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# Synthesis, Fluorescence, and Two-Photon Absorption of Bidentate Phosphane Oxide Derivatives: Complexation with $Pb^{2+}$ and $Cd^{2+}$ Cations

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**Abstract:** A series of fluorescent phosphane oxide derivatives based on diphenylphosphanoethane (DPPE) and diphenylphosphanomethane (DPPM) skeletons has been prepared by means of Grignard reactions and Sonogashira cross-couplings. The photophysical properties and the linear and nonlinear spectra of these compounds have been investigated. An edge-to-face conformation resulting in the formation of an excimer was confirmed by fluorescence lifetime measurements of these multichromophoric derivatives. Upon complexation with heavy metal ions such as $Pb^{2+}$ and $Cd^{2+}$, a red shift of the one- and two-photon excitation spectra was observed in the absorption and emission spectra. Furthermore, enhancement of the electron-withdrawing character of the phosphane oxide resulted in a significant enhancement of the two-photon absorption cross-section, leading to the first biphotonic $Cd^{2+}$ sensors combining high affinity for $Cd^{2+}$, large two-photon absorption cross-sections, and significant enhancement of the two-photon excited fluorescence in the presence of the cation. Such derivatives are highly promising for incorporation into devices for the detection of heavy metal ions in water and effluents.

**Keywords:** charge transfer · fluorescence · phosphane oxide · sensors · two-photon absorption

## Introduction

Over the past few years, significant efforts have been directed towards the development of new fluorescent molecules for various applications, such as molecular labelling, probing environments, and sensing.[1,2] Compared to other analytical methods, the use of fluorescence offers distinct advantages in terms of sensitivity, selectivity, response times, and capacity for local observations (e.g., by fluorescence imaging spectroscopy). The development of one-photon fluorescent molecular sensors[2] has been one of the most studied aspects in the international community. However, such systems have various disadvantages, in particular due to the low excitation wavelength that they require. In contrast, systems based on fluorescence induced by two-photon absorption (TPA)[3] are very attractive and offer many advantages: i) a highly confined excitation and three-dimensional (3D) sub-micrometer spatial resolution can be obtained by employing the technique of two-photon laser scanning microscopy (TPLSM),[4] ii) they permit a non-destructive method that allows the use of excitation wavelengths in the 700–1000 nm region, thus allowing iii) better penetration into cells and tissues (due to reduced scattering losses), and iv) a reduction in the background fluorescence. Of particular importance in this context is the use of fluorophores and fluorescent probes with much higher TPA cross-sections (typically larger by orders of magnitude) than those of endogenous chromophores. This should allow selective excitation of engineered two-photon probes, which is of major importance for biological applications. Indeed, it has recently been shown that suitable "photophysical" molecular engineering can yield photoac-

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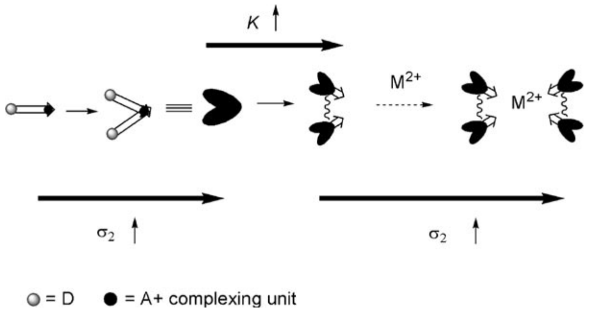
tive molecules for which two-photon excitation allows more selective photo-addressing than the corresponding one-photon excitation.[5] Such selective excitation will reduce the background fluorescence (thus providing an enhanced signal-to-noise ratio and therefore improved sensitivity) and also decrease photo-damage due to concomitant excitation of endogenous compounds. Webb and co-workers, who pioneered the TPLSM technique, have characterised the TPA spectra of endogenous chromophores in the spectral range of interest for biological imaging, showing most of them to have TPA cross-sections considerably lower than a few Göppert–Mayer (GM) ($1\,GM = 10^{-50}\,cm^4\,s\,photon^{-1}$).[4] Hence, the design of "two-photon" or "biphotonic" fluorescent probes with TPA cross-sections of several hundred GM or much higher is a current challenge for bio-related applications. Only recently have a few examples of two-photon fluorescent molecular sensors for metallic cations been described in the literature, focussing mainly on sensors[6] for $Ca^{2+}$, $Mg^{2+}$, and $Zn^{2+}$ in biological matrices. The molecular designs of these TP cation sensors are based on a TP chromophore, most commonly derived from a quadrupolar structure,[6d–i] or occasionally from dipolar[6c] or octupolar derivatives.[6j] The donor moiety,[6d–i] or more rarely the acceptor moiety,[6c,j] is therefore part of the complexing unit. As a result, the presence of the cation can lead either to a decrease (as observed in the case of complexation on the donor side in quadrupolar-type derivatives[6d–f,h,i]) or to an increase in the TPA response (as observed in the case of complexation at the acceptor side in dipolar or octupolar derivatives[6j]). In most reported cases, a quenching of two-photon excited fluorescence (TPEF) is observed upon complexation.[6a,d–f,h,i,7]

Critical issues for the sensitivity of the detection are the complexation strength (as expressed by $pK_D$), its selectivity, and the amplitude of the variation in the fluorescence characteristics as a function of cation concentration. For TP sensors, another critical issue is the magnitude of the response to two-photon excitation (i.e., the TPA cross-section), a higher TPA cross-section being beneficial for both a low detection threshold by reduction of background fluorescence and faster scanning for imaging purposes.

Only rare examples of two-photon sensors for heavy metals such as $Pb^{2+}$ have been reported,[6f,g] and to the best of our knowledge no efficient TP sensors for $Cd^{2+}$ have been described. Within this framework, our goal has been the design of sensitive biphotonic $Pb^{2+}$ and $Cd^{2+}$ fluorescent sensors. Our strategy was to design fluorophores that combine high complexing ability with much larger TPA cross-sections than those of classical fluorophores[4] or "ordinary" fluorescent contaminants,[8] and that show enhancement of their TPEF in the presence of the target cations.

In order to enhance the sensitivity for detection purposes, we decided to prepare fluorescent sensors that could act as *two-photon antennae*. Our idea was to graft *several* TP absorbers (with significant TPA response) bearing the binding unit part onto a multidentate complexing unit (with high affinity for heavy metal ions). Based on these requirements, we decided to focus on dipolar-type two-photon absorber chromophores,[9] in which the acceptor moiety shows affinity for heavy metal cations (Scheme 1).

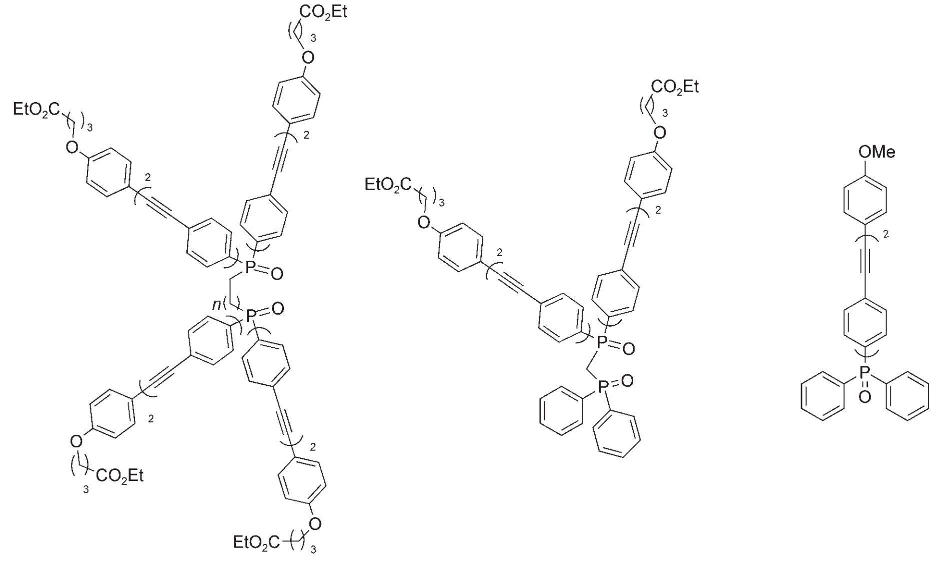


Scheme 1. Design of multidentate biphotonic sensors for heavy metal cations.

It was anticipated that such systems would lead to very large TPA cross-sections and an enhancement of the TPA response in the presence of the cation. In view of the complexing ability of phosphane oxide derivatives with heavy metals,[10] and based on our experience of the synthesis of phosphane oxides,[11] we decided to prepare some analogues of diphenylphosphanoethane (DPPE) and diphenylphosphanomethane (DPPM) and to study their photophysical and complexation properties. In this paper, we report the synthesis of some new derivatives of this type (Scheme 2), and their linear and two-photon spectroscopic properties in the presence of heavy metal ions.

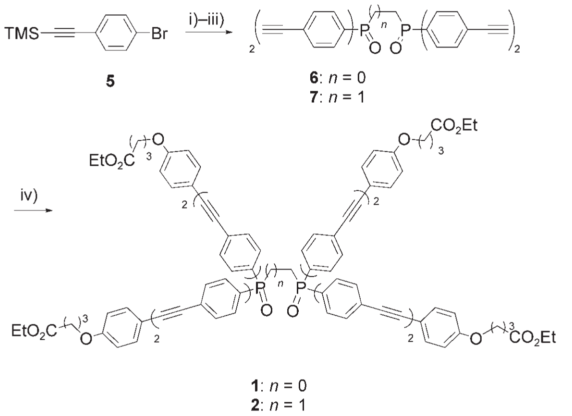
## Results and Discussion

**Synthesis**: The structures of ligands **1** and **2** were designed with a view to increasing the solubility of phosphane oxides in organic solvents while retaining the fluorescent properties of phenylethynyl derivatives. Anticipating that the presence of four phenylethynyl arms was likely to influence the photophysical properties and the stability of the corresponding phosphane oxide, we also decided to prepare the unsymmetrical derivative **3**. A Grignard derivative was first prepared from the commercially available (4-bromophenyl)trimethylsilylacetylene (**5**), phosphorylation of which with bis(dichlorophosphano)methane or 1,2-bis(dichlorophosphano)ethane afforded the respective tetraarylated phosphanes (Scheme 3). The phosphorus atoms were oxidised in the presence of $H_2O_2$, and this was followed by desilylation. The phosphane oxides **6** and **7** were isolated in yields of 31% and 26%, respectively, over the three steps. Sonogashira cross-coupling reactions[12] between the phosphano products **6**/**7** and the iodide **8**[13] were then performed in the presence of 10 mol% of Pd catalyst and 10 mol% of copper iodide. This reaction allowed the introduction of four aryl groups, leading to the desired phosphane oxides, DPPMO (**1**) and



Scheme 2. Phosphane oxide fluorescent probes.

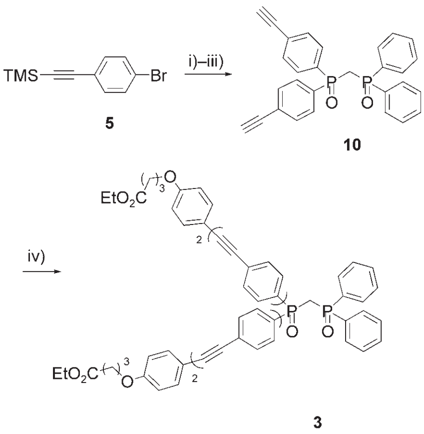
DPPEO (**2**), in isolated yields of 71 and 50 %, respectively (Scheme 3).



Scheme 3. Syntheses of the ligands DPPMO (**1**) and DPPEO (**2**): i) Mg, THF, 50 °C, 1.5 h then $Cl_2PCH_2CH_2PCl_2$ or $Cl_2PCH_2PCl_2$, THF, RT, 20 h, 35 % ($n = 0$), 26 % ($n = 1$); ii) $H_2O_2$, $CH_2Cl_2$/MeOH, RT, 88 % ($n = 0$), 100 % ($n = 1$); iii) $K_2CO_3$, MeOH/$CH_2Cl_2$, RT, 4 h, 70 % ($n = 0$), 80 % ($n = 1$); iv) 10 mol% [Pd(PPh$_3$)$_4$], 10 mol % CuI, I-$C_6H_4$-$C_6H_4$O-$(CH_2)_3CO_2Et$ (**8**), $Et_3N$, toluene, 50 °C, 71 % ($n = 0$), 50 % ($n = 1$).

As a desymmetrisation step was not conceivable starting from **6**, we changed our strategy and decided to take advantage of methyldichlorophosphane oxide (**9**). Scheme 4 outlines the synthesis of the DPPM ligand **3** bearing two fluorescent arms. Here, the Grignard derivative of (4-bromophenyl)trimethylsilylacetylene **5** was quenched with methyldichlorophosphane oxide (**9**). The resulting phosphane oxide was isolated in a modest yield of 44 %. Treatment with a hindered base such as lithium 2,2,6,6-tetramethylpiperidide (LTMP),[14] followed by the addition of chlorodiphenylphosphane oxide as an electrophile, gave the desired phosphane monooxide in an unoptimised 27 % yield. Another route involving the more reactive chlorodiphenylphosphane and a phosphorus oxidation step did not lead to higher yields. Desilylation using $K_2CO_3$ in a mixture of $CH_2Cl_2$/MeOH was followed by the Sonogashira cross-coupling reaction, which led to the desired unsymmetrical phosphane DPPMO-Ph$_2$ (**3**) in 23 % overall yield. Phosphane oxide TPPO (**4**) was prepared according to a previously described procedure.[11]

**Photophysical properties of the ligands**: The absorption and fluorescence properties of the phosphane oxide derivatives are collected in Table 1, and their absorption and emission spectra are shown in Figure 1a) and b), respectively. The fluorophores show an intense absorption band in the



Scheme 4. Synthesis of DPPMO-Ph$_2$ ligand **3**: i) Mg, THF, 50 °C, 1.5 h then $Cl_2POCH_3$ (**9**), THF, RT, 20 h, 44 %; ii) LTMP (2.5 equiv), THF, −40 °C, then ClPOPh$_2$, 27 %; iii) $K_2CO_3$, MeOH/$CH_2Cl_2$, RT, 4 h, 84 %; iv) 10 mol% [Pd(PPh$_3$)$_4$], 10 mol % CuI, **8**, $Et_3N$, toluene, 50 °C, 28 %.

Table 1. Photophysical properties of phosphane oxide derivatives in $CH_3CN/CHCl_3$ 8:2. Maxima of the one-photon absorption $\lambda_{abs}$ [nm] and of steady-state emission $\lambda_{em}$ [nm], molar absorption coefficient $\varepsilon$ [$10^4\,M^{-1}cm^{-1}$], fluorescence quantum yield $\Phi_F$.

| Product | $\lambda_{abs}$ [nm] | $\varepsilon$ [$10^4\,M^{-1}cm^{-1}$] | $\lambda_{em}$ [nm] | $\Phi_F$[a] |
|---|---|---|---|---|
| TPPO (**4**) | 331 | 6 | 426 | 0.77 |
| DPPMO-Ph$_2$ (**3**) | 334 | 12 | 424 | 0.72 |
| DPPEO (**2**) | 334 | 25 | 434 | 0.71 |
| DPPMO (**1**) | 335 | 24 | 455 | 0.54 |

[a] Fluorescence quantum yield with a 10 % random error.

near-UV region. After normalisation to the same number of chromophores, the molar absorption coefficients at the wavelength of maximum absorption are similar for the different fluorophores, indicating weak interaction between the chromophores in the ground state of the multichromophoric fluorophores.[15] All of these molecules show an intense fluorescence emission in the visible region, with fluorescence quantum yields ranging from 0.54 to 0.77. A large Stokes shift and the unresolved vibronic structure of the fluorescence spectra observed for these fluorophores suggest the formation of an intramolecular charge transition. In contrast to the absorption spectra, significant differences are observed in the shapes of the emission spectra and the fluorescence quantum yields for the different compounds. The emission spectra of TPPO (**4**) and DPPMO-$Ph_2$ (**3**) are perfectly superimposable, suggesting weak interaction between the chromophores. Similar behaviour was observed in the case of a star-shaped fluorophore bearing three phenylethynyl fluorescent arms.[11] In contrast, a broadening of the emission and a decrease in the fluorescence quantum yield were observed for the compounds DPPMO (**1**) and DPPEO (**2**). This effect was found to be more pronounced in the case of DPPMO (**1**).

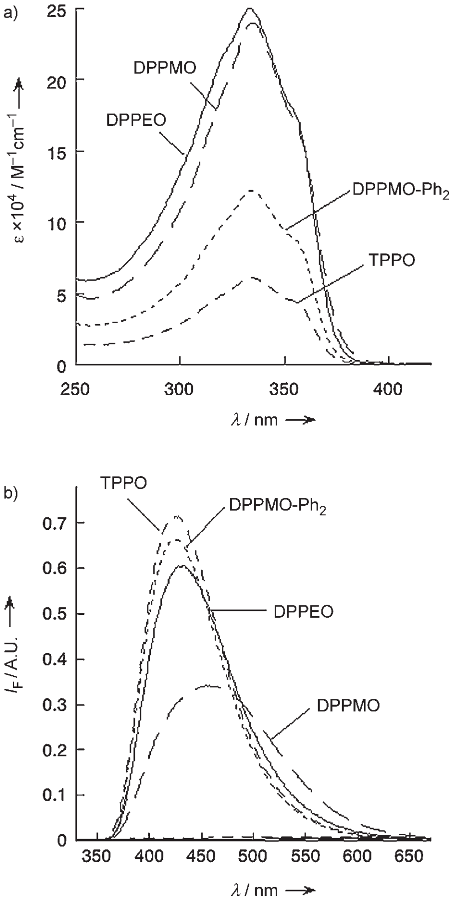


Figure 1. a) Absorptions of phosphane oxide derivatives in $CH_3CN/CHCl_3$ 8:2. b) Corrected normalised emission spectra of the phosphane oxide derivatives in $CH_3CN/CHCl_3$ 8:2.

In order to further characterise the photophysical properties, time-resolved fluorescence measurements were performed by the single-photon counting method with picosecond laser excitation. The fluorescence decays for the different phosphane oxides are shown in Figure 2. For TPPO (**4**) and DPPMO-$Ph_2$ (**3**), the decays are mono-exponential in form. In contrast, satisfactory fits were obtained by considering three exponentials for DPPEO (**2**) and DPPMO (**1**). The fluorescence decays at different emission wavelengths were subjected to global analysis. The time constants thus obtained are reported in Table 2. A component of around 0.9 ns, which is predominant in the region 380–430 nm, may be attributed to the monomer emission. The longest component (5.9 and 6.9 ns, respectively), the contribution of which increases at long wavelengths, may be attributed to excimer formation. It should be noted that the contribution of the longest component is higher in the case of DPPMO (**1**). This may be attributed to a stronger interaction between the chromophores in the excited state of **1** as a result of their proximity. These fluorescence decay measurements thus permit evaluation of the spectra associated with each species. A higher proportion of the excimer band is observed in the case of the methylene chelate. This effect can be ration-

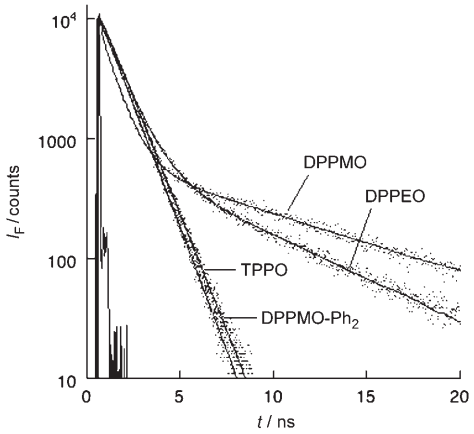


Figure 2. Fluorescence decays of the phosphane oxide derivatives in $CH_3CN/CHCl_3$ 8:2.

Table 2. Fluorescence decay components $\alpha_i$ and $\tau_i$ [ns] of the phosphane oxide derivatives in $CH_3CN/CHCl_3$ 8:2.

| Product | $\lambda_{em}$ [nm] | $\tau_1$ [ns] ($\alpha_1$) | $\tau_2$ [ns] ($\alpha_2$) | $\tau_3$ [ns] ($\alpha_3$) | $\chi_r^2$ |
|---|---|---|---|---|---|
| TPPO (**4**) | 425 | 1.12 (1) | – | – | 1.16 |
| DPPMO-$Ph_2$ (**3**) | 425 | 1.07 (1) | – | – | 1.12 |
| DPPEO (**2**) | 430 | 0.03 (0.1) | 0.99 (0.88) | 5.89 (0.02) | 1.12 |
| | 470 | 0.03 (0.12) | 0.99 (0.84) | 5.89 (0.04) | 1.14 |
| | 510 | 0.03 (0.14) | 0.99 (0.80) | 5.89 (0.05) | 1.14 |
| | 530 | 0.03 (0.16) | 0.99 (0.78) | 5.89 (0.06) | 0.96 |
| DPPMO (**1**) | 450 | 0.12 (0.35) | 0.78 (0.6) | 8.65 (0.05) | 1.27 |
| | 500 | 0.12 (0.28) | 0.78 (0.56) | 8.65 (0.16) | 1.11 |
| | 550 | 0.12 (0.21) | 0.78 (0.50) | 8.65 (0.29) | 1.08 |
| | 640 | 0.12 (0.15) | 0.78 (0.46) | 8.65 (0.39) | 1.17 |

alised in terms of the shorter distance between the fluorophores. The shortest component (0.03 and 0.12 ns), which is predominant in the blue region of the spectra, can be attributed to the decay of the monomer capable of forming an excimer.[16] However, it is important to note that no rise time resulting from excimer formation was detected at 520 nm on the shortest accessible timescale with our instrumentation (around 20 ps); this means that the excimers were preformed, as previously reported for β-cyclodextrins with seven appended naphthalenic fluorophores.[17] All of these data suggest, as previously observed for bichromophoric systems,[18] the presence of different conformers: one may envisage the presence of an expanded structure (leading to monomer emission) and a near-sandwich geometry (responsible for preformed excimer emission).

**Cation-induced photophysical changes**: Complexation studies of the phosphane oxide derivatives with cadmium and lead were performed in $CH_3CN/CHCl_3$ 8:2 in order to ensure good dissociation of the ion pair and good solubility of the complexes. Figure 3 displays the evolution of the absorption spectra (a) and the emission spectra (b) of DPPEO (**2**) upon complexation with cadmium. The absorption spectra a) and emission spectra b) of DPPMO (**1**) upon complexation with cadmium are given in Figure 4. Cation complexation induces a bathochromic shift of the absorption and emission spectra, which may be rationalised in terms of an enhancement of the electron-withdrawing character of the phosphane oxide group due to interaction of its oxygen atoms with the cation. This effect may be correlated with the charge density of the cation, since a more pronounced effect was observed for the $Cd^{2+}$ cation as compared to $Pb^{2+}$. The shifts towards higher wavelength upon cation complexation are much more pronounced for the emission spectra as compared to the absorption spectra, reflecting the efficient intramolecular charge-transfer character of the transition. These spectral characteristics clearly indicate an increase in the electron density on the phosphane oxide moiety in the excited state, thus resulting in stabilisation (and hence a red-shift) of the emissions of the complexed species.

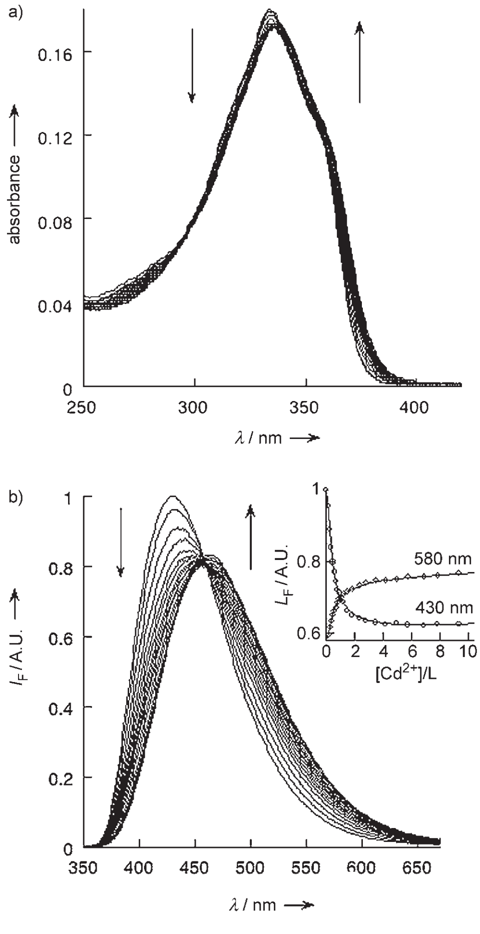


Figure 3. a) Absorption and b) corrected emission spectra of DPPEO ($2\times10^{-6}$ M) ($\lambda_{exc}=338$ nm) in the presence of increasing concentrations of $Cd^{2+}$ in $CH_3CN/CHCl_3$ 8:2.

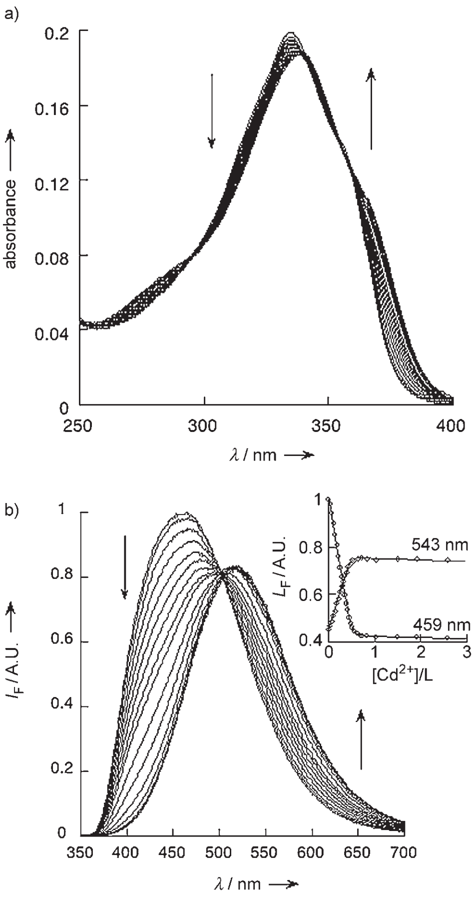


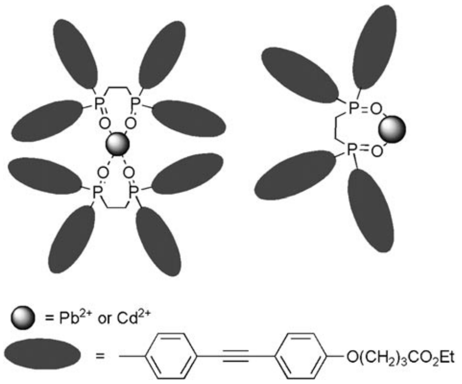
Figure 4. a) Absorption and b) corrected emission spectra of DPPMO ($2\times10^{-6}$ M) ($\lambda_{exc}=338$ nm) in the presence of increasing concentrations of $Cd^{2+}$ in $CH_3CN/CHCl_3$ 8:2.

Careful analysis of the emission spectra upon addition of the cations by means of the SPECFIT program reveals that different complexes are formed with the following different stoichiometries 1:2, 1:1, and 2:1. Table 3 presents the stabili-

Table 3. Stability constants of phosphane oxide derivatives with $Pb^{2+}$ and $Cd^{2+}$.

| Product | Cation | $\log\beta_{12}$ | $\log K_{11}$ | $\log K_{21}$ |
|---|---|---|---|---|
| TPPO | $Pb^{2+}$ | – | 3.7 ± 0.02 | – |
| DPPMO-$Ph_2$ | $Pb^{2+}$ | 14.5 ± 0.2 | 7.8 ± 0.2 | – |
| DPPEO | $Pb^{2+}$ | 13.8 ± 0.4 | 7.6 ± 0.2 | 11.2 ± 0.2 |
| DPPMO | $Pb^{2+}$ | 14 ± 0.2 | 6.6 ± 0.1 | – |
| TPPO | $Cd^{2+}$ | – | 3.0 ± 0.02 | – |
| DPPMO-$Ph_2$ | $Cd^{2+}$ | 15.2 ± 0.2 | 8.0 ± 0.2 | 11.4 ± 0.2 |
| DPPEO | $Cd^{2+}$ | 12.8 ± 0.2 | 6.7 ± 0.1 | 12.4 ± 0.2 |
| DPPMO | $Cd^{2+}$ | 14.2 ± 0.2 | 6.9 ± 0.1 | 10.5 ± 0.2 |

ty constants of the complexes formed with the $Pb^{2+}$ and $Cd^{2+}$ cations, calculated from the results of the titration experiments. Scheme 5 shows the possible complexes formed upon complexation of the cations. It should be noted that very high stability constants are observed upon complexation with the heavy metal ions.



Scheme 5. Model of the binding of DPPEO (**2**) with $Cd^{2+}$ or $Pb^{2+}$.

Time-resolved fluorescence decays were measured upon cation complexation. Figure 5 displays the decays obtained for DPPMO (**1**) upon $Pb^{2+}$ complexation. A sharp decrease in the excimer emission is observed, which may be rationalised in terms of a rigidification of the chelate upon complexation. Similar effects are observed for the phosphane oxide DPPEO (**2**) with both $Pb^{2+}$ and $Cd^{2+}$. This demonstrates that DPPEO (**2**) may be used as a ratiometric fluorescent sensor for $Pb^{2+}$ and $Cd^{2+}$, and may additionally serve as a fluorescence lifetime sensor for these heavy metals. In contrast, a higher proportion of the excimer fluorescence is maintained in the case of the complex between DPPMO (**1**) and $Cd^{2+}$, which may be due to the geometry of the complex. Thus, with regard to imaging applications (FLIM: fluorescence lifetime imaging), the DPPEO ligand **2** shows better characteristics.

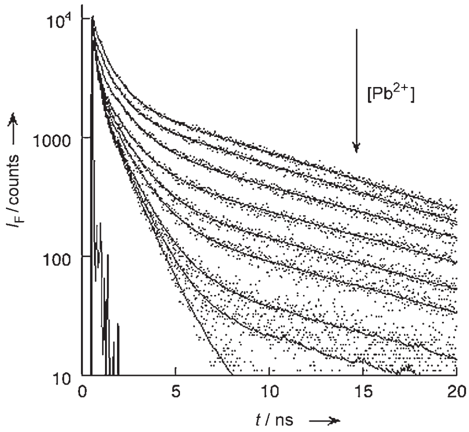


Figure 5. Fluorescence decays of DPPMO ($4.1 \times 10^{-7}$ M) in the presence of increasing concentrations of $Pb^{2+}$ (0 to 2400 equiv). Channel width = 34 ps.

**Two-photon absorption**: The TPA properties of the ligands and complexes were investigated by performing two-photon excited fluorescence (TPEF) measurements in solution according to the methodology developed by Webb and coworkers.[6a] The branched structures of DPPMO (**1**) and DPPEO (**2**) show notable and similar TPA responses in the target spectral region ($\sigma_2$ at $\lambda_{max}^{TPA} \approx 100$ GM), which is consistent with the weak interactions between the two chromophores in the ligands. The first (lowest-energy) TPA band, the maximum for which is located at around 740 nm, is red-shifted with respect to the corresponding one-photon allowed lowest-energy band, and is clearly overlapped by a much more intense blue-shifted band. Such behaviour is typical of a branched system composed of two interacting dipolar chromophores.[9] This is reminiscent of the behaviour of triphenylamine derivatives, except that in their case the dipolar chromophores interact through a common electron-withdrawing moiety[9b] (which is also the complexing unit). This indicates that the phosphane oxide moiety allows coherent coupling between the branches, thus opening an interesting route as an original "joint" module for the molecular engineering of branched systems with enhanced TPA.[9c]

As illustrated in Figure 6, the $ML_2$ complexes show a marked increase in their TPA in the region 700–800 nm. This is consistent with the reinforcement of the photoinduced charge transfer in the chromophoric parts of the ligands as a result of the increased electron-withdrawing ability of the phosphane oxide moiety when complexed with the heavy metal cation. We observe that this leads to an increase in the TPA cross-section at 740 nm (normalised with respect to the number of fluorescent ligands) by a factor 1.7 for $Pb^{2+}$ and by a factor 2.8 for $Cd^{2+}$. The stronger TPA modulation induced by $Cd^{2+}$ in the presence of DPPMO ligand **1** may be correlated with the higher charge density of the $Cd^{2+}$ cation. The bidentate DDPMO ligand **1** is thus found to be a convenient biphotonic fluorescent sensor for $Cd^{2+}$ ions, since it combines high affinity for the cation (large association constant), large TPA cross-sections of the complex species (larger than those of classical fluorophores commonly used in the biology community),[4b,6a] and significant modulation of the TPEF signal in the presence of the $Cd^{2+}$ cation (Table 4).

Table 4. TPA properties of phosphane oxide derivatives in $CH_3CN/CHCl_3$ 8:2. First maximum (lowest energy) of the TPA band [$\lambda_{max}^{TPA}$]. TPA cross-section $\sigma_2$ [1 GM = $10^{-50}$ cm$^4$ s$^{-1}$ photon$^{-1}$].

| Compound | $2\lambda_{max}^{abs}$ [nm] | $\lambda_{max1}^{TPA}$ [nm] | $\sigma_2$ [GM] at $\lambda_{max}^{TPA}$ | $\Phi$ | $\sigma_2\,\Phi$ [GM] at $\lambda_{max}^{TPA}$ |
|---|---|---|---|---|---|
| DPPMO | 670 | ≈740 | 95 | 0.54 | 51 |
| $[DPPMO]_2[Pb]$ | 675 | ≈740 | 320 | 0.03 | 10 |
| $[DPPMO]_2[Cd]$ | 677 | ≈740 | 520 | 0.44 | 229 |

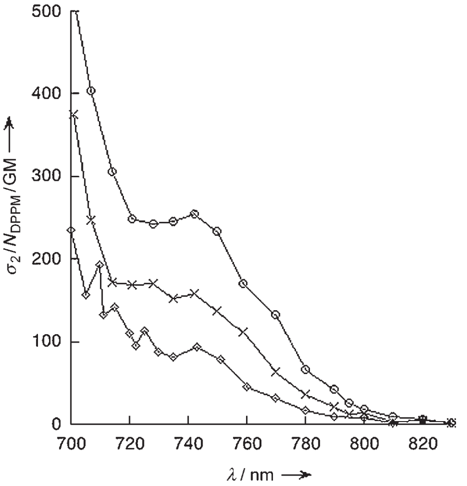


Figure 6. TPA spectra of DPPMO (**1**) and corresponding complexes in $CH_3CN/CHCl_3$ 8:2; ○: $DPPMO_2Cd$, ×: $DPPMO_2PB$, ◇: DPPMO.

## Conclusions

In conclusion, we have synthesised a series of phenylethynyl phosphane oxide derivatives bearing two or four fluorescent arms starting from simple materials. These novel derivatives show an intense absorption band in the near-UV region and an intense fluorescence emission in the visible region, with fluorescence quantum yields ranging from 0.54 to 0.77. We have also shown that weak interactions between the chromophores occur in the ground state for the multichromophoric fluorophores. The observed large Stokes shift and the unresolved vibronic structure of the fluorescence spectra also suggest the formation of an intramolecular charge transition.

Addition of a heavy metal cation such as $Cd^{2+}$ induces a bathochromic shift of the absorption and emission spectra due to an enhancement of the electron-withdrawing character of the complexed phosphane oxide group. As a result, these newly designed fluorophores show considerable promise as ratiometric sensors for $Cd^{2+}$ ions. In addition, these sensors have been found to exhibit large TPA cross-sections, which are significantly enhanced in the presence of the cation. This may be ascribed to the increased electron-withdrawing character of the complexing moieties of the phosphane oxide due to the interaction of the oxygen atom with the cation. These derivatives are therefore of great interest as first prototypical biphotonic sensors for $Cd^{2+}$ cations, and hold much promise for the development of devices for the detection of toxic heavy metals in water and effluents. This is a critical issue with regard to the selective detection of heavy metal ions in enforcing the environmental health official regulations imposed by the World Health Organisation and the European Community.[19]

## Experimental Section

**General**: Reagents were obtained commercially from Acros, Aldrich, or Avocado and were used without further purification unless otherwise stated. Cyclohexane, dioxane, chloroform, dichloromethane, dimethyl sulfoxide, and acetonitrile (spectrometric grade from Aldrich or SDS) were employed as solvents for absorption and fluorescence measurements. Column chromatography was performed on 0.040–0.063 mm Art. 11567 silica gel (E. Merck). $^1H$, $^{13}C$, and $^{31}P$ NMR spectra were recorded on Bruker AV300 or AV400 instruments. All signals ($\delta$) are expressed in units of ppm downfield from $Me_4Si$ for $^1H$ and $^{13}C$ NMR and from $H_3PO_4$ for $^{31}P$ NMR, used as internal standards. Coupling constants ($J$) are reported in Hz and refer to apparent peak multiplicities. Melting points were measured in open capillary tubes. FTIR spectra were recorded on a Thermo-Optek Nexus spectrometer. Mass spectrometric analyses were performed on a Hewlett-Packard HP 5989A instrument at the Ecole Nationale Supérieure de Chimie de Paris. Direct introduction experiments were performed by chemical ionisation with ammonia. Elemental analyses were performed at the Institut de Chimie des Substances Naturelles (Gif-sur-Yvette).

**Bis(4-trimethylsilylethynylphenyl)diphosphanomethane**: A solution of (4-bromophenylethynyl)trimethylsilane (4.66 g, 18.4 mmol) in anhydrous THF (12.5 mL) was added in one portion to activated Mg (485 mg, 20.24 mmol) under argon. The mixture was heated at 55 °C for 2 h and then cannulated into a solution of $DPPMCl_4$ in anhydrous THF (12.5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 18 h. The reaction was then quenched by the addition of saturated aqueous $NH_4Cl$ solution (10 mL) and water (10 mL). After extraction with diethyl ether, the organic phase was dried ($Na_2SO_4$) and filtered, the volatiles were evaporated, and the crude oil was purified by flash chromatography ($CH_2Cl_2$/cyclohexane 1:9→1:1) to afford the desired product as a colorless solid (1.22 g, 35 %). The product was used directly for the synthesis of methylene bis(4-trimethylsilylethynylphenyl)diphosphane oxide. $^1H$ NMR ($CDCl_3$, 300 MHz): $\delta = 0.25$ (s, 36 H), 2.73 (s, 2 H), 7.30 ppm (m, 16 H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): $\delta =$ 0.0, 27.8, 95.7, 104.6, 123.8, 131.8, 132.6, 132.8, 139.0 ppm; $^{31}P$ NMR ($CDCl_3$, 121 MHz): $\delta = -21.6$ ppm.

**Methylene bis(4-trimethylsilylethynylphenyl)diphosphane oxide**: 30 % $H_2O_2$ (1.06 mL) was added dropwise to a solution of bis(4-trimethylsilylethynylphenyl)diphosphanomethane (1.18 g, 1.53 mmol) in dichloromethane (5 mL) and methanol (10 mL). The reaction mixture was stirred at room temperature for 4 h. The excess $H_2O_2$ was then reduced with aqueous $Na_2SO_3$ solution (15 mL) and AcOEt (20 mL) was added. The organic phase was dried over $MgSO_4$ and filtered, and the volatiles were evaporated in vacuo to afford a colorless solid (1.093 g, 88 %). M.p. >250 °C; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 7.62$–7.52 (m, 8 H), 7.44–7.40 (m, 8 H), 3.48 (t, $J = 15$ Hz, 2 H), 0.25 ppm (s, 36 H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta = 132.5$, 132.1, 132.0, 131.9, 131.1, 131.0, 130.9, 127.4, 126.9, 103.8, 98.0, 34.6, 0.0 ppm; $^{31}P$ NMR (121 MHz, $CDCl_3$): $\delta = 24.6$ ppm; MS (ESI): $C_{45}H_{54}O_2P_2Si_4$: $m/z$: 801 $[M+H]^+$; elemental analysis calcd (%) for $C_{45}H_{54}O_2P_2Si_4$: C 67.46, H 6.79; found: C 67.27, H 7.01.

**Methylene bis[bis(4-ethynylphenyl)phosphane oxide] (6)**: $K_2CO_3$ (770 mg, 5.2 mmol) was added to a solution of methylene bis(4-trimethylsilylethynylphenyl)diphosphane oxide (1.093 g, 1.36 mmol) in anhydrous dichloromethane (8 mL) and methanol (16 mL) under argon, and the reaction mixture was stirred for 4 h at room temperature. The reaction was then quenched by the addition of water (10 mL), and dichloromethane (20 mL) was added. After extraction, the organic layer was dried ($Na_2SO_4$) and filtered, and the volatiles were evaporated in vacuo. The resulting pale-yellow solid was then triturated with the minimum volume of AcOEt and diethyl ether (10 mL) to afford the desired desilylated product **6** as a colorless solid (490 mg, 70 %). M.p. >250 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.68$–7.65 (m, 8H), 7.46 (d, $J = 7.0$ Hz, 8H), 3.47 (t, $J = 15$ Hz, 2H), 3.20 ppm (s, 4H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta =$ 133.0, 132.2, 132.1, 132.0, 131.5, 130.9, 130.8, 130.7, 126.2, 82.3, 80.1, 34.6 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta = 23.9$ ppm; MS (ESI): $C_{33}H_{22}O_2P_2$: $m/z$: 513 $[M+H]^+$; MALDI HRMS: $m/z$: calcd for $C_{33}H_{22}O_2P_2Na$: 535.0993; found: 535.0997 $[M+Na^+]$.

**1,2-Bis(4-trimethylsilylethynylphenyl)diphosphanoethane**: A solution of (4-bromophenylethynyl)trimethylsilane (2.183 g, 8.624 mmol) in anhydrous THF (4 mL) was added in one portion to activated Mg (230 mg, 9.48 mmol) under argon. The mixture was heated at 55 °C for 2 h and then cannulated into a solution of $DPPECl_4$ in anhydrous THF (8 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 18 h. Thereafter, the reaction was quenched by the addition of saturated aqueous $NH_4Cl$ solution (10 mL) and water (10 mL). After extraction with diethyl ether, the organic phase was dried ($Na_2SO_4$) and filtered, the volatiles were evaporated, and the crude oil was purified by chromatography on silica gel ($CH_2Cl_2$/cyclohexane 2:8) to afford a pale-yellow solid (424 mg, 26 %). The product was used directly for the synthesis of ethylene 1,2-bis(4-trimethylsilylethynylphenyl)-diphosphane oxide. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 0.24$ (s, 36H), 1.98 (t, $J = 4.1$ Hz, 4H), 7.18 (m, 8H), 7.37 ppm (dd, $J = 0.5$ and 8 Hz, 8H); $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 0.0$, 23.7, 95.7, 104.6, 123.7, 131.9, 132.4, 132.5, 132.6, 138.4 ppm; $^{31}$P NMR ($CDCl_3$, 121 MHz): $\delta = -12.0$ ppm; HRMS (MALDI TOF): $m/z$: calcd for $C_{46}H_{56}P_2Si_4$ $[M^+]$: 783.3007; found: 783.3004.

**Ethylene 1,2-bis(4-trimethylsilylethynylphenyl)diphosphane oxide**: 30 % $H_2O_2$ (360 μL) was added dropwise to a solution of 1,2-bis(4-trimethylsilylethynylphenyl)diphosphanoethane (394 mg, 0.5 mmol) in dichloromethane (1.5 mL) and methanol (6 mL). The reaction mixture was stirred at room temperature for 4 h. The excess $H_2O_2$ was then reduced with aqueous $Na_2SO_3$ solution (6 mL) and dichloromethane (10 mL) was added. The organic phase was dried over $MgSO_4$ and filtered, and the volatiles were evaporated under reduced pressure to afford the desired product as a colorless solid (408 mg, 100 %). M.p. >250 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.55$ (m, 16H), 2.43 (t, $J = 2.4$ Hz, 4H), 0.24 ppm (s, 36H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 132.5$, 132.4, 130.7, 127.6, 103.7, 98.2, 29.9, 27.1, 0.0 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta = 32.2$ ppm; MS (CI, $NH_3$): $C_{46}H_{56}O_2P_2Si_4$: $m/z$: 815 $[M+H]^+$.

**Ethylene 1,2-bis[bis(4-ethynylphenyl)phosphane oxide] (7)**: $K_2CO_3$ (68 mg) was added to a solution of ethylene 1,2-bis(4-trimethylsilylethynylphenyl)diphosphane oxide (393 mg, 0.48 mmol) in anhydrous dichloromethane (4 mL) and methanol (6 mL) under argon, and the reaction mixture was stirred at room temperature for 4 h. The reaction was then quenched with water (10 mL), and AcOEt (20 mL) was added. After extraction, the organic layer was dried ($Na_2SO_4$) and filtered, and the volatiles were evaporated in vacuo to afford the desired desilylated product **7** as a colorless solid (200 mg, 80 %). M.p. >250 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.62$ (m, 16H), 3.20 (s, 4H), 2.47 ppm (d, 4H, $J = 2.7$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 132.6$, 132.5, 132.4, 131.7, 131.1, 130.6, 126.4, 82.3, 80.2, 29.7, 21.4 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta =$ 31.8 ppm; HRMS (ES): $m/z$: calcd for $C_{34}H_{24}O_2P_2Na$: 549.1149; found: 549.1164 $[M+Na]^+$.

**Bis-4-{ethyl 4-[4-di(phenylene ethynylene)phenoxy]butyrate}diphosphanoylmethane (1)**: Iodoaryl building block **8** (434 mg, 1.01 mmol, 6.5 equiv) was added to a solution of **6** (80 mg, 0.16 mmol) in toluene (25 mL) and triethylamine (7 mL). The mixture was degassed under argon, CuI (7.15 mg, 24 mol %) and $[Pd(PPh_3)_4]$ (21 mg, 12 mol %) were added, and the reaction mixture was stirred for 20 h at 50 °C under argon. The volatiles were then evaporated in vacuo and the residue was dissolved in dichloromethane (10 mL). The organic phase was washed with aqueous HCl (10 %), dried ($Na_2SO_4$), filtered, and the solvents were evaporated under reduced pressure. The crude product was then purified by flash chromatography (silica gel; $CH_2Cl_2 \rightarrow CH_2Cl_2$/acetone 1:1) to afford the desired phosphane oxide **1** as a colorless solid (197 mg, 71 %). M.p. 254 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.28$ (m, 8H), 7.52–7.43 (m, 32H), 6.84 (d, $J = 8.7$ Hz, 8H), 4.15 (q, $J = 7.2$ Hz, 8H), 4.02 (t, $J = 6.1$ Hz, 8H), 3.54 (t, $J = 15$ Hz, 2H), 2.52 (t, $J = 7.3$ Hz, 8H), 2.14 (m, 8H), 1.26 ppm (t, $J = 7.4$H, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 173.2$, 159.2, 133.3, 131.8, 131.6, 131.5, 131.1, 127.4, 124.2, 122.0, 115.2, 114.7, 92.3, 91.9, 90.0, 87.9, 67.0, 60.6, 30.9, 24.7, 14.4 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta = 24.3$ ppm; IR (powder): $\tilde{\nu} = 2948$ (m), 2213 (s), 1735 (s), 1595 (s), 1438 (s), 1285 (s), 1245 (s), 1175 (s), 1106 (s), 1035 cm$^{-1}$ (s); HRMS (MALDI): $m/z$: calcd for $C_{113}H_{95}O_{14}P_2$: 1737.6191; found: 1737.6161 $[M+H]^+$; UV/Vis: $\lambda$ ($CH_3CN/CHCl_3$ 8:2) = 335 nm ($\varepsilon = 2.4 \times 10^5$ M$^{-1}$ cm$^{-1}$).

**1,2-Bis-4-{ethyl 4-[4-di(phenylene ethynylene)phenoxy]butyrate}diphosphanoylethane (2)**: Iodoaryl building block **8** (536 mg, 1.235 mmol, 6.5 equiv) was added to a solution of **7** (100 mg, 0.19 mmol) in toluene (30 mL) and triethylamine (10 mL). The mixture was degassed under argon, CuI (4 mg, 5 mol %) and $[Pd(PPh_3)_4]$ (22 mg, 5 mol %) were added, and the reaction mixture was stirred for 20 h at 50 °C under argon. The volatiles were then evaporated in vacuo and the residue was dissolved in dichloromethane (10 mL). The organic phase was washed with 10 % aqueous HCl, dried ($Na_2SO_4$), filtered, and the solvents were evaporated under reduced pressure. The crude product was then purified by flash chromatography (silica gel; $CH_2Cl_2 \rightarrow CH_2Cl_2$/acetone 1:1) to afford the desired phosphane oxide **2** as a colorless solid (50 %). M.p. 256 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.7$–7.6 (m, 16H), 7.48–7.43 (m, 24H), 6.86 (dd, $J = 2$ and 6.9 Hz, 8H), 4.15 (q, $J = 7.2$ Hz, 8H), 4.04 (t, $J =$ 6.1 Hz, 8H), 2.52 (m, 12H), 2.14 (m, 8H), 1.25 ppm (t, $J = 7.2$ Hz, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 173.2$, 159.7, 133.3, 132.1, 131.9, 131.8, 130.9, 127.5, 124.3, 122.0, 115.2, 114.7, 92.4, 91.9, 89.9, 87.9, 67.0, 60.6, 30.9, 29.8, 24.7, 14.3 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta = 32.1$ ppm; IR (powder): $\tilde{\nu} = 2925$ (m), 2207 (s), 1726 (s), 1589 (s), 1472 (s), 1250 (s), 1175 (s), 1105 (s), 1035 cm$^{-1}$ (s); HRMS (MALDI): $m/z$: calcd for $C_{114}H_{97}O_{14}P_2$: 1751.6348; found: 1751.6358 $[M+H]^+$.

**Methylene-bis[4-(trimethylsilylethynyl)phenyl]phosphane oxide**: A solution of (4-bromophenylethynyl)trimethylsilane (19 g, 75.3 mmol) in anhydrous THF (50 mL) was added in one portion to activated Mg (1.98 g, 82.7 mmol) under argon. The mixture was heated at 55 °C for 2 h and then cannulated into a solution of $CH_3P(O)Cl_2$ (5 g, 37.6 mmol) in anhydrous THF (20 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 18 h. The reaction was then quenched by the addition of saturated aqueous $NH_4Cl$ solution (50 mL) and water (10 mL). After extraction with diethyl ether, the organic phase was dried ($Na_2SO_4$) and filtered, the volatiles were evaporated, and the crude oil was purified by flash chromatography ($CH_2Cl_2$/AcOEt 1:1) to afford the product as a colorless solid (6.81 g, 44 %). M.p. 196 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.65$–7.51 (m, 8H), 2.00 (d, $J = 13.2$ Hz, 3H), 0.24 ppm (s, 18H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 134.5$, 133.1, 132.3, 132.1, 130.6, 130.4, 127.1, 103.9, 97.7, 16.5 (d, $J = 74$ Hz), 0.0 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta = 29.5$ ppm; MS: $C_{23}H_{29}OPSi_2$: $m/z$: 409 $[M+H]^+$; UV/Vis ($CH_3CN/CHCl_3$ 8:2): $\lambda = 334$ nm ($\varepsilon = 2.5 \times 10^5$ M$^{-1}$ cm$^{-1}$).

**Bis[4-(trimethylsilylethynyl)phenyl]phosphanoyl-diphenyl phosphanoylmethane**: A solution of LTMP was prepared by treating a solution of TMPH (195 μL, 1.15 mmol) in THF (1 mL) with 2.5 M *n*BuLi (460 μL, 1.15 mmol) for 5 min at 0 °C under argon. This solution was then cannulated at −40 °C into a solution of methylene-bis[4-(trimethylsilylethynyl)-phenyl]phosphane oxide (204 mg, 0.5 mmol) in THF (2 mL) under argon. The mixture was stirred for 1.5 h at 40 °C and then diphenylphosphanic chloride (114 μL, 0.6 mmol) was added dropwise at −40 °C under argon. The resulting mixture was stirred for 4 h at −40 °C and then the reaction was quenched by the addition of saturated aqueous $NH_4Cl$ solution (5 mL). After extraction with dichloromethane (5 mL), the organic layer was dried ($Na_2SO_4$), filtered, and concentrated under reduced pressure. The crude yellow solid was purified by flash chromatography (AcOEt, $R_f = 0.4$) and the solid obtained was triturated with the minimum volume of AcOEt to afford a colorless solid (70 mg, 27 %). M.p. 196 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.67$–7.65 (m, 8H), 7.39–7.36 (m, 8H), 3.50 (t, $J =$ 13.3 Hz, 2H), 0.25 ppm (s, 18H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 133.3$, 132.9, 132.0, 131.8, 131.1, 131.0, 128.8, 128.6, 127.1, 104.0, 97.7, 34.9, 0.0 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta = 24.7$, 24.3 ppm; MS: $C_{23}H_{29}OPSi_2$: $m/z$: 608.

**Bis(4-ethynylphenyl)phosphanoyl-diphenylphosphanoylmethane (10)**: $K_2CO_3$ (26 mg, 0.1 mmol) was added to a solution of bis[4-(trimethylsilylethynyl)phenyl]phosphanoyl-diphenylphosphanoylmethane (50 mg, 0.08 mmol) in a mixture of dichloromethane (1 mL) and MeOH (2 mL), and the reaction mixture was stirred at room temperature for 4 h. After complete conversion, water (4 mL) was added and the mixture was extracted with dichloromethane (5 mL). The organic layer was then dried ($Na_2SO_4$), filtered, and concentrated in vacuo. The crude oil was then triturated with $Et_2O$/cyclohexane 1:1 to afford **10** as a colorless solid (31 mg, 84 %). M.p. 222 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=7.55$–7.45 (m, 8H), 7.44–7.34 (m, 10H), 3.32 (t, $J=14.7$ Hz, 2H), 2.99 ppm (s, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta=133.1$, 132.1, 131.9, 131.1, 130.9, 130.9, 130.7, 128.6, 128.5, 126.0, 82.5, 79.84, 34.3 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta=24.6$ (d, $J=11.5$ Hz), 24.1 ppm (d, $J=11.5$); HRMS (ES): $m/z$: calcd for $C_{29}H_{22}O_2P_2Na$ 487.0993; found 487.0983 $[M+Na]^+$.

**Bis-4-{ethyl 4-[4-di(phenylene ethynylene)phenoxy]butyrate}phosphanoyl-diphenylphosphanoylmethane (3)**: Iodoaryl building block **8** (86 mg) was added to a solution of **10** (31 mg) in anhydrous toluene (4 mL) and triethylamine (2 mL), the mixture was degassed with argon, and then $[Pd(PPh_3)_4]$ (7.7 mg) and CuI (1.5 mg, 10 mol %) were added. The reaction mixture was stirred at 50 °C under argon for 20 h. After cooling to room temperature, the mixture was washed with saturated aqueous $NH_4Cl$ solution (10 mL) and extracted with AcOEt (10 mL). The organic layer was dried ($Na_2SO_4$), filtered, and the volatiles were removed under reduced pressure. The residue was purified by flash chromatography (silica gel; AcOEt) to afford **3** as a colorless solid (20 mg, 28 %). M.p. 210 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=7.77$–7.68 (m, 10H), 7.49–7.34 (m, 24H), 6.87 (d, $J=8$ Hz, 4H), 4.15 (q, $J=7$ Hz, 4H), 4.03 (t, $J=6.1$ Hz), 3.54 (t, $J=15.6$ Hz, 2H), 2.52 (t, $J=7.2$ Hz, 4H), 2.12 (m, 4H), 1.26 ppm (t, $J=7$ Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta=173.1$, 159.1, 133.1, 132.6, 131.9, 131.7, 131.5, 131.4, 131.1, 131.0, 130.9, 130.8, 128.6, 128.5, 127.0, 124.1, 122.0, 115.0, 114.6, 91.9, 91.7, 90.0, 87.8, 66.8, 60.5, 34.8 (t), 30.7, 24.5, 14.2 ppm; $^{31}$P NMR (121 MHz, $CDCl_3$): $\delta=24.6$ (d, $J=14$ Hz), 24.3 ppm (d, $J=14$ Hz); HRMS (MALDI): $m/z$: calcd for $C_{69}H_{59}O_8P_2$:1077.3680; found 1077.3692 $[M+H]^+$.

**Spectroscopic measurements**: UV/Vis absorption spectra were recorded on a Varian Cary 5E spectrophotometer and corrected emission spectra were obtained on a Jobin-Yvon Spex Fluorolog 1681 spectrofluorimeter. Fluorescence quantum yields were determined by using quinine sulfate dihydrate in sulfuric acid (0.5 N; $\Phi_F=0.546$[20]) as a standard. For the emission measurements, the absorbances at the excitation wavelengths were below 0.1 and so the concentrations were below $10^5$ mol L$^{-1}$. Fluorescence intensity decays were obtained by the single-photon timing method with picosecond laser excitation using a Spectra Physics set-up composed of a titanium sapphire Tsunami laser pumped by an argon ion laser, a pulse detector, and doubling (LBO) and tripling (BBO) crystals. Light pulses were selected by optoacoustic crystals at a repetition rate of 4 MHz. Fluorescence photons were detected through a long-pass filter (375 nm) by means of a Hamamatsu MCP R3809U photomultiplier connected to a constant-fraction discriminator. The time-to-amplitude converter was purchased from Tennelec. Data were analysed by a nonlinear least-squares method with the aid of Globals software (Globals Unlimited, University of Illinois at Urbana-Champaign, Laboratory of Fluorescence Dynamics). Complexation constants were determined by global analysis of the evolution of all absorption and/or emission spectra by using the Specfit Global Analysis System V3.0 for 32-bit Windows system. This software uses singular value decomposition and nonlinear regression modelling by the Levenberg–Marquardt method.[21]

**Two-photon excited fluorescence spectroscopy**: Two-photon excited fluorescence spectroscopy was performed using a mode-locked Ti:sapphire laser generating 150 fs wide pulses at a rate of 76 MHz, with a time-averaged power of several hundreds of mW (Coherent Mira 900 pumped by a 5 W Verdi). The laser light was attenuated using a combination of half-wave plates and a Glan laser polariser, and the excitation power was further controlled using neutral density filters of varying optical density mounted in a computer-controlled filter wheel. After five-fold expansion through two achromatic doublets, the laser beam was focused by a microscope objective (10×, NA 0.25, Olympus, Japan) into a standard 1 cm absorption cuvette containing the sample. The average applied laser power arriving at the sample was between 0.5 and 15 mW, leading to a time-averaged light flux in the focal volume of the order of 0.1–1 mW µm$^{-2}$. The generated fluorescence was collected in epi-fluorescence mode, through the microscope objective, and reflected by a dichroic mirror (675dcxru, Chroma Technology Corporation, USA). Residual excitation light was removed using a barrier filter (e650–2p, Chroma) and the fluorescence was coupled into a 600 µm multimode fiber by an achromatic doublet. The fiber was connected to a compact CCD-based spectrometer (BTC112-E, B&WTek, USA), with which the two-photon excited emission spectrum was measured. The emission spectra were corrected for the wavelength-dependence of the detection efficiency using correction factors established through measurements on reference compounds with known fluorescence emission spectra. Briefly, the set-up allowed the recording of corrected fluorescence emission spectra under multiphoton excitation at variable excitation power and wavelength.

Absolute values for the two-photon excitation action cross-sections $\sigma_2\Phi$ were obtained according to the method described by Xu and Webb, using $10^{-4}$ M fluorescein in 0.01 M aqueous NaOH as a reference.[6a] DPPMO (**1**) and DPPEO (**2**) were dissolved in $CH_3CN/CHCl_3$ (80:20) at concentrations of $3.8\times10^{-5}$ and $2\times10^{-5}$ M, respectively. The complexation studies were performed using 0.5 equiv of heavy metal ions in order to ensure a high proportion of the $ML_2$ complex.

**Solvents and salts**: Acetonitrile from Aldrich (spectrometric grade) and Millipore-filtered water (conductivity $<6\times10^8\ \Omega^{-1}$cm$^{-1}$ at 20 °C) were employed as solvents for the absorption and fluorescence measurements. Sodium thiocyanate, potassium thiocyanate, calcium perchlorate, zinc perchlorate, cadmium perchlorate, and lead(II) thiocyanate, from Aldrich or Alfa Aesar, were of the highest quality available and were vacuum-dried over $P_2O_5$ prior to use.

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