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Zn Pyropheophorbide *a*: A β-Face Selective Nicotine Receptor

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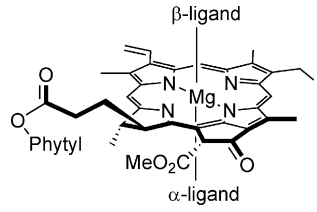
Nicotine binds to a semisynthetic chlorophyll *a* derivative, Zn pyropheophorbide *a*, in a receptor-like manner to form a 1:1 complex with β-face diastereomeric configuration. The geometry of the complex structure was evidenced by ^{1}H NMR, ROESY and DOSY spectroscopic experiments combined with molecular modelling DFT B3LYP calculations at the 6-31G* level. The observed binding between the chlorin and nicotine takes place through a "two-point binding" mechanism, in which the nicotine *N*-pyridyl moiety coordinates to the chlorin zinc atom, whilst the nicotine *N*-methyl pyrrolidine unit forms an ion pair with the chlorin acid group. The binding mechanism was further confirmed by association constants (K_a) obtained from titration experiments in $CDCl_3$ with ^{1}H NMR spectroscopic monitoring (8.0×10^5 M^{-1}) and in toluene with UV/Vis (6.1×10^4 M^{-1}) monitoring. The corresponding binding constants obtained for nicotine with one-point binder, Zn pyropheophorbide *a* methyl ester, were 4.8×10^5 and 2.3×10^4 M^{-1}, respectively.

Introduction

In photosynthetic systems, protein-bound chlorophylls are ligated either in an α- or β-face selective manner by imidazole histidine residues (Scheme 1).[1] The crystal structure of the light-harvesting complex II (LH II)[2] reveals that the α-ligated diastereomer in which the imidazole residue is located below the chlorin plane is predominantly populated with a ratio of 6:1 over the β-diastereomer (Scheme 1).[1] Moreover, the latter mode of ligation is energetically unfavourable, and this has been concluded by statistical analysis and computational studies.[3,4] Distinctly, the protein-bound β-diastereomers are located apart from the α-diastereomers in the inner core of antenna systems.[3] Concomitantly, Balaban suggested that β-ligated chlorophylls have a specific role in light harvesting photosynthetic antennae forming energy traps for the excitation energy transfer.[1,3,5] Recent findings showed that the 13^1-carbonyl of β-ligated chlorophylls in LH II has a strong tendency to H-bond with the neighbouring protein subunits, thus manifesting the key role of the ligation face in the antennae structure.[6]



Scheme 1.

It is very likely that selective chlorin β-face ligation has been formerly achieved by using a covalent approach by trivially replacing the phytyl group of chlorophyll with an *N*-propyl imidazole moiety.[7] In this work, we were intrigued to study whether β-face chlorin ligation could be achieved in a supramolecular manner. We envisioned that selectively α- or β-ligated chlorophyll derivatives could pave the way for their use as stereocontrolled building blocks in the construction of artificial self-assembled photosynthetic systems. The group of D'Souza showed that nicotine can be complexed on face-symmetric zinc porphyrin acid in a receptor-like manner by a "two-point" binding strategy.[8] This encouraged us to extend this strategy to the simple and chemically stable chlorophyll derivative Zn pyropheophorbide *a* (**1**), which has an asymmetric acid residue oriented above the chlorin plane. Our research hypothesis was that the propionic acid group of **1** ($pK_a \approx 4.5$) would form an ion pair with the pyrrolidine nitrogen atom of nicotine ($pK_{aH} \approx 8.1$ in water),[9] and the thus bound nicotine would further coordinate with its pyridine nitrogen atom to the zinc of the chlorin ring from the above side of the plane to form a β-ligated nicotine complex.

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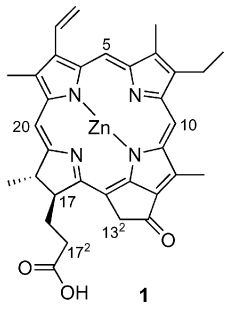

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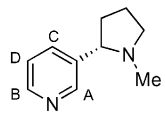

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Beyond this study, compound **1** has a known affinity to substitute heme in the pocket of myoglobin.[10] In general, pyropheophorbides and their metal complexes have been applied as fluorescing intercellular binding probes[11] and tissue accumulating photosensitisers in photodynamic therapy.[12] However, to the best of our knowledge, there are no reports of supramolecular small molecule/free acid metal chlorin complex formation.

Results and Discussion

We prepared semisynthetic chlorophyll derivative **1** from pyropheophorbide *a* methyl ester by using classic methods in acidic hydrolysis of the propionic acid methyl ester[13] and zinc insertion.[14]

The characteristics of the formation of the complex between Zn pyropheophorbide *a* (**1**) and nicotine (**2**) was studied by NMR spectroscopy in $CDCl_3$. In the ^1H NMR spectrum of the mixture of nicotine (**2**) and chlorin **1**, the signals for the A–D pyridine protons are notably shielded relative to the chemical shifts of the signals for these protons in free nicotine (Figure 1). Zn pyropheophorbide *a* methyl ester (**3**) (Figure 1c) was chosen as a reference compound due to the poor solubility of free chlorin acid **1** in less-polar solvents like chloroform. The observed shielding indicates the formation of a strong complex: the pyridine moiety of the nicotine is exposed to the aromatic ring current of the chlorin ring when coordinated to the Zn atom of chlorin **1**, which leads to significant shielding effects.[15] Comparison of the spectrum of the complex with that of Zn pyropheophorbide *a* methyl ester reveals that the 10, 5 and 13^2 chlorin ring protons are clearly deshielded in the complex. These deshielding effects arise evidently from the interaction of Zn chlorin with the nicotine pyridine moiety.

We were able to assign most of the complex signals with the aid of 2D ROESY, DQF-COSY and HSQC NMR spectroscopic techniques.[16] Additionally, the strong- and medium-sized ROE correlations observed between the protons of the propionic acid side chain and the proximate protons were informative in the elucidation of the conformation of the side chain. Even though the broad line shapes in the ^1H NMR spectrum (Figure 1) implies that there is some degree of structural fluctuation in the complex, the ROESY spectrum implies that the supramolecular complex has notable structural specificity. The observed ROE correlations can be expected to arise from the conformation depicted in Scheme 2. Importantly, the correlations between the 18 and 17^2 protons indicate that these protons are located on the same side of the chlorin plane. Evidently, therefore, the propionic acid side chain in the complex is oriented above the tetrapyrrolic plane, which also suggests β-face ligation of nicotine. Finally, the detected weak ROE correlations further support a ligation stereochemistry: the observed ROE between the 13^2 and pyrrolidine protons (grey arrow in Scheme 2) designates the stereochemistry for the β-face.[16]

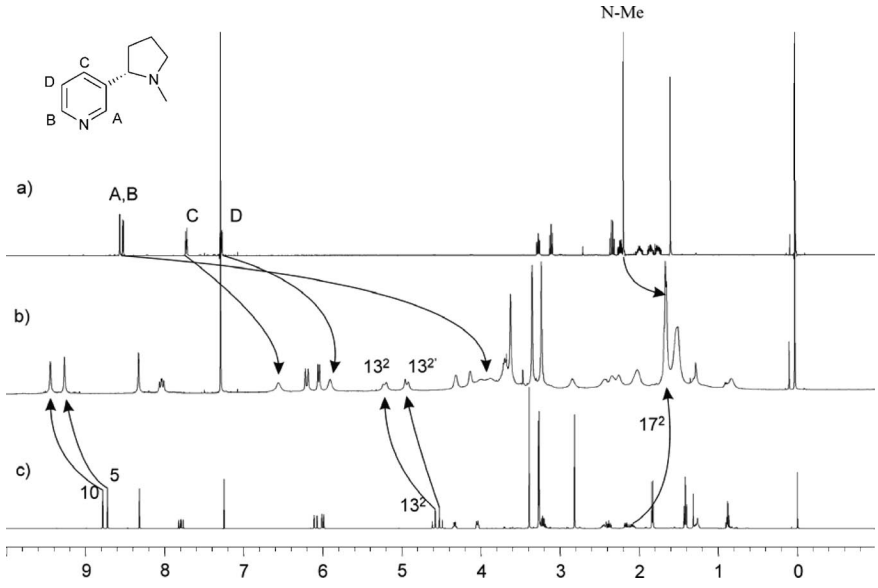
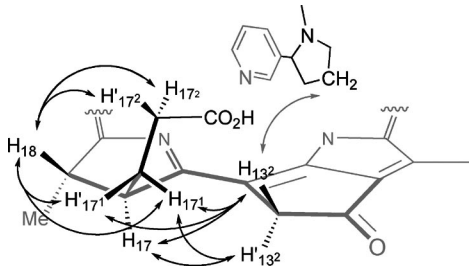


Figure 1. ^1H NMR spectrum (500 MHz) of (a) nicotine (**2**), (b) 1.3:1 mixture of Zn pyropheophorbide *a* (**1**; 39 mM)/nicotine (**2**; 31 mM), (c) Zn pyropheophorbide *a* methyl ester (**3**) measured in $CDCl_3$.



Scheme 2.

The strength of the complex formation was estimated from ^{1}H NMR titration of nicotine (**2**) in $CDCl_3$ by adding variable amounts of chlorin **1** into the solution. By monitoring the pyridine proton (H^A, Figure 1) and by plotting $\Delta\delta$ versus the amount of **1** added, an association constant (K_a) of 8.0×10^5 M^{-1} was obtained for the binding (Figure 2). Furthermore, the titration data was also analysed with a Job's plot type of analysis, which resulted in a 1:1 ratio for the complex formation (Figure 3). A corresponding titration with methyl ester reference compound **3**, which is only capable of a one-point contact, yielded only a slightly weaker K_a of 4.8×10^5 M^{-1}. This indicates that the Zn/pyridine interaction dominates in the complex formation at NMR concentrations [mM].

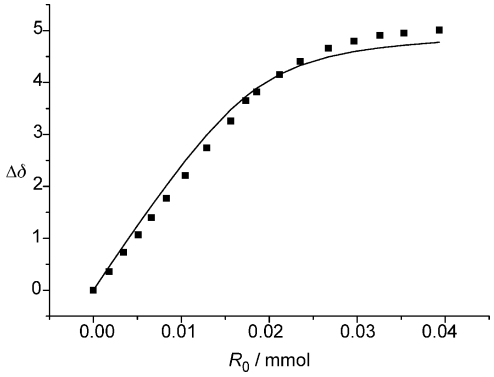


Figure 2. The $\Delta\delta$ of proton A of nicotine is plotted vs. the molar amount of Zn pyropheophorbide *a*. The data was analysed by fitting the curve presented in Equation (1) to the data points (δ_s = δ value of unbound specie, δ_{obs} = observed δ value, $\Delta\delta = \delta_{obs} - \delta_s$, S_0 = [substrate], R_0 = [receptor], $K_a = 1/K_d$).[17]

$$\delta_{obs} = \delta_s + \frac{\Delta\delta}{2S_0}\left[K_d + R_0 + S_0 - \sqrt{(K_d + R_0 + S_0)^2 - 4R_0S_0}\right] \quad (1)$$

In spite of the above molecular ratio evidence (1:1), the possibility of larger aggregate formation (*n*:*n*, $n > 1$) could not be ruled out. Zinc chlorophyll derivatives are known to be prone to aggregation behaviour especially in the presence of polar substituent groups.[19] Therefore, diffusion-ordered NMR spectroscopy (DOSY) measurements were performed to evaluate the molecular weight of the complex.[20] The diffusion coefficients (*D* values) were measured for related compounds and the obtained values were plotted against molecular weight (Figure 4). The measured diffusion coefficient for the Zn pyropheophorbide *a*/nicotine complex is approximately the same as that for monomeric Zn pyropheophorbide *a* methyl ester, that is, it is a 1:1 complex. Interestingly, measurement of pure Zn pyropheophorbide *a* shows a low *D* value (8.6×10^{-10} m^{2}s^{-1}), which corresponds to a molecular aggregate with an average molecular weight of ≈2100 g mol^{-1}, indicating formation of chlorin tetramers or pentamers, as evaluated on the basis of other reference compounds (Figure 4).

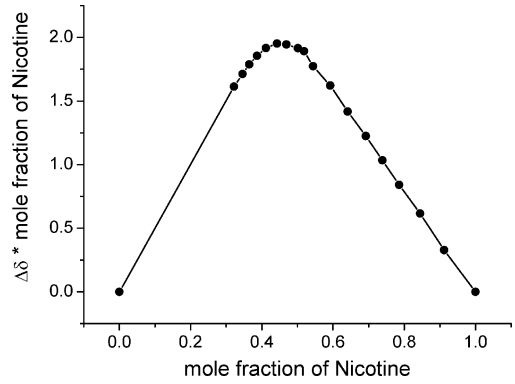


Figure 3. An approximate Job's plot type analysis of the ^{1}H NMR titration data. The top of the curve is located close to 0.5 mol fraction of nicotine denoting 1:1 complex formation.[18]

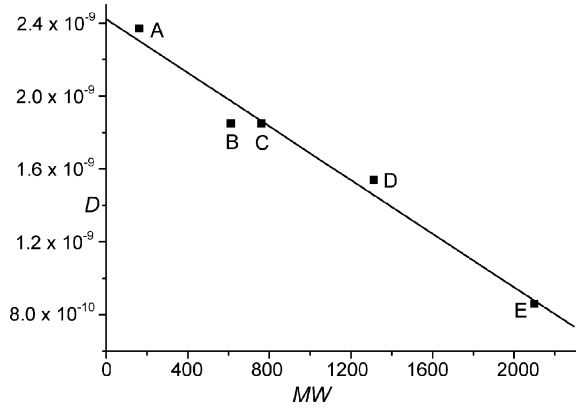


Figure 4. Diffusion coefficients vs. molecular weight for (A) nicotine, (B) Zn pyropheophorbide *a* methyl ester, (C) Zn pyropheophorbide *a*/nicotine complex, (D) Zn pyropheophorbide *a* dyad (linked with amidation by using 2,2′-dithioethamine) and (E) Zn pyropheophorbide *a* aggregate (*MW* is estimated on the basis of the *D* value).

A theoretical examination of the system was carried out by the use of molecular modelling with DFT B3LYP at the 6-31G* level. Prior to the DFT calculations, conformational search was performed with the semiempirical RM1[21] method for the flexible part of the system (Figure 5). The subsequent B3LYP geometry optimisations generated overall 11 structural minima for the "two-point" bound complex below an energy frame of 12 kcal mol^{-1}.[16] Expectedly, the lowest-energy structures were β-ligated and only in two of the found structures was the nicotine α-ligated. Those are also clearly energetically disfavoured and

are 9.1 and 11.5 kcal mol^{-1} higher in energy (ΔH) relative to that of the global minimum structure. The rest of the structures are β-ligated and have some conformational differences in the propionic acid side chain and the orientations of the nicotine pyrrolidine moiety. The obtained global minimum structure lies at an energy level that is 2.9 kcal mol^{-1} lower than the following energy minimum and can be defined as the energetically most favourable structure (Figure 5).[16] In the global minimum structure, the propionic acid side chain is in an approximate *gauche*+ (*g*+) conformation with respect to the C^{17}–C^{17^1} bond, whereas the C^{17^1}–C^{17^2} bond has a *gauche*– (*g*–) conformation. In the next most favourable conformer, the C^{17^1}–C^{17^2} bond is turned to the *g*+ conformation. In contrast, the α-ligated complexes are not only found to lie at a higher energy level, but they are also in a distorted conformation with limited spatial freedom: they are in approximate *g*– and *g*+ conformations for the C^{17}–C^{17^1} and C^{17^1}–C^{17^2} bonds, respectively.[16] Whereas the used calculation method gave binding energies of 15.3 and 12.2 kcal mol^{-1} for the "one-point" bindings chlorin Zn/nicotine pyridine and chlorin COOH/nicotine pyrrolidine, respectively, the found "two-point" bound complex minimum was found to have a binding energy of 23.3 kcal mol^{-1}.[16] Even though the performed modelling omits both solvation and dynamic effects, which can be essential for the formation and stability of a supramolecular structure, the obtained results are in good agreement with the structural data obtained from NMR spectroscopic studies (Scheme 2 and Figure 5).

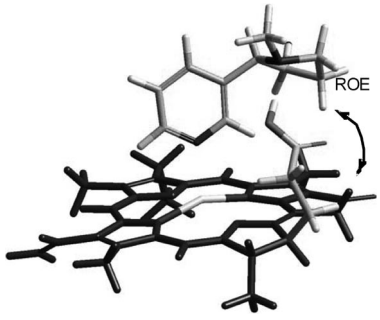


Figure 5. B3LYP 6-31G* geometry-optimised minimum energy structure of Zn pyropheophorbide *a*/nicotine complex. The conformational search was performed for the flexible part of the system (the whole complex apart from the black chlorin moiety).[16]

Direct estimation of the nicotine (**2**)/Zn chlorin **1** population ratios with Bolzmann distribution ($\Delta G = RT\ln[N_2/N_1]$) by using the calculated energy differences and an approximation that $\Delta H \approx \Delta G$ for each complex[22] indicate that the found 2.9 and 9.1 kcal mol^{-1} energy differences between the global minimum and the next advantageous structure, in which the nicotine is above or below the plane, favours the global minimum structure with populations of 1:130 and $1{:}4.7 \times 10^6$, respectively, at 25 °C.

Optical properties of the complex formation were studied with UV/Vis absorption and steady-state fluorescence emission spectroscopy. The UV/Vis titration of Zn pyropheophorbide *a* was performed by adding amounts of nicotine in toluene (Figure 6). A nonpolar solvent was chosen to maximise the noncovalent interactions. Despite the low solubility of compound **1** in toluene, the sensitivity of the optical measurements was an advantage in these experiments in comparison to those performed with NMR spectroscopy. Clear spectral changes can be observed with increasing nicotine concentration. However, the changes are more drastic in the beginning of the titration (the grey-coloured spectra) and can be attributed to the breaking of the oligomeric chlorin acid aggregate structure. After the addition of one equivalent of nicotine, the spectral drifting decreased. The Scatchard analysis[23] above this titration range resulted in $K_a = 6.1 \times 10^4$ M^{-1} (inset Figure 6). In the literature, similar spectral behaviour was reported for a single pyridine molecule coordinated to Zn chlorins in pyridine UV/Vis titrations.[24] A reference UV/Vis titration experiment with "one-point" binding compound **3** yielded a lower value: $K_a = 2.3 \times 10^4$ M^{-1}. This indicates that two-point binding has a more pronounced effect on the formation of the complex at lower concentrations [μM] in nonpolar solvents, as was observed above in $CDCl_3$ in the NMR spectroscopic studies.

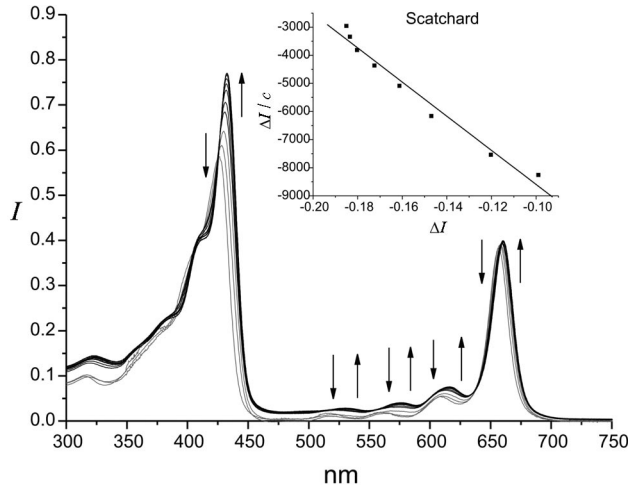


Figure 6. UV/Vis monitored titration of Zn pyropheophorbide *a* (14.2 μM) adding nicotine (0–77.8 μM) in toluene. In the grey-coloured spectra the amount of nicotine is below 1 equiv. with respect to the amount of chlorin. The inset figure shows Scatchard analysis at Soret.

Clear quenching of the fluorescence emission Q_y band (659 nm) was observed in the corresponding fluorescence titration in chloroform (Figure 7).[16] Also, the isosbestic point at 668 nm indicates formation of a new molecular species. Dilution-corrected Benesi–Hildebrandt analysis[25] of quenching resulted in $K_a = 6.1 \times 10^4$ M^{-1}.[16]

Overall, the conducted NMR spectroscopic studies supported by molecular modelling showed that nicotine is tightly β-face ligated on Zn pyropheophorbide *a* with "two-point" binding. The obtained K_a values vary in the range from 6.1×10^4 to 8.0×10^5 M^{-1} and are dependent on concentration; however, the values are still close to that (K_a =

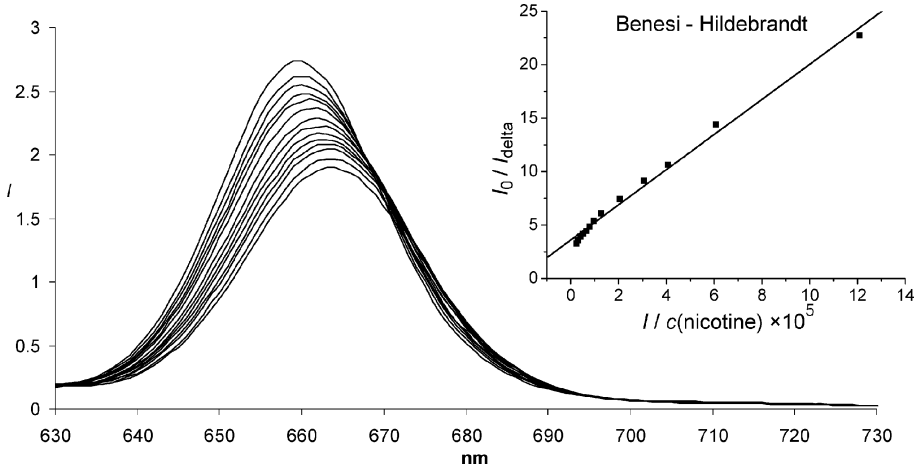


Figure 7. Fluorescence titration of Zn pyropheophorbide *a* (2.75 μM) by adding nicotine (0–44.5 μM) in chloroform (irradiation at 429 nm). The inset chart below shows Benesi–Hildebrandt analysis[25] at 659 nm.

4.6×10^5 M^{-1}) reported earlier for a "two-point" porphyrin-based nicotine chemosensor.[8]

Conclusions

The current results demonstrate that the applied "two-point" binding strategy can be a useful tool to control supramolecular organisation of chlorophyll derivatives in more advanced applications such as the mimicry of chlorophyll components in the photosynthetic antennae or reaction centre. We are currently applying the herein reported phenomenon to construct chlorin-based β-face selective supramolecular photoinduced electron donor–acceptor systems.

Experimental Section

General: NMR experiments were performed at 27 °C with a Varian Unity INOVA 500 NMR spectrometer. UV/Vis spectra were recorded with a Varian Cary5E spectrophotometer. Fluorescence spectra were measured with a BioLogic MOS250 fluorometer. IR spectra were measured with a PE Spectrum One (ATR) FTIR spectrometer.

Molecular Modelling: Geometry optimisation calculations for Zn pyropheophorbide *a*/nicotine complexes were performed with the DFT B3LYP method at the 6-31G* level by using GAUSSIAN03 program. Prior to the DFT calculations conformational search was performed for the propionic acid side chain and for the pyrrolidine moiety of nicotine with semiempirical RM1 method (by HyperChem interface). In the search all the propionic side chain rotamers and nicotine pyrrolidine orientations were systematically studied. Structures showing (pyridine/Zn, COOH/*N*-pyrrolidine) "two-point" coordination were chosen for further optimisations at the DFT level. As a result, 11 different structures were obtained under 12 kcal mol^{-1} energy frame.[16]

Chlorophyll *a* was extracted and purified from *Spirulina Pacifica* by a slightly modified procedure of Smith et al.,[26] and subsequently converted into pyropheophorbide *a* methyl ester.[27] Zn pyropheophorbide *a* (**1**) was synthesised from pyropheophorbide *a* methyl ester according to previously reported procedures.[13,14] The crude product was purified with SiO_2 column ($MeOH/CH_2Cl_2$, 1:10) to afford the product (50 mg, 8.3×10^{-2} mmol, 40%). ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 9.64 (s, 1 H, 10-H), 9.33 (s, 1 H, 5-H), 8.59 (s, 1 H, 20-H), 8.19 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 18.0 Hz, 1 H, 3^1-H), 6.26 (dd, $^2J_{gem}$ = 1.5 Hz, $^3J_{trans}$ = 18.0 Hz, 1 H, $3^2{}_{trans}$-H), 6.08 (dd, $^2J_{gem}$ = 1.5 Hz, $^3J_{cis}$ = 11.5 Hz, 1 H, $3^2{}_{cis}$-H), 5.14 (d, $^2J_{gem}$ = 19.5 Hz, 1 H, 13^2-H), 5.08 (d, $^2J_{gem}$ = 19.5 Hz, 1 H, $13^{2'}$-H), 4.57 (m, 1 H, 18-H), 4.30 (m, 1 H, 17-H), 3.81 (m, 2 H, 8^1-H), 3.62, 3.376, 3.286 (each s, 3 H, 12^1-, 2^1-, 7^1-H), 2.61–1.27 (m, 4 H, 17^1-, 17^2-H), 1.80 (d, $^3J_{18^1,18}$ = 7.0 Hz, 3 H, 18^1-H), 1.70 (t, $^3J_{8^2,8^1}$ = 7.5 Hz, 3 H, 8^2-H) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 1533.86 (1-H), 135.54 (2-H), 13.24 (2^1-H), 138.87 (3-H), 131.20 (3^1-H), 121.17 (3^2-H), 147.74 (4-H), 98.82 (5-H), 151.26 (6-H), 133.99 (7-H), 11.63 (7^1-H), 144.18 (8-H), 19.69 (8^1-H), 18.54 (8^2-H), 145.58 (9-H), 106.69 (10-H), 147.69 (11-H), 133.78 (12-H), 13.16 (12^1-H), 132.30 (13-H), 196.51 (13^1-H), 48.70 (13^2-H), 161.25 (14-H), 105.84 (15-H), 157.51 (16-H), 51.08 (17-H), 30.34 (17^1-H), 31.27 (17^2-H), 175.24 (17^3-H), 48.90 (18-H), 24.22 (18^1-H), 169.21 (19-H), 93.36 (20-H) ppm. IR: $\tilde{\nu}$ = 3600–2400 (br., COOH), 1609 (C=O), 1534 (C=O) cm^{-1}. HRMS (ESI + H): calcd. for $C_{33}H_{32}N_4O_3Zn$ 596.1760; found 596.1746.

Supporting Information (see footnote on the first page of this article): Molecular modelling; ROESY, COSY and HSQC spectra; DOSY parameters.

Acknowledgments

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[1] T. S. Balaban, *Photosynthesis Res.* **2005**, *86*, 251–262.
[2] Z. Liu, H. Yan, K. Wang, T. Kuang, J. Zhang, L. Gui, X. An, W. Chang, *Nature* **2004**, *428*, 287–292.
[3] T. S. Balaban, P. Fromme, A. R. Holzwarth, N. Krauß, V. I. Prokhorenko, *Biochim. Biophys. Acta* **2002**, *1556*, 197–207.
[4] T. Oba, H. Tamiaki, *Photosynth. Res.* **2002**, *74*, 1–10; T. Oba, H. Tamiaki, *J. Photosci.* **2002**, *9*, 362–363; T. Oba, H. Tamiaki, *Bioorg. Med. Chem.* **2005**, *13*, 5733–5739.
[5] T. S. Balaban, *FEBS Lett.* **2003**, *545*, 97–102.

[6] A. Garcia-Martin, L. G. Kwa, B. Strohmann, B. Robert, A. R. Holzwarth, P. Brown, *J. Biol. Chem.* **2006**, *281*, 10626–10634.
[7] I. S. Denniss, J. K. Sanders, *Tetrahedron Lett.* **1978**, *19*, 295–298, in which the reported upfield shifts of $17^2(CH_2)$ imply β-ligation.
[8] G. R. Deviprasad, F. D′Souza, *Chem. Commun.* **2000**, 1915–1916.
[9] W. H. Oldendorf, B. E. Stoller, F. L. Harris, *PNAS* **1993**, *90*, 307–311.
[10] S. G. Boxer, K. A. Wright, *J. Am. Chem. Soc.* **1979**, *101*, 6791–6794; K. A. Wright, S. G. Boxer, *Biochemistry* **1981**, *20*, 7546–7556.
[11] Y. Chen, X. Zheng, M. P. Dobhal, A. Gryshuk, J. Morgan, T. J. Dougherty, A. Oseroff, R. K. Pandey, *J. Med. Chem.* **2005**, *48*, 3692–3695.
[12] E. S. Nyman, P. H. Hynninen, *J. Photochem. Photobiol. B: Biology* **2004**, *73*, 1–28.
[13] P. H. Hynninen, *Acta Chem. Scand.* **1973**, *27*, 1771–1780.
[14] L. J. Boucher, J. J. Katz, *J. Am. Chem. Soc.* **1967**, *89*, 4703–4708.
[15] R. J. Abraham, A. E. Rowan, "Nuclear Magnetic Resonance Spectroscopy of Chlorophyll" in *Chlorophylls* (Ed.: H. Scheer), CRC Press, Boca Raton, **1991**, pp. 797–833.
[16] Data included in the Supporting Information.
[17] C. S. Wilcox in *Frontiers in Supramolecular Organic Chemistry and Photochemistry* (Eds.: H. J. Schneider, H. Dürr), VCH, Weinheim, **1991**, pp. 123–143.
[18] H. Tsukube, H. Furuta, A. Odani, Y. Takeda, Y. Kudo, Y. Inoue, Y. Liu, H. Sakamoto, K. Kimura in *Comprehensive Supramolecular Chemistry* (Eds.: J. E. D. Davies, J. A. Ripmeester), Pergamon Press, Oxford, **1996**, vol. 8, pp. 425–482.
[19] A. Agostiano, L. Catucci, G. Colafemmina, H. Scheer, *J. Phys. Chem. B* **2002**, *106*, 1446–1454.
[20] P. Groves, M. O. Rasmussen, M. D. Molero, E. Samain, F. J. Canada, H. Driguez, J. Jimenez-Barbero, *Glycobiology* **2004**, *14*, 451–456; Y. Cohen, L. Avram T. Evan-Salem, L. Frish in *Analytic Methods in Supramolecular Chemistry* (Ed.: C. A. Schalley), Wiley-VCH, Weinheim, **2007**, pp. 163–220.
[21] G. B. Rocha, R. O. Freire, A. M. Simas, J. J. P. Steward, *J. Comput. Chem.* **2006**, *27*, 1101–1111.
[22] J. M. Goodman in *Chemical Application of Molecular Modelling*, RSC, Cambridge, **2002**.
[23] G. Scatchard, *Ann. N. Y. Acad. Sci.* **1949**, *51*, 660–672.
[24] H. Tamiaki, S. Yagai, T. Miyatake, *Bioorg. Med. Chem.* **1998**, *6*, 2171–2178.
[25] H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707; H. Tsukube, H. Furuta, A. Odani, Y. Takeda, Y. Kudo, Y. Inoue, Y. Liu, H. Sakamoto, K. Kimura in *Comprehensive Supramolecular Chemistry* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. Macnicol, F. Vogtle), Elsevier Science, New York, **1996**, vol. 8.
[26] K. M. Smith, D. A. Goff, D. J. Simpson, *J. Am. Chem. Soc.* **1985**, *107*, 4946–4954.
[27] J. S. Kavakka, S. Heikkinen, I. Kilpeläinen, M. Mattila, H. Lipsanen, J. Helaja, *Chem. Commun.* **2007**, 519–521.

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