Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 3904–3922
Bioorganic & Medicinal Chemistry

Synthesis of substrate analogs of methyltransferases in the vitamin B_{12} biosynthetic pathway and characterization of their enzymatic products

Clotilde Pichon-Santander, Patricio J. Santander and A. Ian Scott*

Department of Chemistry, Texas A&M University, PO Box 30012, College Station, TX 77842-3012, USA

Received 7 October 2005; revised 20 January 2006; accepted 20 January 2006
Available online 7 February 2006

Abstract—The specificity toward substrate analogs of the first two methyltransferases in the vitamin B_{12} biosynthetic pathway was probed with 15 synthetic porphyrinogens. Several novel methylated chlorins and isobacteriochlorins were isolated and characterized, suggesting the same methylation sequence C-2 > C-7 > C-20 as for the natural substrate, uro'gen III. The results allow us to narrow down possible structural requirements concerning substrate recognition by the methyltransferase enzymes.

1. Introduction

Over the last 50 years, several laboratories have explored different aspects of the complex biosynthetic pathway to vitamin B_{12},[1] which utilizes over 25 enzymes. Of these, only the earlier ones, responsible for the formation of the tetrapyrrole macrocycle, have been studied in detail by us and others, and much less is known about the enzymes implicated in the steps after uroporphyrinogen III (**1a**, uro'gen III) (Scheme 1), including C-methylations at positions 2, 7, and 20 to give precorrin-1 (**2a**), precorrin-2 (**3a**), and precorrin-3 (**4a**), respectively. These intermediates were isolated and characterized as their oxidized forms, factor 1 (**6a**),[2–4] factor 2 (**7a**),[5,6] and factor 3 (**8a**).[6,7] A single enzyme (M-1) is responsible for the first two methylations, while a second enzyme (M-2) mediates the third methylation. Both enzymes are *S*-adenosylmethionine (SAM) dependent and have been overexpressed from different sources.[8–11] The natural substrate of M-1 is uro'gen III (**1a**, Scheme 1); however, its isomers uro'gen I, II, and IV (**1b**, **1c**, and **1d** in Scheme 2) have also been shown to serve as substrates.[12–14] In addition, it has been demonstrated that cell-free systems from *Propionibacterium shermanii* and *Pseudomonas denitrificans*, both organisms producing vitamin B_{12}, are able to methylate the 12-methyl analog of uro'gen III **1e** to its precorrin-2 (**3e**) or precorrin-3 (**4e**) analogs.[15,16]

These results suggested the possibility of further exploring the substrate specificity of the first two methyltransferases in order to gain a better understanding of the structural requirements for activity and to utilize these enzymes in a semisynthetic approach to obtain unnatural chlorins and isobacteriochlorins, compounds which in recent years have attracted strong interest as photosensitizers for use in photodynamic therapy.[17,18]

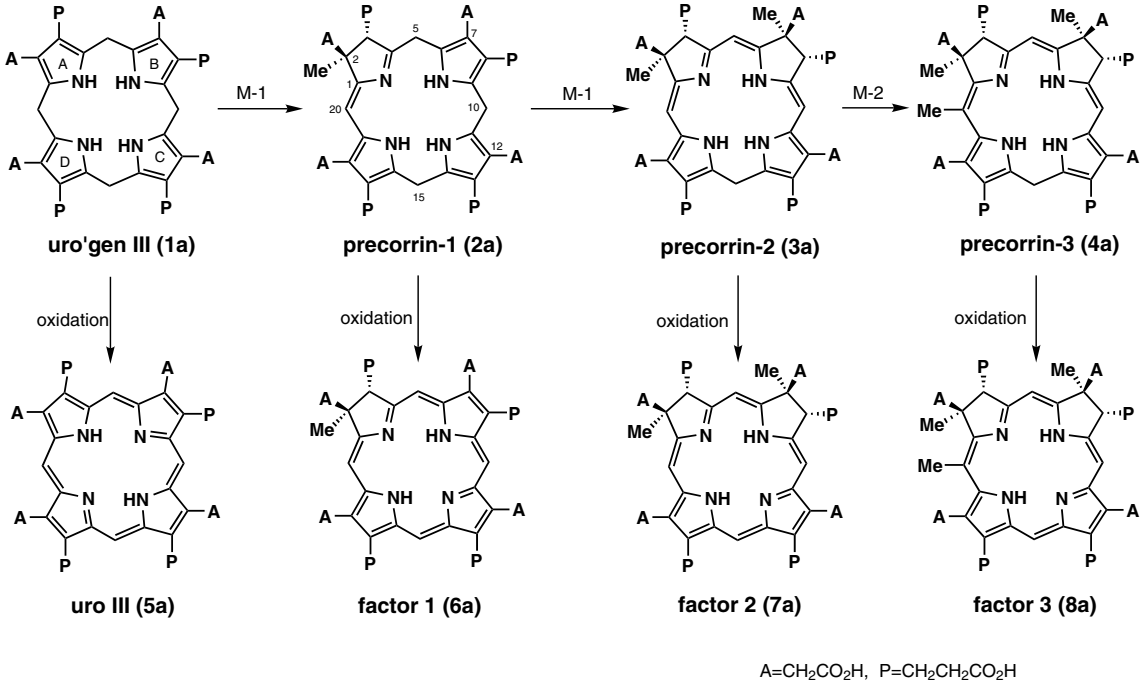
2. Porphyrin syntheses

All the analogs tested in this study are minor variations on the nature and pattern of substitution present on the naturally occurring porphyrinogens uro'gen I and uro'gen III, which exhibit only acetate and propionate side chains (Scheme 2). Since porphyrinogens are oxidized rather easily upon air exposure, all these analogs are more conveniently prepared in their oxidized form (i.e., porphyrin) and reduced back to the porphyrinogen form just before the enzymatic incubation.

The series **5f**–**5h** (Scheme 2) corresponds to analogs of uroporphyrin (uro) I (**5b**), whose propionate substituents have been replaced by butyrate (**5f**), acetate (**5g**) or methyl groups (**5h**). Coproporphyrin (copro) I (**5i**) is the known naturally occurring product of decarboxyl-

Keywords: Vitamin B_{12}; Methyltransferase; Chlorin; Isobacteriochlorin.
* Corresponding author. Tel.: +1 979 845 3243; fax: +1 979 845 5992; e-mail: scott@mail.chem.tamu.edu

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.01.041



Scheme 1. First three methylations in the biosynthetic pathway of vitamin B_{12}.

ation of uro I, which has methyl groups in place of all four acetate side chains. This series of porphyrins was obtained by tetramerization of their parent 2-hydroxymethylpyrroles in buffer pH 7.8–8.0, followed by cyclization and oxidation, as described in a previous report.[19] The preponderance of the type I isomer was over 90% in all cases, based on NMR analysis.

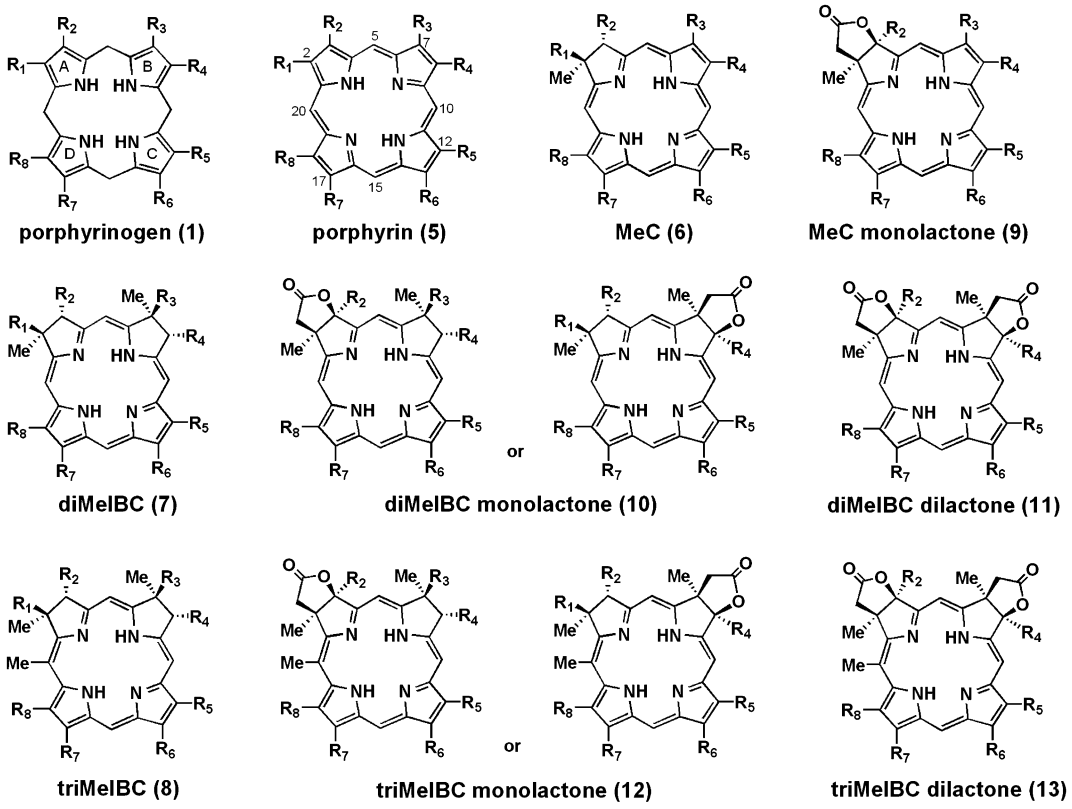
A second series of uro analogs **5j**–**5n** and **5e** (Scheme 2) retains the same acetate and propionate substitution pattern on the 'Northern part' of the molecule (rings A and B) as the natural substrate uro'gen III, but includes variations on the 'Southern part' (rings C and D). From the preparative point of view **5j**–**5l**, which have a symmetrical 'Southern part', were made using the MacDonald approach,[20,21] consisting of the condensation of a diformyldipyrromethane (**14**) with a dipyrromethane-dicarboxylic acid (**15**) (Scheme 3). Porphyrins **5e**, **5m**, and **5n**, which do not present symmetry in the 'Southern part,' had to be made by a different strategy. In order to limit the number of preparative steps by using the building blocks already available in our laboratory, a modification of the MacDonald method was devised for their synthesis, in which each of the dipyrroles carries one aldehyde and one carboxylic acid (Scheme 4); notice that although mixtures of products are obtained, these are easily separated thanks to the different chromatographic behaviors of the alternative products bearing a different number of methyl ester side chains. The side-products, hexamethylester porphyrins **5o** and **5p**, were also included in our activity study.

All dipyrromethanes were prepared following published procedures.[22,23] The benzyl esters were hydrogenated in THF over 10% Pd–C in the presence of Et_3N and after work up, used as such for the coupling reactions (Schemes 2 and 3). The appropriate dipyrromethanes, **14** and **15**, for porphyrins **5j**, **5k**, and **5l** (Scheme 2), **16**, and **17** for porphyrins **5e**, **5m** and **5n** (Scheme 3), were dissolved in CH_2Cl_2 + 10% MeOH and treated with *p*-toluenesulfonic acid under an inert atmosphere in the dark for one to two days. After neutralization with sodium bicarbonate, the coupling products were fully oxidized under an oxygen atmosphere to porphyrins and isolated by preparative TLC, eluting twice with CH_2Cl_2 + 2% MeOH. Their R_F's decrease with the number of carboxylate β-substituents, in the order hexa → hepta → octa-methyl esters. The yields of the target porphyrins vary from 15% to 31%, thus making them comparable with those reported using other methods. Variation in the ratio of dipyrroles utilized (2:1 to 1:2) did not improve the yields of the desired porphyrins and a 1:1 ratio was routinely used.

3. Enzymatic study results

The methyltransferase source chosen for the experiments was CR395, a recombinant strain of *Escherichia coli*, in which two vitamin B_{12} enzymes CobA (M-1) and CobI (M-2) from *P. denitrificans* are overexpressed.[24] The enzymes were used as a clear cell lysate without further purification except for removal of endogenous porphyrinoids.

The methyl esters of the porphyrin analogs were hydrolyzed with 2 N KOH. The pH of the solution was reduced to 8–9 and the free-acid porphyrins were reduced back to porphyrinogens by addition of small pieces of 3% sodium amalgam to the solution until colorless (RT, 2 h). To avoid any oxidation, the reduction, enzymatic incubation, work up, and esterification were all performed under



MeC= monomethylated chlorin
A=CH_2CO_2H, P=$CH_2CH_2CO_2H$, B=$CH_2CH_2CH_2CO_2H$

diMeIBC=dimethylated isobacteriochlorin
triMeIBC= trimethylated isobacteriochlorin

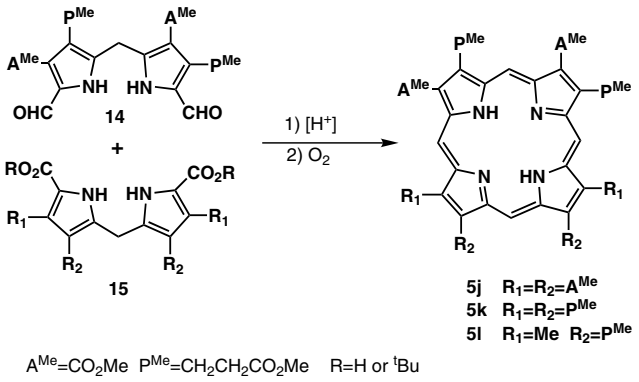
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
a	A	P	A	P	A	P	P	A
b	A	P	A	P	A	P	*A*	*P*
c	A	P	*P*	*A*	A	P	P	A
d	A	P	A	P	*P*	*A*	P	A
e	A	P	A	P	*Me*	P	P	A
f	A	*B*	A	*B*	A	*B*	*A*	*B*
g	A	*A*	A	*A*	A	*A*	*A*	A
h	A	*Me*	A	*Me*	A	*Me*	*A*	*Me*
i	*Me*	P	*Me*	P	*Me*	P	*Me*	*P*
j	A	P	A	P	A	*A*	*A*	A
k	A	P	A	P	*P*	P	P	*P*
l	A	P	A	P	*Me*	P	P	*Me*
m	A	P	A	P	A	P	P	*Me*
n	A	P	A	P	A	P	*Me*	*P*
o	A	P	*P*	*Me*	A	P	P	*Me*
p	A	P	*Me*	P	A	P	*Me*	*P*

Scheme 2. Substrate analogs tested in this study and their enzymatic products.

argon atmosphere in a glove-box. The free-acid porphyrinogen solutions were filtered into a degassed Tris-buffer solution, adjusted to pH 7.5 containing CobA and CobI lysate, and SAM, and incubated at 37 °C under argon overnight. The products were isolated by adsorption on DEAE–Sephadex resin, elution and esterification with MeOH + 5% H_2SO_4, neutralization, extraction into CH_2Cl_2, and chromatography on preparative TLC (benzene/ethyl acetate/MeOH, 70:27:3).

The results are shown in Table 1 and correspond to the isolated yields of the products. Instability to oxidation of the enzyme substrates and products makes it very difficult to perform kinetic studies. Since the main objective of this work was to explore the possible use of these enzymes for synthesis of new unnatural chlorins and isobacteriochlorins, more emphasis was put in their isolation and characterization.

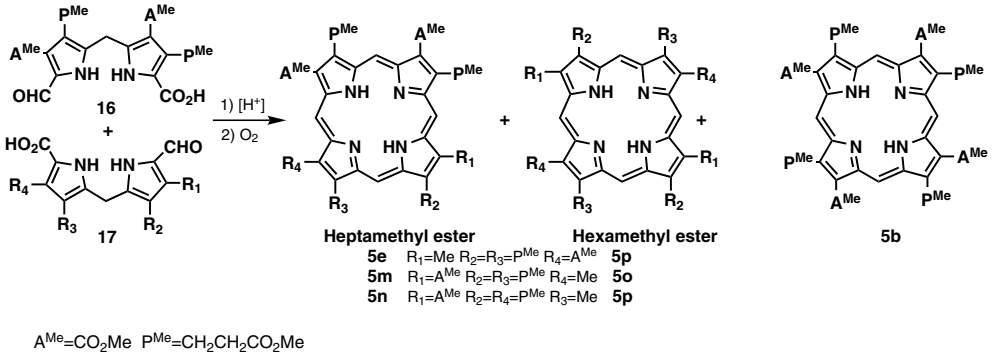
All products were characterized by ^{1}H NMR, UV–vis, and mass spectrometry, and in a few cases by ^{13}C NMR. Some of the products isolated are known (**7a**, **7b**, **7d**, **8a**, **8e**, **10a**, **11a**, **12a** and **13a**) and our NMR data and assignments are in agreement with the published re-



Scheme 3. Synthesis of porphyrins by the MacDonald method.

sults.[6,12,14,16,25,26] Their isolation confirms that the general protocol for enzymatic incubation, and product isolation and purification were adequate. The nature and methylation level of the other products are based on their UV–vis, mass, and NMR data. Their structures are derived by correlation with the NMR of the known compounds, using the sp^2 or sp^3 nature of individual carbons revealed by their NMR as guidance to determine the sites of enzymatic methylation. Often, isomers were also observed by ^{1}H NMR and in most cases, only a partial assignment was possible, since the products could not be separated from the isomeric mixture and/or were present in too small a quantity. Based on their identical mass data and slight differences in their NMR data, they are assumed to be epimerization products at C-3 or C-8, like those described for factor 1 **6a** and factor 2 **7a**.[3,27] The meso protons and methyl ^{1}H NMR assignments of all products are reported in Table 2.

As seen in Table 1, some of the porphyrinogens acted solely as substrates of the first methyltransferase (**1b**, **1d**, **1f**, **1j**, and **1n**); apart from the natural substrate **1a**, only two porphyrinogens (**1e** and **1m**) served as substrates of the two methyltransferases and for most (**1c**, **1g**, **1h**, **1i**, **1k**, **1l**, **1o**, and **1p**), no methylation products could be detected. Although cell lysates expressing simultaneously CobA and CobI were used instead of pure proteins, control experiments with the natural substrate uro'gen III (**1a**) show virtually complete conversion to factor 3 (**8a**) and lactones (**12a** and **13a**), with no factor 1 (**6a**) or factor 2 (**10a**) or respective lactone being observed, which means that both enzymes were sufficiently well expressed and active.[24] Therefore, it can be safely concluded that the absence of product formation with some substrates cannot be attributed to the lack of expression or activity of any of the enzymes, but to other factors. The results of this study indicate that, although a majority of the analogs tested were not methylated by CobA or CobI, these enzymes display a greater tolerance than the earlier enzymes on the vitamin B_{12} biosynthetic pathway, such as PBG deaminase and uro'gen III synthase.[19]



Scheme 4. Synthesis of porphyrins by a modified MacDonald method.

Table 1. Results of porphyrinogen incubations with CobA and CobI

Substrate[a]	Isolated products and yields (%)							
	MeC **6**[b]	MeC monolactone **9**[b]	DiMeIBC **7**[b]	DiMeIBC monolactone **10**[b]	DiMeIBC dilactone **11**[b]	TriMeIBC **8**[b]	TriMeIBC monolactone **12**[b]	TriMeIBC dilactone **13**[b]
1a	—	—	30–36%[c]	4%[c]	2%[c]	22%	8%	6%
1b	—	—	3–5%	4–5%	<1%	—	—	—
1d	19%	7%	1%	Traces[d]	—	Traces[d]	Traces[d]	Traces[d]
1e	2%	—	2%	—	—	28%	9%	—
1f	1–3%	—	—	—	—	—	—	—
1j	5%	—	13%	9%	5%	—	—	—
1m	2–3%	—	—	—	—	8–11%	1%	1%
1n	10%	—	2%	1%	1%	—	—	—

[a] Other porphyrinogens **1c**, **1g**, **1h**, **1i**, **1k**, **1l**, **1o**, and **1p** were tested, but no product of methylation was detected.
[b] In most cases, epimeric forms are present and were observed by ^{1}H NMR (500 MHz) (Table 2).
[c] Incubation run with CobA (M-1) only, instead of CobA (M-1) and CobI (M-2).
[d] Products were isolated in very small quantities and were not confirmed to be derived from uro'gen IV.

Table 2. Characteristic ^{1}H NMR assignments (δ in C_6D_6, 500 MHz) for methylation products

Products	H-15	H-10	H-20	H-5	Me-20	Me(C#)	Me-2	Me-7	Ref.[a]
MeC									
6d	10.09	10.09	9.16	8.97			2.22		3 and 4
	10.08	10.08	9.08	8.92			1.85		
6e	10.09	9.91	9.18	9.00		3.11 (12)	2.24		
6f	10.29	10.19	9.19	9.06			2.28		
6j	10.10	10.04	9.14	8.86			2.20		
6m	10.10	10.00	9.16	8.90		3.10 (18)	2.25		
6n	10.08	9.89	9.17	9.00		3.11 (17)	2.25		
	10.08	9.89	9.10	8.95		3.20 (17)	1.85		
MeC monolactone									
9d	10.21	10.19	9.35	8.80			1.72		
DiMeIBC									
7a	8.85	7.64	7.54	6.78			1.75	1.60	6
	8.82	7.52	7.41	6.68			1.84	1.44	
	8.83	7.65	7.53	6.71			1.59	1.53	
7b	8.84	7.65	7.48	6.80			1.68	1.61	12
	8.80	7.55	7.47	6.71			1.79	1.45	
7d	8.85	7.61	7.55	6.81			1.74	1.63	14
7e	8.83	7.55	7.43	6.72		2.63 (12)	1.85	1.49	
7j	8.82	7.59	7.48	6.76			1.71	1.60	
	8.80	7.61	7.35	6.71			1.59	1.52	
	8.78	7.50	7.47	6.68			1.80	1.45	
7n	8.71	7.69	7.53	6.83		2.78 (17)	1.75	1.65	
DiMeIBC monolactone									
10a	8.96	8.06	7.55	6.71			1.75	1.21	25
	8.98	7.68	7.53	6.97			1.44	1.37	
10b	8.95	8.08	7.48	6.64			1.67	1.22	
	8.94	7.70	7.55	7.02			1.45	1.35	
10j	8.90	7.61	7.45	6.92			1.43	1.34	
	8.86	7.97	7.39	6.56			1.51	1.20	
	8.88	7.99	7.48	6.59			1.71	1.22	
10n	8.85	7.77	7.61	6.99		2.81 (17)	1.50	1.41	
	8.79	7.69	7.57	6.95		2.78 (17)	1.46	1.39	
DiMeIBC dilactone									
11a	9.28	8.25	7.75	7.08			1.42	1.31	26
11b	9.25	8.28	7.79	7.27			1.42	1.31	
11j	9.10	8.13	7.63	7.07			1.38	1.25	
11n	9.15	8.37	7.86	7.37		2.93 (17)	1.46	1.36	
TriMeIBC									
8a	8.73	7.50		6.51	2.77		1.66	1.40	6
	9.05	7.76		6.99	2.79		1.51	1.39	
8e	8.80	7.41		6.64	2.85	2.56 (12)	1.68	1.49	16
	8.72	7.41		6.50	2.78	2.53 (12)	1.85	1.43	
	8.74	7.74		6.56	2.65	2.52 (12)	1.67	1.49	
8m	8.81	7.41		6.64	2.85	2.56 (18)	1.68	1.49	
	8.95	7.79		6.58	2.83	2.47 (18)	1.71	1.26	
	8.85	7.61		6.67	2.84	2.65 (18)	1.75	1.46	
TriMeIBC monolactone									
12a	8.85	7.88		6.40	2.73		1.67	1.16	
12e	8.95	7.79		6.58	2.83	2.47 (12)	1.71	1.26	
12m	8.97	8.00		6.60	2.73	2.64 (18)	1.78	1.21	
TriMeIBC dilactone									
13a	9.46	8.38		7.58	2.99		1.49	1.45	
13m	9.63	8.49		7.75	2.90	2.84 (18)	1.55	1.52	
	9.54	8.29		7.74	3.17	2.63 (18)	1.60	1.46	

[a] In agreement with values reported in the references cited.

4. Discussion

In the series of porphyrinogens which have previously demonstrated substrate activity with M-1 and M-2 (**1a** to **1e**), methylations for the natural substrate uro'gen III (**1a**) have been shown to take place sequentially at C-2, C-7, and C-20.[1–4,6] NMR data for the product of methylation of uro'gen I (**1b**) indicate that the sites of

methylation are also acetate termini on contiguous rings, consistent with the dimethylated isobacteriochlorin (diMeIBC) **7b**.[12] Uro'gen IV (**1d**) also gave a product of dimethylation at C-2 and C-7 **7d**,[14] however the main product was monomethylation at C-2 to form the methylchlorin (MeC) **6d**. For porphyrinogen **1e**, the 12-methyl analog of the natural substrate **1a**, the complete series of mono-, di- and trimethylated products (**6e**, **7e** and **8e**) was isolated, with the trimethylated isobacteriochlorin (triMeIBC) **8e**[16] being the major product, and follows the same methylation sequence as for the natural substrate. The spectroscopic data of all additional products isolated from incubations of **1a**, **1b**, **1d**, and **1e** with CobA and CobI are consistent with products of epimerization at C-3 or C-8,[3,27] or lactonization on ring A and/or ring B.[25,26]

In earlier work with the *E. coli* M-1 CysG, a dimethylation product was isolated from incubation with uro'gen II (**1c**).[14] The NMR data of this methylation product pointed to the second methylation occurring at C-7 on a propionate terminus. However, in our studies, incubations of **1c** with CobA and CobI did not give any methylated product and no evidence of methylation at a propionate terminus with any of the porphyrinogens tested was found.

By comparison with the data discussed above, the spectroscopic data for the products of the four new porphyrinogen, tested in our study (**1f**, **1j**, **1m**, and **1n**) indicate a similar methylation site pattern: C-2 > C-7 > C-20. Although it could be ascertained that the only methylation product from **1f** is monomethylated at an acetate terminus (**6f**), the presence of isomers in the starting porphyrinogen **1f** combined with the very low yield of methylation does not allow determination of its 'isomeric type' (i.e., I–IV). In case of porphyrinogen **1j**, which has the same 'Northern part' as the natural product **1a**, but only acetate substituents on the 'Southern part,' it seems likely that methylations also occur at C-2 and C-7, adjacent to propionate termini on the 'Northern part,' since porphyrinogen **1g** bearing only acetate substituents is not a substrate of these methylases. Finally, for porphyrinogens **1m** (18-methyl analog of uro'gen III **1a**) and **1n** (17-methyl analog of uro'gen I **1b**), NMR data show that the carbon carrying the methyl group originally present remains sp^2 in the enzymatic products, showing that methylations, once again, had taken place on the 'Northern' rings with the natural acetate/propionate substitution pattern. However, the precise position of the methyl group originally present has not been assigned and could be either at C-13 or C-18 for the products derived from **1m** and either C-12 or C-17 for the products derived from **1n**. Products of epimerization on ring A and/or ring B were also observed with all four porphyrinogens.

From this study, some electronic factors governing the methyltransferase CobA and CobI mechanism could be discerned. The first methylation on ring A always happens at C-2 with an acetate terminus next to a propionate substituent at C-3, except for **1f**, which bears a butyrate group at C-3, but for which only very small quantities of MeC **6f** were isolated (Table 1). No methylation was ever observed at a methyl or propionate terminus, nor if C-3 was substituted by an acetate or methyl group. In turn, the second methylation on the adjacent ring B always occurs at C-7 with an acetate terminus next to a propionate terminus at C-8. Only three porphyrinogens acted as substrates of M-2 for the third methylation at C-20: uro'gen III (**1a**), the natural substrate, and porphyrinogens **1e** and **1m**, analogs of **1a** with a methyl replacing the acetate group on ring C for **1e** and ring D for **1m**. From these results, it seems that the sequence APAPXPPY (A, acetate; P, propionate) with X and Y being A or Me would be necessary in order to have methylation at C-20.

However, these structural requirements do not explain all our results. For instance, porphyrinogens **1d** and **1n** possess the substitution sequence APAP on their 'Northern part' and thus, dimethylation was expected, but although the dimethylated products **7d** and **7n** were isolated, the main products in both cases were the monomethylated chlorins **6d** and **6n** (Table 1). Similarly, monomethylation could have been anticipated for the porphyrinogens **1c**, **1k**, **1l**, **1o**, and **1p**, which all have a ring bearing the natural AP substitution pattern, but no product of methylation was detected. It should be mentioned that we had in our hands limited amounts of **1c** and **1l**, so incubations with these two porphyrinogens could not be repeated. As for **1o** and **1p**, solubility problems with their methyl esters may have led to incomplete ester hydrolysis and the subsequent porphyrin to porphyrinogen reductions. Steric hindrance for fitting in the catalytic site of M-1 could explain the lack of reactivity of **1k**. Thus, it seems that the structural requirements outlined above would constitute minimum conditions to be met for substrate activity with the methyltransferases CobA and Cob I.

In addition, it is interesting to note that porphyrinogen **1e** (the 12-methyl analog of uro'gen III) acts as a substrate of CobA and CobI as efficiently as the natural substrate uro'gen III (**1a**) (Table 1). Thus, the trimethylated dihydroisobacteriochlorin (triMediHIBC) **4e** becomes a logical candidate for further methylations. However, prior experiments with ^{14}C-enriched **1e** gave very low incorporation into cobester,[28–30] the stable cobalt containing intermediate of vitamin B_{12} obtained from uro'gen III after eight methylations, one decarboxylation at the C-12 acetate, and one methyl migration from C-11 to C-12. This would imply that **1e** could only be taken up to a certain point along the enzymatic pathway of vitamin B_{12}. The block in the pathway could be at the fifth methylation at C-11 or at the methyl shift from C-11 to C-12, which takes place after the final methylation, as these are the only two steps in the pathway which involve ring C.[1]

5. Conclusion

A number of porphyrinogens have been tested for substrate specificity with the first two methyltransferases

of the vitamin B_{12} biosynthetic pathway. Eight of them gave methylation products, which have been isolated and characterized, and showed to follow the same methylation pattern C-2 > C-7 > C-20 as the natural substrate. This has allowed us to discern the substitution sequence APAPXPPX (with A, acetate; P, propionate; X and Y, A or methyl) as necessary for substrate recognition by the methyltransferase enzymes CobA and CobI. Furthermore, four of the porphyrinogens have never been tested before and through a semisynthetic approach yielded a series of novel chlorins and isobacteriochlorins, two important classes of compounds in the field of photodynamic therapy.

6. Experimental

6.1. Chemistry

6.1.1. General procedures. All reagents were purified and dried when necessary according to standard literature methods. All starting pyrroles come from a large collection of monopyrrole building blocks with acetate, propionate, butyrate, and methyl β-substituents previously made in our laboratory. All reactions were conducted in anhydrous solvents and under argon atmosphere, unless specified otherwise. Column chromatographies were carried out on E. Merk Kieselgel 60 (100–200 mesh), TLC, and preparative TLC (PLC) on Analtech silicagel GF plates.

^{1}H and ^{13}C NMR (500 MHz) were recorded on a Brucker AM-500 and the coupling constants expressed in MHz. Concerning the carbon signals of the porphyrin macrocycle, it should be noted that, as previously postulated by others, the relatively slow exchange on NMR timescale between tautomeric forms of the free-base porphyrin macrocycle leads to the observation of only one broad and weak signal for the α-carbons, often barely distinguishable from the baseline noise, and one broad but sharper signal at higher field for the β-carbons, even in the absence of complete symmetry in the molecule. It is also known that the tautomeric mobility is increased by electron-withdrawing substituents. Thus, observation of the signals for the α- and β-carbons of the porphyrin macrocycle varies with the substituents and degree of symmetry.[31]

UV spectra were obtained on an Ocean Optics USB2000 Miniature Fiber Optic Spectrophotometer and mass spectra on a PE SCIEX QSTAR quadrupole time-of-flight hybrid mass spectrometer using an electrospray ionization source.

6.1.2. Uro III (5a), uro I (5b), uro II (5c), and uro IV (5d). Uro III (**5a**) was obtained by incubation of PBG with PBG deaminase and uro'gen III synthase, and uro I (**5a**) by incubation with PBG deaminase as described in Ref. 19. Uro II (**5c**) and uro IV (**5d**) were made for previous work in our group.[14]

6.1.2.1. Uro III (5a). ^{1}H NMR ($CDCl_3$): δ 10.04, 9.97, 9.95, 9.94 (4s, 4H, 4 meso H); 5.06, 5.04, 5.00, 4.95 (4s, 8H, 4 C$H_2$$CO_2$Me); 4.31 (m, 8H, 4 CH_2C$H_2$$CO_2$Me); 3.83, 3.82, 3.79, 3.78, 3.71, 3.69 (6s, 24H, 8 CO_2CH_3); 3.31 (m, 8H, 4 C$H_2$$CH_2$$CO_2$Me). ^{13}C NMR δ 173.44, 171.81, 8CO_2Me; 140.91, 132.79, 16 C porphyrinic; 97.85, 97.75, 97.69, 97.59, 4 meso C; 52.40, 51.73, 8 $CO_2$$CH_3$; 37.01, 36.90, 4 $CH_2$$CH_2$$CO_2$Me; 32.36, 4 $CH_2$$CO_2$Me; 21.72, 21.68, 21.60, 21.51, 4 $CH_2$$CH_2$$CO_2$Me.

6.1.2.2. Uro I (5b). ^{1}H NMR ($CDCl_3$): δ 10.7 (s, 4H, 4 meso H); 5.06 (s, 8H, 4 C$H_2$$CO_2$Me); 4.38 (t, J = 7.8, 8H, 4 CH_2C$H_2$$CO_2$Me); 3.77, 3.68, (2s, 24H, 8 CO_2CH_3); 3.33 (t, J = 7.8, 8H, 4 C$H_2$$CH_2$$CO_2$Me). ^{13}C NMR δ 173.44, 171.83, 8 CO_2Me; 141.06, 132.96, 16 C porphyrinic; 52.42, 51.75, 8 $CO_2$$CH_3$; 37.05, 4 $CH_2$$CH_2$$CO_2$Me; 32.49, 4 $CH_2$$CO_2$Me; 21.76, 4 $CH_2$$CH_2$$CO_2$Me.

6.1.2.3. Uro II (5c). ^{1}H NMR ($CDCl_3$): δ 10.16, 10.14 (2s, 4H, 4 meso H); 5.10 (s, 8H, 4 C$H_2$$CO_2$Me); 4.43 (t, J = 7.3, 8H, 4 CH_2C$H_2$$CO_2$Me); 3.79, 3.66 (2s, 24H, 8 CO_2CH_3); 3.33 (t, J = 7.3, 8H, 4 C$H_2$$CH_2$$CO_2$Me). ^{13}C NMR δ 173.48, 171.82, 8 CO_2Me; 141.10, 133.20, 16 C porphyrinic; 98.12, 97.82, 4 meso C; 52.48, 51.78, 8 $CO_2$$CH_3$; 36.99, 4 $CH_2$$CH_2$$CO_2$Me; 32.55, 4 $CH_2$$CO_2$Me; 21.74, 4 $CH_2$$CH_2$$CO_2$Me.

6.1.2.4. Uro IV (5d). ^{1}H NMR ($CDCl_3$): δ 10.14 (s, 4H, 4 meso H); 5.10, 5.08 (2s, 8H, 4 C$H_2$$CO_2$Me); 4.42 (t, J = 7.7, 8H, 4 CH_2C$H_2$$CO_2$Me); 3.78, 3.77, 3.68, 3.66 (4s, 24H, 8 CO_2CH_3); 3.35, 3.32 (AB, J = 8.0, 8H, 4 C$H_2$$CH_2$$CO_2$Me). ^{13}C NMR δ 173.50, 173.46, 171.85, 8 CO_2Me; 141.12, 133.13, 16 C porphyrinic; 97.96, 4 meso C; 52.48, 51.79, 8 $CO_2$$CH_3$; 37.10, 37.01, 4 $CH_2$$CH_2$$CO_2$Me; 32.77, 32.51, 4 $CH_2$$CO_2$Me; 21.86, 21.72, 4 $CH_2$$CH_2$$CO_2$Me.

6.1.3. Preparation of the symmetrical porphyrins 5f, 5g, 5h, and 5i. The syntheses of the α-free formylpyrrole precursors of porphyrins **5f**, **5g**, **5h**, and **5i** were performed following standard procedures.[32]

6.1.3.1. 2-Formyl-3-methoxycarbonylmethyl-4-(3-methoxycarbonylpropyl)-pyrrole (precursor of 5f). ^{1}H NMR ($CDCl_3$): δ 10.24 (s br, 1H, NH); 9.55 (s, 1H, CHO); 6.87 (d, J = 2.9, 1H, H-5); 3.70 (s, 2H, C$H_2$$CO_2$Me); 3.64, 3.61 (2s, 6H, 2 CO_2CH_3); 2.44 (t, J = 7.6, 2H, $CH_2$$CH_2CH_2$$CO_2$Me); 2.30 (t, J = 7.4, 2H, C$H_2$$CH_2$$CH_2$$CO_2$Me); 1.82 (m, 2H, CH_2C$H_2$$CH_2$$CO_2$Me). ^{13}C NMR δ 178.03, CHO; 173.79, 171.18, 2 CO_2Me; 129.89, 125.67, 125.23, 124.56, 4 C pyrrolic; 52.16, 51.43, 2 $CO_2$$CH_3$; 32.25, $CH_2$$CH_2CH_2$$CO_2$Me; 29.43, $CH_2$$CO_2$Me; 25.24, CH_2C$H_2$$CH_2$$CO_2$Me; 23.66, $CH_2$$CH_2$$CH_2$$CO_2$Me.

6.1.3.2. 2-Formyl-3,4-dimethoxycarbonylmethyl-pyrrole (precursor of 5g). ^{1}H NMR ($CDCl_3$): δ 10.46, (s br, 1H, NH); 9.52 (s, 1H, CHO); 6.95 (d, J = 3.0, 1H, H-5); 3.73 (s, 2H, C$H_2$$CO_2$Me at C-3); 3.60 (s, 6H, 2 CO_2CH_3); 3.45 (s, 2H, C$H_2$$CO_2$Me at C-4). ^{13}C NMR δ 178.13, CHO; 171.69, 170.90, 2 $CO_2$$CH_3$; 129.82, 125.88 (2), 117.82, 4 C pyrrolic; 52.03, 51.79, 2 $CO_2$$CH_3$; 30.20, 29.31, 2 $CH_2$$CO_2$Me.

6.1.3.3. 2-Formyl-4-methyl-3-methoxycarbonylmethyl-pyrrole (precursor of 5h). ^{1}H NMR ($CDCl_3$): δ 10.52 (s br, 1H, N*H*); 9.51 (s, 1H, C*H*O); 6.82 (d, $J = 2.7$, 1H, H-5); 3.69 (s, 2H, CH_2CO_2Me); 3.62 (s, 3H, CO_2CH_3); 1.98 (s, 3H, CH_3). ^{13}C NMR δ 177.81, *C*HO; 171.16, *C*O_2Me; 129.63, 126.34, 125.41, 121.17, 4 C pyrrolic; 51.97, CO_2CH_3; 29.38, *C*H_2CO_2Me; 9.25, *C*H_3.

6.1.3.4. 2-Formyl-4-methyl-3-(2-methoxycarbonylethyl)-pyrrole (precursor of copro 5i). ^{1}H NMR ($CDCl_3$): δ 10.41 (s br, 1 H, N*H*); 9.51 (s, 1 H, C*H*O); 6.83 (d, $J = 2.8$, 1H, H-5); 3.59 (s, 3H, CO_2CH_3); 2.98 (t, $J = 7.7$, 2H, CH_2CO_2Me); 2.51 (t, $J = 7.7$, 2H, C$H_2CH_2CO_2$Me); 1.97 (s, 3H, CH_3). ^{13}C NMR δ 177.64, *C*HO; 172.83, *C*O_2Me; 133.37, 129.12, 125.74, 120.21, 4 C pyrrolic; 51.48, CO_2CH_3; 35.22, *C*H_2CO_2Me; 18.92, *C*$H_2CH_2CO_2$Me; 9.33, *C*H_3.

The symmetrical porphyrins were obtained by tetramerization of their parent monopyrroles in buffer as described in Ref. 19.

6.1.3.5. Porphyrin 5f (main isomer). ^{1}H NMR ($CDCl_3$): δ 10.27 (s, 4H, 4 meso H); 5.12 (s, 8H, 4 CH_2CO_2Me); 4.16 (t, $J = 7.5$, 8H, 4 $CH_2CH_2CH_2CO_2$Me); 3.73, 3.72 (2s, 24H, 8 CO_2CH_3); 2.74 (t, $J = 6.7$, 8H, 4 C$H_2CH_2CH_2CO_2$Me); 2.66 (m, 8H, 4 $CH_2CH_2CH_2CO_2$Me). ^{13}C NMR δ 174.04, 172.01, 8 *C*O_2Me; 142.09, 132.68, 16 C porphyrinic; 98.00, 4 *C*H meso; 52.38, 51.57, 8 CO_2CH_3; 33.72, 4 $CH_2CH_2CH_2CO_2$Me; 32.54, 4 *C*H_2CO_2Me; 28.12, 4 $CH_2CH_2CH_2CO_2$Me; 25.74, 4 *C*$H_2CH_2CH_2CO_2$Me. MS (ESI) *m*/*z* 999 $[M+H]^+$. HRMS (ESI) $[C_{52}H_{63}N_4O_{16}]^+$, calcd: 999.4239. Found: 999.4217.

6.1.3.6. Porphyrin 5g. ^{1}H NMR ($CDCl_3$): δ 10.86 (s, 4H, 4 meso H); 5.14 (s, 16H, 8 CH_2CO_2Me); 3.66 (s, 24 H, 8 CO_2CH_3). ^{13}C NMR δ 169.75, 8 *C*O_2Me; 142.05, 135.12, 16 C porphyrinic; 101.15, 4 *C*H meso; 52.98, 8 CO_2CH_3; 32.78, 8 *C*H_2CO_2Me. MS (ESI) *m*/*z* 887 $[M+H]^+$.

6.1.3.7. Porphyrin 5h (main isomer). ^{1}H NMR ($CDCl_3$): δ 10.13 (s, 4H, 4 meso H); 5.06 (s, 8H, 4 CH_2CO_2Me); 3.75 (s, 12H, 4 CO_2CH_3); 3.66 (s, 12H, 4 CH_3). ^{13}C NMR δ 174.23, 4 *C*O_2Me; 140.26, 136.12, 16 C porphyrinic; 98.12, 4 *C*H meso; 52.00, 4 CO_2CH_3; 29.77, 4 *C*H_2CO_2Me; 11.83, 4 *C*H_3. MS (ESI) *m*/*z* 655 $[M+H]^+$.

6.1.3.8. Copro (5i) (main isomer). ^{1}H NMR ($CDCl_3$): δ 10.06 (s, 4H, 4 meso H); 4.40 (t, $J = 7.8$, 8H, 4 CH_2CO_2Me); 3.67 (s, 12H, 4 CO_2CH_3); 3.64 (s, 12H, 4 CH_3); 3.26 (t, $J = 7.8$, 8H, 4 C$H_2CH_2CO_2$Me). ^{13}C NMR δ 173.58, 4 *C*O_2CH_3; 138.37, 136.67, 16 C porphyrinic; 96.59, 4 *C*H meso; 51.78, 4 CO_2CH_3; 37.01, 4 *C*H_2CO_2Me; 21.89, 4 *C*$H_2CH_2CO_2$Me; 11.75, 4 *C*H_3. MS (ESI) *m*/*z* 711 $[M+H]^+$.

6.1.4. Preparation of porphyrins 5j, 5k and 5l

6.1.4.1. 5,5′-Diformyl-3′,4-di-(2-methoxycarbonylethyl)-3,4′-dimethoxycarbonylmethyl-2,2′-methylenedi-pyrrole (14). The benzyl-5′-tertbutyl-3′,4-di-(2-methoxycarbonylethyl)-3,4′-dimethoxycarbonylmethyl-2,2′-methylenedipyrrole-5-carboxylate[32] (1.07 g, 1.54 mmol) was dissolved in THF (25 mL) and hydrogenated over 10% Pd–C (100 mg) at RT overnight. The solution was filtered through Celite, the catalyst washed with CH_2Cl_2, and the filtrate evaporated to dryness. The 5′-tertbutyl-3′,4-di-(2-methoxycarbonylethyl)-3,4′-di-methoxycarbonylmethyl-2,2′-methylenedipyrrole-5-carboxylic acid (915 mg, 1.51 mmol, 98%) was used without further purification in the next step. ^{1}H NMR ($CDCl_3$): δ 10.74, 10.49 (2s, 2H, 2N*H*); 4.07 (s, 2H, CH_2 meso) 3.91 (s, 2H, CH_2CO_2Me); 3.72, 3.68, 3.64, 3.60 (4s, 12H, 4 CO_2CH_3); 3.50 (s, 2H, CH_2CO_2Me); 2.98, 2.76 (2t, $J = 7.6$, 6.8, 4H, 2 $CH_2CH_2CO_2$Me); 2.54, 2.47 (2t, $J = 7.6$, 6.8, 4H, 2 C$H_2CH_2CO_2$Me); 1.47 (s, 9H, $CO_2C(CH_3)_3$). ^{13}C NMR δ 173.92, 173.70, 172.88, 172.04, 4 *C*O_2Me; 165.18, *C*O_2CMe_3; 161.47, *C*O_2H; 133.11, 131.28, 130.22, 121.41, 120.53, 119.85, 117.54, 114.54, 8 C pyrrolic; 81.49, *C*Me_3; 52.25, 51.74, 51.38, 4 CO_2CH_3; 34.75, 34.57, 2 $CH_2CH_2CO_2$Me; 30.84, 29.37, 2 *C*H_2CO_2Me; 28.21, C(CH_3)$_3$; 22.29, *C*H_2 meso; 20.36, 18.89, 2 *C*$H_2CH_2CO_2$Me.

The product prepared above was redissolved in TFA (10 mL) and stirred at RT for 4 h. The solution was cooled in ice, trimethylorthoformate (5 mL) was added dropwise, and the reaction was stirred for another 3 h at RT. Ethyl acetate (30 mL) was poured into the reaction and the solution was washed with H_2O (3× 30 mL), 10% $NaHCO_3$ (2× 25 mL), and $NaHCO_3$ until no longer acidic. After evaporation, the product **14** was purified by PLC eluting twice in ethyl acetate/hexanes (2/1) (203 mg, 0.39 mmol, 25%). ^{1}H NMR ($CDCl_3$): δ 10.58, 10.53 (2s br, 2H, 2 N*H*); 9.53, 9.51 (2s, 2H, 2 C*H*O); 4.00 (s, 2H, CH_2 meso); 3.75, 3.71, 3.66, 3.62 (4s, 12H, 4 CO_2CH_3); 3.61, 3.53 (2s, 4H, 2 CH_2CO_2Me); 3.00, 2.78 (2t, $J = 7.6$, 6.8, 4H, 2 $CH_2CH_2CO_2$Me); 2.55 (m, 4H, 2 C$H_2CH_2CO_2$Me). ^{13}C NMR δ 177.64, 177.44, 2 *C*HO; 174.29, 172.95, 172.75, 171.18, 4 *C*O_2Me; 135.45, 134.46, 133.64, 129.39, 128.72, 125.74, 121.02, 8 C pyrrolic; 52.70, 52.31, 52.20, 51.70, 4 CO_2CH_3; 35.86, 34.17, 2 $CH_2CH_2CO_2$Me; 29.92, 29.40, 2 *C*H_2CO_2Me; 22.48, *C*H_2 meso; 19.03, 18.50, 2 *C*$H_2CH_2CO_2$Me.

6.1.4.2. 3,3′,4,4′-Tetramethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylic acid (15j). A solution of the benzyl-2-methyl-3,4-dimethoxycarbonylmethyl-pyrrole-5-carboxylate (1.08 g, 3.0 mL), prepared following standard procedures,[33–35] was dissolved in AcOH/Ac_2O (16 mL/4 mL) and treated with $Pb(OAc)_4$ (2.0 g, 4.6 mmol) at 80 °C for 4 h, then at RT overnight. Ethylene glycol (1 mL) was added to react with the excess of reagent and after 30 min, the reaction mixture was filtered and partitioned between H_2O and CH_2Cl_2. The organic phase was evaporated to dryness by azeotropic distillation with heptane (3× 20 mL). The pale yellow 2-acetoxymethylpyrrole was recrystallized from CH_2Cl_2/hexanes (1.26 g, 3 mmol, 100%). ^{1}H NMR ($CDCl_3$): δ 9.42 (s br, 1H, N*H*); 7.38–7.24 (m, 5H, Ph*H*); 5.26 (s, 2H, CH_2Ph); 5.04 (s, 2H, CH_2O-COCH$_3$); 3.84 (s, 2H, CH_2CO_2Me); 3.63, 3.57 (2s, 6H, 2 CO_2CH_3); 3.49 (s, 2H, CH_2CO_2Me); 2.03 (s, 3H, OCOCH_3). ^{13}C NMR δ 171.48, 171.38, 2 *C*O_2Me and

OCOCH$_3$; 160.42, CO_2CH_2Ph; 135.80, 122.95, 120.01, 117.52, 4 C pyrrolic; 128.88, 128.51, 128.28, 4 C benzylic; 66.11, CH_2Ph; 56.66, CH_2OAc; 52.04, 51.82, 2 CO_2CH_3; 30.45, 29.69, 2 CH_2CO_2Me; 20.77, OCOCH$_3$.

The 2-acetoxymethylpyrrole obtained above was redissolved in CH_2Cl_2 (5 mL) and stirred over Montmorillonite clay (2 g) at RT for 4 days. The solution was filtered through Celite and the catalyst washed with CH_2Cl_2 + 5% MeOH (50 mL). The dibenzyl-3,3′,4,4′-tetramethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylate was isolated by flash chromatography (ethyl acetate/hexanes, 1:1) (819 mg, 1.17 mmol, 78%). ^{1}H NMR ($CDCl_3$): δ 10.44 (s, 2H, 2 N*H*); 7.37–7.24 (m, 10H, 2 Ph*H*); 5.20 (s, 4H, 2 CH_2Ph); 3.86 (s, 4H, 2 CH_2CO_2Me); 3.82 (s, 2H, CH_2 meso); 3.56, 3.54 (2s, 12H, 4 CO_2CH_3); 3.49 (s, 4H, 2 CH_2CO_2Me). ^{13}C NMR δ 173.27, 171.47, 4 CO_2CH_3; 160.20, 2 CO_2CH_2Ph; 135.95, 122.69, 118.90, 114.31, 8 C pyrrolic; 131.89, 128.33, 128.09, 127.75, 8 C benzylic; 65.48, 2 CH_2Ph; 52.21, 51.60, 4 CO_2CH_3; 30.21, 29.45, 4 CH_2CO_2Me; 22.13, CH_2 meso.

Part of the dipyrromethane obtained above (126 mg, 0.18 mmol) was redissolved in THF/MeOH (1/1, 10 mL) and hydrogenated over 10% Pd–C (15 mg) at RT overnight. The solution was filtered and the catalyst washed with warm THF (50 mL), MeOH (50 mL), and CH_2Cl_2 (50 mL) to afford **15j**. The compound was prepared immediately before the preparation of porphyrin **5j** and used without further purification.

6.1.4.3. Porphyrin 5j. Compounds **15j** and **14** (87 mg, 0.17 mmol) were dissolved in CH_2Cl_2/MeOH (20/2 mL). *p*-TsOH·H_2O (264 mg, 1.39 mmol) was added and the reaction stirred in the dark for 48 h. The solution was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$, dried, and evaporated. The residue was redissolved in MeOH, the solution was saturated with O_2 and stirred at RT for 24 h. The porphyrin **5j** was isolated by PLC eluting twice with CH_2Cl_2 + 2% MeOH (34 mg, 0.037 mmol, 22%). ^{1}H NMR ($CDCl_3$): δ 9.77, 9.61, 9.60, 9.47 (4s, 4H, 4 *H* meso); 4.90, 4.89, 4.85, 4.72, 4.66 (5s, 12H, 6 CH_2CO_2Me); 4.22, 4.05 (2t, J = 7.8, 7.8, 4H, 2 $CH_2CH_2CO_2$Me); 3.83, 3.82, 3.76, 3.75, 3.73, 3.71, 3.67, 3.66 (8s, 24H, 8 CO_2CH_3); 3.25, 3.14 (2t, J = 7.8, 7.8, 4H, 2 C$H_2CH_2CO_2$Me). ^{13}C NMR δ 173.38, 173.31, 171.79, 171.73, 171.40, 171.30, 171.26, 8 CO_2Me; 142 (m), 140.7 (br), 133.8 (br), 132.8 (br), 16 C porphyrinic; 98.07, 97.67, 97.24, 4 *C*H meso; 52.34, 51.72, 51.68, 8 CO_2CH_3; 36.89, 36.80, 2 $CH_2CH_2CO_2$Me; 32.20, 32.12, 31.98, 6 CH_2CO_2Me; 21.49, 21.26, 2 $CH_2CH_2CO_2$Me. MS (ESI) *m*/*z* 915 $[M+H]^+$. HRMS (ESI) $[C_{46}H_{51}N_4O_{16}]^+$, calcd: 915.3300. Found: 915.3307.

6.1.4.4. Ditertbutyl-3,3′,4,4′-tetra-(2-methoxycarbonylethyl)-2,2′-methylenedipyrrole-5,5′-dicarboxylate (15k). The compound **15k** was prepared as described for **15j** from the tertbutyl-2-methyl-3,4-di-(2-methoxycarbonylethyl)-pyrrole-5-carboxylate (706 mg, 2 mmol) in a yield of 50% over 2 steps (344 mg, 0.5 mmol). ^{1}H NMR ($CDCl_3$): δ 9.25 (s, 2H, 2 N*H*); 3.85 (s, 2H, CH_2 meso); 3.57, 3.55 (2s, 12H, 4 CO_2CH_3); 2.83, 2.64 (2t, J = 8.2, 7.4, 8H, 4 CH_2CO_2Me); 2.43, 2.30 (2t, J = 8.2, 7.4, 8H, 4 C$H_2CH_2CO_2$Me); 1.40 (s, 18H, 2 C(CH_3)$_3$). ^{13}C NMR δ 173.35, 173.31, 4 CO_2Me; 160.52, 2 $CO_2{}^t$Bu; 129.60, 127.94, 125.79, 119.27, 8 C pyrrolic; 80.42, 2 CMe_3; 51.43, 51.18, 4 CO_2CH_3; 35.21, 35.03, 4 CH_2CO_2Me; 28.09, 2 C(CH_3)$_3$; 22.47, CH_2 meso; 20.53, 18.84, 4 $CH_2CH_2CO_2$Me.

6.1.4.5. Porphyrin 5k. The dipyrromethane **15k** (46 mg, 0.067 mmol) was treated with TFA (500 μL) for 30 min at RT. A solution of the diformyldipyrromethane **14** (33 mg, 0.064 mmol) in CH_2Cl_2/MeOH (10/1 mL) was added, then *p*-TsOH·H_2O (97 mg, 0.51 mmol), and the reaction mixture was stirred in the dark for 48 h. The work up was conducted as for **5j**. Porphyrin **5k** was obtained in 15% yield (9 mg, 0.01 mmol). ^{1}H NMR ($CDCl_3$): δ 10.18, 10.16, 10.14, 10.13 (4s, 4H, 4 C*H* meso); 5.12 (s, 4H, 2 CH_2CO_2Me); 4.44 (t, J = 8.7, 12H, 6 $CH_2CH_2CO_2$Me); 3.77, 3.69, 3.68, 3.67, 3.60 (5s, 24H, 8 CO_2CH_3); 3.32 (m, 12H, 6 C$H_2CH_2CO_2$Me). ^{13}C NMR δ 173.45, 173.37, 171.87, 8 CO_2Me; 147 (m), 144 (m), 140.9 (br), 139.9 (br), 134 (m), 16 C porphyrinic; 97.95, 97.65, 97.32, 4 *C*H meso; 52.44, 51.79, 8 CO_2CH_3; 37.47, 37.13, 37.04, 6 $CH_2CH_2CO_2$Me; 32.58, 2 CH_2CO_2Me; 21.72, 21.64, 6 $CH_2CH_2CO_2$Me. MS (ESI) *m*/*z* 971 $[M+H]^+$. HRMS (ESI) $[C_{50}H_{59}N_4O_{16}]^+$, calcd: 971.3926. Found: 971.3909.

6.1.4.6. 4,4′-Dimethyl-3,3′-di-(2-methoxycarboxylethyl)-2,2′-methylenedipyrrole-5,5′-dicarboxylic acid (15l). The dibenzyl derivative of **15l** was prepared as described for **15j** from the benzyl-2-methyl-3,4-di-(2-methoxycarbonylethyl)-pyrrole-5-carboxylate (1.57 g, 5 mmol) in 55% yield over 2 steps (844 mg, 1.37 mmol). ^{1}H NMR ($CDCl_3$): δ 9.71 (s, 2H, 2 N*H*); 7.30–7.21 (m, 10H, 2 Ph*H*); 5.18 (s, 4H, 2 CH_2Ph); 3.92 (s, 2H, CH_2 meso); 3.57 (s, 6H, 2 CO_2CH_3); 2.73 (t, J = 7.3, 4H, 2 CH_2CO_2Me); 2.42 (t, J = 7.3, 4H, 2 C$H_2CH_2CO_2$Me); 2.26 (s, 6H, 2 CH_3). ^{13}C NMR δ 173.50, 2 CO_2Me; 161.40, 2 CO_2CH_2Ph; 136.18, 130.91, 120.01, 117.66, 8 C pyrrolic; 128.22, 127.72, 127.62, 127.10, 8 C benzylic; 65.36, 2 CH_2Ph; 51.42, 2 CO_2CH_3; 34.37, 2 CH_2CO_2Me; 22.31, CH_2 meso; 19.12, 2 $CH_2CH_2CO_2$Me; 10.60, 2 CH_3.

The dipyrromethane prepared above (65 mg, 0.1 mmol) was redissolved in THF (4 mL) and hydrogenated over 10% Pd–C (10 mg) at RT overnight. The solution was filtered through Celite, the catalyst washed abundantly with THF, MeOH, CH_2Cl_2, and the compound **15l** evaporated to dryness. **15l** was prepared and used without purification immediately before the preparation of porphyrin **5l**.

6.1.4.7. Porphyrin 5l. Compounds **15l** (33 mg, 0.076 mmol) and **14** (39 mg, 0.076 mmol) were dissolved in CH_2Cl_2/MeOH (10/1 mL) and treated with *p*-TsOH·H_2O (100 mg, 0.525 mmol) at RT in the dark for 48 h. The reaction was worked up as for **5j**. Porphyrin **5l** was obtained in 31% yield (19.6 mg, 0.024 mmol). ^{1}H NMR ($CDCl_3$): δ 10.02, 10.01, 9.96 (3s, 4H, 4 C*H*

meso); 5.05, 5.04 (2s, 4H, 2 CH_2CO_2Me); 4.37 (m, 8H, 4 $CH_2CH_2CO_2$Me); 3.78, 3.76, 3.71, 3.70, 3.65 (5s, 18H, 6 CO_2CH_3); 3.60, 3.59 (2s, 6H, 2 CH_3); 3.28 (m, 8H, 4 $CH_2CH_2CO_2$Me). ^{13}C NMR δ 173.49, 172.00, 171.87, 6 CO_2Me; 143.3 (m), 140.4 (br), 138.6 (br), 137.2 (br), 132.4 (br), 16 C porphyrinic; 97.71, 97.22, 97.05, 96.39, 4 CH meso; 52.50, 52.33, 51.85, 51.78, 51.68, 51.57, 6 CO_2CH_3; 37.14, 36.79, 4 $CH_2CH_2CO_2$Me; 32.54, 32.49, 2 CH_2CO_2Me; 21.74, 4 $CH_2CH_2CO_2$Me; 11.63, 2 CH_3. MS(ESI) *m/z* 827 $[M+H]^+$. HRMS (ESI) $[C_{44}H_{51}N_4O_{12}]^+$, calcd: 827.3503. Found: 827.3463.

6.1.5. Preparation of porphyrins 5e, 5m, 5n, 5o, and 5p

6.1.5.1. 5′-Formyl-3′,4-di-(2-methoxycarbonylethyl)-3,4′-dimethoxycarbonylmethyl-2,2′-methylenedipyrrole-5-carboxylic acid (16). The benzyl ester of **16** was prepared as in Ref. 32. Part of it (29 mg, 0.046 mmol) was dissolved in THF (3 mL), Et_3N (200 μL) was added and the solution hydrogenated over 10% Pd–C (5 mg) at RT overnight. The solution was filtered through Celite, the catalyst washed with CH_2Cl_2 (10 mL), and the filtrate neutralized by washing with 0.1 N HCl. **16** was prepared and used without purification immediately prior to the preparation of porphyrins **5e**, **5m**, **5n**, **5o**, and **5p**. ^{1}H NMR ($CDCl_3$): δ 11.40, 10.61 (s br + s, 2H, 2 NH); 9.30 (s, 1H, CHO); 3.94 (s, 2H, CH_2 meso); 3.67, 3.62, 3.61, 3.56 (4s, 12H, 4 CO_2CH_3); 3.63, 3.48 (2s, 4H, 2 CH_2CO_2Me); 2.96, 2.79 (2t, J = 7.4, 7.7, 4H, 2 $CH_2CH_2CO_2$Me); 1.77 (m, 4H, 4 $CH_2CH_2CO_2$Me). ^{13}C NMR δ 177.39, CHO; 173.53, 173.33, 172.49, 170.90, 4 CO_2Me; 164.66, CO_2H; 136.15, 131.64, 131.44, 129.03, 127.80, 121.55, 117.90, 114.85, 8 C pyrrolic; 52.13, 51.60, 51.27, 3 CO_2CH_3; 34.51, 34.44, 2 $CH_2CH_2CO_2$Me; 29.72, 29.44, 2 CH_2CO_2Me; 22.52, CH_2 meso; 20.39, 18.68, 2 $CH_2CH_2CO_2$Me.

6.1.5.2. 5′-Formyl-4′-methyl-3,3′-di-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-2,2′-methylene dipyrrole-5-carboxylic acid (17e). The benzyl ester of dipyrromethane **17e** (867 mg, 1.53 mmol) was prepared following standard procedures described in Refs. 32–35 from the monopyrroles tertbutyl-2,4-dimethyl-3-(2-methoxycarbonylethyl)-pyrrole-5-carboxylate and benzyl-2-methyl-3-(2-methoxycarbonyl-ethyl)-4-(methoxycarbonylmethyl)-pyrrole-5-carboxylate. ^{1}H NMR ($CDCl_3$): δ 10.88, 10.54 (s br + s, 2H, 2 NH); 9.25 (s, 1H, CHO); 7.18–7.07 (m, 5H, PhH); 5.09 (s, 2H, CH_2Ph); 3.92 (s, 2H, CH_2 meso); 3.70 (s, 2H, CH_2CO_2Me); 3.55, 3.52, 3.46 (3s, 9H, 3 CO_2CH_3); 2.68 (m, 4H, 2 $CH_2CH_2CO_2$Me); 2.32 (m, 4H, 2 $CH_2CH_2CO_2$Me); 2.12 (s 3H, CH_3). ^{13}C NMR δ 176.55, CHO; 173.18, 173.08, 171.75, 3 CO_2Me; 160.52, CO_2CH_2Ph; 136.71, 135.84, 132.97, 130.02, 122.74, 121.04, 120.57, 118.71, 8 C pyrrolic; 128.47, 128.12, 127.98, 127.72, 4 C benzylic; 65.40, CH_2Ph; 51.53, 51.42, 3 CO_2CH_3; 34.77, 34.17, 2 $CH_2CH_2CO_2$Me; 30.54, CH_2CO_2Me; 22.36, CH_2 meso; 19.07, 18.92, 2 $CH_2CH_2CO_2$Me; 8.63, CH_3.

The dipyrromethane prepared above was hydrogenated as for preparation of **16** to give **17e** and used without further purification: ^{1}H NMR ($CDCl_3$): δ 11.85, 10.79 (s br + s, 2H, 2 NH); 9.13 (s, 1H, CHO); 3.97 (s, 2H, CH_2 meso); 3.82 (s, 2H, CH_2CO_2Me); 3.66, 3.65, 3.60 (3s, 9H, 3 CO_2CH_3); 2.86, 2.74 (2t, J = 7.5, 7.9, 4H, 2 $CH_2CH_2CO_2$Me); 2.49, 2.29 (2t, J = 7.5, 7.9, 4H, 2 $CH_2CH_2CO_2$Me); 2.25 (s, 3H, CH_3). ^{13}C NMR δ 176.18, CHO; 173.13, 173.00, 172.13, 3 CO_2Me; 165.12, CO_2H; 137.91, 134.64, 130.96, 128.88, 123.64, 121.30, 121.06, 118.93, 8 C pyrrolic; 51.87, 51.67, 51.59, 3 CO_2CH_3; 35.04, 34.67, 2 $CH_2CH_2CO_2$Me; 30.59, CH_2CO_2Me; 22.44, CH_2 meso; 19.54, 19.11, 2 $CH_2CH_2CO_2$Me; 9.01, CH_3.

6.1.5.3. Porphyrin 5e and 5p. The dipyrromethanes **17e** (22 mg, 0.046 mmol) and **16** (25 mg, 0.046 mmol) were dissolved in CH_2Cl_2/MeOH (10/1 mL). *p*-TsOH·H_2O (70 mg, 0.37 mmol) was added and the reaction mixture was stirred at RT in the dark for 48 h. The work up was done as for **5j**. Three products were isolated by PLC. The symmetrical porphyrin **5p** (R_1 = Me, $R_2 = R_3 = CH_2CH_2CO_2$Me, $R_4 = CH_2CO_2$Me) (2 mg, 0.002 mmol, 9%) has the highest R_F: ^{1}H NMR ($CDCl_3$): δ 10.16, 10.09 (2s, 4H, 4 CH meso); 5.07 (s, 4H, 2 CH_2CO_2Me); 4.45, 4.41 (2t, J = 7.7, 7.8, 4H, 2 $CH_2CH_2CO_2$Me); 3.77, 3.70, 3.67 (3s, 18H, 6 CO_2CH_3); 3.64 (s, 6H, 2 CH_3); 3.34, 3.28 (2t, J = 7.8, 7.7, 4H, 2 $CH_2CH_2CO_2$Me). ^{13}C NMR δ 173.64, 173.39, 172.09, 6 CO_2Me; 148.0 (m), 142.6 (br), 139.0 (m), 137.0 (br), 135.2 (br), 134.5 (br), 16 porphyrinic C; 97.25, 97.17; 4 CH meso; 52.42, 51.78, 6 CO_2CH_3; 37.26, 36.88, 4 $CH_2CH_2CO_2$Me; 32.76, 2 CH_2CO_2Me; 21.91, 21.76, 4 $CH_2CH_2CO_2$Me; 11.60, 2 CH_3. MS (ESI) *m/z* 827 $[M+H]^+$. HRMS (ESI) $[C_{44}H_{51}N_4O_{12}]^+$, calcd: 827.3503. Found: 827.3494. The middle band contained the desired porphyrin **5e** (7.6 mg, 0.009 mmol, 20%):[36] ^{1}H NMR ($CDCl_3$): δ 10.11, 10.06, 10.05, 9.97 (4s, 4H, 4 CH meso); 5.13, 5.06, 4.98 (3s, 6H, 3 CH_2CO_2Me); 4.43, 4.39, 4.34 (3m, 8H, 4 $CH_2CH_2CO_2$Me); 3.79, 3.78, 3.76, 3.70, 3.68, 3.63 (6s, 21H, 7 CO_2CH_3); 3.62 (s, 3H, CH_3); 3.33, 3.26 (2m, 8H, 4 $CH_2CH_2CO_2$Me). ^{13}C NMR δ 173.54, 173.49, 173.37, 171.94, 171.86, 171.77, 7 CO_2Me; 147.34–131.42 (m), 16 porphyrinic C; 97.58, 97.45, 96.84, 4 CH meso; 52.39, 51.72, 7 CO_2CH_3; 36.95, 36.61, 4 $CH_2CH_2CO_2$Me; 32.43, 32.30, 32.23, 3 CH_2CO_2Me; 21.59, 21.47, 4 $CH_2CH_2CO_2$Me; 11.41, CH_3. MS (ESI) *m/z* 885 $[M+H]^+$, 907 $[M+Na]^+$. The lowest band was **5b** (9 mg, 0.01 mmol, 21%).

6.1.5.4. 5′-Formyl-4-methyl-3,3′-di-(2-methoxycarbonylethyl)-4′-(methoxycarbonylmethyl)-2,2′-methylene dipyrrole-5-carboxylic acid (17m). The benzyl ester of the dipyrromethane **17m** (753 mg, 1.33 mmol) was prepared as for **17e** from the monopyrroles benzyl-2-methyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonyl methyl-pyrrole-5-carboxylate and benzyl-2,4-dimethyl-3-(2-methoxycarbonylethyl)-pyrrole-5-carboxylate. ^{1}H NMR ($CDCl_3$): δ 10.82, 10.18 (s br + s, 2H, 2NH); 9.39 (s, 1H, CHO); 7.28–7.18 (m, 5H, PhH); 5.18 (s, 2H, CH_2Ph); 3.99 (s, 2H, CH_2 meso); 3.66, 3.64, 3.62, 3.58 (4s, 12H, 4 CO_2CH_3); 2.75 (m, 4H, 2 $CH_2CH_2CO_2$Me); 2.40 (m, 4H, 2 $CH_2CH_2CO_2$Me); 2.24 (s, 3H, CH_3). ^{13}C NMR δ 177.35, CHO; 173.48, 173.25, 171.01, 3 CO_2Me; 161.33, CO_2CH_2Ph; 136.16, 129.74, 128.71, 121.36, 120.28, 117.93, 8 C pyrrolic; 128.22, 127.71, 127.57, 127.04, 4 C benzylic; 65.30, CH_2Ph; 52.14, 51.53, 2

CO_2CH_3; 34.40, 34.32, 2 $CH_2CH_2CO_2Me$; 29.58, CH_2CO_2Me; 22.47, CH_2 meso; 19.23, 18.72, 2 $CH_2CH_2CO_2Me$; 10.58, CH_3.

The dipyrromethane prepared above (36 mg, 0.058 mmol) was hydrogenated as for preparation of **16** to afford **17m**. ^{1}H NMR ($CDCl_3$): δ 11.80, 10.54 (s br + s, 2H, 2N*H*); 9.27 (s, 1H, C*H*O); 3.97 (s, 2H, CH_2 meso); 3.67 (s, 2H, CH_2CO_2Me); 3.62, 3.61, 3.60 (3s, 9H, 3 CO_2CH_3); 2.85, 2.70 (2t, J = 7.5, 7.7, 4H, 2 $CH_2CH_2CO_2Me$); 2.47, 2.30 (2t, J = 7.5, 7.7, 4H, 2 $CH_2CH_2CO_2Me$); 2.23 (s, 3H, CH_3). ^{13}C NMR δ 177.26, *C*HO; 173.11, 172.90, 170.75, 3 CO_2Me; 165.52, CO_2H; 137.23, 130.67, 128.95, 128.29, 121.55, 120.58, 117.78, 8 C pyrrolic; 52.11, 51.42, 51.36, 3 CO_2CH_3; 34.62, 34.52, 2 $CH_2CH_2CO_2Me$; 29.63, CH_2CO_2Me; 22.37, CH_2 meso; 19.47, 18.75, 2 $CH_2CH_2CO_2Me$; 10.48, CH_3.

6.1.5.5. Porphyrin 5m and 5o. The dipyrromethanes **17m** (28 mg, 0.058 mmol) and **16** (31 mg, 0.058 mmol) were dissolved in CH_2Cl_2/MeOH (10/1 mL). *p*-TsOH·H_2O (88 mg, 0.46 mg) was added and the reaction mixture was stirred at RT in the dark for 48 h. The reaction was worked up as for **5j**. Three products were isolated by PLC. The symmetrical **5o** (R_1 = CH_2CO_2Me, R_2 = R_3 = $CH_2CH_2CO_2Me$, R_4 = Me) has the highest R_F (6.7 mg, 0.008 mmol, 28%): ^{1}H NMR ($CDCl_3$): δ 10.14, 10.11 (2s, 4H, 4 C*H* meso); 5.05 (s, 4H, 2 CH_2CO_2Me); 4.44, 4.39 (2t, J = 7.6, 7.8, 8H, 4 $CH_2CH_2CO_2Me$); 3.77, 3.68 (2s, 18H, 6 CO_2CH_3); 3.62 (s, 6H, 2 CH_3); 3.24, 3.27 (2t, J = 7.8, 7.6, 8H, 4 $CH_2CH_2CO_2Me$). ^{13}C NMR δ 173.64, 173.38, 172.18, 6 CO_2CH_3; 148 (m), 142.6 (br), 139.0 (m), 137.0 (br), 135.3 (br), 134.4 (br), 16 porphyrinic C; 97.41, 97.02, 4 *C*H meso; 52.43, 51.75, 6 CO_2CH_3; 37.12, 36.73, 4 $CH_2CH_2CO_2Me$; 32.72, 2 CH_2CO_2Me; 21.84, 21.65, 4 $CH_2CH_2CO_2Me$; 11.53, 2 CH_3. MS (ESI) *m*/*z* 827 $[M+H]^+$, 849 $[M+Na]^+$. HRMS (ESI) $[C_{44}H_{51}N_4O_{12}]^+$, calcd: 827.3503. Found: 827.3542. The middle band was porphyrin **5m** (11.3 mg, 0.013 mmol, 22%):[37] ^{1}H NMR ($CDCl_3$): δ 9.99, 9.93, 9.92 (3s, 4H, 4 C*H* meso); 5.03, 4.99, 4.96 (3s, 6H, 3 CH_2CO_2Me); 4.38, 4.32 (t + m, J = 5.2, 8H, 4 $CH_2CH_2CO_2Me$); 3.79, 3.78, 3.72, 3.71, 3.70, 3.64 (6s, 21H, 7 CO_2CH_3); 3.58 (s, 3H, CH_3); 3.31, 3.24 (m + t, J = 5.2, 8H, 4 $CH_2CH_2CO_2Me$). ^{13}C NMR δ 173.54, 173.50, 173.38, 172.13, 172.02, 171.93, 171.76, 7 CO_2Me; 146.4–133.5 (m), 16 porphyrinic C; 97.64, 97.56, 97.27, 96.97, 4 *C*H meso; 52.40, 51.73, 7 CO_2CH_3; 37.03, 36.98, 36.64, 4 $CH_2CH_2CO_2Me$; 32.52, 32.44, 32.33, 3 CH_2CO_2Me; 21.66, 21.55, 4 $CH_2CH_2CO_2Me$; 11.47, CH_3. MS (ESI) *m*/*z* 885 $[M+H]^+$. **5m** structure was confirmed by isolation of the first product of decarboxylation of uro'gen III (**1a**) during its enzymatic production from PBG in the presence of PBG deaminase and uro'gen III synthase lysates containing decarboxylases. The product isolated from this incubation has identical ^{1}H- and ^{13}C NMR. The lowest band contained **5b** (6 mg, 0.006 mmol, 22%).

6.1.5.6. 5′-Formyl-3-methyl-3′,4-di-(2-methoxycarbonylethyl)-4′-methoxycarbonylmethyl-2,2′-methylene dipyrrole-5-carboxylic acid (17n). The benzyl ester of dipyrromethane **17n** (598 mg, 1.06 mmol) was prepared as for **17e** from the monopyrroles benzyl-2-methyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethyl-pyrrole-5-carboxylate and benzyl-2,3-dimethyl-4-(2-methoxycarbonylethyl)-pyrrole-5-carboxylate. ^{1}H NMR ($CDCl_3$): δ 11.15, 10.50 (s br + s, 2H, 2 N*H*); 9.32 (s, 1H, C*H*O); 7.18–7.06 (m, 5H, Ph*H*); 5.11 (s, 2H, CH_2Ph); 3.85 (s, 2H, CH_2 meso); 3.58, 3.55, 3.51 (3s, 9H, 3 CO_2CH_3); 2.91, 2.69 (2t, J = 8.0, 7.8, 2 $CH_2CH_2CO_2Me$); 2.38, 2.30 (2t, J = 8.0, 7.8, 2 $CH_2CH_2CO_2Me$); 1.95 (s, 3H, CH_3). ^{13}C NMR δ 177.49, *C*HO; 173.41, 173.08, 170.91, 3 CO_2Me; 160.88, CO_2CH_2Ph; 136.79, 135.91, 130.12, 129.35, 128.60, 121.52, 117.47, 116.82, 8 C pyrrolic; 128.17, 127.65, 4 C benzylic; 65.47, CH_2Ph; 52.13, 51.46, 51.20, 3 CO_2CH_3; 34.52, 34.40, 2 $CH_2CH_2CO_2Me$; 29.54, CH_2CO_2Me; 22.53, CH_2 meso; 20.63, 18.77, 2 $CH_2CH_2CO_2Me$; 8.61, CH_3.

The dipyrromethane prepared above (40 mg, 0.071 mmol) was hydrogenated as for preparation of **16** to afford **17n**. ^{1}H NMR ($CDCl_3$): δ 11.98, 10.74 (s br + s, 2H, 2 N*H*); 9.26 (s, 1H, C*H*O); 3.91 (s, 2H, CH_2 meso); 3.68 (s, 2H, CH_2CO_2Me); 3.65, 3.63, 3.60 (3s, 9H, 3 CO_2CH_3); 3.00, 2.85 (2t, J = 7.9, 7.7, 4H, 2 $CH_2CH_2CO_2Me$); 2.51, 2.45 (2t, J = 7.9, 7.7, 4H, 2 $CH_2CH_2CO_2Me$); 2.00 (s, 3H, CH_3). ^{13}C NMR δ 177.38, *C*HO; 173.55, 172.98, 170.80, 3 CO_2Me; 165.40, CO_2H; 137.25, 131.23, 130.55, 128.97, 121.68, 117.44, 117.38, 8 C pyrrolic; 52.26, 51.55, 51.30, 3 CO_2CH_3; 34.80, 34.55, 2 $CH_2CH_2CO_2Me$; 29.72, CH_2CO_2Me; 22.51, CH_2 meso; 20.64, 18.89, 2 $CH_2CH_2CO_2Me$; 8.80, CH_3.

6.1.5.7. Porphyrins 5n and 5p. The dipyrromethanes **17n** (34 mg, 0.071 mmol) and **16** (38 mg, 0.071 mmol) were dissolved in CH_2Cl_2/MeOH (10/1 mL). *p*-TsOH·H_2O (108 mg, 0.57 mmol) was added and the reaction stirred at RT in the dark for 48 h. The reaction was worked up as for **5j**. Three products were isolated by PLC. The compound with the highest R_F was the symmetrical porphyrin **5p** (R_1 = CH_2CO_2Me, R_2 = R_4 = $CH_2CH_2CO_2Me$, R_3 = Me) (11 mg, 0.13 mmol, 18%). The middle band was porphyrin **5n** (16 mg, 0.018 mmol, 26%). ^{1}H NMR ($CDCl_3$): δ 9.91, 9.87, 9.86, 9.79 (4s, 4H, 4 C*H* meso); 5.01, 4.96, 4.86 (3s, 6H, 3 CH_2CO_2Me); 4.30, 4.25 (2m, 8H, 4 $CH_2CH_2CO_2Me$); 3.80, 3.79, 3.77, 3.74, 3.72, 3.71, 3.68 (7s, 21H, 7 CO_2CH_3); 3.56 (s, 3H, CH_3); 3.30, 3.25 (2m, 8H, 4 $CH_2CH_2CO_2Me$). ^{13}C NMR δ 173.49, 173.37, 171.94, 171.87, 171.75, 7 CO_2Me; 147-131 (m), 16 porphyrinic C; 97.61, 97.47, 96.33, 4 *C*H meso; 52.37, 51.73, 7 CO_2CH_3; 37.07, 36.98, 36.77, 4 $CH_2CH_2CO_2Me$; 32.41, 32.27, 3 CH_2CO_2Me; 21.72, 21.60, 4 $CH_2CH_2CO_2Me$; 11.45, CH_3. The lowest band was **5b** (15 mg, 0.016 mmol, 23%).

6.2. Enzymatic studies

6.2.1. Enzymes. *Pseudomonas dinitrificans* cobA and cobI genes were simultaneously overexpressed in an engineered strain of *E. coli*, providing a source of uro'gen III methyl transferase and precorrin-2 methyltrans-

ferase. 4× 1 L Luria–Bertani (LB) medium containing 50 mg/L ampicillin was inoculated and incubated for 16 h in a shaker set at 37 °C and 200 rpm. The cells were collected by centrifugation and resuspended in 150 mL buffer (50 mM Tris–HCl, 75 mM KCl, and 12 mM $MgCl_2{\cdot}H_2O$, pH 7.5). The cells were lysed by sonication maintaining the temperature below 20 °C, the cell debris removed by centrifugation (12,000*g* for 30 min) and the lysate was then treated with 0.5 g DEAE–Sephadex A-25 to remove endogenous porphyrinoids. After addition of 30 mL glycerol, the lysate was kept frozen at −20 °C until use, remaining active for several months.

6.2.2. Enzymatic reactions. General procedures

6.2.2.1. Hydrolysis and reduction of the porphyrin methyl esters. Each porphyrin methyl ester (5–25 mg) was suspended in THF (1 mL per 10 mg substrate) and hydrolyzed with 2 N KOH (1 mL per 10 mg substrate) at RT overnight. The solvent was evaporated and the pH of the residual aqueous solution was decreased by addition of 1 N HCl (1.5 mL per 10 mg substrate). The solution was placed under argon atmosphere and transferred to an argon-purged glove-box. Pieces of 3% sodium amalgam were added to the stirred free-acid porphyrin solution until it became colorless (ca. 2 h) to provide the porphyrinogen.

6.2.2.2. Incubations. A solution of buffer (150 mM Tris–HCl, 100 mM KCl, and 20 mM $MgCl_2{\cdot}6H_2O$, pH 7.5; 100 mL per 10 mg substrate) containing *S*-adenosyl-L-methionine (100 mg per 10 mg substrate) was degassed by several cycles of high vacuum and argon bubbling. The CobA and CobI lysate (20 mL per 10 mg substrate) was added with antifoam 204 (Sigma, 0.5 mL). After several additional cycles of degassing, the buffer solution was transferred to the glove-box. The porphyrinogen solution was filtered through a cotton-plugged pipette into the buffer solution. After a few additional cycles of degassing, the flask was sealed and incubated for 20 h at 37 °C in the dark.

6.2.2.3. Isolation and purification of the products. The incubation mixture was centrifuged to remove precipitated protein. The solution was passed slowly through DEAE–Sephadex A-25 (300 mg per 10 mg of substrate) to trap the porphyrinoid compounds. After washing with argon saturated acetone (20–50 mL), the resin was dried by passing argon through it, then the porphyrinoids were eluted with MeOH + 5% H_2SO_4 (20 mL per 10 mg substrate) and esterified for 18 h. The solution was diluted with CH_2Cl_2 (20–30 mL) and H_2O (50 mL), then neutralized with saturated $NaHCO_3$ and extracted in CH_2Cl_2 (3× 25 mL). After evaporation of the solvent to dryness, MeOH (2–3 mL) was added to the crude material and the soluble fraction was filtered off the solid residue, which was washed with more MeOH (3× 1 mL). The solid residue contained oxidized unreacted starting material (porphyrins). The MeOH-soluble fractions contained the methylation products, chlorins and isobacteriochlorins. They were collected, evaporated, and chromatographed on PLC eluting 2–3 times with benzene/ethyl acetate/MeOH (70:27:3). Products and yields are reported in Table 1. The most important ^{1}H NMR signals, corresponding to the meso protons and methyl groups, are reported in Table 2.

6.2.3. Product characterizations

6.2.3.1. From uro'gen III (1a). (a) When Cob A was used instead of CobA and CobI, 3 products were isolated: factor 2 methyl ester (**7a**) (30–36%), factor 2 monolactone methyl ester (**10a**) (4%), and factor 2 dilactone methyl ester (**11a**) (2%). *Factor 2 **7a*** (major isomer):[6] ^{1}H NMR (C_6D_6): δ 8.85 (s, 1H, H-15); 7.64 (s, 1H, H-10); 7.54 (s, 1H, H-20); 6.78 (s, 1H, H-5); 4.14 (m, 6H, 2 CH_2CO_2Me at C-12, C-18 and H-3, H-8); 3.79 (t, J = 7.6, 4H, 2 $CH_2CH_2CO_2Me$ at C-13 and C-17); 3.42, 3.37, 3.34, 3.33, 3.25 (2), 3.22, 3.16 (7s, 24H, 8 CO_2CH_3); 2.98 (t, J = 7.6, 4H, 2 $CH_2CH_2CO_2Me$ at C-13 and C-17); 2.62, 2.53 (AB, J = 16.3, 2H, CH_2CO_2Me at C-2); 2.52 (d, J = 9.1, 2H, CH_2CO_2Me at C-7); 2.47 (t, J = 8.8, 2H, $CH_2CH_2CO_2Me$ at C-8); 2.25, 2.11, 1.92 (3m, 6H, $CH_2CH_2CO_2Me$ at C-3 and $CH_2CH_2CO_2Me$ at C-8); 1.75, 1.60 (2s, 6H, 2 CH_3). UV λ_{max} (MeOH) 280 (0.23), 368 (sh, 0.65), 373 (1), 405 (sh, 0.55), 505 (0.12), 543 (0.19), 585 (0.27), 627 (0.03) nm. MS (ESI) *m/z* 975 $[M+H]^+$. Starting with **1a** labeled at C-4, C-5, C-9, C-10, C-14, C-15, C-16, and C-20 allows a partial ^{1}H and ^{13}C NMR assignment for the 3 isomers in agreement with published results.[6] The major isomer of **7a** was isolated in the band with the lowest R_F: ^{1}H NMR (C_6D_6): δ 8.85 (d, J_{CH} = 152.4, 1H, H-15); 7.64 (d, J_{CH} = 154.5, 1H, H-10); 7.53 (d, J_{CH} = 153.3, 1H, H-20); 6.77 (d, J_{CH} = 155.0, 1H, H-5); 4.15 (s, 2H, CH_2CO_2Me at C-18); 4.13 (AB + m, J = 13, 4H, CH_2CO_2Me at C-12 and H-3, H-8); 3.78 (m, 4H, 2 $CH_2CH_2CO_2Me$ at C-13, C-17); 3.42, 3.37, 3.34, 3.33, 3.25 (2), 3.22, 3.15 (8s, 24H, 8 CO_2CH_3); 2.98 (t, J = 7.5, 4H, 2 $CH_2CH_2CO_2Me$ at C-13, C-17); 2.62, 2.53 (AB, J = 15, 2H, CH_2CO_2Me at C-2); 2.52 (d, J = 9.0, 2H, CH_2CO_2Me at C-7); 2.47 (t, J = 7.9, 2H, $CH_2CH_2CO_2Me$ at C-18); 2.30, 2.25, 1.90 (3m, 6H, $CH_2CH_2CO_2Me$ at C-3 and $CH_2CH_2CO_2Me$ at C-18); 1.75 (s, 3H, CH_3 at C-2); 1.59 (s, 3H, CH_3 at C-7). ^{13}C NMR δ 164.87 (d, J_{CH} = 71.5, C-4); 152.58 (d, J_{CH} = 77.9, C-9); 138.04 (d, J_{CH} = 72.2, C-14); 135.68 (d, J_{CH} = 69.9, C-19); 108.60 (t, J_{CH} = 71.4, C-15); 95.89 (d, J_{CH} = 77.0, C-10); 94.17 (s, C-20); 89.57 (d, J_{CH} = 75.5, C-5). The middle band contained some of the major isomers and 2 epimers in a 3:2 ratio. The band with the highest R_F contained only the 2 epimers in the inverted ratio 2:3. Analysis of the two bands allowed the following tentative assignments for the 2 epimers by correlation to the data in Refs. 3 and 4. ***7a** epimer 1*: ^{1}H NMR (C_6D_6): δ 8.82 (d, J_{CH} = 152.6, 1H, H-15); 7.52 (d, J_{CH} = 153.5, 1H, H-10); 7.41 (d, J_{CH} = 134.5, 1H, H-20); 6.68 (d, J_{CH} = 155.29, 1H, H-5); 4.13, 4.11 (2s + 2m, 6H, 2 CH_2CO_2Me at C-12, C-18 and H-3, H-8); 3.78 (m, 4H, 2 $CH_2CH_2CO_2Me$ at C-13 and C-17); 3.43, 3.37, 3.36, 3.32, 3.25, 3.24 (2), 3.15 (7s, 24H, 8 CO_2CH_3); 2.98–2.84 (m, 4H, 2 $CH_2CH_2CO_2Me$ at C-13 and C-17); 2.65–2.50 (m, 4H, 2 CH_2CO_2Me at C-2 and C-7); 2.48–2.35 (m, 2H, $CH_2CH_2CO_2Me$ at C-8 or C-3); 2.24, 2.08 (m, 6H, $CH_2CH_2CO_2Me$ at C-3 or C-8 and $CH_2CH_2CO_2Me$ at C-3 or C-8); 1.84 (s, 3H, CH_3 at C-2); 1.44 (s, 3H, CH_3 at C-7). ^{13}C NMR

δ 166.74 (d, J_{CH} = 70.8, C-4); 150.65 (d, J_{CH} = 78.2, C-9); 140.11 (d, J_{CH} = 73.2, C-14); 134.15 (d, J_{CH} = 70.9, C-19); 109.08 (t, J_{CH} = 71.3, C-15); 96.05 (d, J_{CH} = 80.3, C-10); 95.02 (s, C-20); 89.42 (d, J_{CH} = 73.2, C-5). **7a** *epimer 2*: ^{1}H NMR (C_6D_6): δ 8.83 (d, J_{CH} = 152.1, 1H, H-15); 7.65 (d, J_{CH} = 140.3, 1H, H-10); 7.53 (d, J_{CH} = 155.5, 1H, H-20); 6.71 (d, J_{CH} = 155.6, 1H, H-5); 4.13, 4.12 (2s, 4H, 2 CH_2CO$_2$Me at C-12 and C-18); 4.13, 4.11 (2m, 2H, H-3 and H-8); 3.78 (m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.41, 3.38, 3.35, 3.34, 3.26, 3.23, 3.22, 3.00 (8s, 24H, 8 CO$_2$CH_3); 2.98–2.84 (m, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.65–2.50 (m, 4H, 2 CH_2CO$_2$Me at C-2 and C-7); 2.48–2.35 (m, 2H, CH_2CH$_2$CO$_2$Me at C-8 or C-3); 2.24, 2.08 (m, 6H, CH_2CH$_2$CO$_2$Me at C-3 or C-8 and CH_2CH$_2$CO$_2$Me at C-3 or C-8); 1.59 (s, 3H, CH_3 at C-2); 1.53 (s, 3H, CH_3 at C-7). ^{13}C NMR δ 161.11 (d, J_{CH} = 70.1, C-4); 154.55 (d, J_{CH} = 79.1, C-9); 138.73 (d, J_{CH} = 73.1, C-14); 135.25 (d, J_{CH} = 70.6, C-16); 109.08 (t, J_{CH} = 71.3, C-15); 96.99 (d, J_{CH} = 77.2, C-10); 93.00 (s, C-20); 90.04 (d, J_{CH} = 78.6, C-5).

Factor 2 monolactone ***10a*** *(major isomer)*. ^{1}H NMR (C_6D_6): δ 8.96 (s, 1H, H-15); 8.06 (s, 1H, H-10); 7.55 (s, 1H, H-20); 6.61 (s, 1H, H-5); 4.16, 4.04 (s + d, J = 2.5, 4H, 2 CH_2CO$_2$Me at C-12 and C-18); 4.15 (m, 1H, H-3 or H-8); 3.83 (m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.43, 3.35, 3.33, 3.32, 3.25, 3.19, 3.16 (7s, 21H, 7 CO$_2$CH_3); 3.01, 2.93 (2t, J = 9.1, 9.1, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.80, 2.53 (AB, J = 18.2, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.36 (d, J = 18.0, 2H, CH_2CO$_2$Me at C-7 or C-2); 2.62 (m, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.20–2.00 (m, 6H, CH$_2$CH_2CO$_2$Me at C-3 or C-8 and CH_2CH_2CO$_2$Me at C-3 or C-8); 1.75 (s, 3H, CH_3 at C-2); 1.21 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 280 (0.25), 356 (sh, 0.58), 375 (1), 404 (0.48), 504 (0.1), 538 (0.17), 575 (0.19), 629 (0.06) nm. MS (ESI) *m/z* 959 [M+H]$^+$. ***10a*** *epimer*: ^{1}H NMR (C_6D_6): δ 8.98 (s, 1H, H-15); 7.68 (s, 1H, H-10); 7.53 (s, 1H, H-20); 6.97 (s, 1H, H-5); 4.21 (m, 1H, H-3 or H-8); 4.16, 4.12 (2d, J = 8.2, 8.1, 4H, 2 CH_2CO$_2$Me at C-12 and C-18); 3.84, 3.67 (t + m, J = 7.0, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.41, 3.34, 3.33, 3.32, 3.25, 3.23, 3.19 (7s, 21H, 7 CO$_2$CH_3); 2.99 (m, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.54, 2.30 (AB, J = 17.5, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.52 (s, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.59, 2.37 (2m, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.44 (t, J = 7.0, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.21, 2.09, 2.00 (3m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-3 and C-8); 1.44 (s, 3H, CH_3 at C-2); 1.37 (s, 3H, CH_3 at C-7).

Factor 2 dilactone ***11a***. ^{1}H NMR (C_6D_6): δ 9.28 (s, 1H, H-15); 8.25 (s, 1H, H-10) ; 7.75 (s, 1H, H-20); 7.08 (s, 1H, H-5); 4.24, 4.11 (d + s, J = 2.8, 4H, 2 CH_2CO$_2$Me at C-12 and C-18); 4.06, 3.93, 3.82 (3m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.45, 3.32, 3.26, 3.23, 3.19 (2) (6s, 18H, 6 CO$_2$CH_3); 3.04, 2.98 (2t, J = 8.9, 8.9, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.64, 2.37 (AB, J = 17.9, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.53 (s, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.59, 2.20–2.00 (2m, 8H, 2 CH_2CH_2CO$_2$Me at C-3 and C-8); 1.42 (s, 3H, CH_3 at C-2); 1.31 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 279 (0.26), 357 (0.48), 374 (1), 385 (sh, 0.76), 405 (0.62), 487 (0.12), 540 (0.14), 578 (0.16), 624 (0.12). MS (ESI) *m/z* 943 [M+H]$^+$.

(b) Incubation of CobA and CobI with uro III (**1a**) gave 3 isolated products: factor 3 methyl ester (**8a**) (22%), factor 3 monolactone methyl ester (**12a**) (8%), and factor 3 dilactone methyl ester (**13a**) (6%). *Factor 3* ***8a*** *(major isomer)*:[6] ^{1}H NMR (C_6D_6): δ 8.73 (s, 1H, H-15); 7.50 (s, 1H, H-10); 6.51 (s, 1H, H-5); 4.21 (d, J = 19.7, 2H, CH_2CO$_2$Me at C-18); 4.06, 4.04 (2m, 2H, H-3 and H-8); 4.03 (AB, J = 12.8, 2H, CH_2CO$_2$Me at C-12); 3.69 (m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.43, 3.38, 3.37, 3.31, 3.27, 3.26, 3.24, 3.22 (8s, 24H, 8 CO$_2$CH_3); 2.92 (2m, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.77 (s, 3H, CH_3 at C-20); 2.67, 2.50 (AB, J = 9.1, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.65 (s, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.59 (m, 2H, CH_2CH$_2$CO$_2$Me at C-8); 2.29, 2.18, 2.09 (3m, 6H, CH_2CH_2CO$_2$Me at C-3 and CH$_2$CH_2CO$_2$Me at C-8); 1.66 (s, 3H, CH_3 at C-2); 1.40 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 275 (0.24), 361 (sh, 0.59), 381 (1), 410 (sh, 0.52), 500 (0.1), 544 (0.15), 574 (0.17), 633 (0.07) nm. MS (ESI) *m/z* 989 [M+H]$^+$. ***8a*** *epimer*: ^{1}H NMR (C_6D_6): δ 9.05 (s, 1H, H-15); 7.76 (s, 1H, H-10); 6.99 (s, 1H, H-5); 4.40, 4.31 (AB, J = 18.2, 2H, CH_2CO$_2$Me at C-12 or C-18); 4.36, 4.05 (2m, 2H, H-3 and H-8); 4.14, 4.03 (AB, J = 12.8, 2H, CH_2CO$_2$Me at C-12 or C-18); 3.87 (m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.43, 3.39, 3.35, 3.27 (2), 3.25, 3.15 (7s, 24H, 8 CO$_2$CH_3); 2.98 (m, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.79 (s, 3H, CH_3 at C-20); 2.64 (s, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.62, 2.44 (AB, J = 18.2, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.29–2.00 (m, 8H, 2 CH_2CH_2CO$_2$Me at C-3 and C-8); 1.51 (s, 3H, CH_3 at C-2); 1.39 (s, 3H, CH_3 at C-7).

Factor 3 monolactone ***12a***. ^{1}H NMR (C_6D_6): δ 8.85 (s, 1H, H-15); 7.88 (s, 1H, H-10); 6.40 (s, 1H, H-5); 4.19 (AB, J = 17.5, 2H, CH_2CO$_2$Me at C-18); 4.11 (m, 1H, H-3 or H-8); 3.94 (AB, J = 7.7, 2H, CH_2CO$_2$Me at C-12); 3.73, 3.64 (2m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.42, 3.40, 3.37, 3.27, 3.24, 3.19, 3.17 (7s, 21H, 7 CO$_2$CH_3); 2.99 (d, J = 17.5, CH_2CO$_2$Me at C-7); 2.94, 2.88 (2 AB, J = 6.9, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.73 (s, 3H, CH_3 at C-20); 2.64 (s, 2H, CH_2CO$_2$Me at C-2); 2.38 (d, J = 18.2, 2H, CH_2CH$_2$CO$_2$Me at C-3 or CH_2CH$_2$CO$_2$Me C-8); 2.42, 2.31 (AB, J = 18.0, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.18, 2.00 (2m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-3 and C-8); 1.67 (s, 3H, CH_3 at C-2); 1.16 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 274 (0.24), 360 (0.55), 379 (1), 407 (0.52), 500 (0.10), 537 (0.16), 580 (0.17), 631 (0.07) nm. MS (ESI) *m/z* 973 [M+H]$^+$.

Factor 3 dilactone ***13a***. ^{1}H NMR (C_6D_6): δ 9.46 (s, 1H, H-15); 8.38 (s, 1H, H-10); 7.58 (s, 1H, H-5); 4.49 (AB, J = 18.9, 2H, CH_2CO$_2$Me at C-18); 4.16 (s, 2H, CH_2CO$_2$Me at C-12); 4.02-3.91 (m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.43 (2), 3.26, 3.25, 3.20, 3.16 (6s, 18H, 6 CO$_2$CH_3); 3.06, 3.02 (2t, J = 6.8, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17);

2.99 (s, 3H, CH_3 at C-20); 2.84, 2.60, 2.73, 2.36 (2AB, J = 18.0, 18.2, 4H, 2 CH_2CO$_2$Me at C-2 and C-7); 2.80, 2.54 (2m, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.20–2.00 (m, 6H, CH_2CH$_2$CO$_2$Me at C-3 or C-8 and CH$_2$CH_2CO$_2$Me at C-3 or C-8); 1.49 (s, 3H, CH_3 at C-2); 1.45 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 282 (0.14), 359 (sh, 0.33), 377 (1), 387 (sh, 0.53), 407 (0.69), 489 (0.07), 535 (0.08), 578 (0.1), 624 (0.12) nm. MS (ESI) *m/z* 957 $[M+H]^+$.

6.2.3.2. From uro'gen I (1b). Three products were isolated: diMeIBC methyl ester **7b** (3–5%), diMeIBC monolactone methyl ester **10b** (4–5%), and diMeIBC dilactone **11b** (<1%). *DiMeIBC **7b** (major isomer)*:[12] ^{1}H NMR (C_6D_6): δ 8.84 (s, 1H, H-15); 7.65 (s, 1H, H-10); 7.48 (s, 1H, H-20); 6.80 (s, 1H, H-5); 4.32, 4.07 (2m, 2H, H-3 and H-8); 4.27, 4.16 (2m, 4H, 2 CH_2CO$_2$Me at C-12 and C-17); 3.75 (m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-18); 3.42, 3.37, 3.34, 3.33, 3.32, 3.26, 3.22, 3.16 (8s, 24H, 8 CO$_2$CH_3); 2.97 (m, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-18); 2.59, 2.52 (2 AB, J = 13.3, 10.6, 4H, 2 CH_2CO$_2$Me at C-2 and C-7); 2.46 (t, J = 7.9, 2H, CH_2CH$_2$CO$_2$Me at C-8); 2.30, 2.26, 2.01 (3m, 6H, CH_2CH_2CO$_2$Me at C-3 and CH$_2$CH_2CO$_2$Me at C-8); 1.68 (s, 3H, CH_3 at C-2); 1.61 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 277 (0.27), 359 (sh, 0.67), 377 (1), 392 (sh, 0.86), 507 (0.12), 545 (0.18), 588 (0.28), 645 (0.07) nm. MS (ESI) *m/z* 975 $[M+H]^+$, 997 $[M+Na]^+$. HRMS (ESI) $[C_{50}H_{63}N_4O_{16}]^+$, calcd: 975.4239. Found: 975.3826. Starting with **1b** labelled at C-4, C-5, C-9, C-10, C-14, C-15, C-19 and C-20 allows for a partial ^{13}C NMR assignment for the major isomer of **7b** in agreement with Ref. 12. ^{13}C NMR δ 164.57 (d, J_{CH} = 63.8, C-4); 152.94 (d, J_{CH} = 79.3, C-9); 144.69 (d, J_{CH} = 65.4, C-19); 137.94 (d, J_{CH} = 71.0, C-14); 108.30 (d, J_{CH} = 71, C-15); 95.93 (d, J_{CH} = 74.5, C-10); 93.89 (d, J_{CH} = 67.5, C-20); 89.55 (d, J_{CH} = 71.8, C-5).

*DiMeIBC monolactone **10b** (major isomer)*. ^{1}H NMR (C_6D_6): δ 8.95 (s, 1H, H-15); 8.08 (s, 1H, H-10); 7.48 (s, 1H, H-20); 6.64 (s, 1H, H-5); 4.29, 4.08 (m + AB, J = 6.7, 4H, 2 CH_2CO$_2$Me at C-12 and C-17); 4.05 (m, 1H, H-3 or H-8); 3.83, 3.78, 3.72 (3m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-18); 3.43, 3.37, 3.34, 3.32, 3.31, 3.21, 3.16 (7s, 21H, 7 CO$_2$CH_3); 2.96 (AB, J = 5.7, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-18); 2.80, 2.37 (AB, J = 18.3, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.62 (t, J = 7.3, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.53 (d, J = 3.8, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.20, 2.03 (2m, 6H, 2 CH_2CH$_2$CO$_2$Me at C-3 and C-8 and CH$_2$CH_2CO$_2$Me at C-3 or C-8); 1.67 (s, 3H, CH_3 at C-2); 1.22 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 279 (0.26), 356 (sh, 0.61), 377 (1), 403 (0.58), 500 (0.11), 537 (0.16), 577 (0.19), 639 (0.07) nm. MS (ESI) *m/z* 959 $[M+H]^+$, 981 $[M+Na]^+$. HRMS (ESI) $[C_{49}H_{59}N_4O_{16}]^+$, calcd: 959.3926. Found: 959.3205. ***10b** epimer*: ^{1}H NMR (C_6D_6): δ 8.94 (s, 1H, H-15); 7.70 (s, 1H, H-10); 7.55 (s, 1H, H-20); 7.02 (s, 1H, H-5); 4.32 (m, 1H, H-3 or H-8); 4.30 (m, 2H, CH_2CO$_2$Me at C-12 or C-17); 4.14 (AB, J = 9.9, CH_2CO$_2$Me at C-12 or C-17); 4.06 (m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-18); 3.41, 3.37, 3.33, 3.32, 3.27, 3.25, 3.19 (7s, 21H, 7 CO$_2$CH_3); 3.00, 2.88 (2t, J = 6.6, 6.6, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-18); 2.67, 2.37 (AB, J = 24.6, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.51 (s, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.45 (t, J = 7, 2H, CH$_2$CH_2CO$_2$Me at C-3 or C-8); 2.20–2.00 (m, 6H, CH$_2$CH_2CO$_2$Me at C-3 or C-8 and 2 CH_2CH$_2$CO$_2$Me at C-3 and C-8); 1.47 (s, 3H, CH_3 at C-2); 1.35 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 278 (0.25), 362 (sh, 0.60), 379 (1), 406 (0.57), 505 (0.11), 542 (0.16), 586 (0.21), 638 (0.05) nm. MS (ESI) *m/z* 959 $[M+H]^+$, 981 $[M+Na]^+$. HRMS (ESI) $[C_{49}H_{58}NaN_4O_{16}]^+$, calcd: 981.3746. Found: 981.3188.

*DiMeIBC dilactone **11b***. ^{1}H NMR (C_6D_6): δ 9.25 (s, 1H, H-15); 8.28 (s, 1H, H-10); 7.79 (s, 1H, H-20); 7.27 (s, 1H, H-5); 4.41, 4.15 (s + AB, J = 1.8, 4H, 2 CH_2CO$_2$Me at C-7 and C-12); 3.91, 3.83, 3.72 (3m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-18); 3.44, 3.38, 3.26 (2), 3.20 (2), (4s, 18H, 6 CO$_2$CH_3); 3.00, 2.90 (2t, J = 6.8, 6.8, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-18); 2.78, 2.46 (AB, J = 21.1, 2H, CH_2CO$_2$Me at C-2 and C-7); 2.56 (t, J = 9.2, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.68, 2.27 (2m, 2H, CH_2CH$_2$CO$_2$Me at C-3 or C-8); 2.54 (s, 2H, CH_2CO$_2$Me at C-2 or C-7); 2.15, 2.03 (2m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-3 and C-8); 1.42 (s, 3H, CH_3 at C-2); 1.31 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 281 (0.20), 355 (sh, 0.43), 375 (1), 384 (sh, 0.75), 405 (0.75), 487 (0.09), 543 (0.10), 582 (0.13), 625 (0.12) nm. MS (ESI) *m/z* 943 $[M+H]^+$, 965 $[M+Na]^+$. HRMS (ESI) $[C_{48}H_{54}NaN_4O_{16}]^+$, calcd: 965.3433. Found: 965.3245.

6.2.3.3. From uro'gen IV (1d). Three products were isolated: MeC methyl ester **6d**[13] (19%), MeC monolactone methyl ester **9d** (7%), and diMeIBC methyl ester **7d**[14] (1%). The product **MeC 6d** could not be obtained free of the other isomer. However, fractions containing different ratios of the isomers could be analyzed and allowed these tentative assignments by correlation to the data in Refs. 3 and 4. ***6d** (major isomer)*: ^{1}H NMR (C_6D_6): δ 10.09 (s, 2H, H-10 and H-15); 9.16 (s, 1H, H-20); 8.97 (s, 1H, H-5); 4.98 (t, J = 7.5, 1H, H-3); 4.72, 4.71 (m + s, 6H, 3 CH_2CO$_2$Me at C-7, C-13 and C-18); 4.33-4.24 (m, 6H, 3 CH$_2$CH_2CO$_2$Me at C-8, C-12 and C-17); 3.47, 3.41(2), 3.29, 3.28, 3.27, 3.26, 3.10 (8s, 24H, 8 CO$_2$CH_3); 2.95, 2.75 (AB, J = 15.9, 2H, CH_2CO$_2$Me at C-2); 3.23, 3.25 [overlapping with signals of methyl esters] (t + m, 6H, 3 CH_2CH$_2$CO$_2$Me at C-8, C-12 and C-17); 2.46 (m, 2H, CH_2CH$_2$CO$_2$Me at C-3); 2.19 (m, 2H, CH$_2$CH_2CO$_2$Me at C-3); 2.22 (s, 3H, CH_3 at C-2). **6d** *epimer*: ^{1}H NMR (C_6D_6): δ 10.08 (s, 2H, H-10 and H-15); 9.08 (s, 1H, H-20); 8.92 (s, 1H, H-5); 4.73, 4.70 (s + m, 6H, 3 CH_2CO$_2$Me at C-7, C-13 and C-18); 4.62 (m, 1H, H-3); 4.33–4.24 (m, 6H, 3 CH$_2$CH_2CO$_2$Me at C-8, C-12 and C-17); 3.58, 3.51 (AB, J = 15, 2H, CH_2CO$_2$Me at C-2); 3.37, 3.36, 3.31 (2), 3.28, 3.27, 3.26, 3.10 (8s, 24H, 8 CO$_2$CH_3); 3.15, 3.25 [overlapping with signals of methyl esters] (2m, 6H, 3 CH_2CH$_2$CO$_2$Me at C-8, C-12 and C-17); 2.60, 2.11 (2m, 4H, CH_2CH_2CO$_2$Me at C-3); 1.85 (s, 3H, CH_3 at C-2). UV λ_{max} (MeOH) 282 (0.12), 354 (sh, 0.23), 396 (1), 495 (0.1), 589 (0.05), 645 (0.29) nm. MS (ESI) *m/z* 959 $[M+H]^+$, 981 $[M+Na]^+$, 997 $[M+K]^+$.

HRMS (ESI) $[C_{49}H_{50}NaN_4O_{16}]^+$, calcd: 981.3746. Found: 981.3588.

MeC monolactone ***9d***. ^{1}H NMR (C_6D_6): δ 10.21 (s, 1H, H-15); 10.19 (s, 1H, H-10); 9.35 (s, 1H, H-20); 8.80 (s, 1H, H-5); 4.75 (s, 2H, CH_2CO$_2$Me at C-13); 4.70, 4.58 (2 AB, J = 11.4, 8.5, 4H, 2 CH_2CO$_2$Me at C-7 and C-18); 4.33 (AB, J = 6.6, 2H, CH$_2$CH_2CO$_2$Me at C-8); 4.30, 4.06 (2t, J = 7.4, 6.2, 4H, 2 CH$_2$CH_2CO$_2$Me at C-12 and C-17); 3.52, 3.40, 3.34, 3.26 (2), 3.21, 3.20 (6s, 21H, 7 CO$_2$CH_3); 3.29 (m, 6H, 3 CH_2CH$_2$CO$_2$Me at C-8, C-12 and C-17); 2.97, 2.70 (AB, J = 18.0, 2H, CH_2CO$_2$Me at C-2); 2.50 (m, 2H, CH_2CH$_2$CO$_2$Me at C-3); 2.19 (m, 2H, CH$_2$CH_2CO$_2$Me at C-3); 1.72 (s, 3H, CH_3 at C-2). UV λ_{max} (MeOH) 285 (0.14), 347 (sh, 0.23), 393 (1), 495 (0.1), 585 (0.05), 640 (0.23) nm. MS (ESI) *m/z* 943 $[M+H]^+$, 965 $[M+Na]^+$, 981 $[M+K]^+$. HRMS (ESI) $[C_{48}H_{54}NaN_4O_{16}]^+$, calcd: 965.3433. Found: 965.3349.

The product *DiMeIBC* ***7d***[14] was obtained in too small a quantity to allow a full ^{1}H NMR assignment, thus only the most important characterization data are reported: ^{1}H NMR (C_6D_6): δ 8.85 (s, 1H, H-15); 7.61 (s, 1H, H-10); 7.55 (s, 1H, H-20); 6.81 (s, 1H, H-5); 1.74 (s, 3H, CH_3 at C-2); 1.63 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 282 (0.28), 379 (sh, 0.77), 397 (1), 500 (0.11), 545 (0.1), 589 (0.27) nm. MS (ESI) *m/z* 975 $[M+H]^+$. HRMS (ESI) $[C_{50}H_{63}N_4O_{16}]^+$, calcd: 975.4239. Found: 975.3980.

6.2.3.4. From porphyrin (1e). After 2 series of PLC, 4 products were isolated: MeC methyl ester **6e** (2%), diMeIBC methyl ester **7e** (2%), triMeIBC methyl ester and epimers **8e** (28%), and triMeIBC monolactone methyl ester **12e** (9%). *MeC* ***6e***: ^{1}H NMR (C_6D_6): δ 10.09 (s, 1H, H-15); 9.91 (s, 1H, H-10); 9.18 (s, 1H, H-20); 9.00 (s, 1H, H-5); 5.00 (m, 1H, H-3); 4.91 (AB, J = 12.2, 2H, CH_2CO$_2$Me at C-7); 4.72 (s, 2H, CH_2CO$_2$Me at C-18); 4.43, 4.31, 4.19, 4.06 (4m, 6H, 3 CH$_2$CH_2CO$_2$Me at C-8, C-13 and C-17); 3.40, 3.39, 3.31, 3.29, 3.27, 3.24, 3.22 (7s, 21H, 7 CO$_2$CH_3); 3.11 (s, 3H, CH_3 at C-12); 3.29 [overlapping with signals of methyl esters], 3.16 (2m, 6H, 3 CH_2CH$_2$CO$_2$Me at C-8, C-13 and C-17); 2.95, 2.76 (AB, J = 17.5, 2H, CH_2CO$_2$Me at C-2); 2.46 (m, 2H, CH_2CH$_2$CO$_2$Me at C-3); 2.24 (s, 3H, CH_3 at C-2); 2.20 (m, 2H, CH$_2$CH_2CO$_2$Me at C-3). UV λ_{max} (MeOH) 289 (0.10), 348 (sh, 0.21), 393 (1), 496 (0.10), 536 (0.05), 570 (0.04), 649 (0.19) nm. MS (ESI) *m/z* 901 $[M+H]^+$. HRMS (ESI) $[C_{47}H_{57}N_4O_{14}]^+$, calcd: 901.3871. Found: 901.3438.

DiMeIBC ***7e***. ^{1}H NMR (C_6D_6) δ 8.83 (s, 1H, H-15); 7.55 (s, 1H, H-10); 7.43 (s, 1H, H-20); 6.72 (s, 1H, H-5); 4.30, 4.05 (2m, 2H, H-3 and H-8); 4.15 (m, 2H, CH_2CO$_2$Me at C-18); 3.81, 3.67 (2m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.37 (2), 3.33, 3.25, 3.23, 3.21, 3.16 (7s, 21H, 7 CO$_2$CH_3); 3.00, 2.71 (2t, J = 6.8, 6.7, 4H, 2 CH_2CH$_2$CO$_2$Me at C-13 and C-17); 2.70 (m, 2H, CH_2CH$_2$CO$_2$Me at C-8); 2.63 (s, 3H, CH_3 at C-12); 2.63 (d, J = 6.8, 2H, CH_2CO$_2$Me at C-7); 2.67, 2.54 (AB, J = 13.5, 2H, CH_2CO$_2$Me at C-2); 2.30–2.05 (2m, 6H, CH_2CH_2CO$_2$Me at C-3 and CH$_2$CH_2CO$_2$Me at C-8); 1.85 (s, 3H, CH_3 at C-2); 1.49 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 279 (0.27), 357 (sh, 0.76), 381 (1), 405 (sh, 0.70), 500 (0.14), 540 (0.17), 585 (0.22). MS (ESI) *m/z* 939 $[M+Na]^+$. HRMS (ESI) $[C_{48}H_{60}NaN_4O_{14}]^+$, calcd: 939.4004. Found: 939.3731.

TriMeIBC ***8e*** *(major isomer)*[16]. ^{1}H NMR (C_6D_6): δ 8.80 (s, 1H, H-15); 7.41 (d, J = 0.7, 1H, H-10); 6.64 (s, 1H, H-5); 4.27 (AB, J = 17.4, 2H, CH_2CO$_2$Me at C-18); 4.19 (t, J = 6.7, 1H, H-3); 4.13 (AB, J = 3.9, 1H, H-8); 3.80, 3.75, 3.63 (3m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-17); 3.44, 3.34, 3.32, 3.28, 3.27, 3.23, 3.21 (7s, 21H, 7 CO$_2$CH_3); 2.98 (m, 2H, CH_2CH$_2$CO$_2$Me at C-17); 2.85 (s, 3H, CH_3 at C-20); 2.74 (t, J = 7.5, 2H, CH_2CH$_2$CO$_2$Me at C-13); 2.70 (AB, J = 15.3, 2H, CH_2CO$_2$Me at C-7); 2.56 (s, 3H, CH_3 at C-12); 2.54 (AB, J = 14.9, 2H, CH_2CH$_2$CO$_2$Me at C-8); 2.49 (m, 2H, CH_2CO$_2$Me at C-2); 2.30, 2.23, 2.10 (3m, 6H, CH_2CH_2CO$_2$Me at C-3 and CH$_2$CH_2CO$_2$Me at C-8); 1.68 (s, 3H, CH_3 at C-2); 1.49 (s, 3H, CH_3 at C-7). ^{13}C NMR δ 173.27, 173.17, 172.98 (2), 172.26, 171.53, 171.29, 7 *C*O$_2$CH$_3$; 170.17, C-6; 159.46, C-4 and C-9; 153.34, C-1; 151.14, C-19; 143.73, C-11; 141.65, C-17; 136.81, C-13; 135.57, C-16; 131.55, C-14; 125.19, C-18; 123.82, C-12; 107.35, C-15; 104.82, C-20; 94.94, C-10; 90.05, C-5; 55.60, C-3; 51.81, 51.59, 51.10 (2), 51.01, 50.72, 50.56, 7 CO$_2$*C*H$_3$; 44.40, *C*H$_2$CO$_2$Me at C-7; 41.90, *C*H$_2$CO$_2$Me at C-7; 36.85, 36.32, 2 CH$_2$*C*H$_2$CO$_2$Me at C-13 and C-17; 33.95, *C*H$_2$CO$_2$Me at C-18; 32.71, 32.15, 2 CH$_2$*C*H$_2$CO$_2$Me at C-3 and C-8; 25.87, 25.22, 2 *C*H$_2$CH$_2$CO$_2$Me at C-3 and C-8; 21.48, 21.20, 2 *C*H$_2$CH$_2$CO$_2$Me at C-13 and C-17; 20.58, *C*H$_3$ at C-7; 19.97, *C*H$_3$ at C-2; 19.13, *C*H$_3$ at C-20; 10.01, *C*H$_3$ at C-12. UV λ_{max} (MeOH) 274 (0.25), 359 (sh, 0.58), 376 (1), 390 (sh, 0.68), 409 (0.59), 500 (0.09), 550 (0.16), 594 (0.21), 636 (0.08) nm. MS (ESI) *m/z* 931 $[M+H]^+$. HRMS (ESI) $[C_{49}H_{63}N_4O_{14}]^+$, calcd: 931.4341. Found: 931.4225. 2 epimers of **8e** were isolated together and only partial assignments for ^{1}H NMR were possible. ***8e*** *epimer 1*: ^{1}H NMR (C_6D_6): δ 8.72 (s, 1H, H-15); 7.41 (s, 1H, H-10); 6.50 (s, 1H, H-5); 2.78 (s, 3H, CH_3 at C-20); 2.53 (s, 3H, CH_3 at C-12); 1.85 (s, 3H, CH_3 at C-2); 1.43 (s, 3H, CH_3 at C-7). ***8e*** *epimer 2*: ^{1}H NMR (C_6D_6): δ 8.74 (s, 1H, H-15); 7.74 (s, 1H, H-10); 6.56 (s, 1H, H-5); 2.65 (s, 3H, CH_3 at C-20); 2.52 (s, 3H, CH_3 at C-12); 1.67 (s, 3H, CH_3 at C-2); 1.49 (s, 3H, CH_3 at C-7).

TriMeIBC monolactone ***12e***. ^{1}H NMR (C_6D_6): δ 8.95 (s, 1H, H-15); 7.79 (s, 1H, H-10); 6.58 (s, 1H, H-5); 4.27 (AB, J = 19.0, 2H, CH_2CO$_2$Me at C-18); 4.08 (m, 1H, H-3 or H-8); 3.79, 3.60 (2m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 or C-17); 3.43, 3.35, 3.30, 3.27, 3.21, 3.18 (6s, 18H, 6 CO$_2$CH_3); 3.05 (m, 2H, CH_2CH$_2$CO$_2$Me at C-17); 2.99 (AB, J = 8.1, 2H, CH_2CO$_2$Me at C-7); 2.83 (s, 3H, CH_3 at C-20); 2.70 (t, J = 7.0, 2H, CH_2CH$_2$CO$_2$Me at C-13); 2.65, 2.48 (AB, J = 13.4, 2H, CH_2CO$_2$Me at C-2); 2.47 (s, 3H, CH_3 at C-12); 2.44, 2.35 (AB, J = 15.9, 2H, CH_2CH$_2$CO$_2$Me at C-8); 2.25–2.00 (m, 6H, CH_2CH_2CO$_2$Me at C-3 and CH$_2$CH_2CO$_2$Me at C-8); 1.71 (s, 3H, CH_3 at C-2); 1.26 (s, 3H, CH_3 at C-7). ^{13}C NMR δ (most important signals) 107.47 (C-5), 104.15 (C-20), 96.88 (C-10), 94.83 (C-3 or C-8), 90.14 (C-5),

9.75 (CH_3 at C-12). UV λ_{max} (MeOH) 277 (0.25), 358 (sh, 0.50), 377 (1), 393 (sh, 0.70), 412 (0.73), 500 (0.10), 539 (0.13), 583 (0.17), 629 (0.09) nm. MS (ESI) *m/z* 915 $[M+H]^+$. HRMS (ESI) $[C_{48}H_{59}N_4O_{14}]^+$, calcd: 915.4028. Found: 915.3973.

6.2.3.5. From porphyrin (1f). Only one product was isolated in very small quantities: the methyl ester MeC **6f** (1–3%). ^{1}H NMR (C_6D_6): δ (major isomer) 10.29 (s, 1H, H-15); 10.19 (s, 1H, H-10); 9.19 (d, $J = 7.0$, 1H, H-20); 9.06 (d, $J = 5.2$, 1H, H-5); 5.03 (m, 1H, H-3); 4.84 (m, 2H, CH_2CO_2Me at C-7); 4.72, 4.68 (AB + d, $J = 4.9$, 13.8, 4H, 2 CH_2CO_2Me at C-12 and C-18); 4.05, 3.98 (2m, 6H, 3 $CH_2CH_2CH_2CO_2Me$ at C-8, C-13 and C-17); 3.45, 3.44 (3), 3.43 (2), 3.42, 3.31 (8s, 24H, 8 CO_2CH_3); 3.42, 3.41, 3.11 (3m, 6H, 3 $CH_2CH_2CH_2CO_2Me$ at C-8, C-13 and C-17); 3.08, 2.92 (AB, $J = 14.9$, 2H, CH_2CO_2Me at C-2); 2.61 (m, 6H, 3 $CH_2CH_2CH_2CO_2Me$ at C-8, C-13 and C-17); 2.51 (m, 4H, $CH_2CH_2CH_2CO_2Me$ at C-3); 2.28 (s, 3H, CH_3 at C-2); 2.17 (AB, $J = 7.5$, 2H, $CH_2CH_2CH_2CO_2Me$ at C-3). ^{13}C NMR δ (partial assignment) 101.60 (C-15 and C-20), 94.57 (C-10), 92.17 (C-5). UV λ_{max} (MeOH) 288 (0.10), 344 (sh, 0.17), 393 (1), 494 (0.08), 591 (0.03), 643 (0.29) nm. MS (ESI) *m/z* 1015 $[M+H]^+$, 1037 $[M+Na]^+$. HRMS (ESI) $[C_{53}H_{67}N_4O_{16}]^+$, calcd: 1015.4552. Found: 1015.4343.

6.2.3.6. From porphyrin (1j). Four products were isolated: MeC methyl ester **6j** (5%), diMeIBC methyl ester **7j** (13%), diMeIBC monolactone methyl ester **10j** (9%), and diMeIBC dilactone methyl ester **11j** (5%). *MeC **6j***: ^{1}H NMR (C_6D_6): δ 10.10 (s, 1H, H-15); 10.04 (s, 1H, H-10); 9.14 (s, 1H, H-20); 8.86 (s, 1H, H-5); 4.99 (m, 1H, H-3); 4.88, 4.83, 4.78, 4.72 (4s, 10H, 5 CH_2CO_2Me at C-7, C-12, C-13, C-17 and C-18); 4.27 (m, 2H, $CH_2CH_2CO_2Me$ at C-8); 3.47, 3.41 (3), 3.40, 3.32, 3.28, 3.12 (8s, 24H, 8 CO_2CH_3); 3.14 (m, 2H, $CH_2CH_2CO_2Me$ at C-8); 2.94, 2.75 (AB, $J = 1.51$, 2H, CH_2CO_2Me at C-2); 2.56, 2.46, 2.28 (3m, 4H, $CH_2CH_2CO_2Me$ at C-3); 2.20 (s, 3H, CH_3 at C-2). UV λ_{max} (MeOH) 288 (0.16), 358 (sh, 0.25), 396 (1), 496 (0.11), 542 (0.07), 585 (0.09), 645 (0.29) nm. MS (ESI) *m/z* 937 $[M+Li]^+$. HRMS (ESI) $[C_{47}H_{54}LiN_4O_{16}]^+$, calcd: 937.2935. Found: 937.3514.

*DiMeIBC **7j** (major isomer)*. ^{1}H NMR (C_6D_6): δ 8.82 (s, 1H, H-15); 7.59 (s, 1H, H-10); 7.48 (s, 1H, H-20); 6.76 (s, 1H, H-5); 4.35, 4.32, 4.29, 4.24 (s + AB + s + AB, $J = 7.4$, 12.6, 8H, 4 CH_2CO_2Me at C-12, C-13, C-17 and C-18); 4.10, 4.06 (t + m, $J = 6.7$, 2H, H-3 and H-8); 3.41, 3.38, 3.36, 3.35, 3.34, 3.33, 3.23, 3.16 (8s, 24H, 8 CO_2CH_3); 2.61, 2.52 (AB, $J = 15.1$, 2H, CH_2CO_2Me at C-2); 2.52 (AB, $J = 7.9$, 2H, CH_2CO_2Me at C-7); 2.45 (m, 2H, $CH_2CH_2CO_2Me$ at C-8); 2.32, 2.25, 2.21 (3m, 6H, $CH_2CH_2CO_2Me$ at C-3 and $CH_2CH_2CO_2Me$ at C-8); 1.71 (s, 3H, CH_3 at C-2); 1.60 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 280 (0.28), 357 (sh, 0.65), 379 (1), 393 (sh, 0.85), 504 (0.14), 546 (0.18), 589 (0.29) nm. MS (ESI) *m/z* 947 $[M+H]^+$, 969 $[M+Na]^+$. HRMS (ESI) $[C_{48}H_{59}N_4O_{16}]^+$, calcd: 947.3926. Found: 947.3288. 2 minor epimers of **7j** were isolated together in too small a quantity to allow a full ^{1}H NMR assignment, thus only the most important characterization data are reported. ***7j** epimer 1*: ^{1}H NMR (C_6D_6): δ 8.80 (s, 1H, H-15); 7.61 (s, 1H, H-10); 7.35 (s, 1H, H-20); 6.71 (s, 1H, H-5); 4.02, 3.79 (2m, 2H, H-3 and H-8); 1.59 (s, 3H, CH_3 at C-2); 1.52 (s, 3H, CH_3 at C-7). ***7j** epimer 2*: ^{1}H NMR δ 8.78 (s, 1H, H-15); 7.50 (s, 1H, H-10); 7.47 (s, 1H, H-20); 6.68 (s, 1H, H-5); 4.09, 3.69 (2m, 2H, H-3 and H-8); 1.80 (s, 3H, CH_3 at C-2); 1.45 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 278 (0.28), 359 (sh, 0.69), 369 (1), 394 (sh, 0.77), 512 (0.13), 546 (0.21), 589 (0.35). MS (ESI) *m/z* 947 $[M+H]^+$, 969 $[M+Na]^+$. HRMS (ESI) $[C_{48}H_{58}NaN_4O_{16}]^+$, calcd: 969.3746. Found: 969.3166.

*DiMeIBC monolactone **10j** (major isomer)*. ^{1}H NMR (C_6D_6): δ 8.90 (s, 1H, H-15); 7.61 (s, 1H, H-10); 7.45 (s, 1H, H-20); 6.92 (s, 1H, H-5); 4.41, 4.39, 4.28, 4.20 (s + AB + s + AB, $J = 11.8$, 14.1, 8H, 4 CH_2CO_2Me at C-12, C-13, C-17 and C-18); 4.18 (m, 1H, H-3 or H-8); 3.40, 3.27, 3.33 (4), 3.19 (4s, 21H, 7 CO_2CH_3); 2.52 (m, 4H, CH_2CO_2Me at C-2 and C-7); 2.44 (t, $J = 6.7$, 2H, $CH_2CH_2CO_2Me$ at C-3 or C-8); 2.37–1.97 (m, 6H, $CH_2CH_2CO_2Me$ at C-3 or C-8 and $CH_2CH_2CO_2Me$ at C-3 or C-8); 1.43 (s, 3H, CH_3 at C-2); 1.34 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 279 (0.22), 362 (sh, 0.59), 383 (1), 404 (sh, 0.77), 506 (0.11), 542 (0.17), 583 (0.20), 642 (0.05) nm. MS (ESI) *m/z* 953 $[M+Na]^+$. HRMS (ESI) $[C_{47}H_{54}NaN_4O_{16}]^+$, calcd: 953.3433. Found: 953.3226. 2 epimers of **10j** were also isolated. ***10j** epimer 1*: ^{1}H NMR (C_6D_6): δ 8.86 (s, 1H, H-15); 7.97 (s, 1H, H-10); 7.39 (s, 1H, H-20); 6.56 (s, 1H, H-5); 4.32, 4.29, 4.22, 4.17 (AB + s + 2AB, $J = 8.7$, 16.6, 4.8, 8H, 4 CH_2CO_2Me at C-12, C-13, C-17 and C-18); 3.73 (m, 1H, H-3 or H-8); 3.44, 3.37, 3.36, 3.35, 3.34, 3.31, 3.23 (7s, 21H, 7 CO_2CH_3); 2.79, 2.38 (AB, $J = 18.2$, 2H, CH_2CO_2Me at C-2 or C-7); 2.56 (s + m, 4H, CH_2CO_2Me at C-2 or C-7 and $CH_2CH_2CO_2Me$ at C-8); 2.20, 1.97 (2m, 6H, $CH_2CH_2CO_2Me$ at C-3 or C-8 and 2 $CH_2CH_2CO_2Me$ at C-3 and C-8); 1.51 (s, 3H, CH_3 at C-2); 1.20 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 279 (0.29), 358 (sh, 0.63), 378 (1), 405 (sh, 0.6), 503 (0.13), 539 (0.17), 581 (0.23), 640 (0.1). MS (ESI) *m/z* 931 $[M+H]^+$, 953 $[M+Na]^+$. HRMS (ESI) $[C_{47}H_{54}NaN_4O_{16}]^+$, calcd: 953.3433. Found: 953.3249. ***10j** epimer 2*: ^{1}H NMR (C_6D_6) δ 8.88 (s, 1H, H-15); 7.99 (s, 1H, H-10); 7.48 (s, 1H, H-20); 6.59 (s, 1H, H-5); 4.33, 4.30, 4.22, 4.17 (2AB + s + AB, $J = 17.7$, 2.6, 17.3, 8H, 4 CH_2CO_2Me at C-12, C-13, C-17 and C-18); 4.14 (m, 1H, H-3 or H-8); 3.42, 3.37, 3.35 (2), 3.34, 3.32, 3.17 (7s, 21H, 7 CO_2CH_3); 2.79, 2.36, 2.52 (2AB, $J = 18.2$, 14.3, 4H, 2 CH_2CO_2Me at C-2 and C-7); 2.67, 2.59, 2.19 (3m, 8H, 2 $CH_2CH_2CO_2Me$ at C-3 and C-8); 1.71 (s, 3H, CH_3 at C-2); 1.22 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 279 (0.27), 355 (sh, 0.60), 377 (1), 404 (sh, 0.47), 500 (0.12), 538 (0.17), 580 (0.21), 639 (0.08) nm. MS (ESI) *m/z* 931 $[M+H]^+$, 953 $[M+Na]^+$. HRMS (ESI) $[C_{47}H_{54}NaN_4O_{16}]^+$, calcd: 953.3433. Found: 953.3176.

*DiMeIBC dilactone **11j***. ^{1}H NMR (C_6D_6): δ 9.10 (s, 1H, H-15); 8.13 (s, 1H, H-10); 7.63 (s, 1H, H-20); 7.07 (s, 1H, H-5); 4.43, 4.36, 4.30, 4.19 (d + 3AB, $J = 0.9$, 17.5, 5.7,

11.0, 8H, 4 CH_2CO_2Me at C-12, C-13, C-17 and C-18); 3.43, 3.37, 3.34, 3.33, 3.27, 3.19 (6s, 18H, 6 CO_2CH_3); 2.60, 2.35 (AB, $J = 18.4$, 2H, CH_2CO_2Me at C-2); 2.55, 2.23 (AB, $J = 18.3$, 2H, CH_2CO_2Me at C-7); 2.59, 2.41 (2m, 4H, 2 $CH_2CH_2CO_2Me$ at C-3 and C-8); 2.21, 2.08 (2m, 4H, 2 $CH_2CH_2CO_2Me$ at C-3 and C-8); 1.38 (s, 3H, CH_3 at C-2); 1.25 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 278 (0.20), 358 (sh, 0.46), 373 (1), 384 (sh, 0.79), 405 (0.63), 491 (sh, 0.09), 542 (0.14), 582 (0.15), 628 (0.10) nm. MS (ESI) *m/z* 921 $[M+Li]^+$, 937 $[M+Na]^+$. HRMS (ESI) $[C_{46}H_{50}NaN_4O_{16}]^+$, calcd: 937.3120. Found: 937.2852.

6.2.3.7. From porphyrin (1m). Four products were isolated: MeC methyl ester **6m** (2–3%), triMeIBC methyl ester and epimers **8m** (8–11%), triMeIBC monolactone methyl ester **12m** (1%), and triMeIBC dilactone methyl ester **13m** (1%). *MeC **6m***: ^1H NMR (C_6D_6): δ 10.10 (s, 1H, H-15); 10.00 (s, 1H, H-10); 9.16 (s, 1H, H-20); 8.90 (s, 1H, H-5); 4.99 (m, 1H, H-3); 4.76, 4.72 (2s, 4H, 2 CH_2CO_2Me at C-2 and C-7); 4.32, 4.06 (2m, 6H, 3 $CH_2CH_2CO_2Me$ at C-8, C-13 and C-17); 3.47, 3.34, 3.31, 3.28, 3.27 (2), 3.26 (7s, 21H, 7 CO_2CH_3); 3.10 (s, 3H, CH_3 at C-18); 3.26, 3.25 [overlapping with methyl ester signals], 2.97 (2m + t, $J = 8.4$, 6H, 3 $CH_2CH_2CO_2Me$ at C-8, C-13 and C-17); 2.96, 2.78 (AB, $J = 8.3$, 2H, CH_2CO_2Me at C-2); 2.47 (m, 2H, $CH_2CH_2CO_2Me$ at C-3); 2.25 (s, 3H, CH_3 at C-2); 2.04 (m, 2H, $CH_2CH_2CO_2Me$ at C-3). UV λ_{max} (MeOH) 281 (0.1), 350 (sh, 0.21), 396 (1.0), 500 (0.09), 531 (0.04), 587 (0.04), 643 (0.19) nm. MS (ESI) *m/z* 901 $[M+H]^+$, 923 $[M+Na]^+$. HRMS (ESI) $[C_{47}H_{56}NaN_4O_{14}]^+$, calcd: 923.3691. Found: 923.3301.

*TriMeIBC **8m***. ^1H NMR (C_6D_6): δ 8.81 (s, 1H, H-15); 7.41 (s, 1H, H-10); 6.64 (s, 1H, H-5); 4.27 (AB, $J = 17.8$, 2H, CH_2CO_2Me at C-12); 4.18, 4.07 (2m, 2H, H-3 and H-8); 3.77, 3.63 (2m, 4H, 2 $CH_2CH_2CO_2Me$ at C-13 and C-17); 3.44, 3.34, 3.32, 3.29, 3.28, 3.23, 3.20 (7s, 24H, 7 CO_2CH_3); 3.00 (m, 2H, $CH_2CH_2CO_2Me$ at C-17); 2.85 (s, 3H, CH_3 at C-20); 2.74 (m, 2H, $CH_2CH_2CO_2Me$ at C-13); 2.70 (AB, $J = 6.5$, 2H, CH_2CO_2Me at C-7); 2.56 (s, 3H, CH_3 at C-18); 2.54 (AB, $J = 7.5$, 2H, CH_2CO_2Me at C-2); 2.50, 2.31 (2m, 2H, $CH_2CH_2CO_2Me$ at C-8); 2.22, 2.10, 2.04 (3m, 6H, $CH_2CH_2CO_2Me$ at C-3 and $CH_2CH_2CO_2Me$ at C-8); 1.68 (s, 3H, CH_3 at C-2); 1.49 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 276 (0.32), 358 (sh, 0.61), 378 (1.0), 405 (sh, 0.81), 500 (0.12), 540 (0.16), 591 (0.18), 638 (0.07) nm. MS (ESI) *m/z* 931 $[M+H]^+$, 953 $[M+Na]^+$. HRMS (ESI) $[C_{49}H_{63}N_4O_{14}]^+$, calcd: 931.4363. Found: 931.4625. 2 minor epimers of **8m** were isolated together in too small a quantity to allow a full ^1H NMR assignment, thus only the most important characterization data are reported. ***8m** epimer 1*: ^1H NMR (C_6D_6): δ 8.95 (s, 1H, H-15); 7.79 (s, 1H, H-10); 6.58 (s, 1H, H-5); 3.43, 3.34, 3.30, 3.27, 3.25, 3.19, 3.16 (7s, 21H, 7 CO_2CH_3); 2.83 (s, 3H, CH_3 at C-20); 2.47 (s, 3H, CH_3 at C-18); 1.71 (s, 3H, CH_3 at C-2); 1.26 (s, 3H, CH_3 at C-7). ***8m** epimer 2*: 8.85 (s, 1H, H-15); 7.61 (s, 1H, H-10); 6.67 (s, 1H, H-5); 2.84 (s, 3H, CH_3 at C-20); 2.65 (s, 3H, CH_3 at C-18); 1.75 (s, 3H, CH_3 at C-2); 1.46 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 278 (0.23), 359 (sh, 0.57), 380 (1.0), 405 (sh, 0.80), 500 (0.12), 542 (0.15), 582 (0.17), 630 (0.09) nm. MS (ESI) *m/z* 937 $[M + Li]^+$. HRMS (ESI) $[C_{49}H_{62}LiN_4O_{14}]^+$, calcd: 937.3663. Found: 937.4632.

The *TriMeIBC monolactone **12m*** was obtained in too small a quantity to allow a full ^1H NMR assignment, thus only the most important characterization data are reported: ^1H NMR (C_6D_6): δ 8.97 (s, 1H, H-15); 8.00 (s, 1H, H-10); 6.60 (s, 1H, H-5); 3.43, 3.41, 3.37, 3.27, 3.25, 3.16 (6s, 18H, 6 CO_2CH_3); 2.73 (s, 3H, CH_3 at C-20); 2.64 (s, 3H, CH_3 at C-18); 1.78 (s, 3H, CH_3 at C-2); 1.21 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 278 (0.22), 359 (sh, 0.53), 380 (1), 405 (0.74), 500 (0.11), 535 (0.15), 576 (0.16), 629 (0.07) nm. MS (ESI) *m/z* 915 $[M+H]^+$. HRMS (ESI) $[C_{48}H_{59}N_4O_{14}]^+$, calcd: 915.4050. Found: 915.4260.

Two epimers of TriMeIBC dilactone **13m** were isolated together and only partial assignment for ^1H NMR was possible. ***13m** epimer 1*: ^1H NMR (C_6D_6): δ 9.63 (s, 1H, H-15); 8.49 (s, 1H, H-10); 7.75 (s, 1H, H-5); 2.90 (s, 3H, CH_3 at C-20); 2.84 (s, 3H, CH_3 at C-18); 1.55 (s, 3H, CH_3 at C-2); 1.52 (s, 3H, CH_3 at C-7). ***13m** epimer 2*: ^1H NMR δ 9.54 (s, 1H, H-15); 8.29 (s, 1H, H-10); 7.74 (s, 1H, H-5); 3.17 (s, 3H, CH_3 at C-20); 2.63 (s, 3H, CH_3 at C-18); 1.60 (s, 3H, CH_3 at C-2); 1.46 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 286 (0.14), 361 (sh, 0.37), 378 (1), 389 (sh, 0.65), 409 (0.86), 497 (0.08), 536 (0.08), 578 (0.09), 624 (0.12) nm. MS (ESI) *m/z* 899 $[M+H]^+$, 921 $[M+Na]^+$, 937 $[M+K]^+$. HRMS (ESI) $[C_{47}H_{54}NaN_4O_{14}]^+$, calcd: 921.3535. Found: 921.3495.

6.2.3.8. From porphyrin (1n). Four products were isolated: MeC methyl ester **6n** (10%), diMeIBC methyl ester **7n** (2%), diMeIBC monolactone methyl ester **10n** (1%), and diMeIBC dilactone methyl ester **11n** (1%). *MeC **6n** (major isomer)*: ^1H NMR (C_6D_6): δ 10.08 (s, 1H, H-15); 9.89 (s, 1H, H-10); 9.17 (s, 1H, H-20); 9.00 (s, 1H, H-5); 4.99 (m, 1H, H-3); 4.72, 4.69 (2s, 6H, 2 CH_2CO_2Me at C-7 and C-12); 4.27, 4.18 (m + t, $J = 7.4$, 6H, 3 $CH_2CH_2CO_2Me$ at C-8, C-13 and C-18); 3.47, 3.40, 3.32, 3.28, 3.27, 3.24, 3.23 (7s, 21H, 7 CO_2CH_3); 3.27, 3.15 [overlapping the methyl ester signals] (m + t, $J = 7.3$, 6H, 3 $CH_2CH_2CO_2Me$ at C-8, C-13 and C-18); 3.11 (s, 3H, CH_3 at C-17); 2.95, 2.76 (AB, $J = 15.1$, CH_2CO_2Me at C-2); 2.47 (m, 2H, $CH_2CH_2CO_2Me$ at C-3); 2.25 (s, 3H, CH_3 at C-2); 2.24, 2.19 (2m, 2H, $CH_2CH_2CO_2Me$ at C-3). The ***6n** epimer* could not be obtained free of the major isomer and only partial assignment for ^1H NMR was possible: ^1H NMR δ 10.08 (s, 1H, H-15); 9.89 (s, 1H, H-10); 9.10 (s, 1H, H-20); 8.95 (s, 1H, H-5); 4.65 (m, 1H, H-3); 3.73, 3.54 (AB, $J = 16.9$, 2H, CH_2CO_2Me at C-2); 3.20 (s, 3H, CH_3 at C-17); 1.85 (s, 3H, CH_3 at C-2). UV λ_{max} (MeOH) 282 (0.09), 346 (sh, 0.21), 394 (1.0), 495 (0.09), 535 (0.04), 592 (0.04), 650 (0.24) nm. MS (ESI) *m/z* 901 $[M+H]^+$, 923 $[M+Na]^+$. HRMS (ESI) $[C_{47}H_{57}N_4O_{14}]^+$, calcd: 901.3893. Found: 901.3491.

The product *DiMeIBC **7n*** was obtained in too small a quantity to allow a full ^1H NMR assignment, thus only

the most important characterization data are reported: ^{1}H NMR (C_6D_6): δ 8.71 (s, 1H, H-15); 7.69 (s, 1H, H-10); 7.53 (s, 1H, H-20); 6.83 (s, 1H, H-5); 2.78 (s, 3H, CH_3 at C-17); 1.75 (s, 3H, CH_3 at C-2); 1.65 (s, 3H, CH_3 at C-7). MS (ESI) *m/z* 917 $[M+H]^+$. HRMS (ESI) $[C_{48}H_{61}N_4O_{14}]^+$, calcd: 917.4206. Found: 917.3866.

DiMeIBC monolactone ***10n***. Compound **10n** and one epimer were isolated together and only partial assignment for ^{1}H NMR was possible. ***10n*** *(major isomer)*: ^{1}H NMR (C_6D_6) δ 8.85 (s, 1H, H-15); 7.77 (s, 1H, H-10); 7.61 (s, 1H, H-20); 6.99 (s, 1H, H-5); 2.81 (s, 3H, CH_3 at C-17); 1.50 (s, 3H, CH_3 at C-2); 1.41 (s, 3H, CH_3 at C-7). ***10n*** *epimer*: ^{1}H NMR δ 8.79 (s, 1H, H-15); 7.69 (s, 1H, H-10); 7.57 (s, 1H, H-20); 6.95 (s, 1H, H-5); 2.78 (s, 3H, CH_3 at C-17); 1.46 (s, 3H, CH_3 at C-2); 1.39 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 280 (0.22), 351 (sh, 0.54), 386 (1), 405 (sh, 0.85), 500 (0.13), 540 (0.15), 582 (0.17), 644 (0.12) nm. MS (ESI) *m/z* 901 $[M+H]^+$, 923 $[M+Na]^+$. HRMS (ESI) $[C_{47}H_{56}NaN_4O_{14}]^+$, calcd: 923.3691. Found: 923.3378.

DiMeIBC dilactone ***11n***. ^{1}H NMR (C_6D_6): δ 9.15 (s, 1H, H-15); 8.37 (s, 1H, H-10); 7.86 (s, 1H, H-20); 7.37 (s, 1H, H-5); 4.16 (s, 2H, CH_2CO$_2$Me at C-12); 3.90, 3.83, 3.70, 3.59 (4m, 4H, 2 CH$_2$CH_2CO2Me at C-13 and C-18); 3.44, 3.25, 3.21, 3.20, 3.16 (5s, 15H, 5 CO$_2$CH_3); 2.93 (s, 3H, CH_3 at C-17); 2.93, 2.71 (2t, J = 8.0, 7.3, 4H, 2 CH$_2$CH_2CO$_2$Me at C-13 and C-18); 2.81, 2.49 (AB, J = 18.3, 2H, CH_2CO$_2$Me at C-2); 2.72 (m, 2H, CH_2CH$_2$CO$_2$Me at C-8); 2.60, 2.28 (AB, J = 18.5, 2H, CH_2CO$_2$Me at C-7); 2.57 (m, 2H, CH_2CH$_2$CO$_2$Me at C-3); 2.28, 2.13, 2.02 (3m, 4H, 2 CH$_2$CH_2CO$_2$Me at C-3 and C-8); 1.46 (s, 3H, CH_3 at C-2); 1.36 (s, 3H, CH_3 at C-7). UV λ_{max} (MeOH) 282 (0.15), 357 (sh, 0.34), 375 (1.0), 383 (sh, 0.65), 405 (0.81), 485 (0.09), 576 (0.11), 621 (0.16) nm. MS (ESI) *m/z* 885 $[M+H]^+$, 907 $[M+Na]^+$. HRMS (ESI) $[C_{46}H_{52}Na\ N_4O_{14}]^+$, calcd: 907.3378. Found: 907.3082.

Acknowledgments

We thank the NIH (NIDDK) for financial support and Dr. Mario D. Gonzalez for uro II and IV samples. Use of the TAMU/LBMS-Applications Laboratory is also acknowledged.

References and notes

1. Scott, A. I.; Roessner, C. A.; Santander, P. J. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Genetic and mechanistic exploration of the two pathways of vitamin B12 biosynthesis; Academic Press: San Diego, CA, 2003; Vol. 12, pp 211–228.
2. Deeg, R.; Kriemler, H. P.; Bergmann, K. H.; Müller, G. Z. *Physiol. Chem.* **1977**, *358*, 339–352.
3. Battersby, A. R.; Seo, S. *J. Chem. Soc., Perkin Trans. 1* **1983**, *1*, 3049–3052.
4. Bykhovskii, V. Y.; Santander, P. J.; Stupperikh, E.; Zaitseva, N. I.; Pusheva, M. A.; Detkova, E. N.; Valyushok, D. S.; Scott, A. I. *Prikl. Biokhim. Mikrobiol.* **1996**, *32*, 185–193.
5. Scott, A. I.; Irwin, A. J.; Seigel, L. M.; Shoolery, J. N. *J. Am. Chem. Soc.* **1978**, *100*, 316–318.
6. Gonzalez, M. D.; Williams, H. J.; Santander, P. J.; Ozaki, S.; Stolowich, N. J.; Scott, A. I. *Tetrahedron* **1992**, *48*, 5951–5960.
7. Müller, G.; Gneuss, K. D.; Kriemler, H.-P.; Irwin, A. J.; Scott, A. I. *Tetrahedron* **1981**, *37*, 81–90.
8. Warren, M. J.; Roessner, C. A.; Santander, P. J.; Scott, A. I. *Biochem. J.* **1990**, *265*, 725–729.
9. Blanche, F.; Debussche, L.; Thibaut, D.; Crouzet, J.; Cameron, B. *J. Bacteriol.* **1989**, *171*, 4222–4231.
10. Warren, M. J.; Roessner, C. A.; Ozaki, S.; Stolowich, N. J.; Santander, P. J.; Scott, A. I. *Biochemistry* **1992**, *31*, 603–609.
11. Thibaut, D.; Couder, M.; Crouzet, J.; Debussche, L.; Cameron, B.; Blanche, F. *J. Bacteriol.* **1990**, *172*, 6245–6251.
12. Scott, A. I.; Williams, H. J.; Stolowich, N. J.; Karuso, P.; Gonzalez, M. D.; Blanche, F.; Thibaut, D.; Müller, G.; Savvidis, E.; Hlineney, K. *J. Chem. Soc. Chem. Commun.* **1989**, *1*, 522–525.
13. Blanche, F.; personal communications; 1989.
14. Warren, M. J.; Gonzalez, M. D.; Williams, H. J.; Stolowich, N. J.; Scott, A. I. *J. Am. Chem. Soc.* **1990**, *112*, 5343–5345.
15. Battersby, A. R.; Bladon, C.; Huang, J.-J.; Seo, S. *J. Chem. Soc. Chem. Commun.* **1986**, *1*, 1492–1495.
16. Blanche, F.; Handa, S.; Thibaut, D.; Gibson, C. L.; Leeper, F. J.; Battersby, A. R. *J. Chem. Soc. Chem. Commun.* **1988**, *1*, 1117–1119.
17. Pandey, R. K.; Zheng, G. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Photosensitizers in Photodynamic Therapy; Academic Press: San Diego, CA, 2000; Vol. 6, pp 157–230.
18. Chen, Y.; Li, G.; Pandey, R. K. *Curr. Org. Chem.* **2004**, *8*, 1105–1134.
19. Pichon-Santander, C.; Scott, A. I. *Tetrahedron Lett.* **2005**, *46*, 8669–8672.
20. Vicente, M. G. H.; Smith, K. M. *Curr. Org. Chem.* **2000**, *4*, 139–174.
21. Shanmugathasan, S.; Edwards, C.; Boyle, R. W. *Tetrahedron* **2000**, *56*, 1025–1046.
22. Pichon, C.; Atshaves, B. P.; Stolowich, N. J.; Scott, A. I. *Bioorg. Med. Chem.* **1994**, *2*, 153–168.
23. Pichon, C.; Atshaves, B. P.; Xue, T.; Stolowich, N. J.; Scott, A. I. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1105–1110.
24. Roessner, C. A.; Park, J.-H.; Scott, A. I. *Bioorg. Med. Chem.* **1999**, *7*, 2215–2219.
25. Battersby, A. R.; Jones, K.; McDonald, E.; Robinson, J. A.; Morris, H. R. *Tetrahedron Lett.* **1977**, *25*, 2213–2216.
26. Battersby, A. R.; McDonald, E.; Morris, H. R.; Thompson, M.; Williams, D. C. *Tetrahedron Lett.* **1977**, *25*, 2217–2220.
27. Battersby, A. R.; McDonald, E.; Neier, R.; Thompson, M. *J. Chem. Soc. Chem. Commun.* **1979**, *1*, 960–961.
28. Scott, A. I.; Georgopapadakou, N.; Ho, K. S.; Klioze, S.; Lee, E.; Lee, S. L.; Temme, G. H., III; Townsend, C. A.; Armitage, I. M. *J. Am. Chem. Soc.* **1975**, *97*, 2548–2550.
29. Battersby, A. R.; McDonald, E.; Hollenstein, R.; Ihara, M.; Satoh, F.; Williams, D. C. *J. Chem. Soc., Perkin Trans. 1* **1977**, 166–178.
30. Scott, A. I.; Williams, H. J.; Stolowich, N. J.; Karuso, P.; Gonzalez, M. D.; Müller, G.; Hlineney, K.; Savvidis, E.; Schneider, E.; Traub-Eberhard, U.; Wirth, G. *J. Am. Chem. Soc.* **1989**, *111*, 1897–1900.
31. Shaw, S. J.; Shanmugathasan, S.; Clarke, O. J.; Boyle, R. W.; Osborne, A. G.; Edwards, C. *J. Porphyrins Phthalocyanines* **2001**, *5*, 575–581.

32. Battersby, A. R.; Fookes, C. J. R.; Gustafson-Potter, K. E.; McDonald, E.; Matcham, G. W. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2413–2427.
33. Kenner, G. W.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc. Chem. Commun.* **1973**, 43–44.
34. Smith, K. M. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; pp 758–759.
35. Battersby, A. R.; McDonald, E.; Wurziger, H. K. W.; James, K. J. *J. Chem. Soc. Chem. Commun.* **1975**, 493–494.
36. Battersby, A. R.; McDonald, E.; Hollenstein, R.; Ihara, M.; Satoh, F.; Williams, D. C. *J. Chem. Soc. Perkin Trans. 1* **1977**, 166–178.
37. Battersby, A. R.; Hunt, E.; McDonald, E.; Paine, J. B., III; Saunders, J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1008–1018.