Hydrogen bond driven self-assembled C_2-symmetric chlorin *syn* dimers; unorthodox models for chlorophyll 'special pairs' in photosynthetic reaction centres†

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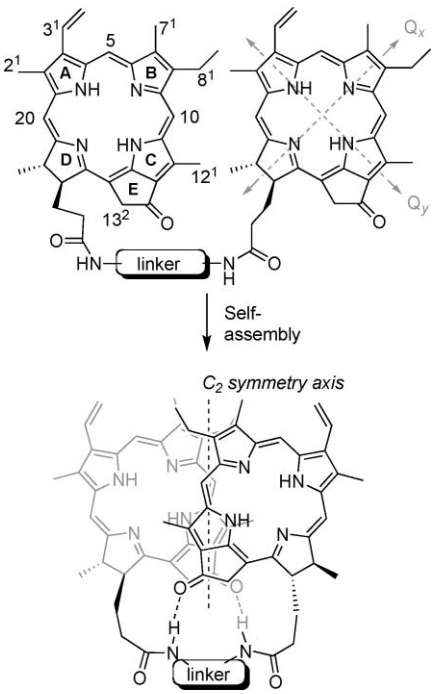
Amide linked *pyro*-pheophorbide *a* dimers, equipped with a suitable length of linkage, assemble in non-polar solvents by internal hydrogen bonding into C_2-symmetric stacked structures as evidenced by UV-vis, fluorescence, IR, CD, ^{1}H NMR spectroscopy and DFT molecular modelling studies.

Introduction

In both green plant and bacterial photosynthetic reaction centres (RC) stacked chlorophyll (Chl) dimers *i.e.* special pairs (SP) act as primary electron donors in exciton to charge energy conversion processes.[1] The specific dimer geometry varies depending on the RC,[2–4] but in each case at least two closely spaced chromophores have a strong excitonic interaction, which is optically observed as a characteristic red shifted Q_y band absorption and electrochemically as a lowered oxidation potential compared to the monomer.

Several synthetic covalently linked chlorin dimers have been reported as RC SP models.[5,6] Katz and collaborators have perhaps most elegantly constructed a glycol linked chlorophyllide dimer,[5a] which self-assembles into a folded conformer by two hydrate bridges *via* oxygen metal coordination and carbonyl hydrogen bonding. The assembling strategy utilized is based on Chls' known tendency to self-associate to form dimers or larger aggregates in non-polar solvents in the presence of small amounts of water.[7]

We were intrigued to study whether folded dimers could be constructed by a self-assembling approach without hydrate bridges. In peptide chemistry conformational folding is typically stabilized by hydrogen bonds formed in between amide proton hydrogen donors and amide carbonyl electron pairs as hydrogen bond acceptors. We envisioned that a related folding strategy could also be viable for chlorophyll derivatives. It has been shown previously that pheophorbides can be converted to diamide pheophorbide dimers, which have the high fluorescence quantum yields required in photodynamic therapy.[8] In our current study the working hypothesis is that in the dimers the amide protons are potential hydrogen bond donors whereas the 13^1 carbonyls of the neighbouring dimer molecules are the potential hydrogen bond acceptors as visualized in Scheme 1. To explore this idea we synthesized *pyro*-pheophorbide *a* derived propyl amide monomer **1** and a set of dimers **2–12** including varying linker lengths and types. The syntheses of the amides were carried out *via* pentafluorophenol ester activated *pyro*-pheophorbide *a*, resulting in dimers in high yields. The dimer folding was studied by UV-vis absorbance, fluorescence emission, IR, circular dichroism (CD), ^{1}H NMR spectroscopy and finally examined theoretically with molecular modelling on the DFT level.



Scheme 1

Results and discussion

The linker effect was studied at first with UV-vis absorption spectroscopy in chloroform (Table 1). In analogy with SP photosynthetic RC it would be logical to consider the Q_y band red shifts (bathochromic shift) as a measure of folding. However as the expected folded dimers (Scheme 1) are only weakly overlapped in the y-axis dimension, the Soret band shift values can be considered more indicative in this respect. The largest Q_y band red shifts in chloroform are measurable (4–6 nm), but reasonably

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Table 1 UV-vis absorption Soret and Q_y bands (in nm) measured in $CHCl_3$

Compound	Linker	Soret	ΔSoret [c]	Q_y	ΔQ_y [c]
1	$-CH_2CH_2CH_3$ [a]	415		669	
2	$-(CH_2)_3-$	415	0	669	0
3	$-(CH_2)_4-$	415	0	674	5
4	$-(CH_2)_5-$	413	−2	670	1
5	$-(CH_2)_6-$	401	−14	673	4
6	$-(CH_2)_7-$	401	−14	672	3
7	$-(CH_2)_8-$	413	−12	671	2
8	$-(CH_2)_{10}-$	414	−1	670	1
9	$-(CH_2)_{12}-$	415	0	669	0
10	$-(CH_2)_2O(CH_2)_2-$	414	−1	670	1
11	$(-CH_2CH_2S)_2$	413	−2	671	2
12	$-CH_2Ar^bCH_2-$	402	−13	675	6

[a] Reference monomer. [b] Ar = 1,3-phenyl. [c] Δ = (compound **X** − reference).

weak. More pronounced effects are observed in the Sorets, which maximally show a 14 nm blue shift (hypsochromic) for dimers **5** and **6** equipped with hexamethylene and heptamethylene linkers. Correspondingly, these compounds have also clear red shifts in the Q_y bands (4 and 3 nm). Dimer **12** tethered with a *m*-xylene linker shows similar shifts in both Soret and Q_y bands.

In order to probe the nature of folding the dimer **5** was titrated with an electron donating solvent, DMSO, to break the hydrogen bonds that stabilize the folding (Fig. 1). Expectedly, upon the addition of DMSO the Soret and Q_y band absorptions red and blue shifted, respectively to resemble the unfolded spectrum. The emission fluorescence titration of dimer **5** was somewhat more surprising: upon addition of DMSO the peak top was not only slightly blue shifted, but the emission intensity was notably diminished (Fig. 1). This implies that fluorescence quantum yield is significantly higher for the folded dimer geometry than the open one. Similar behaviour has though been reported for the classic special pair models.[5a,6]

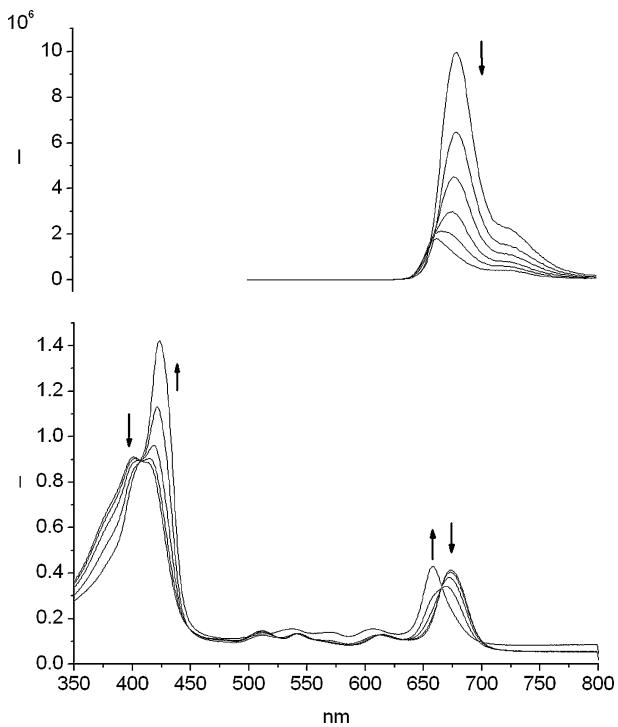

Fig. 1 Fluorescence emission (irradiated at 410 nm) (upper spectra) and UV-vis absorbance titration spectra (lower spectra) of **5** adding amounts of DMSO (UV-vis: 5.65 μM of **5**, DMSO 0–50 mM; Fluorescence: 56.6 nm of **5**, DMSO 0–1.0 mM).

The CD spectra measured for the compounds **1–12** indicated an increased Q_y band rotation value for the dimers that were also the most shifted in the UV-vis measurements. Stacked plot CD spectra of the dimers **2–9** in Fig. 2 show that the Q_y band intensity is steadily increased on going from dimer **2** to **5**, while it decreases with an oscillating trend from **6** to **9**. The maximally oriented Q_y dipole moment of dimer **5** correlates with the strongest shifts observed in the UV-vis (vide supra) and ^{1}H NMR spectra (vide infra), originating obviously from the folding of **5**.

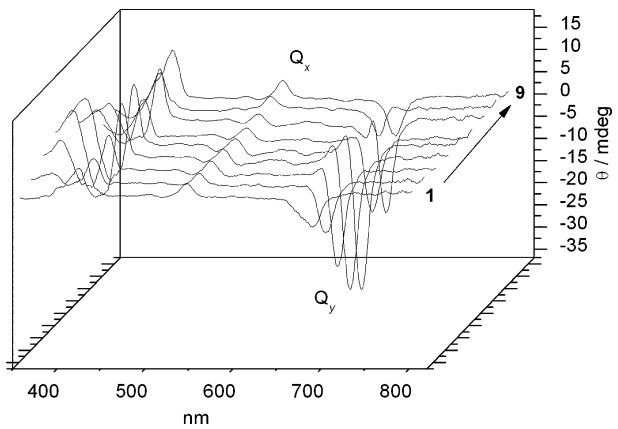

Fig. 2 Stacked plot CD spectra of monomer **1** (0.35 mM) and dimers **2–9** (0.17 mM) (in $CHCl_3$) presented in increasing order of chain length from front to back.

^{1}H NMR spectroscopy studies were conducted to reveal the folding geometry. The chlorin ring stacking is known to induce prominent spectral shieldings for the protons that are exposed to the ring current of the neighbouring macrocycle.[9] The construction $\Delta\delta_H$ value map of dimer **5** in Fig. 3 shows that spectral changes are localized on the right side of the chlorin macrocycle. While the strongest shielded protons are H12^1 (−1.89 ppm) and H10 (−1.68 ppm), only the amide proton is distinctly shielded

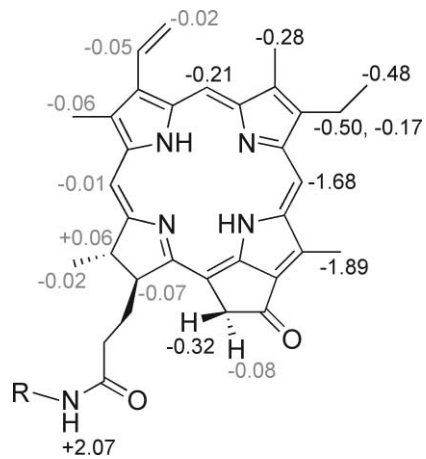

Fig. 3 $\Delta\delta_H$ value map of dimer **5** built with the reference monomer ($\Delta\delta_H = \delta_{5H} - \delta_{1H}$).

(+2.07 ppm). Hydrogen bonding typically induces chemical deshielding for amide protons, whereas chlorin macrocycle stacking is characteristically observed as spectral shieldings. Together these effects imply concomitant chlorin macrocycle stacking and amide bonding as depicted in Scheme 1.

Dynamic ^{1}H NMR was studied to elucidate the strength of folding. The variable temperature ^{1}H NMR studies of dimer **5** show also that the δ_H values of H10, H12^1 and amide protons are strongly dependent on T (Fig. 4). This result corroborates with the optical spectroscopy conclusion that the macrocycle stacking and hydrogen bonding are interconnected features.

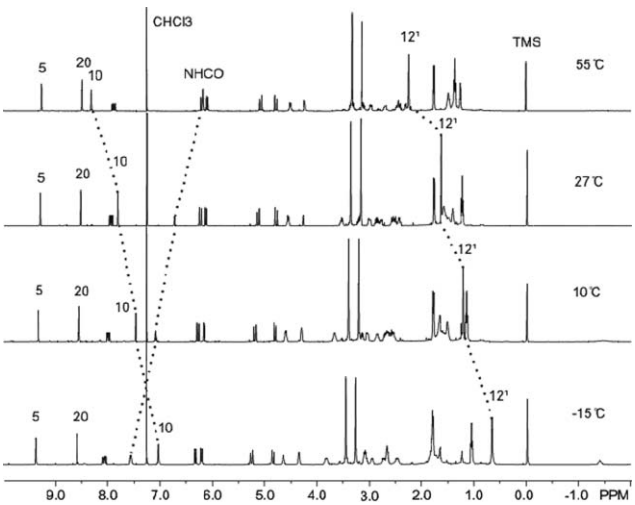

Fig. 4 ^{1}H NMR spectra of dimer **5** (–$(CH_2)_6$-linker) measured at various temperatures (–15 – +55 °C) in $CDCl_3$. The most T sensitive protons (H10, H12^1 and N*H*CO) are linked with dashed lines.

The dynamic ^{1}H NMR studies on compounds **1–12** are analysed by the graphs in Fig. 5, in which the most temperature dependent protons are charted. Unfortunately, the temperature range used (–15 – +55 °C) is too narrow to cover either the fully hydrogen bonded or the non bonded states, which can be concluded from the linear change of the δ values in all the cases. The absence of these extremes makes quantitative van't Hoff analysis[10] complicated in the present case. However, the graphs are as such qualitatively informative when the line slope *vs.* temperature dependence and δ_H values are simply compared to the ones of reference monomer **1**.

The protons of the reference monomer **1** have only a minor T dependence and the absolute δ_H values differ from those of the linked dimers **2–12** (Fig. 5). In the case of all the dimers it is obvious that H-bonding character is connected to macrocycle stacking induced shieldings, while line slope is approximately of opposite magnitude for the amide and macrocycle protons, respectively. A qualitative inspection of the lines shows that the dimer protons of **2**, **9** and **10** are closest to the reference, whereas those of dimers **5** and **12** deviate correspondingly by the largest amount. The behaviour of dimers **2** and **10** indicates that short and long linker lengths do not favour folding. In contrast to that the strong folding observed in dimers **5**, **6**, **7** and **12** implies that in the case of alkyl chains a linker length of 6–8 carbons is optimal, while the more rigid *m*-xylene linkage in **12** shows strong folding with a 5 carbon backbone. Additionally, heteroatoms in the linker can somewhat

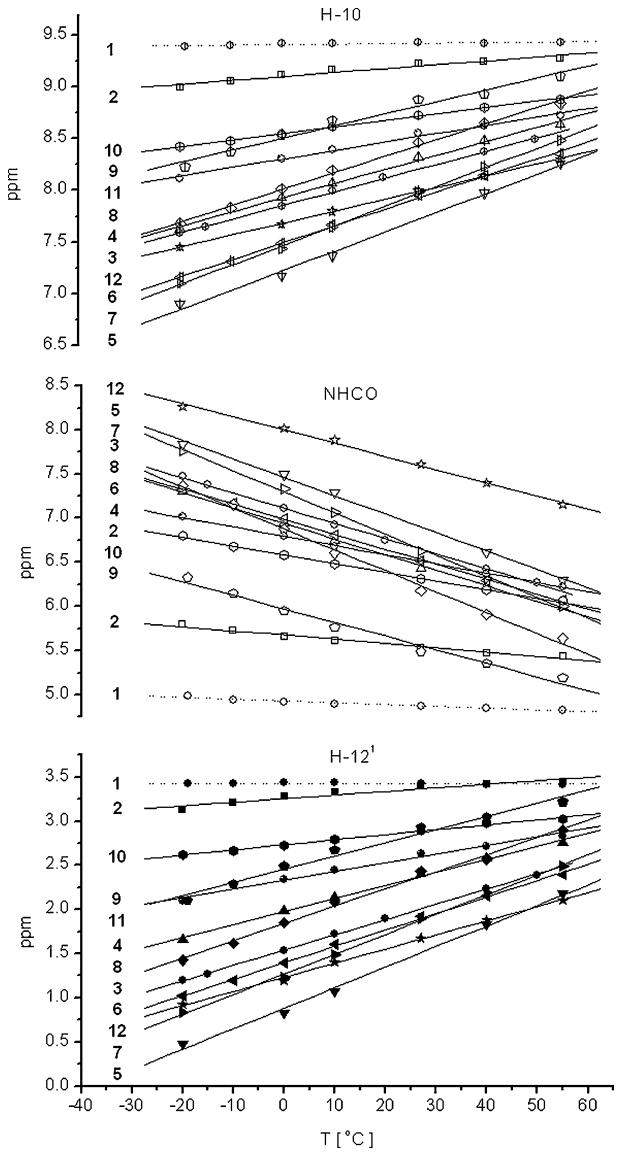

Fig. 5 δ_H temperature dependence of H12^1, H10 and N*H*CO protons of compounds **1–12** (1.3 mM in $CDCl_3$).

interfere with the folding as indicated by a comparison between similar chain length dimers **5** and **11**, and **4** and **10**.

To further ensure that the observed folding effects are truly amide hydrogen bond related, we measured IR spectra in $CHCl_3$ for compounds **1–12**, Table S1 (ESI†). In the case of monomer **1**, the measured dominating shift was at 3448 cm^{-1}, which is characteristic of a non bonded amide. In contrast to that all the dimers **2–12** showed additionally shifts at 3325–3375 cm^{-1} with variable intensities, which are typical resonance frequencies for hydrogen bonded amides.[11] The associated shifts were dominating for structures **3–6**, **11** and **12**. These dimers exhibited also the strongest folding tendency as documented above by other spectroscopic means. This undoubtedly confirms that the observed folding effect and hydrogen bonding are associated phenomena.

In order to get theoretical insight into the studied dimer folding we performed molecular modelling for dimer **5** with the DFT B3LYP/6-31G(d) method. Geometry optimization yielded a folded minimum energy structure, which exhibits an approximate

C_2-symmetry (Fig. 6). In the structure the interplanar distance of the slipped macrocycles is ca. 3.6 Å, whereas both amide proton–13[1] carbonyl hydrogen bond lengths are 1.85 Å.

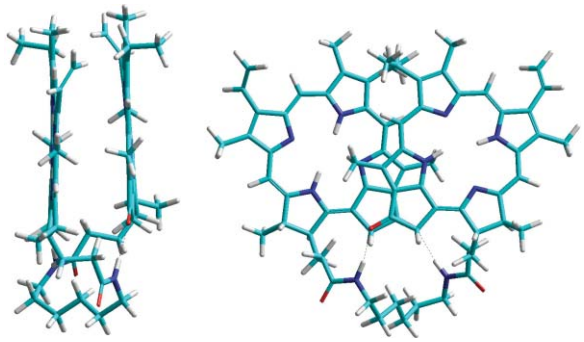

Fig. 6 B3LYP 6-31G(d) geometry optimized model of **5** side (left) and on top (right) view.

The approximate C_2-symmetry found for the dimer **5** gives us a basis to estimate its compatibility with the optical spectral data. In absorption spectroscopy it is known that the mutual orientation of the Q_y transition dipoles of both chlorins falls within a typical range for so-called J-dimers.[12] (Orientation of the Q_y transition dipole is given by a line connecting nitrogens in the A and C pyrrole rings (Scheme 1).) J-dimers are characterized by a red shift of the main Q_y transition compared to that of the monomer and excitonic CD signal with a ± pattern,[13] as indeed observed. Thus both absorption and CD spectra are in a qualitative agreement with the presented optimized structure.

Visualization of the two highest and lowest occupied MO's in Fig. 7 shows that the MO's are occupied simultaneously at both macrocycles. Moreover, a closer insight on the pyrrole D-ring region reveals that in the case of LUMO and HOMO-1 the macrocycles share MO's in this region. This indicates chemical bonding. The result is in agreement with the classical Hunter's π–π interaction model,[14] but is rarely observed at uncorrelated Hartree–Fock and DFT levels.[15]

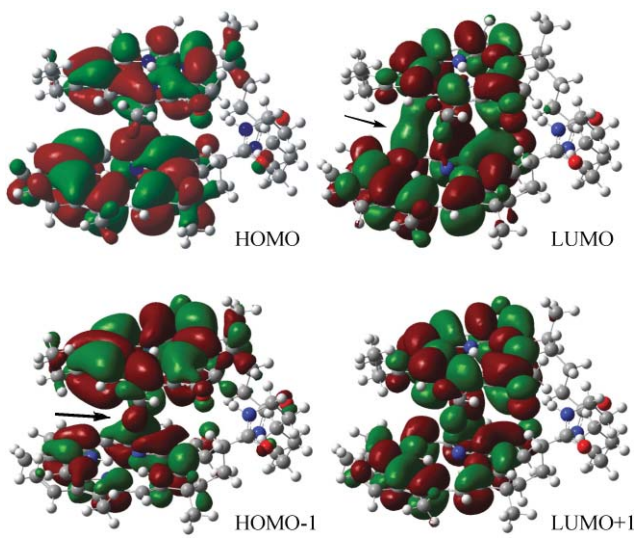

Fig. 7 HOMO, HOMO-1, LUMO and LUMO+1 visualization of the B3LYP 6-31Gd optimized **5**. (Interplanar D-ring MOs shown by arrows.)

Table 2 Calculated electronic energies and ionization potentials (eV) of folded and opened chlorin dimer. Structural parameters have been optimized with B3LYP/6-31G(d) and the electronic structure refined with B3LYP/6-311+G(d) methods in benzene ($\varepsilon = 2.2$, approximated with IEF-PCM)

	Energy (eV)			
Compound	neutral	cation	ΔIP (eV)	$\Delta IP_{f\text{-}o}$ [a]
5 folded	–99081.352	–99075.681	+5.672	
5 open	–99081.368	–99075.344	+6.024	0.353
1	–50627.135	–50621.329	+5.806	0.135

[a] IP(**5** folded) – IP(**5** open) or IP(**5** folded) – IP(**1** monomer).

Finally, ionization potentials (ΔIP) were calculated for the B3LYP/6-31G(d) geometry optimized open and folded dimer **5** and reference **1** structures using 6-311+G(d) basis set and benzene solvation model (Table 2). The ΔIP's were obtained by subtraction of electronic energies that were calculated for the neutral and cationic states. A ΔIP difference of 353 meV was obtained between the folded and open dimer **5** (Table 2). This is, however, a rather unrealistic value while an inspection of atom charges reveals that the positive charge is evenly delocalized over both macrocycles in the folded and opened dimer **5**. In the latter case this is not a probable physical phenomenon neither in *in vivo* nor *in vitro* systems, but rather a computational deficiency. Therefore, the use of the ΔIP of the reference compound **1** (monomer) in the comparison gives straightforwardly the energy difference that folding induces. This kind of comparison ΔIP yields an ΔIP potential difference of 135 meV, which is very close to that (140 meV) computed recently for the Chl SP in photosystem II using equivalent calculation parameters.[16]

Conclusions

In summary, we have shown that diamide linked *pyro*-pheophorbide *a* dimers fold into C_2-symmetric stacked structures, driven by amide proton–carbonyl hydrogen bonding, when the linkers are of suitable length. Even though the synthesized folded dimers differ notably from the genuine SP's in the photosynthetic RC[2–4] by being metal free species and also by their different stacking geometries, the folded dimers have similarities with the genuine ones. The conducted optical studies imply that in the folded dimers the chlorin units are in excitonic interaction and the electronic calculations predict also that the macrocycle MO's are in close interaction. Additionally, the calculated ΔIP's agree with the ones reported for the SP of PSII.

In preliminary experiments we have observed that similar folding also takes place for pheophorbides other than the *pyro*-pheophorbide *a*, whereas in the case of metal ligated chlorins an external ligand is required to maintain the folding. In fact, it has been reported that amide functionalized metallated chlorins form C_2-symmetric aggregates with slight ring D overlap,[17] however with a rather different mechanism than we have described herein for the dimers. We are currently extending this study to electron acceptor conjugated folded dimers to explore the *in vitro* electron donor capabilities of these unorthodox SP's models.

Experimental section

Synthesis of compounds 1–12

The general synthetic strategy was to extract Chl *a* from algae, *Spirulina pacifica*, and modify it with standard procedures to give *pyro*pheophorbide *a* (*pyro*Pheo *a*).[5k,18] This compound was functionalized to the corresponding pentafluoroester (Fig. 8) and used as a synthetic precursor in all the syntheses of **1–12**.

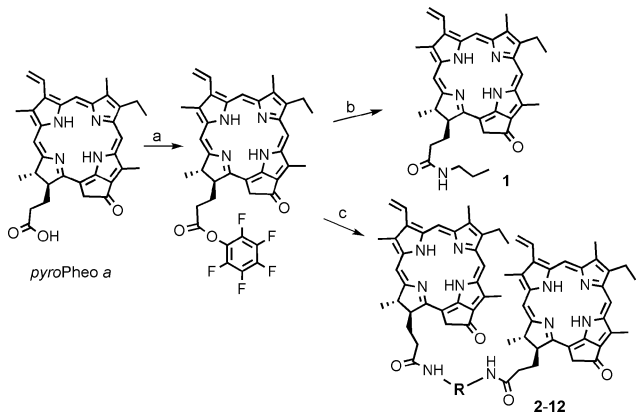

Fig. 8 Preparation of amide linked *pyro*-pheophorbide *a* monomer **1** and dimers **2–12**. Reagents: a) Pentafluorophenol, DMAP, EDC, CH_2Cl_2; b) H_2N-R, TEA, CH_2Cl_2; c) H_2N-R-NH_2, TEA, CH_2Cl_2.

Pentafluorophenol ester activated *pyro*-pheophorbide *a*, pentafluorophenyl *pyro*-pheophorbide *a*

Pyro-pheophorbide *a* acid (247 mg, 0.46 mmol), pentafluorophenol (101 mg, 0.55 mmol) and 4-dimethylaminopyridine (DMAP) (11 mg, 0.09 mmol) were dissolved in dichloromethane (50 ml) in an argon atmosphere. The solution was cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) (124 mg, 0.65 mmol) was dissolved in dichloromethane (1 ml) and added to the mixture. According to TLC (eluent 10:1 dichloromethane-methanol), the reaction was finished after 18 h stirring at rt. The reaction mixture was filtered through a silica pad eluting with 10:1 dichloromethane-methanol and the filtrate was evaporated to dryness to give the product (295 mg, 92%). R_f = 0.9 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.47 (s, 1H, 5-H) 9.37 (s, 1H, 10-H) 8.56 (s, 1H, 20-H) 7.98 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.9 Hz, 1H, 3^1-H) 6.28 (dd, $^2J_{gem}$ = 1.6 Hz, $^3J_{trans}$ = 17.9 Hz, 1H, $3^2{}_{trans}$-H) 6.16 (dd, $^2J_{gem}$ = 1.6 Hz, $^3J_{cis}$ = 11.5 Hz, 1H, $3^2{}_{cis}$-H) 5.23 (d, $^2J_{gem}$ = 20.0 Hz, 1H, 13^2-H) 5.12 (d, $^2J_{gem}$ = 20.0 Hz, 1H, $13^{2'}$-H) 4.54 (m, 1H, 18-H) 4.37 (m, 1H, 17-H) 3.67 (m, 2H, 8^1-H) 3.65 (s, 3H, 7^1-H) 3.41 (s, 3H, 12^1-H) 3.22 (s, 3H, 2^1-H) 2.88 (m, 1H, 17^2-H) 2.82 (m, 1H, 17^1-H) 2.60 (m, 1H, $17^{2'}$-H) 2.40 (m, 1H, $17^{1'}$-H) 1.84 (d, $^3J_{18}1_{-18}$ = 7.2 Hz, 3H, 18^1-H) 1.68 (t, $^3J_82_{-8}1$ = 7.8 Hz, 3H, 8^2-H) 0.45 (br s, 1H, NH) −1.7 (br s, 1H, NH). HRMS (ESI $+H^+$): calcd. for $C_{39}H_{34}F_5N_4O_3$ 701.2546; found 701.2563.

Propyl amide linked *pyro*-pheophorbide *a*, monomer 1

Pentafluorophenol ester activated *pyro*-pheophorbide *a* (20mg, 0.029 mmol), propylamine (2 mg, 0.034 mmol) and triethylamine (4µl, 0,029mmol) were dissolved in dichloromethane (4 ml). The reaction mixture was stirred under argon at rt for 4 hours. The crude product was purified with SiO_2 column (40:1→10:1; DCM:MeOH, gradient) to give **1** (16 mg, 100%). R_f = 0.5 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.33 (s, 1H, 5-H) 9.22 (s, 1H, 10-H) 8.52 (s, 1H, 20-H) 7.97 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 1H, 3^1-H) 6.27 (dd, $^2J_{gem}$ = 1.5 Hz, $^3J_{trans}$ = 17.8 Hz, 1H, $3^2{}_{trans}$-H) 6.16 (dd, $^2J_{gem}$ = 1.5 Hz, $^3J_{cis}$ = 11.5 Hz, 1H, $3^2{}_{cis}$-H) 5.21 (m, 1H, 17^4-NH) 5.21 (d, $^2J_{gem}$ = 19.5 Hz, 1H, 13^2-H) 5.06 (d, $^2J_{gem}$ = 19.5 Hz, 1H, $13^{2'}$-H) 4.50 (m, 1H, 18-H) 4.32 (m, 1H, 17-H) 3.58 (m, 2H, 8^1-H) 3.39 (s, 3H, 7^1-H) 3.35 (s, 3H, 12^1-H) 3.21 (s, 3H, 2^1-H) 2.96 (m, 2H, 17^5-H) 2.67–1.90 (m, 4H, 17^1-, 17^2-H) 1.79 (d, $^3J_{18}1_{-18}$ = 7.3 Hz, 3H, 18^1-H) 1.62 (t, $^3J_82_{-8}1$ = 7.7 Hz, 3H, 8^2-H) 1.19 (m, 2H, 17^6-H) 0.67 (t, $^3J_{17}7_{-17}6$ = 7.4 Hz, 3H, 17^7-H) 0.3–0.5 (br s, 1H, NH) −1.65 (br s, 1H, NH) calcd. for $C_{36}H_{42}N_5O_2$ 575.3333; found 576.3343.

Amide linked *pyro*-pheophorbide *a* dimers 2–12

General procedure.

Synthesis of dimer 2. Pentafluorophenol ester activated *pyro*-pheophorbide *a* (50 mg, 0,071 mmol), 1,3-diaminopropane (2,4 mg, 0,032 mmol) and triethylamine (10 µl, 0,071 mmol) were dissolved in dichloromethane (10 ml). The reaction mixture was stirred under argon at rt over night. Subsequently, the mixture was diluted with dichloromethane and washed with sodium bicarbonate, water and brine. The organic layer was dried over anhydrous sodium sulphate, filtrated and evaporated. The crude product was purified with SiO_2 column (40:1→10:1; DCM:MeOH, gradient) to give **2** (24 mg, 68%) R_f = 0.4 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.23 (s, 2H, 5-H) 9.18 (s, 2H, 10-H) 8.47 (s, 2H, 20-H) 7.77 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.04 (d, $^3J_{trans}$ = 17.8 Hz, 2H, $3^2{}_{trans}$-H) 5.95 (d, $^3J_{cis}$ = 11.5 Hz, 2H, $3^2{}_{cis}$-H) 5.53 (t, $^3J_{17}4_{-17}5$ = 5.8 Hz, 2H, 17^4-NH) 4.97 (d, $^2J_{gem}$ = 19.3 Hz, 2H, 13^2-H) 4.81 (d, $^2J_{gem}$ = 19.3 Hz, 2H, $13^{2'}$-H) 4.43 (m, 2H, 18-H) 4.16 (m, 2H, 17-H) 3.53 (q, $^3J_81_{-8}2$ = 7.6 Hz, 4H, 8^1-H) 3.40 (s, 6H, 2^1-H) 3.26 (s, 6H, 7^1-H) 3.09 (s, 6H, 12^1-H) 2.59 (m, 2H, 17^5-H) 2.51 (m, 2H, $17^{5'}$-H) 2.41 (m, 2H, 17^1-H) 2.30 (m, 2H, $17^{1'}$-H) 2.20 (m, 2H, 17^2-H) 1.95 (m, 2H, $17^{2'}$-H) 1.72 (d, $^3J_{18}1_{-18}$ = 7.4 Hz, 6H, 18^1-H) 1.60 (t, $^3J_82_{-8}1$ = 7.6 Hz, 6H, 8^2-H) 0.77 (m, 2H, 17^6-H) 0.27 (br s, 2H, NH) −1.85 (br s, 2H, NH) calcd. for $C_{69}H_{75}N_{10}O_4$ 1107.5967; found 1107.5936.

Dimer 3. (7 mg, 15%) R_f = 0.3 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.22 (s, 2H, 5-H) 8.50 (s, 2H, 20-H) 8.28 (s, 2H, 10-H) 7.76 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.41 (t, $^3J_{17}4_{-17}5$ = 5.7 Hz, 2H, 17^4-NH) 5.94 (dd, $^2J_{gem}$ = 1.1 Hz, $^3J_{trans}$ = 17.9 Hz, 2H, $3^2{}_{trans}$-H) 5.89 (dd, $^2J_{gem}$ = 1.1 Hz, $^3J_{cis}$ = 11.5 Hz, 2H, $3^2{}_{cis}$-H) 4.77 (d, $^2J_{gem}$ = 18.8 Hz, 2H, 13^2-H) 4.56 (d, $^2J_{gem}$ = 18.8 Hz, 2H, $13^{2'}$-H) 4.56 (m, 2H, 18-H) 4.12 (m, 2H, 17-H) 3.38 (m, 2H, 17^5-H) 3.32 (m, 2H, 8^1-H) 3.21 (s, 6H, 2^1-H) 3.15 (s, 6H, 7^1-H) 3.09 (m, 2H, $8^{1'}$-H) 2.55–2.41 (m, 8H, 17^1-, 17^2-H) 2.46 (m, 2H, $17^{5'}$-H) 2.12 (s, 6H, 12^1-H) 1.77 (d, $^3J_{18}1_{-18}$ = 7.2 Hz, 6H, 18^1-H) 1.35 (t, $^3J_82_{-8}1$ = 7.6 Hz, 6H, 8^2-H) 1.26 (m, 4H, 17^6-H) 0.33 (br s, 2H, NH) −1.61 (br s, 2H, NH) calcd. for $C_{70}H_{76}N_{10}O_4$ 1121.6129; found 1121.6145.

Dimer 4. (35 mg, 88%) R_f = 0.4 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.10 (s, 2H, 5-H) 8.39 (s, 2H, 20-H) 8.32 (s, 2H, 10-H) 7.77 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.43 (m, 2H, 17^4-NH) 6.15 (d, $^3J_{trans}$ = 17.8 Hz, 2H,

3^2_{trans}-H) 6.05 (d, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 4.92 (d, $^2J_{gem}$ = 19.0 Hz, 2H, 13^2-H) 4.60 (d, $^2J_{gem}$ = 19.0 Hz, 2H, $13^{2'}$-H) 4.34 (m, 2H, 18-H) 3.99 (m, 2H, 17-H) 3.27 (s, 6H, 2^1-H) 3.27 (m, 2H, 8^1-H) 3.24 (m, 2H, 17^5) 3.06 (s, 6H, 7^1-H) 3.05 (m, 2H, $8^{1'}$-H) 2.96 (m, 2H, $17^{5'}$) 2.41 (s, 6H, 12^1-H) 2.32–2.14 (m, 8H, 17^1-, 17^2-H) 1.67 (d, $^3J_{18}1_{-18}$ = 7.3 Hz, 6H, 18^1-H) 1.35 (t, $^3J_82_{-8}1$ = 7.6 Hz, 6H, 8^2-H) 1.32 (m, 4H, 17^6-H), 1.24 (m, 2H, 17^7-H) 0.51 (br s, 2H, NH) −1.68 (br s, 2H, NH) calcd. for $C_{71}H_{79}N_{10}O_4$ 1135.6286; found 1135.6289.

Dimer ***5***. (29 mg, 78%). R_f = 0.4 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.28 (s, 2H, 5-H) 8.51 (s, 2H, 20-H) 7.94 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.9 Hz, 2H, 3^1-H) 7.80 (s, 2H, 10-H) 6.72 (17^4-NH) 6.23 (dd, $^2J_{gem}$ = 1.0 Hz, $^3J_{trans}$ = 17.9 Hz, 2H, 3^2_{trans}-H) 6.12 (dd, $^2J_{gem}$ = 1.0 Hz, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 5.12 (d, $^2J_{gem}$ = 19.0 Hz, 2H, 13^2-H) 4.77 (d, $^2J_{gem}$ = 19.0 Hz, 2H, $13^{2'}$-H) 4,55 (m, 2H, 18-H) 4,26 (m, 2H, 17-H) 3.53 (m, 2H, 17^5-H) 3.36 (s, 6H, 2^1-H) 3.20 (m, 2H, 8^1-H) 3.17 (s, 6H, 7^1-H) 3.00 (m, 2H, $17^{5'}$-H) 2.85 (m, 2H, $8^{1'}$-H) 2.77 (m, 2H, 17^1-H) 2.75 (m, 2H, $17^{1'}$-H) 2.51 (m, 2H, 17^2-H) 2.43 (m, 2H, $17^{2'}$-H) 1.76 (d, $^3J_{18}1_{-18}$ = 7.4 Hz, 6H, 18^1-H) 1.62 (s, 6H, 12^1-H) 1.57–1.48 (m, 8H, 17^6-, 17^7-H) 1.22 (t, $^3J_82_{-8}1$ = 7.7 Hz, 6H, 8^2-H) 0.42 (br s, 2H, NH) −1.52 (br s, 2H, NH). HRMS (ESI +Na^+): calcd. for $C_{72}H_{81}N_{10}O_4$ 1149.6437; found 1149.6458.

Dimer ***6***. (27 mg, 73%) R_f = 0.3 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.20 (s, 2H, 5-H) 8.49 (s, 2H, 20-H) 7.95 (s, 2H, 10-H) 7.86 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.51 (t, J = 5.7 Hz, 2H, 17^4-NH) 6.19 (dd, $^2J_{gem}$ = 1.1 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^2_{trans}-H) 6.08 (dd, $^2J_{gem}$ = 1.1 Hz, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 5.15 (d, $^2J_{gem}$ = 19.1 Hz, 2H, 13^2-H) 4.80 (d, $^2J_{gem}$ = 19.1 Hz, 2H, $13^{2'}$-H) 4.54 (m, 2H, 18-H) 4.24 (m, 2H, 17-H) 3.43 (m, 2H, 17^5-H) 3.33 (s, 6H, 2^1-H) 3.20 (m, 2H, 8^1-H) 3.12 (s, 6H, 7^1-H) 2.97 (m, 2H, $17^{5'}$-H) 2.93 (m, 2H, $8^{1'}$-H) 2.73–2.28 (m, 8H, 17^1-, 17^2-H) 1.93 (s, 6H, 12^1-H) 1.77 (d, $^3J_{18}1_{-18}$ = 7.3 Hz, 6H, 18^1-H) 1.50–1.30 (m, 10H, 17^6-, 17^8-H) 1.26 (t, $^3J_82_{-8}1$ = 7.8 Hz, 6H, 8^2-H) 0.18 (br s, 2H, NH) −1.65 (br s, 2H, NH) calcd. for $C_{73}H_{83}N_{10}O_4$ 1163.6599; found 1163.6561.

Dimer 7. (8 mg, 21%) R_f = 0.4 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.29 (s, 2H, 5-H) 8.53 (s, 2H, 20-H) 7.99 (s, 2H, 10-H) 7.95 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.9 Hz, 2H, 3^1-H) 6.63 (m, 2H, 17^4-NH) 6.26 (dd, $^2J_{gem}$ = 1.0 Hz, $^3J_{trans}$ = 17.9 Hz, 2H, 3^2_{trans}-H) 6.15 (dd, $^2J_{gem}$ = 1.0 Hz, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 5.24 (d, $^2J_{gem}$ = 19.0 Hz, 2H, 13^2-H) 4.90 (d, $^2J_{gem}$ = 19.0 Hz, 2H, $13^{2'}$-H) 4.59 (m, 2H, 18-H) 4.31 (m, 2H, 17-H) 3.38 (s, 6H, 2^1-H) 3.37 (m, 2H, 17^5-H) 3.26 (m, 2H, 8^1-H) 3.18 (s, 6H, 7^1-H) 2.99 (m, 2H, $17^{5'}$-H) 2.97 (m, 2H, $8^{1'}$-H) 2.79–2.29 (m, 8H, 17^1-, 17^2-H) 1.90 (s, 6H, 12^1-H) 1.81 (d, $^3J_{18}1_{-18}$ = 7.4 Hz, 6H, 18^1-H) 1.51–1.27 (m, 12H, 17^6–17^8-H) 1.29 (t, $^3J_82_{-8}1$ = 7.8 Hz, 6H, 8^2-H) 0.51 (br s, 2H, NH) −1.50 (br s, 2H, NH) calcd. for $C_{74}H_{85}N_{10}O_4$ 1177.6755; found 1177.6770.

Dimer ***8***. (26 mg, 68%) R_f = 0.3 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.21 (s, 2H, 5-H) 8.47 (s, 2H, 20-H) 8.40 (s, 2H, 10-H) 7.87 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.25 (m, 2H, 17^4-NH) 6.20 (dd, $^2J_{gem}$ = 0.8 Hz, $^3J_{trans}$ = 17.9 Hz, 2H, 3^2_{trans}-H) 6.09 (dd, $^2J_{gem}$ = 0.8 Hz, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 5.20 (d, $^2J_{gem}$ = 19.1 Hz, 2H, 13^2-H) 4.92 (d, $^2J_{gem}$ = 19.1 Hz, 2H, $13^{2'}$-H) 4.53 (m, 2H, 18-H) 4.26 (m, 2H, 17-H) 3.33 (m, 2H, 8^1-H) 3.33 (s, 6H, 2^1-H) 3.15 (m, 2H, $8^{1'}$-H) 3.12 (s, 6H, 7^1-H) 3.11 (m, 2H, 17^5-H) 2.91 (m, 2H, $17^{5'}$-H) (m, 8H, 17^1-, 17^2-H) 2.38 (s, 6H, 12^1-H) 1.77 (d, $^3J_{18}1_{-18}$ = 7.4 Hz, 6H, 18^1-H) 1.39 (t, $^3J_82_{-8}1$ = 7.7 Hz, 6H, 8^2-H) 1.29–1.18 (m, 16H, 17^6–17^9-H) 0.3–0.5 (br s, 2H, NH) −1.60 (br s, 2H, NH) calcd. for $C_{76}H_{89}N_{10}O_4$ 1205.7068; found 1205.7081.

Dimer ***9***. (16 mg, 42%) R_f = 0.4 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.26 (s, 2H, 5-H) 8.87 (s, 2H, 10-H) 8.49 (s, 2H, 20-H) 7.89 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.21 (dd, $^3J_{trans}$ = 17.8 Hz, 2H, 3^2_{trans}-H) 6.10 (dd, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 5.49 (m, 2H, 17^4-NH) 5.19 (d, $^2J_{gem}$ = 19.3 Hz, 2H, 13^2-H) 4.98 (d, $^2J_{gem}$ = 19.3 Hz, 2H, $13^{2'}$-H) 4.50 (m, 2H, 18-H) 4.28 (m, 2H, 17-H) 3.46 (m, 2H, 8^1-H) 3.39 (m, 2H, $8^{1'}$-H) 3.34 (s, 6H, 2^1-H) 3.15 (s, 6H, 7^1-H) 2.93 (s, 6H, 12^1-H) 2.92 (m, 4H, 17^5-H) 2.66 (m, 2H, 17^1-H) 2.48 (m, 2H, $17^{1'}$-H) 2.19 (m, 2H, 17^2-H) 1.95 (m, 2H, $17^{2'}$-H) 1.76 (d, $^3J_{18}1_{-18}$ = 7.4 Hz, 6H, 18^1-H) 1.51 (t, $^3J_82_{-8}1$ = 7.7 Hz, 6H, 8^2-H) 1.16–0.98 (m, 20H, 17^6–17^{10}-H) 0.3–0.5 (br s, 2H, NH) −1.63 (br s, 2H, NH) calcd. for $C_{78}H_{93}N_{10}O_4$ 1233.7381; found 1233.7374.

Dimer ***10***. (33 mg, 91%) R_f = 0.3 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 8.98 (s, 2H, 5-H) 8.72 (s, 2H, 10-H) 8.31 (s, 2H, 20-H) 7.62 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.31 (t, J = 5.7 Hz, 2H, 17^4-NH) 6.05 (dd, $^2J_{gem}$ = 1.1 Hz, $^3J_{trans}$ = 17.9 Hz, 2H, 3^2_{trans}-H) 5.96 (dd, $^2J_{gem}$ = 1.1 Hz, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 4.76 (d, $^2J_{gem}$ = 19 Hz, 2H, 13^2-H) 4.48 (d, $^2J_{gem}$ = 18.8 Hz, 2H, $13^{2'}$-H) 4.20 (m, 2H, CH-18) 3.82 (m, 2H, 17-H) 3.33–3.23 (m, 8H, 17^5–17^6-H) 3.31 (m, 2H, 8^1-H) 3.19 (s, 6H, 2^1-H) 3.12 (m, 2H, $8^{1'}$-H) 2.99 (s, 6H, 7^1-H) 2.90 (s, 6H, 12^1-H) 2.32–1.87 (m, 8H, 17^1-, 17^2-H) 1.60 (d, $^3J_{18}1_{-18}$ = 7.4 Hz, 6H, 18^1-H) 1.46 (t, $^3J_82_{-8}1$ = 7.7 Hz, 6H, 8^2-H) 0.3–0.5 (br s, 2H, NH) −1.91 (br s, 2H, NH) calcd. for $C_{70}H_{77}N_{10}O_5$ 1137.6078; found 1137.6064.

Dimer ***11***. (41 mg, 31%) R_f = 0.4 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.08 (s, 2H, 5-H) 8.55 (s, 2H, 20-H) 8.37 (s, 2H, 10-H) 7.75 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 6.52 (m, 2H, 17^4-NH) 6.12 (d, $^3J_{trans}$ = 17.8 Hz, 2H, 3^2_{trans}-H) 6.03 (d, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 4.90 (d, $^2J_{gem}$ = 19.2 Hz, 2H, 13^2-H) 4.61 (d, $^2J_{gem}$ = 19.2 Hz, 2H, $13^{2'}$-H) 4.33 (m, 2H, 18-H) 3.95 (m, 2H, 17-H) 3.41 (m, 2H, 17^5-H) 3.32 (m, 4H, 8^1-H) 3.25 (s, 6H, 2^1-H) 3.22 (m, 2H, $17^{5'}$-H) 3.21 (m, 2H, $8^{1'}$-H) 3.05 (s, 6H, 7^1-H) 2.63 (s, 6H, 12^1-H) 2.60 (t, $^3J_{17}6_{-17}5$ = 6.2 Hz, 4H, 17^6-H) 2.38 (m, 4H, 17^2-H) 2.26 (m, 4H, 17^1-H) 1.64 (d, $^3J_{18}1_{-18}$ = 7.3 Hz, 6H, 18^1-H) 1.42 (t, $^3J_82_{-8}1$ = 7.7 Hz, 6H, 8^2-H) 0.3–0.5 (br s, 2H, NH) −1.93 (br s, 2H, NH) calcd. for $C_{70}H_{77}N_{10}O_4S_2$ 1185.5571; found 1185.5563.

Dimer ***12***. (32 mg, 86%) R_f = 0.4 (10:1; DCM:MeOH); δ_H(500 MHz; $CDCl_3$; 27 °C) 9.24 (s, 2H, 5-H) 8.52 (s, 2H, 20-H) 8.00 (s, 2H, 10-H) 7.87 (dd, $^3J_{cis}$ = 11.5 Hz, $^3J_{trans}$ = 17.8 Hz, 2H, 3^1-H) 7.60 (m, 2H, 17^4-NH) 7.31 (d, $^4J_{4'\text{-}5'}$ = 7.4 Hz, $^4J_{6'\text{-}5'}$ = 7.4 Hz, 2H, 4′-H and 6′-H) 7.24 (s, 1H, 2′-H) 7.15 (t, $^4J_{5'\text{-}4'}$ = 7.4 Hz, $^4J_{5'\text{-}6'}$ = 7.4 Hz, 1H, 5′-H) 6.19 (d, $^3J_{trans}$ = 17.8 Hz, 2H, 3^2_{trans}-H) 6.09 (d, $^3J_{cis}$ = 11.5 Hz, 2H, 3^2_{cis}-H) 4.87 (m, 2H, 17^5-H) 4.75 (d, $^2J_{gem}$ = 19.5 Hz, 2H, 13^2-H) 4.53 (m, 2H, 18-H) 4.15 (d, $^2J_{gem}$ = 19.5 Hz, 2H, $13^{2'}$-H) 4.06 (m, 2H, 17-H) 4.06 (m, 2H, $17^{5'}$-H) 3.34 (s, 6H, 2^1-H) 3.23 (q, $^3J_81_{-8}2$ = 7,6 Hz, 2H, 8^1-H) 3.16 (s, 6H, 7^1-H) 2.96 (q, $^3J_81'_{-8}2$ = 7,6 Hz, 2H, $8^{1'}$-H) 2.79–2.36 (m, 8H, 17^1-, 17^2-H) 1.81 (d, $^3J_{18}1_{-18}$ = 7.4 Hz, 6H, 18^1-H) 1.67 (s, 6H, 12^1-H)

1.29 (t, $^3J_{8\text{-}8}2_{\text{-}8}1 = 7.6$ Hz, 6H, 8^2-H) 0.30 (br s, 2H, NH) −1.56 (br s, 2H, NH) calcd. for $C_{74}H_{77}N_{10}O_4$ 1169.6129; found 1169.6123.

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