# Metallotetrabenzoporphyrins. New Phosphorescent Probes for Oxygen Measurements

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Tetrabenzoporphyrins (TBP) of Zn, Pd, Lu, Y, Sn and Pb have been synthesized and Pd, Sn and Lu complexes characterized by $^1$H NMR spectroscopy. Phosphorescence at room temperature in DMF solutions was measured for Pd and Lu complexes giving quantum yields of 7.9% and 3.5% and lifetimes of 250 μs and 870 μs, respectively. Pd *meso*-tetraphenyltetrabenzoporphyrin **1** ($PdPh_4TBP$) was synthesized, characterized by $^1$H NMR and showed a red shift of the Q-band to 628 nm. Electronic absorption spectra of synthesized complexes are briefly discussed with relation to their structures. Complex **1** reacted with $ClSO_3H$ and the obtained chlorosulfonato derivative **2** was converted into two water soluble chromophores: Pd *meso*-tetra(sulfophenyl)tetrabenzoporphyrin [$PdPh_4(SO_3Na)_4TBP$] **3** and the corresponding sulfonamide [$PdPh_4(PEG)_4TBP$] **4** with aminopoly-(ethyleneglycol) ($M_{av}$ 5000). Electronic absorption and phosphorescence spectra of **1** and its derivatives were recorded and phosphorescence quantum yields and lifetimes were measured for deoxygenated solutions. The oxygen quenching constants were measured for water-soluble complexes and the effect of binding to a large protein, serum albumin, on the phosphorescence characteristics was examined.

Metallotetrabenzoporphyrins were found to be useful phosphors for oxygen measurements in biological systems.

In past years metalloporphyrins have been widely studied and used in different areas of technology and medicine due to their unique optical properties. A new and very important application appeared recently, namely determination of oxygen in tissue and other biological materials by measuring oxygen-dependent quenching of phosphorescence.[1] Some Pt and Pd porphyrins have high extinction coefficients in the visible range and relatively large phosphorescence quantum yields, as well as lifetimes of triplet states and quenching constants well suited to use in measuring oxygen. Two different approaches are currently being used in developing phosphorescence-based oxygen measuring systems. One is the direct introduction of the porphyrins, either free or bound to other molecules, into the medium of interest, *i.e.*, biological materials,[1b] and the other is to incorporate the porphyrins into plastic or polymer films where the phosphorescence can be used as a measure of oxygen in the medium surrounding the film.[2] Our research group has focused on the former approach with emphasis on development of a method for non-invasive determination of oxygen in tissue.[1]

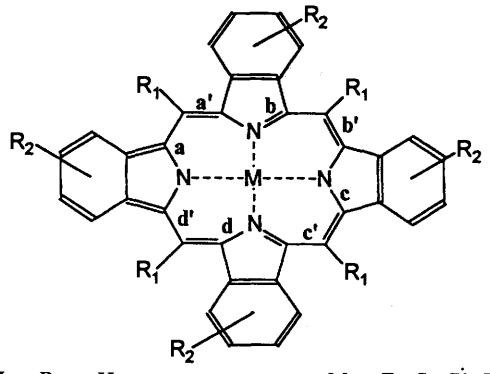
Most of the phosphorescent probes used in biological experiments at the present time are based on a few chromophores with well known absorption and emission properties: Pd *meso*-porphyrin, Pd coproporphyrin and Pd-(*meso*-tetracarboxyphenyl)porphyrin. All these compounds have strong phosphorescence in the near infrared region and can be excited either at Soret or Q bands which lie at about 380–430 nm and 500–560 nm, respectively. However, in tissue other chromophores, such as haemoglobin, myoglobin, and cytochromes, are present in high concentrations and they also absorb light at the same wavelength range. This 'background' absorption limits penetration of the excitation light into the tissue to 50–100 μm near 400 nm and to 500–1000 μm around 560 nm. Oxygen measurements are therefore limited only to the surface layer of tissue or optically clear tissue such as the eye. The absorbance of the natural chromophores of the tissue decreases to very low values at wavelengths greater than about 600 nm. Thus, new phosphors with both their absorption and emission bands located in the near IR window (> 600 nm and < 1300 nm wavelength region where light is only very slightly absorbed by tissue) would permit oxygen measurements through a much greater depth of tissue.

Metal complexes of tetrabenzoporphyrins (TBP) (for a recent review see ref. 3) present an interesting class of chromophores which are potentially suitable for oxygen measurements. Their Q-band absorption occurs at the blue border of the near IR window of tissue (> 600 nm) and extinction coefficients are two–three times larger than for corresponding porphyrins. Phosphorescence at 77 K has been detected for Pd,[4] Pt,[4,5] Lu, Cd and Zn[6,7] tetrabenzoporphyrins and also for Mg, Zn, Cd and Pd diphenyltetrabenzoporphyrins.[8] Room temperature phosphorescence has been observed for PdTBP and ZnTBP in deoxygenated pyridine solutions with lifetimes of a few milliseconds[9] however quantum yields have not been reported.†

In this study tetrabenzoporphyrin complexes of several metals (Sn, Pb, Pd, Y and Lu) have been synthesized and tested for the presence of oxygen-dependent phosphorescence at room temperature. PdTBP and LuTBP showed long lived triplet states. The Pd complex has the higher phosphorescence quantum yield (7.9%).

Phosphorescent probes for biological applications have to be water soluble. Therefore further derivatization has to be performed after the chromophore molecule is synthesized, or (and) functional groups have to be introduced into the structure at the stage of formation of the macrocycle. The latter approach, however, is limited to only a few inert substituents[10] because of the very extreme reaction conditions required for tetrabenzoporphyrin macrocycle formation. Tetraphenyltetrabenzoporphyrins ($Ph_4TBP$), on the other hand, seem to be more appropriate for modification than non-substituted TBP because the phenyl rings are more reactive toward various electrophylic agents. In addition, recent spectroscopic measurements of mono-, di-, tri- and tetra-phenyl substituted Zn tetrabenzoporphyrins[11] showed that the bathochromic shift of the Q-band is dependent

† The existence of room-temperature phosphorescence of perfluorotetrabenzoporphyrins of Pt and Pd has been pointed out in ref. 2(*b*).




$R_1$ = H $R_2$ = H M = Zn, Sn, Pb, Y, Lu, Pd
$R_1$ = Ph $R_2$ = H (**1**) M = Zn, Pd
$R_1$ = Ph $R_2$ = $SO_2Cl$ (**2**), $SO_3Na$ (**3**), $SO_2NH$–(PEG) (**4**) M = Pd

**Fig. 1** Compounds synthesized in this paper. **a**–**a'**, **b**–**b'**, **c**–**c'** and **d**–**d'** are pairs of bonds used to estimate macrocycle planarity (see the text).

on the number of phenyl groups in the macrocycle. Considering that non-substituted PdTBP has an absorption maximum[4] at 607 nm, near the IR window of tissue, shift of the Q-band farther to the red is highly desirable. Thus $PdPh_4TBP$ porphyrin has been synthesized and its Q-band absorption maximum was determined as 628 nm in dimethylformamide (DMF) solution. The phosphorescence lifetime and quantum yield of $PdPh_4TBP$ in degassed DMF solution has been measured, giving values of 250 μs and 8.0%, respectively. Finally two water-soluble derivatives of $PdPh_4TBP$ were prepared and their phosphorescence characteristics (lifetimes in deoxygenated solutions and phosphorescence Stern-Volmer quenching constants for **3**) are also reported.

## Results and Discussion

*Synthesis.*—Fig. 1 summarizes the compounds studied in this paper. The discussion of methods for the preparation of ZnTBP, which is the starting compound for most of all related syntheses, has been given by Edwards *et al.*[12] A method based on template condensation of carboxymethylphthalimidine has also been suggested[12] and used for preparation of high purity ZnTBP. A more complete review of reactions leading to the TBP macrocycle has appeared recently.[3] Three other condensations, which are of practical interest, have been used: starting from potassium phthalimide,[13] isoindole[14] and 2-acetylbenzoic acid.[4] The method suggested by the Kopranenkov *et al.*[13] has been chosen in our study because of the commercial availability and low cost of starting materials (potassium phthalimide) and a reasonably high yield (26%). ZnTBP, synthesized according to ref. 13, was initially purified as suggested in ref. 12 and introduced into the following transformations.

A few methods for demetallation of metallotetrabenzoporphyrins using different acidic agents have been described previously. However, displacement of Zn with sulfuric acid[15] can lead to the partial sulfation of the tetrabenzoporphyrin macrocycle and use of HCl–$CHCl_3$, proposed in ref. 16, is inconvenient owing to the necessity of using gaseous HCl. On the other hand, we failed to obtain demetallation of the Zn complex with acetic acid, as suggested in ref. 10(*a*). A mixture of trifluoroacetic acid in $CHCl_3$, successfully employed for demetallation of CdTBP[17] and $ZnPh_4TBP$,[18b] did not completely demetallate ZnTBP even after heating for long periods of time. Dissolving ZnTBP in a hot mixture of acetic and phosphoric acids (1:5) lead to complete removal of Zn in about 2 h.

Insertion of metals into metal free TBP was performed using either DMF or an imidazole melt as the reaction medium. We failed to obtain good yields of metallation in refluxing DMF using such metal chlorides as $PdCl_2$, and $LuCl_3{\cdot}6H_2O$. When TBP was refluxed in DMF with an excess of $PdCl_2$,[4] the visible spectrum of the reaction mixture showed a small peak at 665 nm characteristic of free TBP, even after 4 days. However, $LuCl_3$ and $Pd(OAc)_2$ are much more reactive and their insertion could be carried out quantitatively in hot DMF within a few hours.

PdTBP has been synthesized and purified following the method suggested for ZnTBP by Edwards *et al.*[12] PdTBP is outstandingly inert towards strong acids. Thus overnight heating (60–70 °C) in $ClSO_3H$ did not lead to the removal of Pd from the coordination sphere of the macrocycle. On the other hand, all of the other metallotetrabenzoporphyrins studied could be relatively easily demetallated using HCl, $CF_3COOH$ or even acetic acid in case of Y and Lu complexes. Probably the residual chlorine atom on Lu was at least partially replaced by $^-OH$ during the aqueous treatments of the reaction mixture, because of the well known oxophilicity of lanthanides.

Insertion of Sn into TBP occurs easily and the formed complex has good stability in solution. Considering that $Sn^{II}$ is strongly reducing and that the spectrum of the synthesized complex (see below) does not show the split of the Soret band typical for $Sn^{II}$ porphyrins, the TBP complex probably contains tin in the oxidation state VI.* The picture is far more complicated in the case of PbTBP. Heating of an excess of $Pb(OAc)_2$ with $H_2TBP$ in DMF leads to very fast and complete change in the absorption spectrum (see below). However, cooling the solution results in rapid formation of a white residue, partial decompositions of PbTBP and the initial appearance of the absorption bands of $H_2TBP$. This pattern of the spectrum remains stable for days when the solution is concentrated. The most intriguing feature of the system is that simple dilution of the mixture sample with a larger amount of DMF at room temperature again produces the spectrum of pure PbTBP, not contaminated with lines of free $H_2TBP$. This behaviour of the system is highly reproducible and it did not allow us to separate the pure Pb complex for the stoichiometric analysis. However, diluted solutions of the complex are stable and therefore suitable for further spectroscopic studies. The relatively high stability of $Pb^{II}$ towards oxidation suggests that the synthesized complex most likely contains $Pb^{II}$.

$^1H$ NMR spectra of Pd and Sn complexes are similar to that reported for CdTBP,[17] and PdTBP shows a *ca.* 0.1 ppm shift of all signals towards high field. The spectrum of LuTBP [Fig. 2(*a*)] is shifted even further (almost 0.5 ppm) and we were unable to obtain resolved resonances of the benzo ring protons.

As reported by Ichimura *et al.*,[18a] and recently repeated in ref. 11, all procedures originally proposed for synthesis of *meso*-phenyl substituted tetrabenzoporphyrin systems[10a,14] usually give complicated mixtures of products. Zinc acetate, used as a template, can also act as a donor of unsubstituted methine bridges in the macrocycle, and therefore reaction mixtures contain mono-, di- and tri-phenyl substituted tetrabenzoporphyrins. On the other hand, using Zn benzoate instead of

* In the initial review of our manuscript a reviewer suggested the possible structure $Sn(OH)_2TBP$. This enforced us to check additionally the stoichiometry of the complex. Subsequent laser desorption mass spectrometry of the sample showed a main peak with $m/z$ = 662, the molecular weight of the suggested dihydroxy complex. An additional, smaller, peak appeared at $m/z$ = 679, and it was attributed to $SnCl(OH)TBP(H)^+$. Peaks with $m/z$ 644 and 628, have much lower intensity, and these are probably the result of molecular ion fragmentation: $Sn(OH)TBP(H)^+$ and $SnTBP(H)^+$. We also failed to obtain any VIS spectral changes when benzene solutions of the complex were treated with a strong oxidant such as tetrachloro-1,4-benzoquinone, although these would have been expected if the compound contained the strongly reducing $Sn^{II}$.

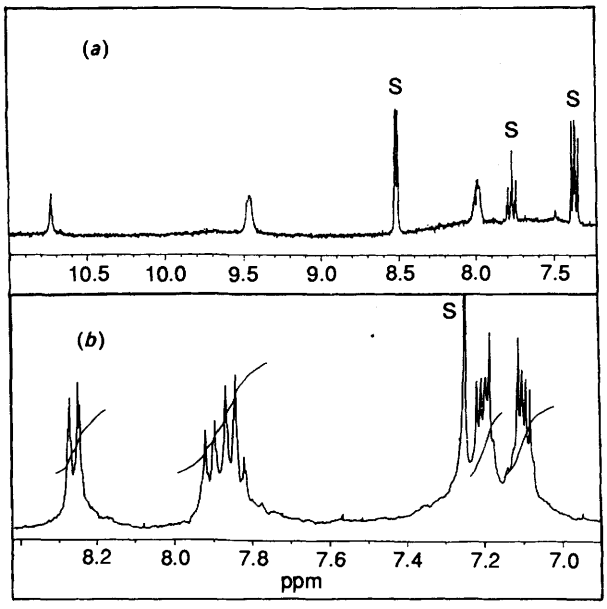
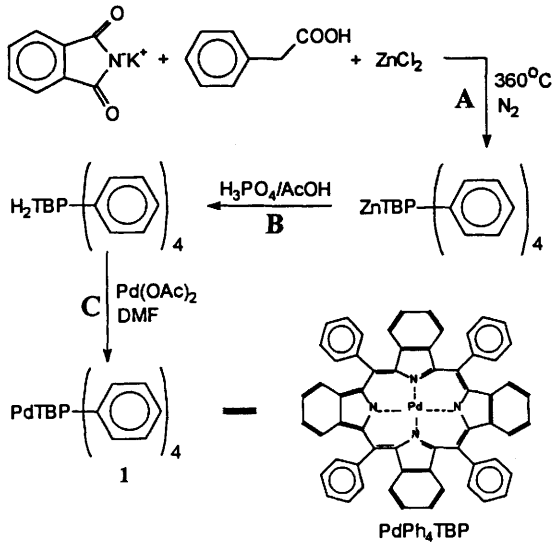



**Fig. 2** $^1$H NMR spectra of LuTBP in $(CD_3)_2SO$ (*a*) and $PdPh_4TBP$ in $CDCl_3$ (*b*); Peaks marked with S originate from solvents




**Scheme 1**

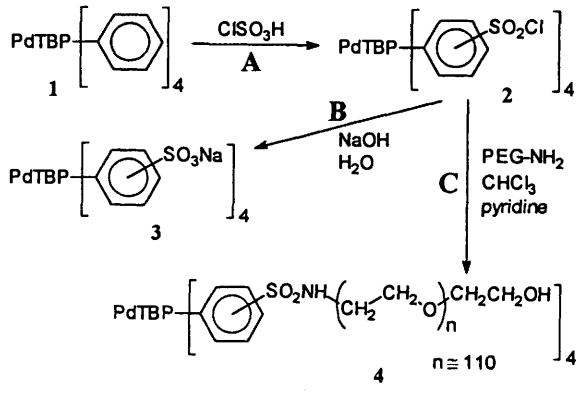
Zn acetate has been reported to increase the relative yield of tetraphenyltetrabenzoporphyrin in the template condensation of 3-benzylidenephthalimidine.[18b] We adopted the same strategy and in our case $ZnPh_4TBP$ was synthesized by the condensation of potassium phthalimide with phenylacetic acid, the method suggested in ref. 10(*a*), in the presence of $ZnCl_2$ (see Scheme 1**A**). Prepared $ZnPh_4TBP$, was used in further transformations without additional purification. [A sample of $ZnPh_4TBP$ was purified as described in ref. 18(*b*)].

Demetallation of $ZnPh_4TBP$, carried out in a mixture of $H_3PO_4$–AcOH (Scheme 1**B**), turned out to be much more rapid than for ZnTBP, as was insertion of Pd into free $H_2Ph_4TBP$ (Scheme 1**C**). The latter took only about 30 min in refluxing DMF, while formation of PdTBP required a few hours of refluxing. Insertion of other metals into free $Ph_4TBP$ was not as successful as for Pd. Thus, heating of $PtBr_2$ with $H_2Ph_4TBP$ in either DMF or an imidazole melt resulted in only traces of the corresponding platinum complex even after a few days. The same lack of reactivity was obtained for $LuCl_3$ and $YCl_3$, but in contrast the reaction with $Pb(OAc)_2$ occurred within a few minutes. It should be noted that attempts to use $PdCl_2$ in the case of phenylated TBP also gave only traces of $PdPh_4TBP$ (**1**).

The $^1$H NMR spectrum of **1** [Fig. 2(*b*)] is consistent with that reported for $ZnPh_4TBP$,[11,18a] and also shows about 0.2 ppm shifts of all macrocycle resonances to high field.

It is known that the closest analogues of tetrabenzoporphyrins, the phthalocyanines, form aggregates in the presence of even tiny amounts of water. The luminescent properties of aggregates are significantly different from those for monomeric species. Thus the phosphorescence quantum yield might be dramatically decreased by self-quenching when the distance between individual macrocycles decreases due to aggregation. Ehrenberg *et al.*[19] reported that in the concentration range 0.05–20 μmol $dm^{-3}$ there was no evidence for aggregation of Mg and Zn tetrabenzoporphyrins in water solutions, but the absorption spectra still showed significant broadening. It also has been pointed out that fluorescence quantum yields were significantly increased when the complexes were placed in a lipophilic environment, such as lipid vesicles. In order to determine whether Pd tetrabenzoporphyrins still showed reasonable phosphorescence in aqueous media, two water soluble derivatives of **1** were synthesized: $Pd(SO_3H)_4Ph_4TBP$ (**3**) and poly(ethyleneglycol)-modified $Pd(PEG)_4Ph_4TBP$ (**4**) and their spectroscopic properties were examined.

The chlorosulfonated derivative of **1** was prepared by treatment of **1** with chlorosulfonic acid at room temperature (Scheme 2**A**). This yielded a compound which was then




**Scheme 2**

converted into the water-soluble sulfonato derivative by hydrolysis in aqueous NaOH (Scheme 2**B**). $^1$H NMR spectra of the compound isolated after chlorosulfation showed the presence of a mixture of substituted products. However the signals of the benzo rings' protons appeared practically unchanged, which suggests substitution mainly into phenyl rings.

Purification of water-soluble derivative **3** from inorganic salts after hydrolysis was performed using dialysis and ultrafiltration through the molecular membranes with a cut-off molecular weight of 3000. It should be mentioned here that, for reasons that are not well understood, tetraphenylporphyrins containing sulfonato-, as well as carboxy-groups, and sulfonated phthalocyanines do not readily penetrate the pores of dialysis membranes, even if the pore size of the latter is substantially larger than required by the molecular weight of macrocycle. This effect has been used for initial purification of **3**, since it allows removal of the NaCl formed during neutralization of the reaction mixture after hydrolysis. Attempts to pass **3** through the column with Sephadex-G25 resulted in complete absorption of dye in the upper part of chromatographic column, suggesting very high affinity of Sephadex for this ionic porphyrin.

The chlorosulfonic derivative **2** formed sulfonamide **4** with $NH_2$-modified poly(ethylene glycol) ($M_{av}$ 5000) (Scheme 2**C**). Deep green aqueous solutions of **4** could be purified by gel

**Table 1** Absorption maxima of metallotetrabenzoporphyrins (solutions in DMF at room temperature)

| Compound | λ[Soret (0,0)]/nm (log ε) | λ(Other)/nm | λ[Q (0,0)]/nm (log ε) | λ(Other)/nm |
|---|---|---|---|---|
| ZnTBP[a] | 433 (5.50) | 408 | 628 (4.99) | — |
| SnTBP[b] | 430 (4.81) | 413 | 638 (4.63) | 593 |
| PbTBP[c] | 483 (—) | 429 | 659 (—) | 622 |
| YTBP[d] | 426 (4.96) | 403 | 627 (4.49) | 581 |
| LuTBP[a,d] | 426 (4.98) | 399 | 627 (4.49) | 580 |
| PdTBP[a] | 407 (5.25) | 384 | 606 (5.02) | 556 |

[a] Absorption data for these complexes have been reported earlier (see the text for references) and reproduced in this study. [b] log ε is given assuming the molecular formula $Sn(OH)_2TBP$. [c] Extinction coefficients could not be measured. See the text for the details. [d] log ε values are given assuming molecular formulas Y(OH)(pyridine)TBP and Lu(OH)(pyridine)TBP, respectively.

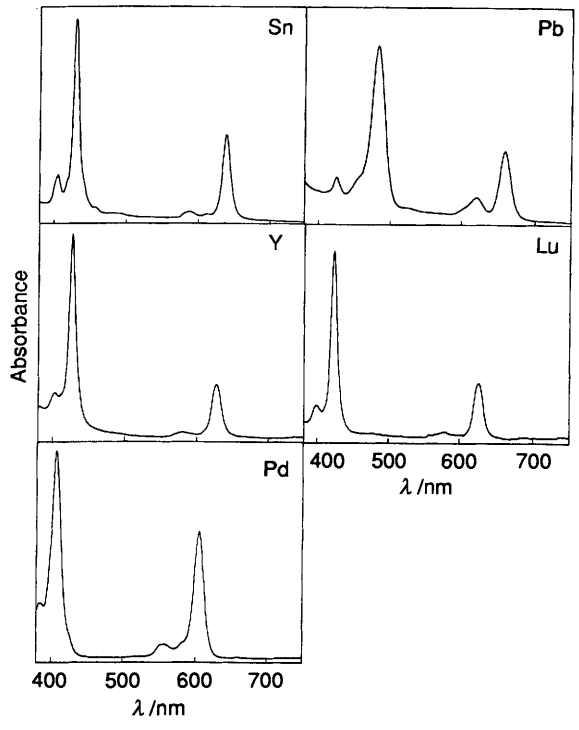



**Fig. 3** Electronic absorption spectra of metallotetrabenzoporphyrins

filtration on a Sephadex-G25 column. The entire green fraction eluted with the solvent front, suggesting a high degree of conversion into the PEG derivative. Spectroscopic determination, based on absorbance relative to parent $Ph_4TBP$ gave a molecular weight greater than 30 000, which suggests that the compound was not completely freed from $NH_2$-PEG present in excess in the reaction mixture. However, because this purity is completely optically inactive in the wavelength range of interest it could not affect further spectroscopic study.

*Absorption Properties.*—Absorption spectra of Sn, Pb, Pd, Y and Lu tetrabenzoporphyrins are shown in Fig. 3 and the positions of absorption maxima are summarized in Table 1.

The data on Zn, Pd and Lu compounds are similar to those reported earlier (refs. 12, 4 and 7, respectively). All spectra show good agreement with Gouterman's four-orbital model,[20] which predicts strong degenerate $S_1 \longleftarrow S_0$ (Q-band) and $S_2 \longleftarrow S_0$ (Soret band) absorptions. Owing to the extended π-electron system of tetrabenzoporphyrins *vs.* the parent porphyrins, both bands are red shifted as expected. Usually an increase in stability of the metal complex is correlated with a blue shift of absorption spectra together with a relative increase of Q-band intensity. Thus, in the case of the Pd complex (highest stability in the series) the Q-band is most shifted to the blue (606 nm). The integral of the Q band is approximately 65% of the integral of the Soret band as compared to 38% for SnTBP and 26% for ZnTBP. However, PbTBP does not exactly follow this general tendency, being rather unstable. At the same time the integral of the Q-band absorption for PbTBP is 56% of that of the Soret band.

Lu and Y complexes are characterized by almost identical locations of the absorption bands. The difference in atomic weights of almost 100 units and the filled f-shell in the Lu atom has practically no effect on the absorption spectra of the metal complex. On the other hand, the close similarity in ionic radius of Y and Lu seems to be a much more important factor. Attempts to prepare the TBP complex of another diamagnetic lanthanide $La^{3+}$ were not successful, probably due to the large size of $La^{3+}$ (1.05 Å) compared with $Y^{3+}$ and $Lu^{3+}$ (0.90 Å and 0.86 Å, respectively). These observations are consistent with the general trend in lanthanide coordination chemistry, when steric effects often dominate over electronic factors.

Fig. 4 shows the absorption spectra of four *meso*-tetraphenylated Pd tetrabenzoporphyrins. Absorption band maxima are given in Table 2. Our data on the absorption of $ZnPh_4TBP$ are in a very good agreement with those published earlier.[18] The molar extinctions of $PdPh_4TBP$ are given in Table 3 together with literature data on ZnTBP, $ZnPh_4TBP$ and PdTBP for comparison.

Both absorption bands of the $PdPh_4TBP$ are shifted 20 nm to the red relative to the unsubstituted complex. The similar shift takes place for the case of ZnTBP *vs.* $ZnPh_4TBP$ complexes and also for free base *meso*-phenyl and *meso*-naphtho substituted porphyrins[21] and their Zn derivatives.[22] Absorption bands of diphenyl substituted Zn and Pd tetrabenzoporphyrins[8] occupy an intermediate position between non- and tetra-substituted compounds. Thus, for example, the Q-bands of PdTBP, $PdPh_2TBP$ and $PdPh_4TBP$ are at 606, 615 and 628 nm, respectively. Unlike non-extended Zn porphyrins,[22] neither Zn or Pd tetrabenzoporphyrins show a tendency towards increased intensity of the Q-band at the expense of Soret absorption when bulky substituents are introduced into the *meso*-positions. On the contrary, both Zn and Pd complexes show a decrease in total absorption and an increase in relative intensity of Soret bands.

Fonda *et al.*[21] attributed the red shift in the absorption bands within a series of *meso*-substituted porphyrins to two major factors: distortion of macrocycle planarity and extended conjugation of π-electrons of *meso*-substituents with the cyclic tetrapyrrole system. In case of tetrabenzoporphyrin systems this interpretation is certainly also valid. MM2 molecular-mechanics calculations carried out for $MPh_4TBP$[23] (where M is a hypothetical two-valent cation) showed even stronger ring distortion than in the case of a non-extended porphyrin system with *meso*-phenyl substituents. At the same time, the structure of non-substituted MTBP is almost completely planar. The

**Table 2** Absorption maxima of Pd tetraphenyltetrabenzoporphyrins

| Sample | λ[Soret (0,0)]/nm | λ[Q (0,0)]/nm | Width of Q-band/nm | λ(Other)/nm |
|---|---|---|---|---|
| $PdPh_4TBP$ (**1**)[a] | 442 | 628 | 22 | 579 |
| $Pd(SO_2Cl)_4Ph_4TBP$ (**2**)[b] | 443 | 629 | 31 | 578 |
| $Pd(SO_3H)_4Ph_4TBP$ (**3**)[b] | 443 | 631 | 30 | 582 |
| $Pd(PEG)_4Ph_4TBP$ (**4**)[b] | 446 | 634 | 33 | 583 |

[a] Solution in DMF. [b] Solution in $H_2O$.

**Table 3** Molar extinctions of Zn and Pd tetrabenzoporphyrins

| Compound | Solvent | ε(Soret) | ε(Q) | Ratio ($\varepsilon_{Soret}/\varepsilon_Q$) | Ref. |
|---|---|---|---|---|---|
| ZnTBP | Pyridine | 314 000 | 98 000 | 3.2 | 19 |
| $ZnPh_4TBP$ | $CHCl_3$ | 263 000 | 72 500 | 3.6 | 10(*a*) |
| PdTBP | DMF | 179 000 | 105 000 | 1.7 | 4 |
| PdTPhTBP | DMF | 119 000 | 51 000 | 2.3 | This work |

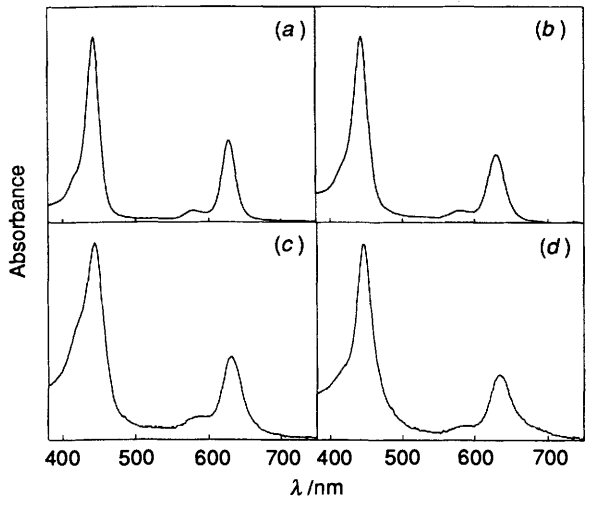



**Fig. 4** Electronic absorption spectra of Pd *meso*-phenyl substituted tetrabenzoporphyrins: **1**, **2**, **3** and **4**

average dihedral angle, characteristic of macrocycle non-planarity (average of angles **a**–**a'**, **b**–**b'**, **c**–**c'** and **d**–**d'** on Fig. 1), was calculated for $MPh_4TBP$ as 10.6°, and the structure as a whole has a 'saddle' shape with the *meso*-phenyl rings twisted at an angle of 51.2° (average value). The corresponding values determined for M tetraphenylporphyrin were found to be 1.2° and 55.3°, respectively. (The angles calculated in ref. 21 are 0.55° and 60.0°, and they are in a better agreement with experimentally found values.) Recent X-ray crystallographic data for $Zn(THF)Ph_4TBP$[24] showed a very strong non-planarity of the substituted macrocycle, with the phenyl rings twisted at 61.9°. Hence correlations of structural data with electronic spectra show the same effect of *meso*-phenyl substitution for benzoporphyrin systems as for non-extended porphyrins.

As seen from Table 3 chlorosulfonation of the phenyl rings does not significantly shift the positions of absorption peaks, whereas the absorption bands in the spectra of all three substituted compounds (Fig. 4) are about 10 nm broader than for $PdPh_4TBP$. Because chlorosulfonation of $PdPh_4TBP$ is a non-regioselective process, compound **2** is in fact a mixture of large number of isomers, each with its own absorption maximum. Therefore, the spectrum is a superposition of these multiple absorptions. The fact that spectra of hydrophilic derivatives are not substantially different in width from the spectrum of **2** in DMF suggests there is minimal aggregation of compounds **3** and **4** in water media, which is very important for phosphorescence applications.

*Emission Properties.*—Phosphorescence spectra of deoxygenated solutions of Pd and Lu tetrabenzoporphyrins, measured in DMF at room temperature are shown in Fig. 5. Both aerated and deaerated solutions were examined and the emission which appeared only in absence of oxygen was attributed to phosphorescence. The maxima of the emission bands for the compounds examined in this study are summarized in Table 4.

Phosphorescence at 77 K of non-substituted and diphenyl-substituted Zn tetrabenzoporphyrins has been reported.[8] There is also a report of ZnTBP triplet-state emission in pyridine solution at room temperature.[9] We were not, however, able to reproduce the latter result with either steady-state or time-correlated measurements. Both ZnTBP and $ZnPh_4TBP$, synthesized in this study, show strong $S_0 \longleftarrow S_1$ fluorescence from the first excited state, with peak positions being in good agreement with those reported previously.

$S_0 \longleftarrow S_1$ fluorescence of LuTBP in pyridine solution at room temperature was earlier observed[7] with a quantum yield of 0.21%. We have found, however, that part of it belongs to delayed fluorescence, because deoxygenated samples showed about a twofold stronger signal at 632 nm than the corresponding aerated samples. This indicates that $S_1$ and $T_1$ excited states have relatively close energies and that an equilibrium exists between them. The intensity of $S_0 \longleftarrow T_1$ phosphorescence for LuTBP is still much higher (see below), which is expected because of the high yield of intersystem crossing introduced by the heavy-atom effect. The phosphorescence maximum found for LuTBP is at the longest wavelength, 803 nm, of the compounds observed in this study. Despite the similarities between Lu and Y in the chemical behaviour and light absorption properties of corresponding TBP complexes, their emission spectra are distinctively different. In fact we were not able to detect any phosphorescence from YTBP under steady-state illumination conditions, though all deoxygenated samples of YTBP showed a very low intensity decay with relatively long (> 700 μs) lifetimes. These signals are probably due to very weak phosphorescence, which could barely be detected even with the sensitive equipment for time-correlated

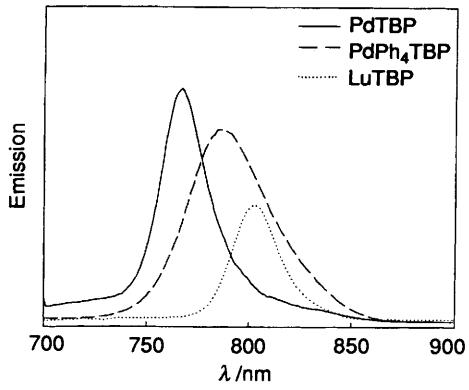



**Fig. 5** Phosphorescence spectra of Lu and Pd tetrabenzoporphyrins; excitation at maxima of Q-bands

**Table 4** Emission data for metallotetrabenzoporphyrins (f and p indicate fluorescence and phosphorescence, respectively)

| Compound | Emission maximum/nm |
|---|---|
| SnTBP[a] | 649, 698 (f) |
| PdTBP[a] | 767 (p) |
| LuTBP[a] | 632 (f);[b] 803 (p) |
| $ZnPh_4TBP$[a] | 630, 695 (f) |
| $PdPh_4TBP$[a] | 785 (p) |
| **3** in $H_2O$[c] | 790 (p) |
| **3** + BSA[c,d] in $H_2O$ | 790 (p) |
| **4** in $H_2O$[c] | 793 (p) |
| **4** + BSA[c,d] in $H_2O$ | 793 (p) |

[a] Measured in degassed DMF solutions at room temperature. [b] This fluorescent band becomes much weaker when solutions were equilibrated with air, which indicates dynamic equilibrium between $S_1$ and $T_1$ states. [c] Water solutions contained glucose and were deoxygenated with glucose oxidase–catalase system, as described in the Experimental section. [d] Probes were prepared using a buffered (pH = 7.2) 2% solution of bovine serum albumin.

measurements. Fluorescence from YTBP was also of very low intensity, as in the case of the Lu complex.

SnTBP shows fluorescence at 649 nm with the theoretically predicted mirror-image shape of the spectrum, but no phosphorescence. We were unable to observe any emission from PbTBP, which is most likely due to the presence of low-energy empty orbitals located on metal ions which support charge-transfer states.

Pd complexes were found to be the most phosphorescent among all of the studied compounds. Phosphorescence emission by **1** was found to have a maximum at 785 nm, which is a wavelength 10 nm longer than for $PdPh_2TBP$ (775 nm),[8] and 18 nm longer than for PdTBP (767 nm).[4] Similar shifts were observed for the absorption bands. The emission peak of **1** (48 nm in half height) is 20 nm wider than for PdTBP (28 nm) and LuTBP (27 nm). This might be due to the presence of a larger number of vibronic states: a consequence of the high non-planarity of the phenylated macrocycle. The normalized phosphorescence spectra of water-soluble Pd tetraphenyltetrabenzoporphyrins are presented in Fig. 6. As seen from the figure, the widths of the absorption bands are similar for all the compounds and the positions of the maxima shift only very slightly towards longer wavelength with addition of substituents in the phenyl rings.

In order to determine the suitability of the synthesized complexes for the measurement of oxygen, the phosphorescence quantum yields and lifetimes were measured and compared with the values for two of the phosphors already being used to measure oxygen in biological systems: Pd *meso*-tetra(4-carboxyphenyl)porphyrin (PdTCPP) and Pd mesoporphyrin (PdmP). The comparative data are presented in Table 5. The quantum yields of Pd porphyrin phosphorescence at 77 K are reported to be in the range 20–90%.[25] Our measured quantum yields at room temperature are in the range 2–20%, with the tetrabenzoporphyrins comparable to the corresponding non-extended porphyrin compounds. The quantum yields for phosphorescence of Pd *meso*-tetra(4-carboxyphenyl)porphyrin and Pd mesoporphyrin are 6.8% and 19.6%, respectively. $PdPh_4TBP$ has the highest phosphorescence quantum yield (8%) among the tetrabenzoporphyrin derivatives, though this is very close to that of the unsubstituted PdTBP (7.9%). The quantum yield of *meso*-tetraphenylated non-extended porphyrin (PdTCPP) is about 2%. For both non-extended and tetrabenzoporphyrins of Pd in organic solutions, the phosphorescence lifetimes* follow changes in quantum yields, however LuTBP, which is characterized by the lowest yield among organic soluble complexes (3.5%), has a longest phosphorescence decay (850 μs).

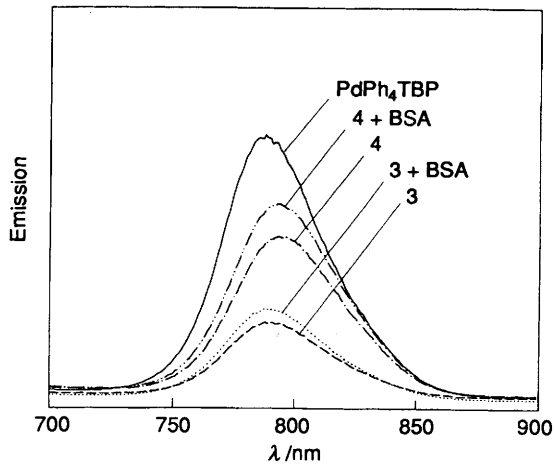



**Fig. 6** Phosphorescence spectra of **1**, its water-soluble derivatives **3** and **4**, and their adducts with bovine serum albumin (BSA). All spectra are corrected by relative absorbances at maxima of the Q-bands, used for excitation.

**Table 5** Phosphorescence quantum yields and lifetimes of some Pd porphyrins[a]

| Compound | Solvent | $\tau_p$/μs | $\varphi_p$ (abs.) (%)[d] | $\varphi_p$ rel.[e] |
|---|---|---|---|---|
| PdTCPP[b] | DMF | 220 | 6.8 | 1.0 |
| PdmP[c] | DMF | 670 | 19.2 | 2.8 |
| PdTBP | DMF | 260 | 7.9 | 1.1 |
| $PdPh_4TBP$ | DMF | 250 | 8.0 | 1.2 |
| LuTBP | DMF | 870 | 3.5 | 0.5 |
| **3** | $H_2O$ | 310 | 2.3 | 0.3 |
| **3** + BSA[f] | $H_2O$ | 320 | 2.8 | 0.4 |
| **4** | $H_2O$ | 260 | 5.1 | 0.7 |
| **4** + BSA[f] | $H_2O$ | 280 | 6.2 | 0.9 |

[a] Measured from deoxygenated solutions (see the Experimental section for details). [b] Pd (*meso*-tetracarboxyphenyl)porphyrin. [c] Pd *meso*-porphyrin. [d] Measured relative to $H_2TPP$ or ZnTPP fluorescence quantum yields (13% and 3.3% in deaerated benzene[24]), for which our relative values were to within ± 5% of published. [e] Given relative to the phosphorescence quantum yield of PdTCPP in degassed DMF. [f] BSA indicates 2% of bovine serum albumin present in the probe solution.

* The phosphorescence lifetime for PdmP in deoxygenated THF solution is reported to have a value of 1330 μs,[2a] which is significantly higher than that obtained in our study. This might be due to residual oxygen, present in the DMF even after multiple freezing–degassing–thawing cycles, and which could not be removed using chemical traps such as Na–benzophenone ketyl or Na–K alloy due to the nature of the solvent.

As mentioned above, PdTCPP has been extensively used in biological experiments. To our knowledge, the quantum yield of this phosphor has not been determined previously and according to our measurements has a value of 6.8%. The fact that quantum yields determined for TBP based complexes lie within the same range suggests that these chromophores may be useful for biological applications without significant reconstruction of measurement equipment.

Quantum yields of the water-soluble tetraphenyltetrabenzoporphyrin derivatives **3** and **4** were measured in water solutions and have values of, respectively, 4 and 1.7 times lower than for the parent $PdPh_4TBP$. We assume that this is mostly due to media quenching effects,[2a] because, as mentioned above, the absorption spectra do not show significant aggregation in water solutions. Addition of long PEG chains to the chromophore molecule increases the quantum yield by more than a factor of two, although the lifetime decreases by almost 20%. Phosphorescence quantum yields of both water-soluble derivatives increase by about 30% in the presence of bovine serum albumin. This protein is known to have relatively non-specific binding sites for hydrophobic molecules. Binding of the phosphor to a large protein might diminish its vibrational freedom and limit its interaction with the solvent. Both factors tend to cause a decrease in the probability of non-radiative decay of the triplet state, and hence could increase the phosphorescence quantum yield. A similar effect has been detected for the fluorescence of Zn and Mg TBP, when the latter was measured from micelles with probe molecules *vs.* water solutions.[19]

Phosphorescence of Pd porphyrins is quenched by molecular oxygen according to Stern–Volmer equation [eqn. (1)] where

$$\frac{1}{\tau} = \frac{1}{\tau_o} + k_q[O_2] \qquad (1)$$

$\tau_o$ is phosphorescence lifetime in the absence of oxygen, while $k_q$ is related to the collision frequency between triplet-state probe molecules and the quencher and is a second-order rate constant. These two parameters are characteristic of the probe and its environment and thus need to be measured as part of the calibration. It should be pointed out, however, that the Stern–Volmer relationship holds only if the quenching process is purely dynamic. This assumes, in turn, that (*i*) the quencher is present in the system in a large excess and (*ii*) probabilities of secondary processes such as triplet–triplet and singlet–triplet quenching are negligibly small compared with the probability of interactions between triplet-state probe and quencher molecules.* In the conditions typical for biological applications, such as measuring oxygen in living tissue, concentrations of excited phosphor are about (0.2–5) × $10^{-9}$ mol $dm^{-3}$, which is three to four orders of magnitude lower than the lowest oxygen concentration to be measured, and thus the Stern–Volmer equation is entirely appropriate to use.†

The quenching constant $k_q$ is strongly affected by the local environment of chromophore and oxygen diffusion rates in the media. It was found[1a] that binding of PdTCPP to bovine serum albumin decreases $k_q$ from about 3 × $10^9$ $dm^3$ $mol^{-1}$ $s^{-1}$ to about 3 × $10^8$ $dm^3$ $mol^{-1}$ $s^{-1}$. This effect is probably due to the protection which the protein gives to a porphyrin when the latter is placed inside a hydrophobic cavity. The quenching constants ($k_q$) were determined for the water-soluble derivative **3** at room temperature using samples equilibrated with air, with two mixtures of gases containing 2.89% and 10% of oxygen, and enzymatically deoxygenated solutions. The obtained values are 2.6 × $10^9$ $dm^3$ $mol^{-1}$ $s^{-1}$ and 2.1 × $10^8$ $dm^3$ $mol^{-1}$ $s^{-1}$ for aqueous saline solutions without and with 2% BSA, respectively. These values are consistent with changes in phosphorescence quantum yields and indicate interaction of the complex with BSA.

The measured values of $k_q$ are comparable with those reported for non-extended Pd porphyrin complexes,[1a] when the lifetimes are slightly shorter. However, further modification of the described compounds may significantly improve both lifetimes and quenching constants. Work on derivatization of PdTBP-based chromophores to optimize them for oxygen measurements is currently in progress.

## Experimental

*Physical Measurements.*—$^1$H NMR spectra were obtained with a Bruker AM-300 (300 MHz) instrument. UV–VIS spectra were recorded using a Beckman DU-64 spectrophotometer. Static phosphorescence spectral measurements were performed on an SPF-500C spectrofluorimeter (SLM Instruments, Inc., USA). Time-correlated phosphorescence measurements were obtained with an Oxyspot light-guide phosphorimeter (Medical Systems Corp., Greenvalue, NY). This phosphorescence measurement instrument has been described in detail in ref. 4, however, for the present studies the instrument was modified to use either a silicon photodiode (SPD) for steady-state or an avalanche photodiode (APD) (Hammamatsu) for time-correlated measurements. In both cases the measured values were corrected for the instrument response function and relative intensity of the excitation source. A tungsten–$I_2$ lamp and a Bausch and Lomb 250 mm monochromator with a bandwidth at half height 6.7 nm was used for excitation during steady-state measurements. Phosphorescence decay curves were collected using a 12 bit 1 MHz A/D converter (Metrabyte, DAS-50) mounted in a 386 16 MHz AT microcomputer, and stored on the disk for further analysis. Phosphorescence decay curves were analysed with a lifetime distribution analysis algorithm.[26] Calculations were performed on a Gateway 2000 P5-60 MHz microcomputer.

Electronic absorption spectra were recorded for dilute (*ca.* 5 μmol $dm^{-3}$) solutions. Molar extinction coefficients were determined with carefully weighed portions of solutes diluted in volumetric flasks (reproducibility ± 5%).

Emission spectra were obtained using dilute solutions (absorbance at excitation maximum < 0.2 *A*). Right angle detection was used throughout all measurements. Phosphorescence was detected by comparison of emission spectra of degassed and air saturated samples. In all cases excitation spectra were recorded with the emission monochromator set at the emission maximum for phosphorescence in order to reject any light originating from possible highly fluorescent impurities (*e.g.*, free-base porphyrins).

Phosphorescent samples were degassed by several freeze–pump–thaw cycles of DMF or benzene solutions and sealed under vacuum. All samples for the phosphorescence measurements had sufficiently low absorbance (0.1–0.15 *A* at Q-band maximum). Binding of Pd porphyrins to BSA is described elsewhere.[1a] Water solutions of phosphors were deoxygenated using glucose (0.1 mol $dm^{-3}$) and catalytic amounts of glucose oxidase and catalase. Small amounts of the enzymes were added to the solutions of porphyrins in buffered media (10 mmol $dm^{-3}$ MOPS, 10 mmol $dm^{-3}$ $KH_2PO_4$, 10 mmol $dm^{-3}$ Tris and 120 mmol $dm^{-3}$ NaCl; pH = 7.2) containing 0.020 to 0.1 mol $dm^{-3}$ glucose, and vials were immediately sealed.

Samples equilibrated with nitrogen–oxygen gas mixtures

* Oxygen measurements are still possible even when plot of $1/\tau$ *vs.* $[O_2]$ is not linear, but then additional calibration is needed.

† This estimation is based on oxygen pressures from 10 to about 100 Torr, a range typical for biological samples, concentrations of phosphorescent probe of about 5 μmol $dm^{-3}$, phosphorescence quantum yields of 0.2–0.4, extinction coefficients of about 25 000 mol $cm^{-1}$ and intensities and lifetimes of typically used excitation sources.

containing 2.89% $O_2$ and 10% $O_2$ were prepared by bubbling with the gas mixture until there was no further change in phosphorescence lifetime.

Quantum yields were determined according to ref. 27. Steady-state excitation light intensity was measured directly using SPD and neutral density optical filters. It was corrected according to SPD relative wavelength response. The relative amount of absorbed photons was calculated as an integral of the absorption spectrum of the phosphor and intensity of the excitation source over the monochromator slit (6.7 nm) range. The corrected intensity of emitted light was compared with fluorescence of reference samples: solutions of *meso*-tetraphenylporphyrin ($H_2$TPP for free base) or its Zn derivative (ZnTPP) in degassed benzene. The reported values of fluorescence quantum yields ($\varphi_f$) for $H_2$TPP and ZnTPP in deaerated benzene solutions at room temperature are 0.13 and 0.033 respectively.[28]

*Materials.*—Tanks with mixtures of nitrogen and oxygen containing 2.89% and 10% of $O_2$ were purchased from Airco, Co. (USA). All solvents were obtained from Aldrich. DMF was distilled over $K_2CO_3$ and stored over activated 4 Å molecular sieves. All other solvents (reagent grade) were used as purchased. Deionized and degassed water was used throughout.

Phenylacetic acid, potassium phthalimide, imidazole and all inorganic salts were purchased from Aldrich. Alumina for column chromatography (neutral, activity grade I), glucose, methoxypoly(oxyethylene amine) (PEG-$NH_2$) ($M_{av}$, 5000), bovine serum albumin (BSA), catalase (bovine liver, $H_2O_2$–$H_2O_2$ oxidoreductase, EC 1.11.1.6) and glucose oxidase [type II, (+)-β-D-glucose–$O_2$ oxidoreductase, EC 1.1.3.4] were obtained from Sigma. $H_2$TPP and ZnTPP were obtained from Porphyrin Products, Inc. (Logan, Utah).

Zn (*Pyridine*)*tetrabenzoporphyrin* (ZnTBP).—ZnTBP was synthesized according to ref. 10(*a*). A well-ground mixture of potassium phthalimide (10 g, 0.054 mol), zinc acetate dihydrate (5.9 g, 0.027 mol) and anhydrous sodium acetate (35.4 g, 0.432 mol) was placed into a two-necked round-bottomed flask and a slow flow of nitrogen was passed through the vessel. The mixture was heated in the sand bath up to 350–360 °C, and the temperature was held at that level for 3 h. After cooling the black–violet solid was washed with 1 $dm^{-3}$ of hot water on the glass filter and dried under vacuum. The following procedure followed ref. 12. The black powder was extracted with 100 $cm^3$ of hot pyridine in a Soxhlet extractor. After cooling, the resulting deep green solution was mixed with 300 $cm^3$ of diethyl ether and allowed to stand for a few hours. It was filtered on a fritted glass filter and volume was reduced to 20 $cm^3$. 100 $cm^3$ of methanol were added and the mixture was placed in the freezer at −20 °C. Shiny, violet crystals formed and they were recovered by filtration and dried under vacuum. The yield of crude product was 2.3 g, 26%. This material was used for demetallation without additional purification.

*Tetrabenzoporphyrin* ($H_2$TBP).—Crude ZnTBP(pyridine) (2.0 g) was treated with 30 $cm^3$ of mixture of acetic and phosphoric acids (AcOH–$H_3PO_4$ = 1:5) at 100 °C for 1 h. The progress of the reaction was monitored by VIS spectroscopy. After cooling, the mixture was poured into 500 $cm^3$ of water. A precipitate formed and it was recovered by filtration, washed extensively with water, then with aq. $Na_2CO_3$ and then again with water until the pH of filtrate became neutral. The resulting solid was collected and dried in vacuum. $H_2$TBP was purified as described in ref. 17. The overall yield of pure tetrabenzoporphyrin was estimated as 11% (based on amount of potassium phthalimide used for synthesis of ZnTBP). The absorption spectrum of $H_2$TBP agreed well with that reported in ref. 22.

*Metal Insertion using an Imidazole Melt.*—Insertion of metals into free tetrabenzoporphyrin was carried out in an imidazole melt at 150 °C, using the appropriate chlorides ($SnCl_2$, $YCl_3·6H_2O$, $LuCl_3·6H_2O$) or acetates [$Pd(OAc)_2$, $Pb(OAc)_2$, $Lu(OAc)_3·6H_2O$] as the metal-ion source. In a typical procedure 2 g of imidazole, 7–10 mg, of $H_2$TBP and an excess of metal salt were mixed together and heated with stirring under a nitrogen atmosphere. The reaction progress was monitored by changes in absorption spectra. When the reaction was complete, the temperature was lowered and 5–10 $cm^3$ of water were added before crystallization of imidazole started. The resulting mixture was centrifuged and the precipitate, containing the metallotetrabenzoporphyrin, was washed with water and ethanol, dried under vacuum and used for optical measurements without further purification. Spectroscopic data of complexes are given in Table 1.

Pd *Tetrabenzoporphyrin* (PdTBP).—PdTBP was synthesized according to ref. 4, except that $Pd(OAc)_2$ was used instead of $PdCl_2$, which significantly improves the rate of insertion. Roughly 1 g of $Pd(OAc)_2$ was added in small portions over a period of 1 h to a refluxing DMF solution of 0.5 g $H_2$TBP, until the absorption bands of unsubstituted tetrabenzoporphyrin in the visible spectrum disappeared. The mixture was cooled and poured into 200 $cm^3$ of water. A precipitate formed which was collected by filtration and washed and dried under vacuum. The resulting greenish black powder was extracted with 20 $cm^3$ of DMF in a Soxhlet extractor. The solution was reduced in volume to 10 $cm^3$ and methanol (70 $cm^3$) was added. The mixture was cooled and the resulting precipitate was collected by filtration. Further purification was performed according to ref. 12. Crude PdTBP was kept at 400 °C for 2 h in a stream of nitrogen and then extracted with hot DMF, until the solution was colourless. 480 mg of pure complex (81%) were obtained by precipitation with methanol. $\delta$[$(CD_3)_2SO$] 11.19 (s, 4 H), 9.78 (m, 8 H) and 8.27 (m, 8 H).

Sn *Tetrabenzoporphyrin* (SnTBP).—20 mg of $H_2$TBP and an excess of $SnCl_2$ were refluxed in 5 $cm^3$ of DMF until the spectrum showed full conversion of the free porphyrin into a metal complex. The solution was cooled and filtered and 20 $cm^3$ of ether were added. The mixture was then kept overnight at −20 °C. The residue formed was centrifuged, washed with ether and dried under vacuum. Yield: 19 mg, 73% [calculated for $Sn^{IV}(OH)_2$TBP complex]. $\delta$[$(CD_3)_2SO$] 11.99 (s, 4 H), 10.28 (m, 8 H) and 8.6 (m, 8 H).

Lu(Cl) *Tetrabenzoporphyrin* (LuTBP).—LuTBP was synthesized using 200 mg of $H_2$TBP, $LuCl_3·6H_2O$ and 5 $cm^3$ of DMF as the reaction medium. Purification was performed on an $Al_2O_3$ column, using pyridine–ether mixture (1:3) as the eluent. Yield: 120 mg, 38% (calculated for the mono-pyridine adduct). $\delta$[$(CD_3)_2SO$] 10.73 (s, 4 H), 9.47 (br s, 8 H) and 8.01 (br s, 8 H).

$PdPh_4$TBP (**1**).—A well-ground mixture of potassium phthalimide (10 g, 0.054 mol), $ZnCl_2$ (3.7 g, 0.027 mol) and phenylacetic acid (15 g, 0.11 mol) was heated to 350–360 °C for 3 h under a slight flow of nitrogen. After cooling, the greenish–black solid was washed with hot water and dried under vacuum. The absorption spectrum in the visible region of the spectrum in DMF showed the absorption maxima of $ZnPh_4$TBP.[8] The solid was treated with $H_3PO_4$–AcOH mixture as described above and the resulting black powder, containing unsubstituted $H_2Ph_4$TBP as suggested by VIS spectroscopy, was extracted with 100 $cm^3$ of $CHCl_3$ in a Soxhlet extractor. The extraction solvent was evaporated off and resulting solid was dissolved in 25 $cm^3$ of DMF. Small portions of $Pd(OAc)_2$ (approximately

2 g) were added to a refluxing DMF solution of crude $H_2Ph_4TBP$ until the VIS spectrum indicated the reaction had gone to completion. The usual washes with hot water and drying was followed by chromatographic purification on an $Al_2O_3$ column, using benzene–light petroleum (1:1) as the eluent. Subsequent drying under vacuum at 150 °C gave $PdPh_4TBP$ (1.53 g, 12.3%) (Found: C, 78.9; H, 4.0. Calc. for $C_{60}H_{36}N_4Pd$: C, 78.4; H, 3.9%); $\delta(CDCl_3)$ 8.27 (d, 8 H), 7.91 (d, 4 H), 7.86 (d, 8 H), 7.21 (m, 8 H) and 7.11 (m, 8 H).

$PdPh_4(SO_2Cl)_4TBP$ (**2**).—0.5 g (0.54 mmol) of **1** were dissolved in 5 $cm^3$ of $ClSO_3H$ and allowed to react for 12 h at room temperature. The mixture was added dropwise to a saturated NaCl water solution at −5 °C and the precipitate which formed was rapidly filtered off, washed with ice-cold water and dried under vacuum. Yield 665 mg, 93% (Found: C, 55.1; H, 2.4. Calc. for $C_{60}H_{32}Cl_4N_4O_8PdS_4$: C, 54.9; H, 2.6%).

$PdPh_4(SO_3Na)_4TBP$ (**3**).—0.2 g (0.15 mmol) of **2** were hydrolysed by being refluxed in aqueous NaOH for 5 h and neutralized with aqueous HCl after cooling. The excess of inorganic salts was removed by molecular filtration/centrifugation of the resulting solution through membrane filters (Centriprep-3, Amicon, Inc., USA), followed by extensive dialysis. The resulting solution was analysed using a gradient HPLC system (Spectra Physics SP8800; reversed-phase Macrosphere C4 column; mobile phase MeOH–water = 1:9) and showed one broad peak. Yield: 190 mg, 94% (Found: C, 53.7; H, 2.3. Calc. for $C_{60}H_{32}N_4Na_4O_{12}PdS_4$: C, 54.3; H, 2.4%).

$PdPh_4(PEG)_4TBP$ (**4**).—0.2 g (0.22 mmol) of **1** were treated with $ClSO_3H$ as described above. The mixture was added to a saturated aqueous NaCl solution at −5 °C and the precipitate which formed was rapidly filtered, washed with ice-cold water and dried under vacuum. It was dissolved in 25 $cm^3$ of $CHCl_3$ and 5 g of PEG-$NH_2$ ($M_{av}$ 5000) and 0.5 $cm^3$ of pyridine were added. The mixture was allowed to react for 24 h at room temperature, then the solvent was removed under vacuum and the resulting green solid was dissolved in water, filtered a few times through molecular membranes (Centriprep-10, Amicon, Inc.) and dialysed for 5 days. Finally it was purified on a Sephadex G25 column. The green fraction eluting with the solvent front was collected and analysed by HPLC (as described for **3**). It showed one broad peak with a substantially longer retention time than the corresponding sulfonato derivative **3**. The solution volume was reduced to 10–15 $cm^3$, frozen, and dried under vacuum. The total weight of recovered solid was 2.4 g.

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