Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4207–4211
Bioorganic & Medicinal Chemistry Letters

# Amino porphyrins as photoinhibitors of Gram-positive and -negative bacteria

V. Sol,* P. Branland, V. Chaleix, R. Granet, M. Guilloton, F. Lamarche, B. Verneuil and P. Krausz

*Université de Limoges, Laboratoire de Chimie des Substances Naturelles, 123, Avenue Albert Thomas, 87060 Limoges, France*

Received 23 March 2004; revised 31 May 2004; accepted 8 June 2004
Available online 26 June 2004

**Abstract**—Twenty four aminoporphyrin derivatives have been tested in vitro for their antibacterial photoactivity against *Escherichia coli* and *Staphylococcus aureus*. Two of these compounds, bearing polyamine units, exhibited a significant activity especially against Gram-negative bacteria (*E. coli*).


## 1. Introduction

PDT is based upon the selective accumulation of photoreactive compounds (photosensitizers) in tumour tissue and on the production of singlet oxygen by irradiation of the sensitizer-enriched tumour with visible light.[1] Formation of cytotoxic singlet oxygen directly in tumour cells causes cell death and often total tumour necrosis. Photosensitization can represent a useful approach for the killing of microbial cells since it has been shown that several porphyrins and related compounds display phototoxicity against bacteria and yeast.[2] Indeed, the appearance of bacteria, which show multiple resistance to antibiotics is a growing concern in hospitals due to the difficulty to eradicate them (nosocomial infection...). In order to overcome these resistance problems, there is an urgent need for discovering novel antibacterial agents belonging to structural classes distinct from existing antibiotics. Porphyrins are molecules of considerable interest due to their ability to act as photosensitizers when irradiated with visible light and recent studies have shown that porphyrins could be used in PACT (photodynamic antimicrobial chimiotherapy).[3] Moreover, it has been shown that the nature and position of substituents around the macrocyclic core influence the pharmacokinetics and the biodistribution of porphyrins used in PDT.[4] Also, structure–activity relationships (SARs) for photodynamic sensitizers is not very studied compared to other pharmaceuticals because it is difficult to synthesize porphyrins susbstituted with a wide diversity of substituents.

In an effort to identify new antibacterial compounds and in connection with our research programme on porphyrins, we report, herein, synthesis and initial structure–activity relationship (SAR) studies of 24 porphyrins bearing amino functions and glucosyl or methyl units on the *meso*phenyl group. Indeed, many studies have shown that Gram-positive bacteria are sensitive to photoinactivation with porphyrin, chlorine or phthalocyanine photosensitizers.[5] However, Gram-negative bacteria have been reported to be relatively unsensitive unless the photosensitizers bear cationic groups or are administrated in the presence of additional substances as, for example, polymyxin B nonapeptide (PMBN) that alter the permeability of the outer membrane.[6] So, in these last examples, the presence of an amine function seems to be required for activity. The purpose of this study was to determine if the variation (number, position and nature) of the amino function directly grafted on the macrocycle influence the photobiocidal activity of porphyrins against *Staphylococcus aureus* and *Escherichia coli* in absence of added PMBN. For this purpose, we have synthesized different porphyrins where $NH_2$ function is borne either directly by the phenyl group of the macrocycle, an amino acid (alanine or serine), a small peptide (RGD) or polyamines (spermine or spermidine). Elsewhere, a number of

*Keywords*: Porphyrin derivatives; Polyamines; Photodynamic antimicrobial chemotherapy; *Escherichia coli*.
* Corresponding author. Tel.: +33(0)-5-5545-7490; fax: +33(0)-5-5545-7202; e-mail: vincent.sol@unilim.fr

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.06.016

studies[7] have shown that *E. coli* is equipped with polyamine uptake systems, which allow compounds bearing spermine or spermidine to enter the cell membrane. In order to produce conjugates with photobactericidal effect against Gram-negative bacteria, a synthetic strategy was devised that enabled precisely a protoporphyrin ring to be attached to polyamines.

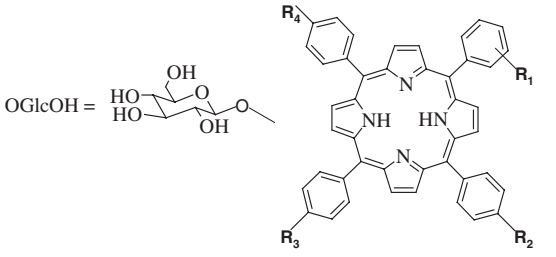
## 2. Chemistry

Syntheses of porphyrins **1**–**14** (Schemes 1 and 2) was conducted in our laboratory and are described in previous papers.[1j,8] Two tritolylporphyrins **13**–**14** bearing only one unit of spermidine or spermine were synthesized.[8b] In connection with our research programme on porphyrin–polyamine derivatives and in order to increase the number of amino functions on the macrocycle, we have synthesized two new porphyrins **17**–**18** linked by spacer arms with two units of spermidine or spermine. The synthetic route followed for the preparation of the desired compounds is depicted in Scheme 3. Protoporphyrin IX reacted with $N^4$-(4-aminobutyl)-$N^1$,$N^8$-bis-*t*-butoxycarbonylspermidine or with $N^4$-(4-aminobutyl)-$N^1$,$N^8$,$N^{12}$-tris-*t*-butoxycarbonylspermine in presence of DCC and HOBt in DMF. After purification by PLC, protected polyamine porphyrin conjugates **15** and **16** were obtained in 80% and 85% yield, respectively. The protecting groups (Boc) were removed with standard method in high yields with TFA in $CH_2Cl_2$ at room temperature (2 h). Compounds **17** and **18** were obtained in quantitative yields (Scheme 3).

UV–vis absorption, MS MALDI, $^1H$ (400 MHz) NMR spectra of these compounds showed the expected signals.[9]

## 3. Photoinhibition of bacterial growth

Compounds **1**–**14** and **17**, **18** were evaluated for their antimicrobial activity against two strains, which were obtained from 'Institut Pasteur, Paris': (a) *Escherichia coli* CIP 368548, class 2 and (b) *Staphylococcus aureus* CIP 35053156, class 2. These strains are DNase positive, Phosphatase positive. The antibiotic resistance profiles



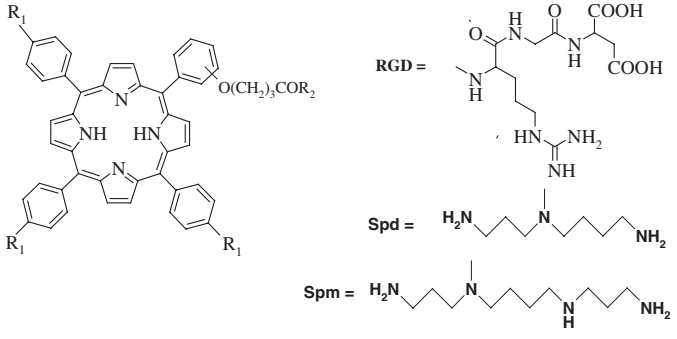

**1a,b** : $R_1$ = *o* or *p* $NH_2$; $R_2 = R_3 = R_4$ = OGlcOH
**2** : $R_1 = R_3$ = *p* $NH_2$; $R_2 = R_4$ = OGlcOH
**3** : $R_1 = R_2$ = *p* $NH_2$; $R_3 = R_4$ = OGlcOH
**4a,b** : $R_1$ = *o* or *p* OGlcOH; $R_2 = R_3 = R_4$ = $NH_2$
**5a,b** : $R_1$ = *o* or *p* NH-Alanine; $R_2 = R_3 = R_4$ = OGlcOH
**6** : $R_1 = R_3$ = *p* NH-Alanine; $R_2 = R_4$ = OGlcOH
**7** : $R_1 = R_2$ = *p* NH-Alanine; $R_3 = R_4$ = OGlcOH
**8a,b** : $R_1$ = *o* or *p* OGlcOH; $R_2 = R_3 = R_4$ = NH-Alanine

**9a,b** : $R_1$ = *o* or *p* $NH_2$; $R_2 = R_3 = R_4$ = $CH_3$
**10a,b** : $R_1$ = *o* or *p* NH-Serine-OGlcOH ; $R_2 = R_3 = R_4$ = $CH_3$

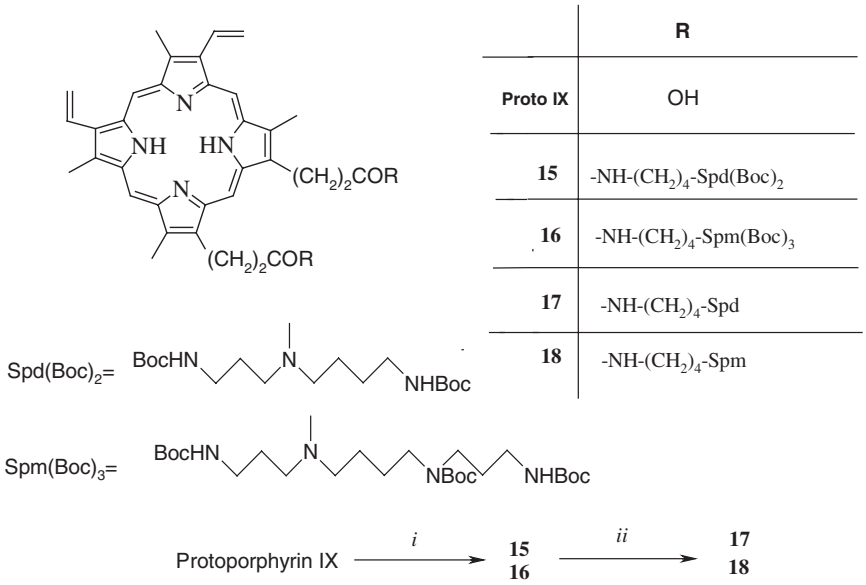
a and b refer to *ortho* and *para* position of $R_1$ group respectively

**Scheme 1.**

| | 11a,b | 12a,b | 13 | 14 |
|---|---|---|---|---|
| $R_1$ | $CH_3$ | OGlcOH | $CH_3$ | |
| $R_2$ | -RGD | -RGD | Spd | Spm |




**Scheme 2.**




**Scheme 3.** Reaction conditions: (i) (a) $N^4$-(4-aminobutyl)-$N^1$,$N^8$-bis-*t*-butoxycarbonylpermidine (2.2 equiv), DCC (2.2 equiv), HOBt (2.2 equiv), DMF, rt, 72 h, (b) $N_4$-(4-aminobutyl)-$N^1$,$N^8$,$N^{12}$-tris-*t*-butoxycarbonylspermine (2.2 equiv), DCC (2.2 equiv), HOBt (2.2 equiv), DMF, rt, 72 h; (ii) TFA/$CH_2Cl_2$(1/1), rt, 2 h.

are described in Table 1. These two strains were grown 24 h at 37 °C in 10 mL peptone water medium. 0.2 mL of a dilution to 1/20 of the broth is used as inoculum of a Müeller–Hinton agar. A stock solution of each porphyrin (**1**–**14**, **17**, **18**) was prepared in pure DMSO at concentrations of 500–2700 $\mu g\,mL^{-1}$ and kept in the dark until use. The tests were realized during 24 h at room temperature with illumination by two 60 W tungsten lamps placed 20 cm away and 45° above both sides of the plates. Cultures treated in the dark as reference were covered with aluminum foil during incubation. A screening was first realized: a Whatman paper disk, impregnated by 50 μL of a 200 $\mu g\,mL^{-1}$ porphyrin diluted solution, was placed on the nutrient agar seeded with the target strain. A DMSO reference was conducted in the same conditions. Inhibition was quantified by measuring the halo diameter formed around the paper disk (a diameter larger than 1 cm is considered as a positive response). MIC and MBC were determined for each photosensitizer giving positive responses. Duplicate experiments were performed at least three times.

### 3.1. MIC determination

MIC of the porphyrins giving a positive reaction in peptone water medium were quantified by measuring the absorbance at 570 nm for a 0.3–1.5 $\mu g\,mL^{-1}$ porphyrin concentration range, after 24 h of illumination at room temperature.

### 3.2. MBC determination

MBC was estimated for porphyrin concentrations giving a negative culture reaction (first tube). 0.2 mL of the corresponding peptone water medium was used as an inoculum of a Müeller–Hinton agar. After 24 h of growth at 37 °C, a bacterial numeration was carried out.

A comparative analysis of the results obtained in these conditions (Tables 2 and 3) has shown that (i) *meso*arylporphyrins **1**–**14** displayed a photobactericidal effect only against Gram-positive bacteria and an enhanced activity is observed for porphyrin structures carrying simultaneously glucosyl units and amino groups. So, for example, *meso*arylporphyrins **1a**,**b** present MIC and MBC values lower than **9a**,**b**, respectively. In the same way, **12a**,**b** structure bearing three *O*-β-D glucosyl units and one RGD group express a greater photobiocidal activity than **11a**,**b** whose glucosyl units were substituted by $CH_3$ groups. (ii) Protoporphyrins **17**–**18** bearing polyamines were the only products that exhibited activity against the Gram-negative bacterium *E. coli*. Moreover, two polyamine functions

**Table 1.** Antibiotic resistance profiles of *E. coli* and *S. aureus*

| Antibiotics | Str | Chl | Sul | Ery | Rif | Tet | Pen | Kan | Nal | Van | Amo | Cep | Cef | Pri |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain CIP 368548, class 2. | S | S | S | L | S | S | — | — | — | — | S | — | L | R |
| Strain CIP 35053156, class 2. | S | L | S | S | S | S | S | S | R | S | — | — | — | — |

S: sensitive, L: limit, R: resistant, Str: streptomycin, Chl: chloramphenicol, Sul: sulfamide, Ery: erythromycin, Rif: rifampicin, Tet: tetracycline, Pen: penicillin, Kan: kanamycin, Nal: nalidixic acid, Van: vancomycin, Amo: amoxillin, Cep: cephalotin, Cef: cefamandole, Pri: pristinamycin, —: not tested.

**Table 2.** Minimal inhibitory concentration (MIC, $\mu g\,mL^{-1}$) and minimal bactericidal concentration (MBC, $\mu g\,mL^{-1}$) against *S. aureus*

| Porphyrins | *S. aureus* MIC ($\mu g\,mL^{-1}$) | *S. aureus* MBC ($\mu g\,mL^{-1}$) |
|---|---|---|
| **1a** | 0.5–1.0 | >1.5 |
| **1b** | 1.0–1.5 | 1–1.5 |
| **2** | 0–0.5 | 1–1.5 |
| **3** | >3 | NE[a] |
| **4a** | 1.5–2.0 | >1.5 |
| **4b** | 1.0–1.5 | >1.5 |
| **5a** | 1.5–2.0 | >1.5 |
| **5b** | 1.0–1.5 | >1.5 |
| **6** | 0.5–1.0 | 1–1.5 |
| **7** | 0.5–1.0 | 1–1.5 |
| **8a** | 1.5–2.0 | >1.5 |
| **8b** | 1.0–1.5 | >1.5 |
| **9a** | >2.5 | >3 |
| **9b** | >2.5 | >3 |
| **10a** | >3 | NE[a] |
| **10b** | >3 | NE[a] |
| **11a** | >2.5 | >3.0 |
| **11b** | >2.5 | >3.0 |
| **12a** | 0.5–1.0 | 1–1.5 |
| **12b** | 0.5–1.0 | 1–1.5 |
| **13** | 0.5–1.0 | 1–1.5 |
| **14** | 1.0–1.5 | >1.5 |
| **17** | 1.5–2.0 | 2.0–2.5 |
| **18** | 0.5–1.0 | 1–1.5 |

[a] NE, not evaluated.

**Table 3.** Minimal inhibitory concentration (MIC, $\mu g\,mL^{-1}$) and minimal bactericidal concentration (MBC, $\mu g\,mL^{-1}$) against *E. coli*

| Porphyrins[a] | *E. coli* MIC ($\mu g\,mL^{-1}$) | *E. coli* MBC ($\mu g\,mL^{-1}$) |
|---|---|---|
| **17** | 1.5–2.0 | 2.0–2.5 |
| **18** | 1.0–1.5 | 1.5–2.0 |

[a] Porphyrins **1**–**14** exhibited no significative activity.

(spermidine or spermine) seem to be necessary to obtain such an inhibition.

In conclusion, and for the first time, we have shown that porphyrin derivatives bearing two units of polyamines (spermine or spermidine) are able to inhibit the growth of Gram-negative bacteria without assistance of a membrane destabilizing agent (polymyxin nonapeptide,...) to permeabilize the outer membrane. Moreover, our results showed that the photoinhibition activity against the Gram-positive bacteria *S. aureus* is, in most cases, increased when amino*meso*arylporphyrins were substituted by sugar units.

## References and notes


1. (a) Czuchajowski, L.; Li, H. *Tetrahedron Lett.* **1994**, *35*, 1629–1630; (b) Cornia, M.; Casiraghi, G.; Binacchi, S.; Zanardi, F.; Rassu, G. *J. Org. Chem.* **1994**, *59*, 1226–1230; (c) Momenteau, M.; Oulmi, D.; Maillard, Ph.; Croisy, A.; Spie *Photodynamic Therapy of Cancer II* **1994**, *2325*, 13–23; (d) Oulmi, D.; Maillard, Ph.; Guerquin-Kern, J. L.; Heul, C.; Momenteau, M. *J. Org. Chem.* **1995**, *60*, 1554–1564, and references cited therein; (e) Hombrecher, H. K.; Ohm, S.; Koll, D. *Tetrahedron* **1996**, *52*, 5441–5448; (f) Hombrecher, H. K.; Schell, C.; Thiem, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1199–1202; (g) Sol, V.; Blais, J. C.; Bolbach, G.; Carré, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. *Tetrahedron Lett.* **1997**, *38*, 6391–6394; (h) Mikata, Y.; Onchi, Y.; Tabata, K.; Ogura, S.; Okura, I.; Ono, N.; Yano, S. *Tetrahedron Lett.* **1998**, *39*, 4505–4508; (i) Schell, C.; Hombrecher, H. K. *Chem. Eur. J.* **1999**, *5*, 587–598; (j) Chaleix, V.; Sol, V.; Huang, H.-M.; Guilloton, M.; Granet, R.; Blais, J. C.; Krausz, P. *Eur. J. Org. Chem.* **2003**, 1486–1493.
2. (a) Nitzan, Y.; Balzam-Sudakevitz, A.; Ashkenazi, H. *J. Photochem. Photobiol., B: Biol.* **1998**, *42*, 211–218; (b) Soukos, N. S.; Ximenez-Fyvie, L. A.; Hamblin, M. R.; Socransky, S. S.; Hasan, T. *Antimicrob. Agents Chemother.* **1998**, *42*, 2595–2601; (c) Valduga, G.; Breda, B.; Giacometti, G. M.; Jori, G.; Reddi, E. *Biochem. Biophys. Res. Commun.* **1999**, *256*, 84–88; (d) Bertoloni, G.; Lauro, F. M.; Cortella, G.; Merchat, M. *Biochim. Biophys. Acta* **2000**, *1475*, 169–174; (e) Minnock, A.; Vernon, D. I.; Scofield, J.; Griffiths, J.; Parish, J. H.; Brown, S. B. *Antimicrob. Agents Chemother.* **2000**, *44*, 522–527; (f) Rovaldi, C. R.; Pievsky, A.; Sole, N. A.; Friden, P. M.; Rothstein, D.; Spacciapoli, P. *Antimicrob. Agents Chemother.* **2000**, *44*, 3364–3367; (g) Polo, L.; Segalla, A.; Bertoloni, G.; Jori, G.; Schaffner, K.; Reddi, E. *J. Photochem. Photobiol., B: Biol.* **2000**, *59*, 152–158; (h) Stojiljkovic, I.; Evavold, B. D.; Kumar, V. *Exp. Opin. Invest. Drugs.* **2001**, *10*, 309–320; (i) Nitzan, Y.; Ashkenazi, H. *Curr. Microbiol.* **2001**, *42*, 408–414; (j) Hamblin, M. R.; O'Donnell, D. A.; Murthy, N.; Rajagopalan, K.; Michaud, N.; Sherwood, M. E.; Hasan, T. *J. Antimicrob. Chemother.* **2002**, *49*, 941–951.
3. Wainwright, M. *J. Antimicrob. Chemother.* **1998**, *42*, 13–28.
4. Boyle, R. W.; Dolphin, D. *Photochem. Photobiol.* **1996**, *64*, 469–485.
5. (a) Sol, V.; Branland, P.; Granet, R.; Kaldapa, C.; Verneuil, B.; Krausz, P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3007–3010; (b) Wilson, M.; Pratten, J. *Lasers Surg. Med.* **1995**, *16*, 272–276; (c) Ehrenberg, B.; Malik, Z.; Nitzan, Y. *Photochem. Photobiol.* **1984**, *41*, 429–435.
6. Nitzan, Y.; Gutterman, M.; Malik, Z.; Ehrenberg, B. *Photochem. Photobiol.* **1992**, *55*, 89–96; Gabor, F.; Szocs, K.; Maillard, P.; Csik, G. *Radiat. Environ. Biophys.* **2001**, *40*, 145–151.
7. (a) Igarashi, K.; Kashiwagi, K. *Biochem. J.* **1999**, *344*, 633–642; (b) Igarashi, K.; Kiyoshi, I.; Kashiwagi, K. *Res. Microbiol.* **2001**, *152*, 271–278.
8. (a) Sol, V.; Blais, J. C.; Carré, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. *J. Org. Chem.* **1999**, *64*, 4431–4444; (b) Lamarche, F.; Sol, V.; Huang, Y.-M.; Granet, R.; Guilloton, M.; Krausz, P. *J. Porphyrins Phthalocyanines* **2002**, *6*, 130–134.
9. Selected data: For **15**, $^1$H NMR ($CDCl_3$, 400.13 MHz): $\delta$ (ppm) = 9.76, 9.69, 9.54 (s, 4H, *H-meso*), 8.14 (dd, 2H, $J$ = 11.6–17.7 Hz, C*H* vinyl), 6.34 (d, 2H, $J$ = 17.8 Hz, C$H_2$–vinyl), 6.21 (b d, 2H, $J$ = 10.7 Hz, C$H_2$–vinyl), 5.27 (s, 4H, N*H*Boc), 4.79 (s, 2H, –N*H*CO), 4.26 (m, 4H, Proto-C$H_2$–CH$_2$–CO), 3.50 (m, 16H, Proto-CH$_2$–C$H_2$–CO and C$H_3$ β-pyrrole), 3.1–2.2 (m, 24H, C$H_2$–N, C$H_2$–NH–), 1.43–1.20 (m, 20H, –CONHCH$_2$($CH_2$)$_2$ CH$_2$N(CH$_2$)$_2$, (CH$_2$)$_2$NCH$_2$C$H_2$C$H_2$CH$_2$NHBoc and –(CH$_2$)$_2$NCH$_2$-C$H_2$CH$_2$NHBoc), 1.36 (s, 36H, C$H_3$Boc), −3.69 (s, 2H, N*H* pyrrole). UV–vis spectrum in $CH_2Cl_2$: $\lambda_{max}$, nm ($\varepsilon$, $L\,cm^{-1}\,mol^{-1}\times10^3$): 407 (113.9); 504 (8.8); 541 (8.4); 575 (5.3); 629 (3.2). MS (MALDI) $m/z$ 1360.43 ([M+H$^+$] monoisotopic). Anal. Calcd for $C_{76}H_{118}N_{12}O_{10}$: C, 912.80; H, 119.00; N, 168.00; found: C, 912.69; H, 118.64; N, 167.15. For **16**, $^1$H NMR ($CDCl_3$, 400.13 MHz): $\delta$ (ppm) = 9.94, 9.90, 9.88, 9.75 (s, 4H, *H-meso*), 8.19 (dd, 2H, $J$ = 11.6–17.7 Hz, C*H* vinyl), 6.36 (d, 2H,

$J = 17.8$ Hz, C$H_2$–vinyl), 6.21 (d, 2H, $J = 11.4$ Hz, C$H_2$–vinyl), 5.25 (b s, 4H, N*H*Boc), 4.75 (b s, 2H, –N*H*CO), 4.33 (m, 4H, Proto-C$H_2$–$CH_2$–CO), 3.71–3.46 (m, 16H, C$H_3$ β-pyrrole and Proto-$CH_2$–C$H_2$–CO), 3.22–2.88 (m, 32H, C$H_2$–N, –(C$H_2$)$_2$N–CO and C$H_2$NHCO), 1.45–1.26 (m, 74H, C$H_3$Boc, CONHC$H_2$(C$H_2$)$_2$C$H_2$ N($CH_2$)$_2$, ($CH_2$)$_2$NC$H_2$C$H_2$C$H_2$NHBoc, –($CH_2$)$_2$N $CH_2$C$H_2$C$H_2$NHBoc and ($CH_2$)$_2$NC$H_2$C$H_2$C$H_2$N($CH_2$)), −3.71 (s, 2H, N*H*-pyrrole). UV–vis spectrum in $CH_2Cl_2$: $\lambda_{max}$, nm ($\varepsilon$, L cm$^{-1}$ mol$^{-1}$ × 10$^3$): 406 (133.5); 504 (10.5); 540 (9.3); 574 (6.6); 629 (3.7). MS (MALDI) *m*/*z* 1673.70 ([M+H$^+$] monoisotopic). Anal. Calcd for $C_{92}H_{148}N_{14}O_{14}$: C, 1104.98; H, 149.18; N, 196.09; found: C, 1103.73; H, 148.01; N, 194.92. For **17**, $^1$H NMR ($CD_3OD$, 400.13 MHz): $\delta$ (ppm) = 9.46 (b s, 3H, *H-meso*), 9.34 (b s, 1H, *H-meso*), 8.17 (dd, 1H, $J = 11.6$–17.4 Hz, C*H* vinyl), 8.09 (dd, 1H, $J = 12.0$–17.1 Hz, C*H* vinyl), 6.40–6.30 (m, 4H, C$H_2$–vinyl), 4.29 (m, 4H, Proto-C$H_2$–$CH_2$–CO), 3.50 (m, 12H, C$H_3$ β-pyrrole), 3.34 (s, 4H, Proto-$CH_2$–C$H_2$–CO), 3.01–2.64 (m, 16H, C$H_2$–N, C$H_2$–NH–), 1.55–1.16 (m, 20H, –CONHC$H_2$(C$H_2$)$_2$C$H_2$N($CH_2$)$_2$, ($CH_2$)$_2$NC$H_2$ C$H_2$C$H_2$C$H_2$N$H_2$ and –($CH_2$)$_2$NC$H_2$C$H_2$C$H_2$N$H_2$), UV–vis spectrum in $CH_3OH$: $\lambda_{max}$, nm ($\varepsilon$, L cm$^{-1}$ mol$^{-1}$ × 10$^3$): 403 (27.0); 503 (2.5); 538 (2.1); 575 (1.3); 629 (0.9). MS (MALDI) *m*/*z* 959.73 ([M+H$^+$] monoisotopic). For **18**, $^1$H NMR ($CD_3OD$, 400.13 MHz): $\delta$ (ppm) = 9.52–9.41 (s, 4H, *H-meso*), 8.33–8.17 (m, 2H, C*H* vinyl), 6.36 (m, 4H, C$H_2$–vinyl), 4.33 (m, 4H, Proto-C$H_2$–$CH_2$–CO), 3.50 (m, 12H, C$H_3$ β-pyrrole), 3.22–2.88 (m, 36H C$H_2$)$_3$N, –(C$H_2$)$_2$NH, CONHC$H_2$–, C$H_2$N$H_2$ and Proto-$CH_2$–C$H_2$–CO), 1.45–1.26 (m, 24H, CONHC$H_2$(C$H_2$)$_2$C$H_2$N($CH_2$)$_2$, ($CH_2$)$_2$ NC$H_2$C$H_2$C$H_2$C$H_2$NH–C$H_2$C$H_2$C$H_2$N$H_2$ and ($CH_2$)$_2$ NC$H_2$C$H_2$C$H_2$N$H_2$). UV–vis spectrum in $CH_3OH$: $\lambda_{max}$, nm ($\varepsilon$, L cm$^{-1}$ mol$^{-1}$ × 10$^3$): 402 (29.7); 503 (2.6); 538 (2.1); 575 (1.4); 629 (0.9). MS (MALDI) *m*/*z* 1073.92 ([M+H$^+$] monoisotopic).