient absorption spectra were detected by Edinburgh analytical instruments (LP 980, Edinburgh Instruments, U.K.). The lifetime were obtained with the LP900 software. The samples were deaerated with N₂ delivered by needle for ca. 15 min before measurement.

Sub-Picosecond Transient Absorption Spectroscopy: The apparatus used for the transient absorption spectroscopy (TAS) measurements is based on a Ti:sapphire regenerative amplifier (BMI Alpha 1000) system pumped by a Ti:sapphire oscillator (Spectra Physics Tsunami). The system produces 100 fs pulses at 785 nm, 1 kHz repetition rate and average power of 450-500 mW. The pump beam polarization has been set to magic angle with respect to the probe beam by rotating a $\lambda/2$ plate. Excitation powers were on the order of 30-50 nJ. The probe pulse was generated by focusing a small portion of the fundamental laser output radiation on a 3 mm thick calcium fluoride window, which was kept under continuous movement during the measurements. Pump-probe delays were introduced by sending the probe beam through a motorized stage. Multichannel detection was achieved by sending the white light continuum after passing through the sample to a flat field monochromator coupled to a home-made CCD detector. TAS measurements were carried out in a guartz cell (2 mm thick) mounted on a movable stage to avoid sample photo degradation and multiple photon excitation. The recorded kinetic traces and transient spectra have been analysed by using a global analysis.^[69] The number of kinetic components has been estimated by performing a preliminary singular values decomposition (SVD) analysis.[83] Global analysis was performed using the GLOTARAN package (http://glotaran. org),[70] and employing a linear unidirectional "sequential" model.

Time-Resolved Electron Paramagnetic Resonance (TREPR) Spectroscopy: TREPR measurements were performed using a homemade continuous wave/TREPR setup based on an X-band Bruker EMX spectrometer (9 GHz) equipped with a N₂-cooled temperature control system ($T \sim 80 - 300$ K). In all variable-temperature experiments the sample was first shock-frozen in liquid nitrogen and then transferred into the cavity and equilibrated with its temperature for several minutes. A Nd:YaG laser LOTIS-TII with the excitation wavelength of 532 nm was used. All simulations of TREPR spectra were done using EasySpin.^[84] The concentrations of all samples were adjusted to have the full light absorption at the width of the quartz EPR tube, 2.8 mm. For compounds BDP-Pery-1 and BDP-Pery-4, the typical concentration was $c \approx 1 \times 10^{-3}$ M; for

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FULL PAPER

compounds BDP-Pery-2 and BDP-Pery-3, the typical concentration was *c* $\approx 1 \times 10^{-4}$ M. In all cases, solution was placed into the quartz EPR tube, evacuated (10^{-2} Torr pressure), and sealed off.

Photodynamic therapy (PDT): The cytotoxicity of BDP-Pery-2 in the presence and absence of light irradiation was assessed by 3-(4,5dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) assays. HeLa cells were seeded in 96-well plates (5.000 cells per well) cultured in standard Dulbecco's Modified Eagle Medium (DMEM) for 24 h incubation (37 °C, 5% CO2). The cells were then incubated with BDP-Pery-2 in DMEM suspension at various concentrations in the dark for 24 h. The mixtures were washed with PBS and added with fresh standard DMEM before exposure to green light (4 mW cm⁻²) for 40 min. The cells were further cultured for 4 h, and then 10 μ L of freshly prepared MTT (5 mg mL⁻¹) solution was added into each well. The MTT solution was carefully removed after 3 h of incubation, and DMSO (150 $\mu\text{L})$ was added into each well to dissolve all the formazan formed. The absorbance of MTT at 490 nm was measured by the microplate reader (Varioskan LUX, Thermo Scientific, USA). Cell viability was expressed by the ratio of the absorbance of the cells incubated with BDP-Pery-2 in the presence and absence of light irradiation, respectively.

Acknowledgements

J. Zhao thanks the NSFC (21673031, 21761142005, 21911530095 and 21421005), State Key Laboratory of Fine Chemicals (ZYTS201901) and the Fundamental Research Funds for the Central Universities (DUT2019TA06) for financial support. M. V. F. and M. Yu. I. thank the Ministry of High Education and Science of Russia. We thank Dr. Peter Sherin (ITC) for helpful discussions.

Keywords: Bodipy • Electron Spin Polarization • Perylene • Photodynamic Therapy • Spin-Orbit Charge Transfer Intersystem Crossing (SOCT-ISC)

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Accepted Manuscrit

FULL PAPER

Entry for the Table of Contents

FULL PAPER

Long Live the Triplet State! We report heavy atom-free triplet photosensitisers (BDP-Perylene) with strong absorption of visible light, high ISC efficiency (60%) and ultra long triplet lifetime (437 µs). The ISC mechanism and design rationales of SOCT-ISC photosensitiser were studied. These novel photosensitisers are demonstrated as potent, completely new type of PDT reagents, achieving significantly reduced undesired dark toxicity, in stark contrast to the conventional heavy atom-containing triplet photosensitisers.



Photochemistry

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Page No. – Page No.

Spin-Orbit Charge Transfer Intersystem Crossing (SOCT-ISC) in Compact Electron Donor-Acceptor Dyads: ISC Mechanism and Application as Novel and Potent Photodynamic Therapy Reagents