Biosynthesis of Uroporphyrinogens. Synthesis of α-Aminomethylbilanes and Their Interaction with the Enzymatic System

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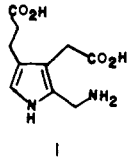
Abstract: The synthesis of α-aminomethylbilanes of biosynthetic interest is described. It is based on synthesis of conveniently prepared *b*-bilene hydrobromides in which the α-unsubstituted position was protected by suitable esters, and the aminomethyl group was protected in the form of a six-membered lactam ring with a terminal acetic acid residue. Hydrogenation of the bilenes, cleavage of the protecting esters, and saponification of the lactam rings and side-chain esters led to α-aminomethylbilanes. An α-aminomethylbilane that is formally derived from the self-condensation of four units of porphobilinogen was examined as a possible biosynthetic intermediate of uroporphyrinogen biosynthesis. It was not found to be a substrate of either porphobilinogen deaminase, uroporphyrinogen III cosynthase, or the deaminase–cosynthase system. The same amount of total uroporphyrinogens was formed by the chemical cyclization of the bilane in the absence or presence of enzymes. The chemical cyclization of the bilane was found to give almost exclusively uroporphyrinogen I (93–100%). No enzymatic formation of uroporphyrinogen III at expense of the bilane was detected. An inhibitory effect of the bilane on the enzymatic formation of uroporphyrinogen III from porphobilinogen was observed.

The enzymatic polymerization of porphobilinogen (PBG, **1**) to form uroporphyrinogen (uro'gen) III (**2**) is the reaction by which porphyrins are made in nature. A dual enzyme system consisting of porphobilinogen deaminase and uro'gen III cosynthase catalyzes the reaction; the deaminase consumes the substrate **1** but forms only uro'gen I (**3**), while the cosynthase does not consume **1** but in its presence the reaction forms uro'gen III (**2**).[1] The early outstanding work of Bogorad, Granick, and others firmly established that the deaminase–cosynthase reaction is entirely intramolecular,[1] and therefore the mechanism of the reaction was investigated with the help of synthetic isomeric α-aminomethylpyrrylmethanes.[2-5] It was found that the dipyrrylmethane **4** is enzymatically incorporated in the presence of **1** only into uro'gen I (**3**) and not into uro'gen



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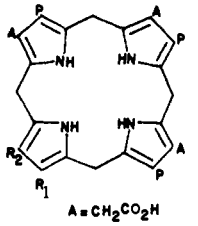
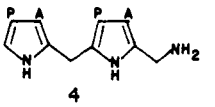


A = CH_2CO_2H
P = $CH_2CH_2CO_2H$
2, R_1 = P ; R_2 = A
3, R_1 = A ; R_2 = P

III (**2**), while it simultaneously dimerizes during a parallel chemical reaction to give uro'gen I (**3**) and uro'gen IV.[1,2,3,6] It was also found that dipyrrylmethane **4** is not dimerized by the deaminase to form uro'gen I (**3**); it only reacts with additional PBG **1** to form **3**.[2,3,7] The biosynthesis of uro'gen III (**2**) from **1** was found to follow an entirely different pathway.[1,5,8] These results were recently challenged by reports[9,10,11] informing that the α-aminomethylbilane **5** (a formal dimerization product of **4**) is enzymatically incorporated into uro'gen III (**2**). These reports are also self-conflicting. One report[9] claims that **5** is a substrate of the deaminase, since more uro'gen I (**3**) is formed in the presence of the latter than during the chemical cyclization of **5** to **3**. In the presence of deaminase–cosynthase this enzymatic surplus was 80% uro'gen III (**2**). The other reports[10,11] reported that while **5** is not a substrate of the deaminase (the same amount of total uro'gens was formed in the absence of the enzymes), by incubation of **5** with deaminase–cosynthase 80% of the total reaction product was



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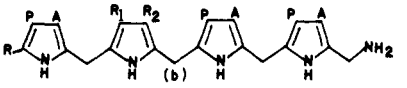

P = $CH_2CH_2CO_2H$; A = CH_2CO_2H
5, R = H ; R_1 = P ; R_2 = A
6, R = CO_2H; R_1 = P ; R_2 = A
7, R = CO_2H; R_1 = A ; R_2 = P

uro'gen III (**2**). The reports also outline the synthetic methods used to obtain **5**, either by a pH-controlled condensation of 2-aminomethyldipyrrylmethanes[9] or by the reduction of an *a,c*-biladiene.[10]

In order to examine the interaction of **5** (and its isomers) with the uro'gen-forming enzymes, we developed a synthetic approach which we found useful to prepare a number of bilanes of biosynthetic interest.

Synthesis of α-Aminomethylbilanes

Bilanes are rather exotic compounds, reputed as unstable and prone to self-isomerization.[12] The α-carboxybilane **6** was obtained by the reduction of an α-benzyloxycarbonyl *a*-bilene.[13] The recently described syntheses of *a,c*-biladienes[14,15] are potentially useful for bilane synthesis, since *a,c*-biladienes are reduced to bilanes.[10] The synthesis of *a,c*-biladienes using acid-sensitive pyrroles is not, however, easy to achieve. The synthesis of bilanes by the pH-controlled condensation of unprotected 2-aminomethyldipyrrylmethanes[9] gave, in our hands, mixtures of isomers.

With the expectation that suitable protected *b*-bilenes will allow the cleavage of the protecting groups and the hydrogenation to bilanes of type **5**, the former were our first synthetic objectives. The synthesis of the necessary 2-formyldipyrrylmethanes was hence explored (Scheme I). The readily obtained acetate **7** was condensed with the α-unsubstituted pyrrole **8**[16] as described elsewhere.[17] The asymmetric dipyrrylmethane **9** was thus prepared in 75% yield, but it was in most cases contaminated with the symmetric 2,5-diethoxycarbonyldipyrrylmethane **10** (see Scheme II). The formation of symmetric dipyrrylmethanes in this type of synthesis is not unusual and has been discussed elsewhere.[18] A complete purification of the

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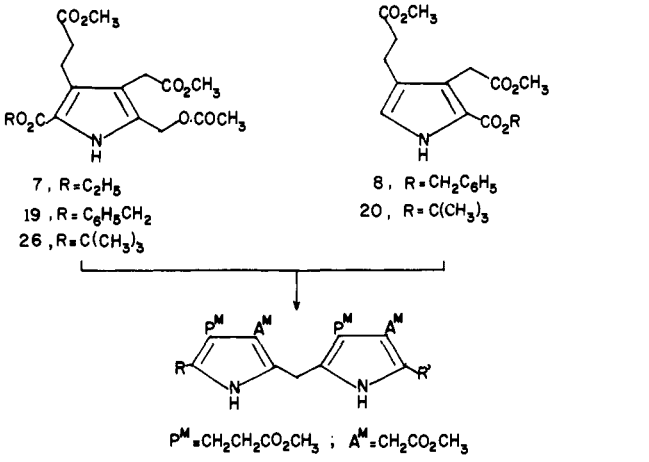
Scheme I. Synthesis of Asymmetric Formyldipyrrylmethanes



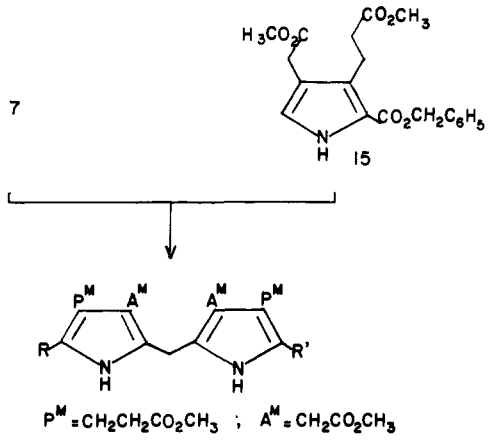
7, R=C_2H_5
19, R=$C_6H_5CH_2$
26, R=$C(CH_3)_3$

8, R=$CH_2C_6H_5$
20, R=$C(CH_3)_3$

P^M=$CH_2CH_2CO_2CH_3$; A^M=$CH_2CO_2CH_3$

9, R=$CO_2C_2H_5$; R'=CO_2Ph
11, R=$CO_2C_2H_5$; R'=CO_2H
12, R=$CO_2C_2H_5$; R'=H
13, R=$CO_2C_2H_5$; R'=CHO
21, R=CO_2CH_2Ph ; R'=$CO_2C(CH_3)_3$
22, R=CO_2CH_2Ph ; R'=CO_2H
24, R=CO_2CH_2Ph ; R'=H
25, R=CO_2CH_2Ph ; R'=CHO
29, R=$CO_2C(CH_3)_3$; R'=CO_2CH_2Ph
30, R=$CO_2C(CH_3)_3$; R'=CO_2H
32, R=$CO_2C(CH_3)_3$; R'=H
33, R=$CO_2C(CH_3)_3$; R'=CHO

Scheme II. Synthesis of a Symmetric Formyldipyrrylmethane



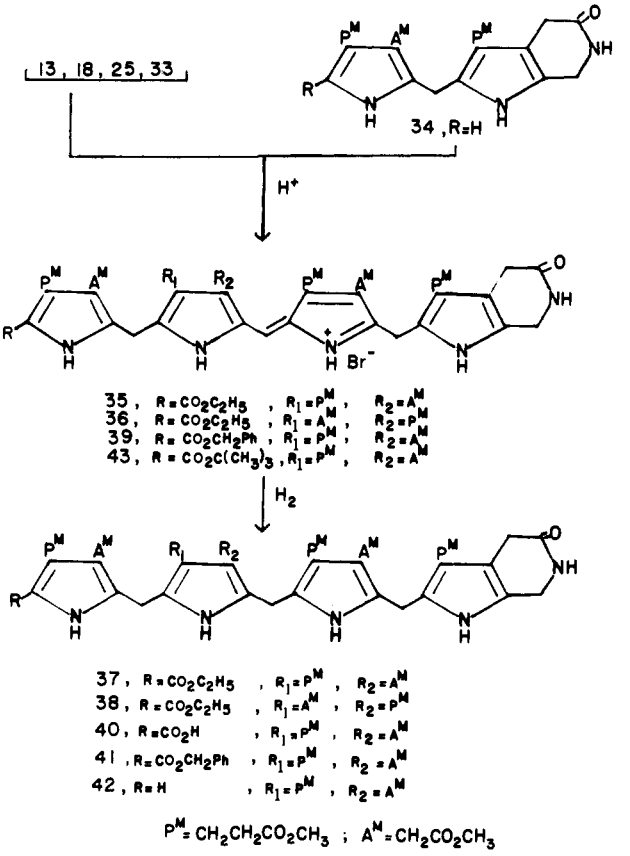
7

15

P^M=$CH_2CH_2CO_2CH_3$; A^M=$CH_2CO_2CH_3$

10, R=$CO_2C_2H_5$; R'=$CO_2C_2H_5$
14, R=$CO_2C_2H_5$; R'=CO_2CH_2Ph
16, R=$CO_2C_2H_5$; R'=CO_2H
17, R=$CO_2C_2H_5$; R'=H
18, R=$CO_2C_2H_5$; R'=CHO
23, R=R'=CO_2CH_2Ph
31, R=R'=$CO_2C(CH_3)_3$

Scheme III. Synthesis of *b*-Bilenes and Bilanes



13, 18, 25, 33

34, R=H

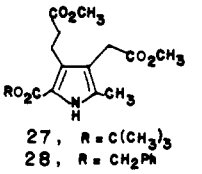
35, R=$CO_2C_2H_5$, $R_1=P^M$, $R_2=A^M$
36, R=$CO_2C_2H_5$, $R_1=A^M$, $R_2=P^M$
39, R=CO_2CH_2Ph, $R_1=P^M$, $R_2=A^M$
43, R=$CO_2C(CH_3)_3$, $R_1=P^M$, $R_2=A^M$

37, R=$CO_2C_2H_5$, $R_1=P^M$, $R_2=A^M$
38, R=$CO_2C_2H_5$, $R_1=A^M$, $R_2=P^M$
40, R=CO_2H, $R_1=P^M$, $R_2=A^M$
41, R=CO_2CH_2Ph, $R_1=P^M$, $R_2=A^M$
42, R=H, $R_1=P^M$, $R_2=A^M$

P^M=$CH_2CH_2CO_2CH_3$; A^M=$CH_2CO_2CH_3$

asymmetric dipyrrylmethanes was achieved by the hydrogenolysis of **9** to **11**, followed by the chromatographic separation of **11** and **10**. Thermal decarboxylation of **11** afforded **12** (74% yield), which was formylated with dimethylformamide–benzoyl chloride.[19] The resulting immonium chloride was extracted into water, and was hydrolyzed at pH 8 to give the aldehyde **13**. The synthesis of dipyrrylmethane **14** was achieved in a similar manner, by condensing the acetate **7** with the α-unsubstituted pyrrole **15**. The contaminant **10** was easily separated from the acid **16** after hydrogenolysis, and the latter was decarboxylated at 200 °C in vacuo to **17**. The formyldipyrrylmethane **18** was obtained from **17** as described above (Scheme II). The condensation of the acetate **19** and the α-unsubstituted pyrrole **20** gave the asymmetric dipyrrylmethane **21**. The *tert*-butyloxycarbonyl pyrrole **20** was obtained by hydrogenolysis of the benzyl ester **8** and subsequent esterification of the resulting unstable acid with *tert*-butyl alcohol in the presence of dicyclohexylcarbodiimide (DCC),[20] Treatment of **21** with 40% hydrogen bromide in glacial acetic acid at 10 °C gave the acid **22** (74% yield), which could then be separated from the small amounts of the contaminant dibenzyloxycarbonyldipyrrylmethane **23**. Decarboxylation of **22** in vacuo at 200 °C gave the α-unsubstituted dipyrrylmethane **24**. The latter can also be obtained directly from **21** by the usual treatment with trifluoroacetic acid, but the separation of **24** from **23** is very difficult. Formylation of **24** gave **25** in 60% yield.

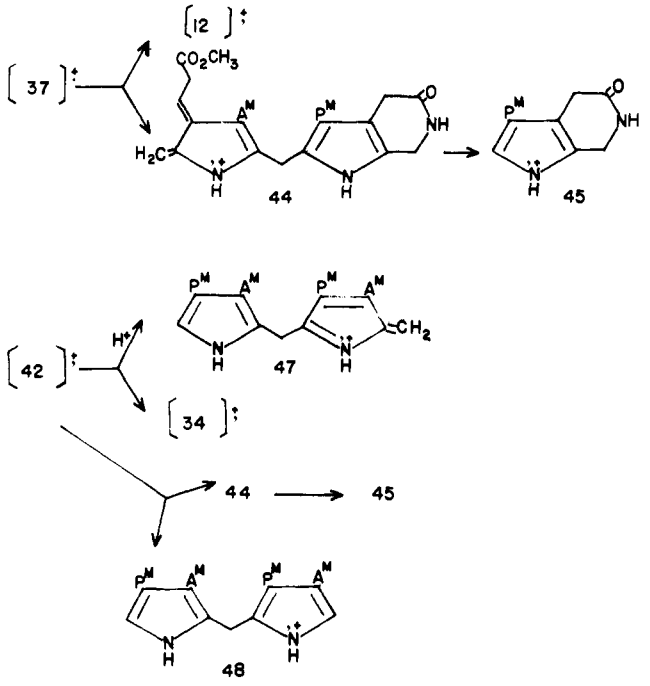
To prepare the *tert*-butyloxycarbonyl acetate **26**, the pyrrole **27** was obtained by hydrogenolysis of **28**, followed by esterification of the acid with *tert*-butyl alcohol–DCC. The acetate **26** was obtained from **27** with the usual lead tetraacetate treatment. The condensation of **26** with **8** gave the dipyrrylmethane **29**, which was hydrogenated to give the dipyrrylmethane acid **30**. The latter was purified by chromatography to separate the contaminant **31**, and then decarboxylated to **32**. Formylation of **32** gave **33** in 70% yield.



27, R=$C(CH_3)_3$
28, R=CH_2Ph

The available formyldipyrrylmethanes **13**, **18**, **25**, and **33** were condensed with the dipyrrylmethane lactam **34**[2] in the search for conveniently protected *b*-bilenes. The condensations were performed in methanol–48% hydrobromic acid and the *b*-bilene hydrobromides readily precipitated from the solution. The ethoxycarbonyl *b*-bilene hydrobromides **35** and **36** (Scheme III) were hydrogenated over 10% Pd on charcoal in the presence of morpholinomethylpolystyrene to give the bilanes **37** and **38**. The fragmentation patterns of the MS-EI spectra[21] of **37** and **38** (Scheme IV) were similar to those of the analogous tripyrranes[22] and helped to secure their struc-

Scheme IV. Mass Spectral Fragmentations of Bilanes 37 and 42



ture. Hydrogenation of the benzyloxycarbonyl *b*-bilene hydrobromide **39** gave a mixture of the carboxybilane **40** (32% yield) and the benzyloxycarbonylbilane **41** (17% yield). The thermal decarboxylation of **40** at 220 °C in vacuo led to the desired α-unsubstituted bilane **42**, but no conditions were found to achieve the total hydrogenolysis of **39** to **40**. The search included the use of PtO_2 instead of palladium catalysts, the use of bases (triethylamine, sodium acetate) other than the polystyrene resin, and even high-pressure (1000 psi) conditions. Hence an alternate pathway was followed. The bilene hydrobromide **43** was treated with 20% hydrogen bromide in glacial acetic acid to cleave the *tert*-butyloxycarbonyl residue and the resulting hydrobromide was hydrogenated as described above. The bilane **42** was the only pyrrylmethane obtained from **43** as judged from multiple TLC analyses. Its MS-EI spectrum (Scheme IV) also lent support to structure **42**. The bilane lactams **42**, **37**, and **38** were saponified to **5**, **6**, and **7** by following the procedure used with analogous pyrrylmethanes.[2,22] They were dissolved in a deaerated 2 N potassium hydroxide solution in 50% ethanol that was kept at 20 °C. The course of the saponification was monitored by NMR and was complete after 72 h. The ethanol was evaporated and, after the solution was adjusted to the desired pH (HCl or IRC 50-H^+ resin), it was used directly for the chemical and enzymatic studies.

Interaction among the Bilanes and the Enzymatic System

To examine if bilanes are possible substrates of deaminase, cosynthase, or deaminase-cosynthase, it is first necessary to find out the pattern of their chemical cyclization to uroporphyrinogens, since the latter reaction also takes place under the conditions used for the enzymatic studies.

The reactions were carried out at the desired conditions of pH, time, and temperature, and they were then stopped by addition of a 1% aqueous iodine solution that oxidized the uro'gens to uroporphyrins and the unreacted bilanes to biladienes. The excess of iodine was destroyed by addition of a 2% sodium thiosulfate solution, and the uroporphyrins were measured spectrophotometrically.[23] To determine the isomer composition of the products, the reaction mixtures were evaporated to dryness after oxidation, the residue was esterified with 5% sulfuric acid in methanol (17 h at 20 °C), and the resulting uroporphyrin octamethyl esters were decarboxylated to the corresponding coproporphyrins.[24] The coproporphyrin I, II, and III/IV isomers were separated by TLC on cellulose-coated plates (E. Merck, DC-fertigplatten, 0.1-μm schichtdicke) using 2,6-lutidine-11 N ammonia solution (40:25, v/v). The coproporphyrin isomers were located by fluorescence and were eluted with 0.7 M ammonium hydroxide. The porphyrin concentration in the eluates was measured by both spectrophotometric and spectrofluorometric methods. Isomers III and IV were measured together, although the presence of isomer IV in the mixture was determined by a separate TLC of the coproporphyrin III/IV fraction as described elsewhere.[6]

The chemical cyclization of **5** at pH 7.4 and 37 °C during 1 h gave 9-13% of uroporphyrinogens, and the yield increased to 20-24% when the reaction was carried out at 55 °C. Uroporphyrinogen I was the main isomer (93-100%) in the reaction products, the rest being uro'gens III/IV. When the reaction was carried out at pH 4.5 and 37 °C during 1 h the total amount of uroporphyrinogens formed decreased to 5%. The proportion of uro'gen I in the reaction products also decreased to 90%, and the rest were uro'gens III/IV. By heating the α-carboxybilane **6** at pH 7.4 and 37 °C for 1 h it formed 3% of total uro'gens of which 75-80% was uro'gen I and the rest were uro'gens III/IV (see also ref 13). No uro'gen II was detected in the reaction products. The uro'gen yields increased with temperature and time (6% was formed at 55 °C in 1 h), but the isomer composition of the reaction products remained fairly constant. When the cyclization was carried out at pH 10 (37 °C, 1 h), 1% of total uro'gens was formed, of which 90% was uro'gen I and 10% were uro'gens III/IV. At pH 5.5 (37 °C, 1 h) 3% of total uro'gens was formed, of which 60% was uro'gen I and 40% were uro'gens III/IV. At pH 2.2 (37 °C, 1 h) the proportion of uro'gen I in the reaction products decreased to 40%, while that of uro'gens III/IV increased to 60%.

The formation of uro'gen I during the chemical cyclization of **5** and **6** is the expected reaction, while the formation of uro'gens III/IV must be due to a rupture at the methane (b) of **5** followed by a recombination of both dipyrrylmethane fragments to give the latter isomers (such reactions are known[5]). The cyclization of **7** lends support to this assertion, since it gave 13% of uro'gen formation at pH 7.4 and 37 °C in 1 h, of which 90% were uro'gens III/IV, 5% was uro'gen I, and 5% was uro'gen II. At lower pH (pH 5.5, 37 °C, 1 h), 13% of uro'gens was also formed, but the proportion of uro'gens III/IV in the reaction product decreased to 80% while that of uro'gens II and I increased in the same proportion. This is what could be expected if a cleavage of **7** at the methane (b) was followed by a recombination of both dipyrrylmethanes to form uro'gens II and I. It can be concluded that, while the expected cyclization of **5** to give uro'gen I is the main chemical reaction under the conditions used in the enzymatic incubations, a secondary reaction arising from the fragmentation of the bilane chain can also afford other uro'gen isomers.

To determine if bilane **5** was a substrate of the deaminase or the deaminase-cosynthase system, the enzymes isolated from wheat germ and from human erythrocytes were used. Different concentrations of bilane **5** and of enzymes were assayed (Table I). Two different amounts of wheat germ deaminase and of deaminase-cosynthase of human erythrocytes were used. One of the latter entirely consumed porphobilinogen **1** at the given incubation conditions (Table I). No increase in the amounts of uro'gens formed could be detected in any case as compared to the values obtained for uro'gen formation when the bilane was incubated in the absence of the enzymes. Therefore, bilane **5** is not a substrate of either deaminase or deaminase-cosynthase, since the same amount of uro'gens (12-13% yield) was formed by **5** in the presence or in the absence of the enzymes (Table I). To further check that the deaminase and the deaminase-cosynthase system were

Table I. Interaction of Bilane **5** and Porphobilinogen Deaminase and Uro'gen III Cosynthase[a]

enzymatic system	addition	uro'gen formed, nmol
	bilane (14 nmol)	1.80
deaminase (25 μL)	bilane (14 nmol)	1.75
deaminase (25 μL)	bilane (14 nmol) + PBG	3.50
deaminase (25 μL)	PBG	1.90
deaminase (75 μL)	PBG	4.00
deaminase (75 μL)	bilane (14 nmol)	1.70
	bilane (28 nmol)	3.90
deaminase-cosynthase (50 μL)	bilane (28 nmol)	3.80
deaminase-cosynthase (50 μL)	bilane (28 nmol) + PBG	4,70
deaminase-cosynthase (50 μL)	PBG	1.06
deaminase-cosynthase (100 μL)	PBG	3.80
deaminase-cosynthase (100 μL)	bilane (28 nmol)	3.70

[a] The incubation mixture contained in a final volume of 150 μL: 15 μmol of phosphate buffer (pH 7.4), bilane **5** (the indicated amount), PBG (16 nmol, when indicated), and enzyme. The deaminase used was purified from wheat germ[23] and gave only uro'gen **I**. The deaminase-cosynthase was isolated from human erythrocytes.[25] Similar results were obtained when wheat germ deaminase-cosynthase was used. The incubations were carried out at 37 °C during 1 h. Uroporphyrins were estimated spectrophotometrically.

active in the presence of **5**, their activity toward the natural substrate (PBG, **1**) was assayed in the presence of the former. Uro'gen formation was found to be only a little less (3%) than the added amounts of the uro'gen formed enzymatically from **1** and that formed chemically from **5** (Table I). Incubations carried out by using a higher amount of bilane **5** (56 nmol) and an enzyme which consumed 32 nmol of **1** in 1 h gave similar results.

Bilane **5** was also incubated with deaminase at 50 °C, i.e., under conditions where the cosynthase is inactive,[1] the chemical cyclication of **5** gives higher uro'gen yields (see above), and the deaminase is more active.[23] No increase in the amount of the uro'gens formed in the presence of the enzymes was found as compared to the amount formed by the chemical cyclization of **5** in the absence of enzyme. Enzymatic formation of uro'gen from PBG **1** in the presence of **5** proceeded as usual at 50 °C.

Longer incubation times (4 h at 37 °C) did not essentially change the results discussed above. Only higher amounts of uro'gens were formed, either chemically from **5** or **5** plus enzyme, or enzymatically from PBG.

Since the bilane **5** was not found to be a substrate for either the deaminase or the deaminase-cosynthase, it was unlikely to be a substrate of the cosynthase.[26] Nevertheless, this possibility was examined by incubating **5** with cosynthase or cosynthase-deaminase, and by analyzing the isomer composition of the mixture (see above). No variation in the relative proportion of the isomer III/IV fraction in the reaction products was found when the bilane **5** was incubated alone, in the presence of cosynthase, or in the presence of cosynthase-deaminase (Table II; only the relative proportion of the isomers is depicted since the total porphyrin formation was already discussed above). The amount of deaminase-cosynthase used was sufficient to entirely transform PBG into uro'gens under the conditions used (Table II). The same negative results regarding the possible interaction on **5** and the cosynthase were found when the incubations were carried out in anaerobiosis or in aerobiosis.

Table II. Effect of Bilane **5** on Uro'gen III Formation[c]

enzymatic system	addition	isomers formed, % I	III (and IV)
	bilane	97	3
cosynthase[a]	bilane	97.8	2.2
deaminase-cosynthase[a]	bilane	98	2
deaminase-cosynthase[a]	bilane + [14]PBG	80	20
deaminase-cosynthase[a]	[14C]PBG	18	82
	bilane	98.2	1.8
cosynthase[b]	bilane	98.4	1.6
deaminase-cosynthase[b]	bilane	98.3	1.7
deaminase-cosynthase[b]	bilane + [14C]PBG	74	26
deaminase-cosynthase[b]	[14C]PBG	20	80

[a] The incubations were carried out at 37 °C during 1 h in aerobiosis. [b] The incubations were carried out at 37 °C during 4 h in anaerobiosis. Isomer estimation was performed by decarboxylating the uro'gens to coproporphyrins followed by TLC analysis of the latter. Under the conditions used all the PBG was transformed into uro'gens. [c] The incubation mixture contained in a final volume of 150 μL: 15 μmol of phosphate buffer (pH 7.4), bilane **5** (14 nmol), [14C]PBG (16 nmol, 6000 dpm/nmol), dithiothreitol (10 mM), and enzyme (100 μL). Deaminase-cosynthase as well as cosynthase was isolated from human erythrocytes.

The incubations of **5** and the deaminase-cosynthase system were also carried out in the presence of [14C]porphobilinogen. This provided a control of the viability of the enzymatic system used, and it allowed us to distinguish between the amount of isomer III/IV formed from the bilane and that formed enzymatically from PBG. It was found that the bilane **5** had an inhibitory effect of the enzymatic isomer III formation from PBG (Table II). Since the total amount of uro'gens formed from [14C]PBG was constant, this effect produced an increase in the [14C]uro'gen I fraction in the presence of **5**, as compared with the amount of [14C]uro'gen I formed from **1** alone. However, under the conditions used, enough cosynthase activity still remains to convert **5** into uro'gen III if such an enzymatic reaction took place. The results depicted in Table II were carried out using a deaminase-cosynthase system from wheat germ or from human erythrocytes, and also by using higher concentrations of bilane **5**. Anaerobic incubations were carried out for 18 h and similar results to those discussed above were found. After 18 h an increase in the proportion of the isomer III/IV fraction was found (4–5%), but in these cases the results were the same whether the bilane **5** was incubated alone or in the presence of deaminase-cosynthase.

The data summarized in Tables I and II clearly indicate that **5** is not a substrate of either deaminase or deaminase-cosynthase. They also lend support to early work[27] concerning the isolation of a bilane (by inhibition of the deaminase with ammonium ions) to which the structure **5** had been attributed and whose properties very much resemble those described above. The results are also in agreement with our early suggestion[2] that it is unlikely that the bilane **5** will serve as a substrate of either deaminase or cosynthase. It is further proof that the enzymatic polymerization of **1** takes place on the enzymatic surface, with no liberation of discrete intermediates.[1,5]

Experimental Section

General. Melting points were determined on a Kofler melting point apparatus and are uncorrected. Microanalyses were performed by the Alfred Bernhardt Mikroanalytisches Laboratorium (Elbach, BRD). NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) on a Perkin-Elmer R-12 spectrometer with internal Me_4Si (organic solvents) or sodium γ-trimethylsilylpropanesulfonate (D_2O) as reference. The results are given in δ parts per million. Mass spectra were obtained with a Varian CH-7 spectrometer. The silica gel used for

column chromathography was TLC Kieselgel (Fluka AG). TLC was performed on precoated silica gel 60 F-254 plaques (Merck, 0.25-mm layer thickness). The substances were spotted by spraying with Ehrlich's reagent (2% *p*-dimethylaminobenzaldehyde in 6 N HCl), or with bromine vapors which gave orange or red colors with dipyrrylmethanes and green or brown colors with bilanes.

Ethyl 2-Acetoxymethyl-3-methoxycarbonylmethyl-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylate (7). Ethyl 2-methyl-3-methoxycarbonyl-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylate (1 g)[16,28] was dissolved in 10 mL of anhydrous methylene chloride and 0.26 mL of sulfuryl chloride was added under moisture exclusion conditions. The stirred solution was kept during 30 min at 20 °C, it was evaporated to dryness, and the residue was dissolved in 30 mL of a 10% anhydrous sodium acetate solution in glacial acetic acid. The mixture was kept at 20 °C during 2 h; it was then poured into 500 mL of ice water and filtered, and the residue was crystallized from benzene–cyclohexane, 990 mg (83%), mp 106–108 °C. Anal. ($C_{17}H_{23}NO_8$) C, H, N. NMR: δ 1.35 (t, 3 H), 2.0 (s, 3 H), 2.8 (m, 4 H), 3.55 (s, 2 H), 3.7 (s, s, 6 H), 4.3 (q, 2 H).

Benzyl 2-acetoxymethyl-3-methoxycarbonylmethyl-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylate (19) was prepared from **28** (1 g)[16] following the procedure described for **7**; 1.05 g (94%) of **19** was obtained, mp 107–109 °C. Anal. ($C_{22}H_{25}NO_8$) C, H, N. NMR: δ 2.1 (s, 3 H), 2.8 (m, 4 H), 3.55 (s, 2 H), 3.65, 3.70 (s, s, 6 H), 5.1 (s, 2 H), 5.35 (s, 2 H), 7.4 (b, 5 H).

***tert*-Butyl 2-Methyl-3-methoxycarbonylmethyl-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylate (27).** A solution of **28** (3 g) in 100 mL of methanol was reduced with hydrogen over 300 mg of 10% Pd on charcoal at 50 psi during 1 h. The catalyst was filtered, the solution was evaporated to dryness, the residue (2.2 g) was dissolved in 10 mL of dry *tert*-butyl alcohol and 9 mL of dry tetrahydrofuran, 900 mg of DCC was added, and the mixture was stirred during 17 h under moisture exclusion conditions. The precipitated urea was then filtered, the solution was evaporated to dryness, and the residue was crystallized from benzene-cyclohexane; 1.8 g (66%) of **27** was obtained, mp 127–128 °C.[29] Anal. ($C_{17}H_{25}NO_6$) C, H, N. NMR: δ 1.55 (s, 9 H), 2.2 (s, 3 H), 2.8 (m, 4 H), 3.4 (s, 2 H), 3.7 (s, 6 H). Mass spectrum: *m/e* 339 (M^+, 20%), 283 (M^+ − isobutylene, 35%).

***tert*-Butyl 2-Acetoxymethyl-3-methoxycarbonylmethyl-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylate (26).** Lead tetraacetate (3 g) was added in small portions over a period of 3 h to a stirred solution of pyrrole **27** (2 g) in 30 mL of glacial acetic acid. The mixture was stirred for a further 17 h; it was then poured into 500 mL of water, the solution was extracted with chloroform (3 × 25 mL), the extracts were evaporated to dryness, and the residue was dissolved in a small volume of 2% methanol in benzene. It was then adsorbed on a silica gel column (3 × 30 cm) prewashed with the same solvent. The latter was also used to elute the acetoxymethylpyrrole **26**; 2.1 g (95%); NMR δ 1.55 (s, 9 H), 2.0 (s, 3 H), 2.7[9] (m, 4 H), 3.55 (s, 2 H), 3.65 (s, 6 H), 5.05 (s, 2 H, CH_2O–); mass spectrum *m/e* 397 (M^+, 50%), 341 M^+ − isobutylene, 25%). It was an oily residue which crystallized on long standing.

***tert*-Butyl 3-(methoxycarbonylmethyl)-4-(β-methoxycarbonylethyl)-2-pyrrolecarboxylate (20)** was obtained from **8** (1.8 g) by following the procedure described for **27**; 780 mg (47%) of **20** was obtained, mp 84–85 °C (from benzene–cyclohexane). Anal. ($C_{16}H_{23}NO_6$), C, H, N. NMR: δ 1.55 (b, 9 H), 2.65 (m, 4 H), 3.7 (b, 6 H), 3.85 (s, 2 H), 6.7 (b, 1 H, H-5). Mass spectrum: *m/e* 325 (M^+, 40%).

Ethyl 3,4′-Di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-benzyloxycarbonyl-5-dipyrrylmethanecarboxylate (9). A solution of 510 mg (1.3 mmol) of the acetate **7**, 430 mg (1.2 mmol) of pyrrole **8**, and 25 mg of *p*-toluenesulfonic acid in 12 mL of glacial acetic acid was heated at 45 °C during 3 h. The solution was poured into 200 mL of water, the mixture was extracted with chloroform (3 × 25 mL), and the extracts were washed with a 5% sodium bicarbonate solution, then with water, dried (Na_2SO_4), and evaporated in vacuo. The residue **9** was crystallized from methanol–water, 700 mg (75%), mp 106–108 °C. Anal. ($C_{34}N_{40}H_2O_{12}$) C, H, N. NMR: δ 1.3 (t, 3 H, CH_3CH_2), 2.8 (m, 8 H, CH_2CH_2CO), 3.6, 3.65, 3.7, 3.75, 3.8 (s, s, b, 16 H, CH_2CO_2, OCH_3), 3.95 (s, 2 H, pyrr–CH_2–pyrr), 4.3 (q, 2 H, CH_2CH_3), 5.25 (s, 2 H, CH_2Ph), 7.4 (b, 5 H, Ph).

TLC analysis (5% methanol in benzene) showed contamination with small amounts of **10** (**9**, R_f 0.60, orange color to bromine vapors; **10**, R_f 0.65, cherry color).

Ethyl 3,3′-di(methoxycarbonylethyl)-4,4′-di(β-methoxycarbonylmethyl)-5′-benzyloxycarbonyl-5-dipyrrylmethanecarboxylate (14) was prepared from 460 mg (1.2 mmol) of acetate **7** and 38.5 mg (1.1 mmol) of the α-unsubstituted pyrrole **15**,[16] following the procedure described for **9**; 670 mg (93%) of **14** was obtained, mp 125–127 °C. Anal. ($C_{34}H_{40}N_2O_{12}$) C, H, N. NMR: δ 1.3 (t, 3 H), 2.8 (m, 8 H), 3.55 (b, 12 H), 3.8 (b, 6 H, CH_2CO_2, pyrr–CH_2–pyrr), 4.25 (q, 2 H), 5.25 (s, 2 H), 7.4 (b, 5 H).

TLC analysis (2% methanol in benzene) showed that it was contaminated with small amounts of **10**.

Benzyl 3,4′-Di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-*tert*-butyloxycarbonyl-5-dipyrrylmethanecarboxylate (21). A solution of 612 mg (1.4 mmol) of the acetate **19**, 460 mg (1.4 mmol) of pyrrole **20**, and 30 mg of *p*-toluenesulfonic acid in 30 mL of anhydrous methylene chloride was heated at 35 °C during 6 h. The solution was cooled, washed with a 5% solution of sodium bicarbonate, then with water, dried (Na_2SO_4), and evaporated to dryness. The oily residue was dissolved in a small volume of 2% methanol in benzene and was purified by filtration through a TLC silica gel packed column (4 × 50 cm) using the same solvent as eluant: 640 mg (64%); NMR δ 1.5 (s, 9 H, $C(CH_3)_3$), 2.7 (m, 8 H, CH_2CH_2), 3.5, 3.55, 3.65, 3.75 (s, s, b, b, 16 H, OCH_3, CH_2CO), 3.9 (s, 2 H, pyrr–CH_2–pyrr), 5.25 (s, 2 H, CH_2Ph), 7.4 (b, 5 H, Ph); mass spectrum *m/e* 696 (M^+, 1%), 639 (M^+ − isobutylene, 3%), 605 (M^+ − CH_2Ph, 2%), 372 (12%),[22] 325 (20, 15%), 282 (22%), 237 (100%).[22]

***tert*-Butyl 3,4′-di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-benzyloxycarbonyl-5-dipyrrylmethanecarboxylate (29)** was prepared from 194 mg (2 mmol) of the acetate **26** and 718 mg (2 mmol) of the pyrrole **8** following the procedure described for the obtention of dipyrrylmethane **21**; 809 mg (64%) of **29** was thus obtained, mp 98 °C (from methanol, lit.[15] mp 95–97 °C). Anal. ($C_{36}H_{44}N_2O_{12}$) C, H, N. NMR: δ 1.5 (s, 9 H), 2.5 (m, 8 H), 3.55 (s, 2 H), 3.70–3.75 (b, 12 H), 3.85 (s, 2 H), 3.95 (s, 2 H), 5.25 (s, 2 H), 7.35 (b, 5 H); mass spectrum *m/e* 696 (M^+, 5%). The mother liquors of the crystallizations contained the symmetrical **31**; NMR δ 1.5 (s, 18 H), 2.75 (m, 8 H), 3.55 (s, 4 H), 3.65 (s, 6 H), 3.75 (b, 8 H).

Ethyl 3,4′-Di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-carboxy-5-dipyrrylmethanecarboxylate (11). Dipyrrylmethane **9** (670 mg) dissolved in 100 mL of glacial acetic acid was reduced with hydrogen over 400 mg of 10% Pd on charcoal at 50 psi during 90 min. After the catalyst was separated and the solution was evaporated to dryness in vacuo, the residue was dissolved in a small amount of 5% methanol in chloroform and was filtered through a TLC silica gel column (2 × 30 cm) that was packed and eluted with the same solvent. The first fractions contained the contaminant **10** (R_f, 0.80), while the latter fractions contained the acid **11** (R_f, 0.20), 350 mg (61%), mp 165–167 °C (methanol–water). Anal. ($C_{27}H_{34}N_2O_2$), C, H, N. NMR: δ 1.3 (t, 3 H), 2.55 (m, 8 H), 3.55 (s, 2 H), 3.65 (b, 12 H), 3.8 (s, 2 H), 3.95 (s, 2 H), 4.3 (q, 2 H).

Ethyl 3,3′-di(methoxycarbonylmethyl)-4,4′-di(β-methoxycarbonylethyl)-5′-carboxy-5-dipyrrylmethanecarboxylate (16) was obtained from **14** (670 mg) following the procedure described for **11**, 350 mg (61%), mp 178–179 °C (methanol–water). Anal. ($C_{27}H_{34}N_2O_{12}$) C, H, N. NMR (pyridine-d_6): δ 1.1 (t, 3 H), 3.2 (m, 8 H), 3.6 (b, 16 H), 4.2 (q, 2 H), 4.35 (s, 2 H).

***tert*-Butyl 3,4′-di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-carboxy-5-dipyrrylmethanecarboxylate (30)** was obtained from **29** (800 mg) following the procedure described for **11**, 490 mg (70%), mp 140–141 °C (methylene chloride–hexane). Anal. ($C_{29}H_{38}N_2O_{12}$) C, H, N.

Benzyl 3,4′-Di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-carboxy-5-dipyrrylmethanecarboxylate (22). Benzyloxycarbonyldipyrrylmethane **21** (320 mg) was dissolved in 2 mL of glacial acetic acid and 0.2 mL of 40% HBr in glacial acetic acid was added. The solution was kept at 10 °C during 15 min, and was then evaporated to dryness of 20 °C. The residue was then filtered through a silica gel column following the procedure described for **11**. The fractions eluted first contained a small amount of the dibenzyl dimer **23** (TLC, 5% methanol in chloroform, R_f 0.90), while the acid **22** was eluted next (TLC, 5% methanol in chloroform, R_f 0.20), 220 mg (74%), mp 160–162 °C. Anal. ($C_{32}H_{36}N_2O_{12}$) C, H, N.

Ethyl 3,4′-Di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5-dipyrrylmethanecarboxylate (12). The acid **11** (350 mg) was heated at 200 °C in vacuo (0.050 Torr) during 2 min. The residue was dissolved in 2% methanol in benzene and filtered through a silica gel column (2 × 30 cm) that had been packed with the same solvent. The eluted fractions containing **12** were combined and evaporated to

dryness; 240 mg (74%) of **12** was thus obtained. NMR: δ 1.3 (t, 3 H), 2.8 (m, 8 H), 3.45 (s, 2 H), 3.6 (s, 2 H), 3.75 (b, 12 H), 3.9 (s, 2 H), 4.3 (q, 2 H), 6.6 (b, 1 H, H-5′); mass spectrum *m/e* 534 (M^+, 100%), 462 (535 − $CO_2C_2H_5$, 20%), 310 (15%).

Ethyl 3,3′-di(methoxycarbonylmethyl)-4,4′-di(β-methoxycarbonylethyl)-5-dipyrrylmethanecarboxylate (17) was obtained following the procedure described for **12;** 350 mg of **16** afforded 280 mg (86%) of **17,** mp 70–71 °C (benzene–hexane). Anal. ($C_{26}H_{34}N_2O_{10}$) C, H, N. NMR: δ 1.3 (t, 3 H), 2.8 (m, 8 H), 3.5 (s, 4 H), 3.75 (b, 14 H), 4.2 (q, 2 H), 6.4 (b, 1 H).

Benzyl 3,4′-di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5-dipyrrylmethanecarboxylate (24) was obtained from **22** (200 mg) following the procedure described for **12,** except for the heating time, that was 5 min; 130 mg (70%) of **24** was obtained. NMR: δ 2.7 (m, 8 H), 3.4 (s, 2 H), 3.50, 3.55, 3.60, 3.65 (b, 14 H), 3.9 (s, 2 H), 5.3 (s, 2 H), 6.6 (b, 1 H), 7.4 (b, 5 H). Mass spectrum: *m/e* 596 (M^+, 1%), 505 (M^+ − CH_2Ph, 10%), 373 (34%), 282 (373 − CH_2Ph, 90%), 91 (100%).

***tert*-Butyl 3,4′-di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5-dipyrrylmethanecarboxylate (32)** was obtained from **30** (490 mg) by following the procedure described for **12,** but heating was at 170 °C during 5 min; 346 mg (76%) of **32** was obtained. NMR (pyridine-d_5): δ 0.75 (s, 9 H), 2.4 (m, 8 H), 2.9 (b, 14 H), 3.25 (s, 2 H, C-4′-CH_2CO), 3.5 (s, 2 H, pyrr–CH_2–pyrr), 6.15 (b, 1 H, H-5′). Mass spectrum: *m/e* 562 (M^+, 32%), 505 (M^+ − isobutylene, 100%), 461 (505 − CO_2, 41%).

Ethyl 3,4′-Di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-formyl-5-dipyrrylmethanecarboxylate (13), A solution of 290 mg of dipyrrylmethane **12** in 1.5 mL of dimethylformamide was kept at 5 °C while 0.3 mL of benzoyl chloride was added in one portion. The mixture was kept under moisture exclusion conditions at 20 °C during 1 h and was then diluted with 10 mL of ethyl ether. The solution was extracted with water (3 × 3 mL) and the aqueous extracts were in turn reextracted with ether (2 × 2 mL). The aqueous solution was adjusted to pH 8 with sodium carbonate and kept at 20 °C during 4 h. After cooling at 5 °C, it was filtered, and the precipitate was dried and crystallized from ethanol; 215 mg (70%) of **13** was thus obtained, mp 115–117 °C. Anal. ($C_{27}H_{34}N_2O_{11}$) C, H, N. NMR: δ 1.3 (t, 3 H), 2.75 (m, 8 H), 3.55 (s, 2 H), 3.7 (b, 14 H), 4.0 (s, 2 H), 4.25 (q, 2 H), 9.6 (s, 1 H, CHO).

Ethyl 3,3′-di(methoxycarbonylmethyl)-4,4′-di(β-methoxycarbonylethyl)-5′-formyl-5-dipyrrylmethanecarboxylate (18) was prepared from **17** (280 mg) following the procedure described for **13;** 240 mg (78%) of **18** was thus obtained, mp 126–127 °C. Anal. ($C_{27}H_{34}N_2O_{11}$) C, H, N. NMR: δ 1.4 (t, 3 H), 2.85 (m, 8 H), 3.65 (s, 4 H), 3.8 (b, 12 H), 3.95 (s, 2 H), 4.35 (q, 2 H), 9.65 (s, 1 H, CHO).

Benzyl 3,4′-di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5-formyl-5-dipyrrylmethanecarboxylate (25) was prepared from **24** (470 mg) following the procedure described for **13;** 300 mg (60%) of **25** was obtained, mp 120–121 °C. Anal. ($C_{32}H_{36}N_2O_{11}$) C, H, N. NMR: δ 2.7 (m, 8 H), 3.6, 3.65, 3.75, 3.80 (b, 16 H), 4.0 (s, 2 H), 5.3 (s, 2 H), 7.4 (b, 5 H), 9.6 (s, 1 H, CHO).

***tert*-Butyl 3,4′-di(methoxycarbonylmethyl)-4,3′-di(β-methoxycarbonylethyl)-5′-formyl-5-dipyrrylmethanecarboxylate (33)** was prepared from **32** (360 mg) following the procedure described for **13;** 260 mg (69%) of **33** was thus obtained, mp 106–107 °C. Anal. ($C_{29}H_{38}N_2O_{11}$) C, H, N. NMR: δ 1.6 (s, 9 H), 2.8 (m, 8 H), 3.65 (s, 2 H, C-3′-CH_2CO), 3.85 (b, 14 H), 4.05 (s, 2 H), 9.75 (s, 1 H, CHO).

1,3,5,7-Tetra(β-methoxycarbonylethyl)-2,4,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-ethoxycarbonyl-8′-aminomethyl-*b*-bilene Hydrobromide Lactam (35), A mixture of 80 mg of aldehyde **13** and 72 mg of dipyrrylmethane lactam **34** was dissolved in 5 mL of hot, anhydrous methanol. The solution was cooled to 20 °C and 0.5 mL of 48% HBr was added. The red *b*-bilene hydrobromide crystals precipitated after 15 min. The mixture was cooled and filtered, and the collected hydrobromide **35** was recrystallized from methanol, 130 mg (84%), mp 128–130 °C. Anal. ($C_{50}H_{62}N_5O_{17}Br$) C, H, N, Br. NMR: δ 1.3 (5, 3 H, CH_2CH_3), 2.4 (m, 16 H, CH_2CH_2CO), 2.85 (m, 2 H, CH_2CONH), 3.51, 3.59, 3.61, 3.63, 3.70 (b, 27 H, OCH_3, CH_2CO), 4.3 (m, 6 H, CH_2CH_3, pyrr–CH_2–pyrr), 4.5 (b, 2 H, CH_2NH), 6.5 (b, 1 H, CONH), 7.6 (b, 1, CH=).

1,4,5,7-Tetra(β-methoxycarbonylethyl)-2,3,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-ethoxycarbonyl-8′-aminomethyl-*b*-bilene hydrobromide lactam (36) was prepared following the procedure described for **35,** The condensation of 100 mg of **18** and 90 mg of **34** gave 160 mg (84%) of **36,** mp 103–105 °C. Anal. ($C_{50}H_{62}N_{15}O_{17}Br$) C, H, N, Br. NMR: δ 1.4 (t, 3 H), 2.9 (m, 18 H), 3.7 (b, 27 H), 4.50, 4.52 (m, b, 8 H), 7.85 (b, 1 H, CH=).

1,3,5,7-Tetra(β-methoxycarbonylethyl)-2,4,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-benzyloxycarbonyl-8′-aminomethyl-*b*-bilene hydrobromide lactam (39) was prepared following the procedure described for **35,** The condensation of 150 mg of aldehyde **25** and 103 mg of **34** gave 230 mg (83%) of **39,** mp 115–120 °C. Anal. ($C_{55}H_{64}N_5O_{17}Br$) C, H, N, Br. NMR: δ 2.7 (m, 16 H, CH_2CH_2), 3.3 (m, 2 H, CH_2CONH), 3.7 (b, 27 H, OCH_3, CH_2COO), 4.35, 4.40 (b, 4 H, pyrr–CH_2–pyrr), 4.45 (b, 2 H, CH_2NH), 5.3 (s, 2 H, CH_2Ph), 6.2 (b, 1 H, NHCO), 7.35 (b, 5 H, Ph), 7.6 (b, 1 H, CH=). UV max (ethanol): 470 nm (ε 30 000).

1,3,5,7-Tetra(β-methoxycarbonylethyl)-2,4,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-*tert*-butyloxycarbonyl-8′-aminomethyl-*b*-bilene hydrobromide lactam (43) was prepared following the procedure described for **35,** The condensation of 140 mg of aldehyde **33** and 100 mg of **34** gave 190 mg (72%) of **43,** mp 110–112 °C. Anal. ($C_{52}H_{66}N_5O_{17}Br$) C, H, N, Br. NMR: δ 1.55 (s, 9 H), 2.5 (m, 16 H), 3.35 (b, 2 H), 3.6 (b, 27 H), 4.40 (b, 6 H), 6.2 (b, 1 H, NHCO), 7.6 (b, 1 H).

1,3,5,7-Tetra(β-methoxycarbonylethyl)-2,4,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-ethoxycarbonyl-8′-aminomethylbilane Lactam (37), The *b*-bilene hydrobromide **35** (130 mg) was dissolved in 80 mL of chloroform and 40 mL of methanol, and was reduced with hydrogen at 40 psi during 1 h over 50 mg of 10% Pd on charcoal and 260 mg of morpholinomethylpolystyrene (Fluka AG). The catalyst and resin were then filtered, the solution was evaporated to dryness, and the pink, crystalline residue was dissolved in 4% methanol in chloroform and was adsorbed on a TLC silica gel column (2 × 30 cm). The column had been packed by suspending the silica gel in the same solvent and by applying enough nitrogen pressure to get a steady 0.5 mL/min flow. The bilane was eluted by using the same solvent and pressure and by analyzing the column effluent by TLC (bromine vapors were used for visualization). The fractions containing the bilane were combined and evaporated to dryness, 70 mg (58%), mp 225–230 °C (methanol). Anal. ($C_{50}H_{63}N_5O_{17}$) C, H, N. Mass spectrum–FD: 1005 (M^+). Mass spectrum: *m/e* 1008 (M^+ + 3 H, 1.3%), 534 (**12,** 53%), 471 (**44,** 26%), 461 (**12** − $CH_2CO_2CH_3$, 100), 222 (**45,** 33%).

1,4,5,7-Tetra(β-methoxycarbonylethyl)-2,3,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-ethoxycarbonyl-8′-aminomethylbilane lactam (38) was prepared following the procedure described for **37,** The hydrogenation of 160 mg of **36** gave 50 mg (33%) of **38;** mass spectrum–FD 1005 (M^+); mass spectrum *m/e* 1008 (M^+ + 3 H, 1%), 534 (73%), 471 (22%), 461 (100%), 222 (35%); NMR δ 1.3 (t, 3 H, CH_2CH_3), 2.6 (m, 16 H, CH_2CH_2), 3.4 (s, 2 H, CH_2CO), 3.49, 3.60, 3.64 (s, s, s, 27 H, CH_2CO_2, OCH_3), 3.70, 3.73 (s, s, 6 H, pyrr–CH_2–pyrr), 4.2 (q, 2 H, CH_2CH_3), 4.3 (b, 2 H, CH_2NH), 6.1 (b, 1 H, NHCO).

1,3,5,7-Tetra(β-methoxycarbonylethyl)-2,4,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-8′-aminomethylbilane Lactam (42), The bilene hydrobromide **43** (200 mg) was dissolved in 5 mL of glacial acetic acid and 5 mL of 40% HBr in glacial acetic acid was added. The solution was kept at 20 °C during 90 min and then freeze-dried. The residue was dissolved in 35 mL of chloroform and 15 mL of methanol and was hydrogenated at 40 psi during 30 min over 100 mg of 10% Pd on charcoal and 200 mg of morpholinomethylpolystyrene. The catalyst and resin were then filtered, and the residue that was left after evaporation of the solvent was purified through a silica gel column following the procedure described for **37;** 30 mg (18%) of the pure **42** was thus obtained. Mass spectrum–FD: 933 (M^+). Mass spectrum: *m/e* 933 (M^+, 26%), 475 (**47,** 100%), 471 (**44,** 41%), 462 (**48,** 63%), 459 (**34,** 55%), 222 (**45,** 55%). NMR (pyridine-d_5): δ 2.5 (b, 2, CH_2CO), 2.9 (m, 16 H, CH_2CH_2), 3.6 (b, 27 H, CH_2CO, OCH_3), 3.9, 4.0, 4.4 (s, b, s, 6 H, pyrr–CH_2–pyrr), 6.6 (b, 1 H, H-1′), 10.5, 10.6, 10.7, 10.9 (s, s, s, s, 4 H, NH).

1,3,5,7-Tetra(β-methoxycarbonylethyl)-2,4,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-benzyloxycarbonyl-8′-aminomethylbilane Lactam (41) and 1,3,5,7-Tetra(β-methoxycarbonylethyl)-2,4,6-tri(methoxycarbonylmethyl)-8-carboxymethyl-1′-carboxy-8′-aminomethylbilane Lactam (40), A solution of bilene **39** (180 mg) in 100 mL of chloroform and 50 mL of methanol was hydrogenated at 40 psi during 30 min over 180 mg of morpholinomethylpolystyrene

and 180 mg of 10% Pd on charcoal. After filtration of the catalyst and evaporation of the solvent to dryness, a crystalline residue was obtained. TLC analysis (4% methanol in chloroform) showed the presence of **41** (R_f 0.60) and of **40** (R_f 0.30, positive Ehrlich reaction on heating). They were separated following the procedure described for **37**; 30 mg (17%) of **41** and 50 mg (32%) of **40** were obtained. Bilane **41** had mp 220–225 °C; mass spectrum-FD 1067 (M^+); mass spectrum *m/e* 1067 (M^+, 0.6%), 596 (**24**$^+$, 13%), 505 (596 − 91, 100), 459 (**34**$^+$, 48%), 222 (**45**$^+$, 55%).

Carboxybilane **40**, mp 230–235 °C (Anal. ($C_{48}H_{59}N_5O_{17}$) C, H, N), was decarboxylated by heating at 220 °C and 0.050 Torr during 1 min. The decarboxylation product was purified by silica gel chromatography as described for **37**, and 7.1 mg (15%) of **42** was obtained.

1,3,5,7-Tetra(β-carboxyethyl)-2,4,6,8-tetra(carboxymethyl)-8′-aminomethylbilane (5). The lactam **42** (25 mg) was dissolved in 0.25 mL of warm ethanol-d_6 and 0.25 mL of a 4 N KOD solution was added. The solution was degassed in vacuo by repeated freezing and thawing and sealed off in vacuo (0.1 Torr). After keeping it for 24 h at 20 °C the ethanol was chased away with nitrogen, 0.25 mL of water was added, and the solution was again sealed off in vacuo and kept for a further 48 h at 20 °C. The NMR spectrum indicated complete saponification: NMR (KOD) δ 2.5 (m, 16 H, CH_2CH_2CO), 3.7 (b, 8 H, CH_2CO_2), 3.85 (b, 2 H, CH_2NH_2), 3.95 (b, 6 H, $-CH_2-$), 6.8 (b, 1 H, H-1′). The solution was adjusted to pH 7.4 with HCl or IRC 50-H^+ for enzymatic studies. Below pH 7 the aminomethylbilane was very unstable, but the solution could be kept at pH 10 and −15 °C for several days.

1,3,5,7-Tetra(β-carboxyethyl)-2,4,6,8-tetra(carboxymethyl)-1′-carboxy-8′-aminomethylbilane (6) was prepared from **37** following the procedure described for **5**; NMR (KOD) δ 2.45 (m, 16 H), 3.7 (b, 8 H), 3.90 (b, 2 H), 4.0 (b, 6 H). It was kept in solution at pH 7–8 and −15 °C before use.

1,4,5,7-Tetra(β-carboxyethyl)-2,3,6,8-tetra(carboxymethyl)-1′-carboxy-8′-aminomethylbilane (7) was prepared from **38** following the procedure described for **5**; NMR (KOD) δ 2.45 (m, 16 H), 3.6 (b, 8 H), 3.80 (b, 2 H), 3.70 (s, 2 H), 3.90 (b, 4 H). It was stable in solution at pH 8 and −15 °C for several days.

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