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SHORT COMMUNICATION

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Homo- and Heterogeneous α-Pinene Photooxidation Using a Protoporphyrin-Derived Amide

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6,7-Bis[3-(N^{ε}-*tert*-butyloxycarbonyllysine methyl ester)]-1,3,5,8-tetramethyl-2,4-divinylporphyrin (**3**) was synthesized and successfully immobilized in a silica matrix by a sol–gel method. Protoporphyrin (PP)-derived amide **3** showed much higher photostability than its parent PP-IX. Its UV/Vis absorption, excitation, and fluorescence spectra as well as its ability to generate $^1O_2$ were measured both in solution and in the matrix. Subsequently, free and immobilized porphyrins **3** were used as sensitizers in the photooxidation of α-pinene, and their photocatalytic properties were compared.

Introduction

Oxidation of α-pinene, (*R*)-limonene, and other terpenes, as well as terpenols, has led to a broad range of oxygenated derivatives present in nature. As a result of their strong fragrance and anticancer properties, they have recently attracted much interest.[1] In nature, these compounds are oxidized by metalloenzymes by using molecular oxygen. However, in the laboratory, the oxygenation of, for example, β-cytronellol to rose oxide is achieved using singlet oxygen.[2] Photooxidation reactions generally consist of generating singlet oxygen ($^1O_2$) through photosynthetic methods, even by utilizing visible light, provided that a suitable dye is used. Porphyrins are photoactive pigments, and thus, they are very efficient photocatalysts.[3,4] The ability of these compounds to mimic enzymes with monooxygenase activity supports the biooxidation of organic substrates by using molecular oxygen as the terminal oxidant and visible light as the energy source for biocatalysis. We have already shown that α-pinene could be oxidized to pinocarvone by singlet oxygen generated by partially protonated octaethylporphyrin.[5] Other examples of these extraordinary compounds (which play a crucial role in biological systems as prosthetic groups) with the potential to be efficiently used as photocatalysts include: hematoporphyrin and protoporphyrin IX (PP-IX). These compounds are poorly soluble in the common organic solvents that are used in photocatalysis; therefore, introduction of substituents that are capable of enhancing their solubilities in hydrophobic solvents is desirable.

Furthermore, photoexcited porphyrins in solution, in general, undergo easy deactivation, and together with their photodecomposition derivatives can contribute to the contamination of the reaction mixture, which makes the purification step troublesome.[6] The use of heterogeneous catalytic systems based on porphyrins immobilized in inorganic solid supports avoids these difficulties, which facilitates the reusability of the active catalyst. The sol–gel route is one the most commonly employed methods for the preparation of organic–inorganic hybrids at the microscale and even at a molecular level under mild conditions.[7,8] This process is an ideal way for the synthesis of materials having controlled pore sizes. The procedure consists of the preparation of the proper alkoxide sol, transformation to a gel, and then drying the gel to a xerogel. The most popular precursors are metal alkoxides that react readily with water, such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS), under acid–base conditions. Aluminates, titanates, and borates are also applied.[9,10] During gelation, different substances can be inserted into the solution to give the gel unique properties, such as mechanical or chemical resistance.[4,11]

Silica sol–gels have been reported to improve the properties of organic pigments effectively,[8,11–13] and with respect to photocatalysis, they have many advantages, including optical transparency, chemical inertness, and superior durability.[14–16] Moreover, negligible swelling of these materials in organic solvents was noticed relative to that observed for organic polymers. Silica aerogels also exhibit many intri-

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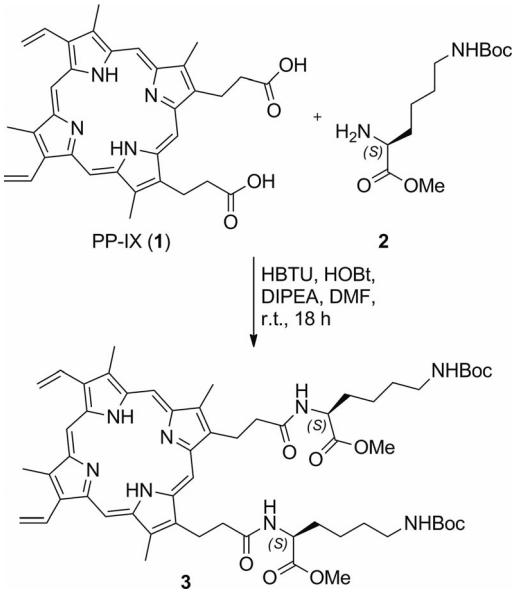
guing properties, which include very low density (0.2–0.01 $g cm^{-3}$), high porosity (80–98%), high inner surface area (500–1000 $m^2 g^{-1}$), and extremely low thermal conductivity (0.02 $W m K^{-1}$).[17,18]

The close proximity of immobilized porphyrins within the pores of silica gel allows easy energy transfer between the pigment molecules, which is important in catalytic processes. Porphyrins in silica gel, in general, are known to retain their luminescence properties; however, their catalytic activity depends on the porphyrin encapsulated. Recently, a substantial difference in the biomimetic photooxidation of α-pinene in the presence of octaethylporphine (OEP) and hematoporphyrin (HmP) was found. When immobilized in porous silica gel, both showed good fluorescence intensity, whereas the biocatalytic activity was only preserved for OEP/SiO_2. Moreover, the immobilization of porphyrins usually enhances their photostability and, thus, their resistance towards reactive oxygen species released during photocatalysis, for example, $^1O_2$ and free radicals.[19]

The main objective of the present work was to obtain an amino acid derivative of PP-IX for the purpose of photocatalysis and to examine its photostability relative to that of PP-IX. Also, the effect of its encapsulation in the sol–gel matrix on its photosensitizing properties was determined.

Results and Discussion

Our new catalyst was prepared by the coupling reaction of PP-IX (**1**) with N^{ε}-Boc-protected lysine methyl ester (**2**) by using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent and *N*,*N*-diisopropylethylamine (DIPEA) as the base (Scheme 1). After purification, desired product **3** was obtained in 97% yield.



Scheme 1. Synthesis of amide **3**.

It is well known that light has a negative effect on porphyrins, as it leads to their irreversible degradation. Therefore, the stabilities of porphyrin **3** and precursor **1** in DMSO solution was examined. Their absorption and fluorescence spectra revealed that porphyrin **3** was significantly more stable than **1** (Figures 1 and 2). A substantial decrease in the Soret and Q bands of **1** was observed after exposure to light for 10 min, whereas the decomposition of photocatalyst **3** occurred only after 2 h of irradiation. Furthermore, the absorption and luminescence spectra of porphyrin **3** showed no changes in shape, for example, peak position, during illumination, which suggested a lack of a transient form of this pigment during degradation. Similarly, protonation of the central nitrogen atoms was not observed. The lack of photobleaching suggested that porphyrin **3** could be a potential photocatalyst for the biotransformation of α-pinene and of other electron-rich hydrophobic compounds.

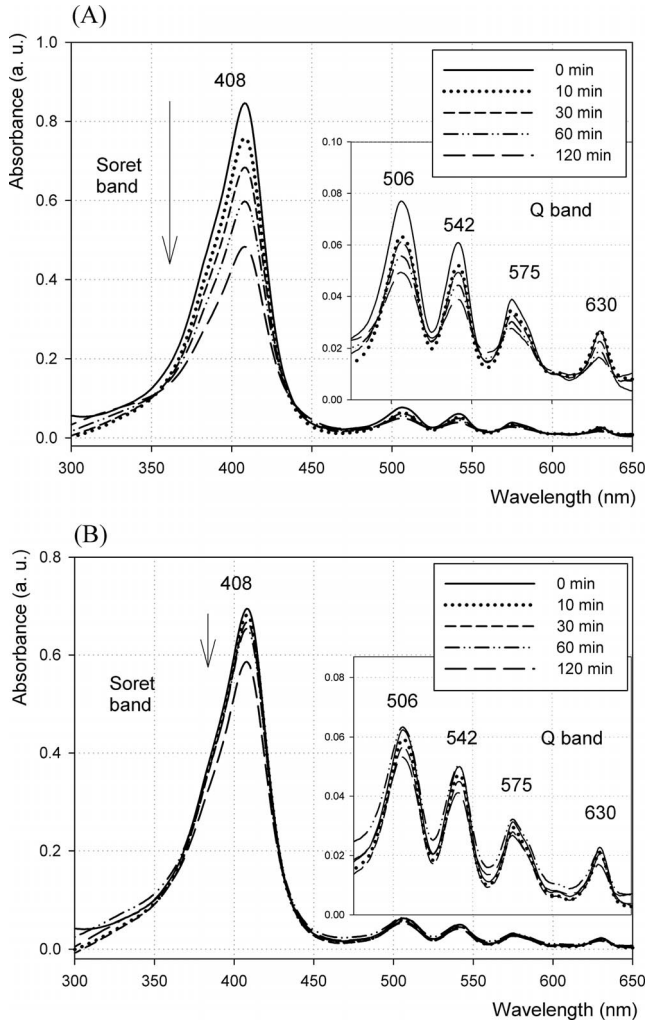


Figure 1. Absorption spectra of (a) PP-IX (**1**) and (b) its amino acid derivative (**3**) in DMSO over a 2 h period of illumination with visible light showing differences in their photostabilities.

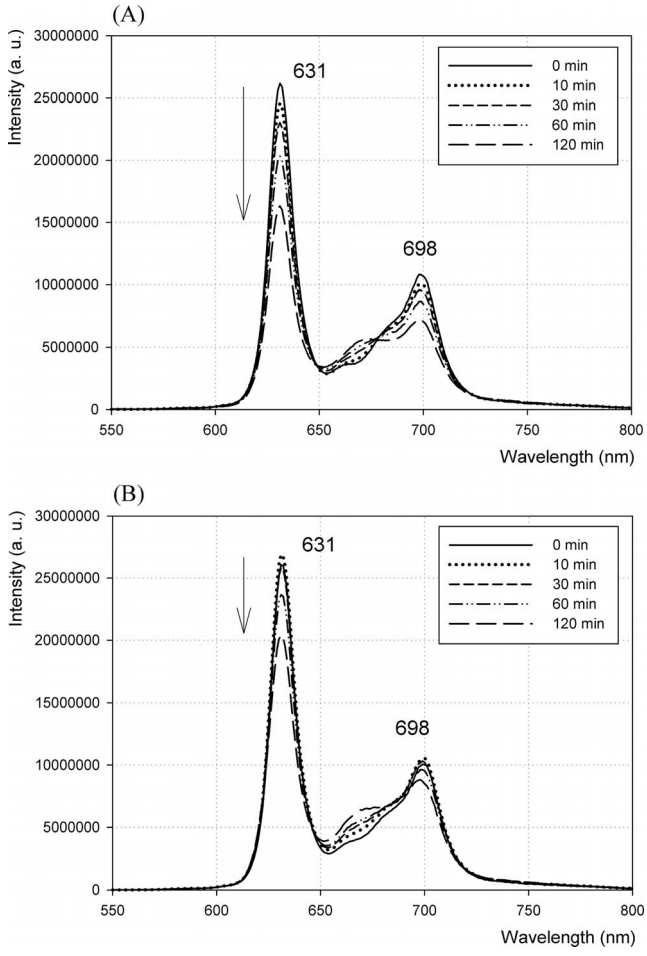


Figure 2. Emission spectra of (a) PP-IX (**1**) and (b) its amino acid derivative (**3**) in DMSO over a 2 h period of illumination with visible light showing differences in their photostabilities. The excitation wavelength was 408 nm.

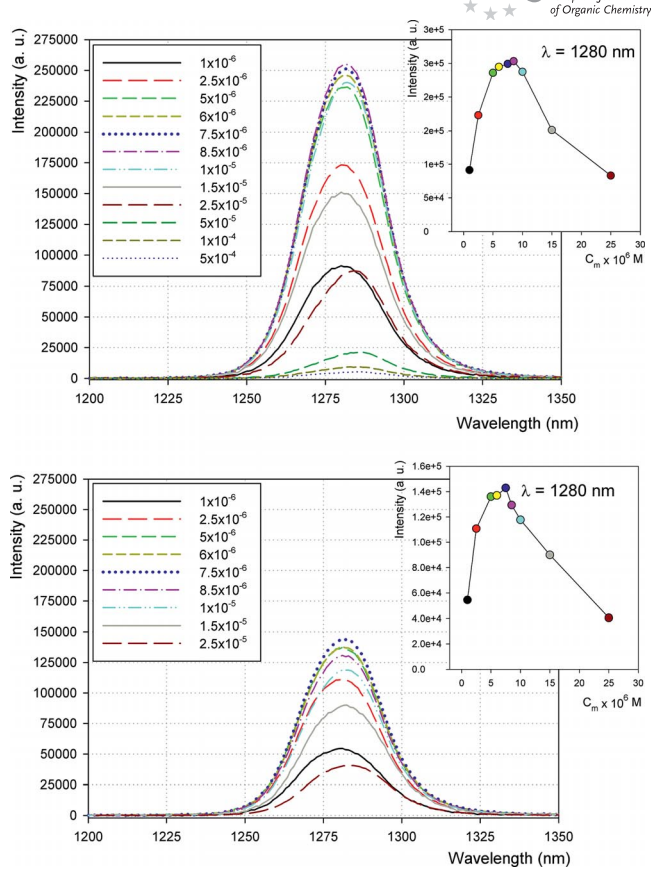


Figure 3. The emission spectrum of $^1O_2$ generated at various concentrations of **3** in chloroform with (bottom) or without (top) α-pinene by light excitation at 410 nm.

The catalytic properties of porphyrin **3** were tested in the light-promoted oxidation of α-pinene. Initially, the ability of **3** to generate $^1O_2$ in chloroform was evaluated at various concentrations both in the absence and in the presence of α-pinene. The results were monitored by infrared fluorescence spectroscopy (at 1280 nm) and the molar concentration of **3** was varied from 1×10^{-6} to 5×10^{-4} M (Figure 3). The maximum intensity of the fluorescence band of $^1O_2$ in a solution without α-pinene was reached at a concentration of 8.5×10^{-6} M for **3**. A slightly lower concentration of **3** (7.5×10^{-6} M) was the best for the solution containing α-pinene. However, upon the addition of this monoterpene to the photocatalytic system, a significant decrease in the intensities of the fluorescence bands of singlet oxygen for the solution was observed, and this is unambiguous evidence for the interaction of $^1O_2$ with α-pinene.

In both cases, an increase in the concentration of porphyrin **3** resulted in a decrease in the generation of $^1O_2$. Presumably, agglomeration of the pigment molecules was responsible for the decrease in their photosensitizing abilities.[20] This study allowed us to select the optimal concentration of the porphyrin (7.5×10^{-6} M) for further catalytic experiments. To the best of our knowledge, the most favorable amount of a pigment in a photooxidation reaction has not been determined by near-IR techniques.

Subsequently, the immobilization of catalyst **3** in silica gel by the sol–gel method was attempted. Because the spectral characteristics of porphyrins are extremely sensitive to processes such as protonation, metalation, ring oxidation, and aggregation, the absorption and emission spectra of porphyrin **3** in chloroform, silica sol, alcogel, and xerogel were registered and compared (Figure 4). In chloroform and in silica sol, similar characteristic absorption bands in the blue (Soret band) and red (Q bands) spectral regions were observed. A difference in the absorption spectrum of xerogel was observed relative to the spectra registered for the sol and alcogel samples. The Soret band was shifted from 402 to 408 nm, whereas in the Q band region, a new, very intense band at 555 nm was observed. We assumed that these changes were a result of the protonation of the porphyrin inside the acidic SiO_2 network with consequent formation of a dication. This was also confirmed by a reduction in the number of bands in the Q region from four to two, which is directly related to the well-known change in the symmetry of porphyrins from D_{2h} for the free-base H_2P porphyrin to D_{4h} for the H_4P^{2+} dication. Probably, xerogel contains a ratio of both the protonated and neutral forms of porphyrin **3**. This ratio could have been altered

during the drying process in air or influenced by the nature of the solvent. The concentration of the free-base form was low, and thus, the Q bands originating from these species overlapped with those derived from the dications. In the emission spectra of porphyrin **3**, a shift in λ_{max} from 630 to 602 nm was observed during the transformation of alcogel to xerogel, and this confirms the formation of a dication in the dried silica gel. We assume that the appearance of a new band near 660 nm was a result of porphyrin aggregates.

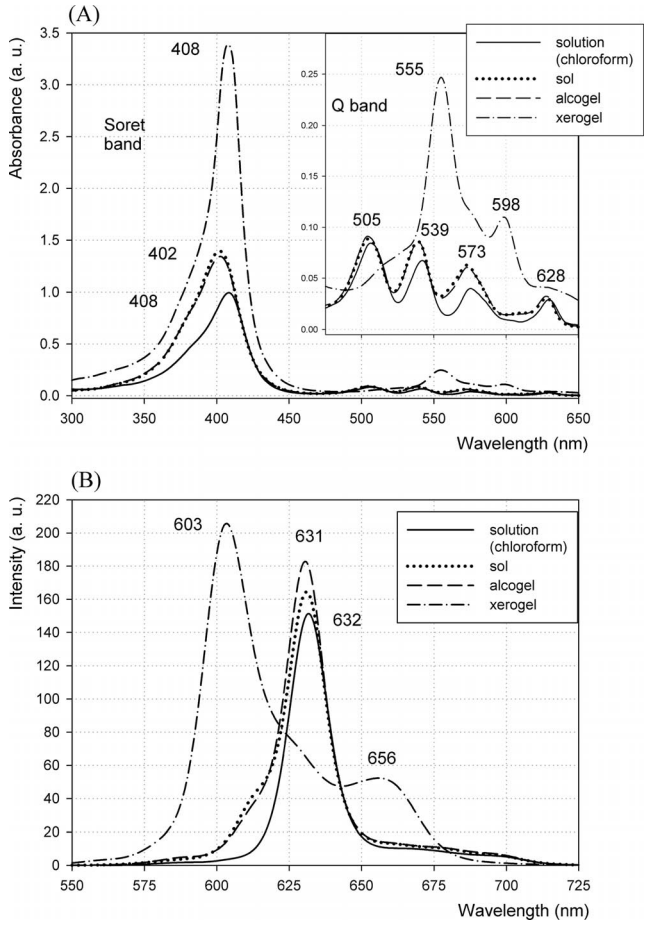
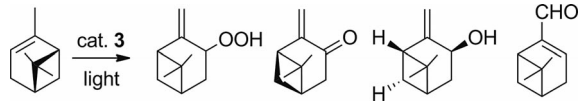


Figure 4. Comparison of the (a) UV/Vis absorption and (b) emission spectra of porphyrin **3** in chloroform, sol, alcogel, and silica xerogel.

Photooxidation of α-pinene was carried out in a homogeneous and heterogeneous system by using the same total equimolar amounts of porphyrin **3**. The oxidation reaction was monitored periodically by using GC–MS after 1, 2, 4, 10, 22, and 30 h (Table 1).

The free-base amide derivative of PP-IX, that is, **3**, in chloroform was shown to be an efficient photocatalyst for the production of commercially important compounds by using light in the visible region. A major terpenoid produced during the reaction was pinocarveyl hydroperoxide, whereas *trans*-pinocarveol, pinocarvone, and myrtenal were obtained in substantially lower amounts (Table 1). The total number of turnovers (the total number of mol of products produced per mol of catalyst used in the phototransformation) was reached after 30 h with 60% conversion of α-pinene. Unexpectedly, porphyrin **3** intercalated in the sol–gel matrix (despite good fluorescence intensity) showed only slight activity, approximately 36-fold lower than that observed under the homogeneous conditions. This fact might be connected to the protonation of porphyrin **3** in silica, which is known to decrease the catalytic efficacy of many photoexcited porphyrins.[21] In contrast, the poor catalytic activity of organic–inorganic hybrid **3**/SiO_2 might result from its hindered ability to generate the reactive oxygen species, probably because of strong interactions between catalyst **3** and the silica matrix. Indeed, only a weak fluorescence signal of $^1O_2$ was observed for samples of xerogel dotted with porphyrin **3**. Gratifyingly, during the course of photocatalysis, no leaking from the silica was observed (the reaction solution remained colorless). Recently, hematoporphyrin was found to preserve luminescence in silica pores, whereas its biocatalytic activity was reduced, and this

Table 1. Photooxidation of α-pinene with amide derivative **3** in homogeneous system and heterogeneous system based on the porphyrin intercalated in the silica matrix.[a]



Time of catalysis [h]	Side products [TN][b]	Pinocarveyl hydroperoxide [TN]	TTN[c]	α-Pinene conversion [%]
1	110.6 (10.9)	428.6 (0)	539.2 (10.9)	4.5 (0.2)
2	190.2 (51.0)	683.1 (0.8)	873.3 (51.8)	8.6 (0.7)
4	282.1 (20.7)	1273.2 (3.1)	1555.3 (23.8)	15.6 (0.8)
10	486.7 (35.4)	2872.3 (22.4)	3359.0 (57.8)	37.2 (0.9)
22	738.8 (46.0)	3676.5 (60.3)	4415.3 (106.3)	59.7 (1.5)
30	944.1 (56.8)	4025.0 (79.2)	4969.1 (136)	60.1 (1.8)

[a] Yields are given as turnover numbers [TN]. Results for the heterogeneous conditions are given in parentheses. [b] Defined as the total turnover number of side products (e.g. pinocarveol, *trans*-pinocarvone and myrtenal). [c] Calculated as the total of the turnover numbers (TTN). Concentration of **3** used in the homogeneous system was 7.5×10^{-6}; the heterogeneous reactions were performed with an equivalent molar amount (2.25×10^{-8} mol) of encapsulated **3** in powder form (550 mg) under identical conditions; α-pinene concentration in chloroform (3 mL): 50 mM.

has been assigned to the interaction of its carboxyl groups with the SiO_2 structure.[5]

Conclusions

Reported porphyrin **3** showed better resistance to the reactive species released during photocatalysis relative to that shown by PP-IX. The porphyrin can serve as an enticing target for potential application in biocatalysis and for the oxidation of natural terpenes. Although the newly synthesized compound retained good luminescence properties in silica gel, unfortunately it showed low catalytic activity in the oxidation of α-pinene, presumably as a result of strong interactions with the silica network. Therefore, future work will consider the possibility of modifying the porphyrin structure by attaching electronegative substituents onto the macrocyclic core, which should result in a decrease in the number of porphyrin–silica interactions. This modification should also increase the resistance of the porphyrin to undergo protonation.

Experimental Section

General Methods: Analytical-grade solvents were used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature with a 500 MHz instrument. DCVC (dry column vacuum chromatography) was performed by using 200–300 mesh silica gel. Flash column chromatography was performed by using 60 mesh silica gel. Thin-layer chromatography (TLC) was performed by using silica gel GF254, 0.20 mm thickness. Room-temperature UV/Vis absorption spectra were recorded with a V-660 (JASCO) spectrophotometer in the 300–750 nm spectral region.[22] A spectral resolution of 1 nm was preserved. The fluorescence spectra were obtained with a FP-6300 spectrofluorometer JASCO. UV/Vis reflectance spectra were registered with a Horizontal Sampling Integrating Sphere (Model: PIV-756) connected to a V-660 spectrophotometer. Singlet oxygen luminescence spectra were registered by using a Photon Technology International, Inc., spectrofluorometer equipped with an infrared module (NIR PMT Module with InP/InGaAs photocathode material, Hamamatsu Photonics K. K.) operating at a spectral response range of 950–1700 nm.

6,7-Bis[3-(*N*$^{\varepsilon}$-*tert*-butyloxycarbonyllysine methyl ester)]-1,3,5,8-tetramethyl-2,4-divinylporphyrin (3): PP-IX (30 mg, 0.05 mmol), *N*$^{\varepsilon}$-Boc-L-lysine methyl ester hydrochloride (90 mg, 0.3 mmol), HBTU (60 mg, 0.16 mmol), and 1-hydroxybenzotriazole (HOBt; 29 mg, 0.21 mmol) were dissolved in dry DMF (4 mL), and then DIPEA (41 μL, 0.32 mmol) was added by syringe. The resulting mixture was stirred overnight at room temperature under an argon atmosphere. The mixture was then poured into DCM and washed consecutively with aqueous HCl (5%), saturated aqueous $NaHCO_3$, water, and brine. The crude product was purified by DCVC (2% methanol in DCM). Recrystallization (hexane/DCM) gave porphyrin **3** as a red solid (55 mg, 97%). R_f = 0.75 (SiO_2, 5% MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.86–9.62 (m, 4 H), 8.18–8.11 (m, 2 H), 6.90 (s, 2 H), 6.34–5.16 (m, 4 H), 4.55–4.34 (s, 2 H), 4.32–4.05 (m, 4 H), 3.50–3.40 (m, 12 H), 3.36 (br. s, 6 H), 3.20 (br. s, 2 H), 2.96–2.89 (m, 2 H), 2.60–2.29 (m, 2 H), 1.74–0.37 (m, 28 H), –0.06 to –0.19 (m, 6 H), –4.90 (s, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 172.5, 155.2, 138.6, 136.0, 130.2, 121.0, 97.6, 97.1, 96.8, 52.0, 51.8, 39.5, 38.5, 30.9, 28.3, 23.2, 21.0, 12.7, 11.6 ppm. HRMS (ESI): calcd. for $C_{58}H_{78}N_8O_{10}$ [M + Na]$^+$ 1069.5746; found 1069.5733. UV/Vis (CH_2Cl_2): λ_{max} (ε, L mol^{-1} cm^{-1}) = 668 (1.45×10^3), 629 (4.24×10^3), 574 (7.19×10^3), 541 (1.06×10^4), 505 (1.22×10^4), 406 (1.59×10^5). $C_{58}H_{78}N_8O_{10}$ (1047.30): calcd. C 66.52, H 7.51, N 10.7; found C 66.50, H 7.50, N 10.52.

Preparation of Transparent Monolithic Silica Gels: Porphyrin **3** was immobilized in silica gel according to a literature procedure.[22] Appropriate amounts of mother solutions of porphyrin **3** in THF were added (by using Hamilton microsyringes) to the sol in the disposable spectrofluorimetric cells. Two parallel series of doped sols were prepared in which the concentrations of the porphyrins were varied from 1×10^{-6} to 2.5×10^{-5} M. The prepared hybrid materials were dried in the absence of light. The name alcogel was used in reference to samples obtained without a mass change, whereas the term xerogel was used in reference to the material after shrinking due to solvent evaporation.

Photocatalytic Studies: The resulting dry monolith samples containing **3** were broken and powdered in a mill to an average particle size of 0.3 mm. Prior to the catalytic cycle, the powder was washed extensively with methanol and dried by using a rotary evaporator with a water bath at 60 °C. The procedure was repeated twice with chloroform to remove any released porphyrin. In a standard photooxidation procedure, the reaction vessels were regularly irradiated with four fluorescent visible lamps at 20 °C. After the appropriate time intervals, and quick sedimentation of dispersed silica particles (about 1 min) in the case of heterogeneous catalysis, each solution was sampled. The products were detected by GC–MS. Details of the biotransformation experiment and chromatographic analyses experiment can be found elsewhere.[22]

Supporting Information (see footnote on the first page of this article): ^{1}H NMR and ^{13}C NMR spectra of porphyrin **3**.

Acknowledgments

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