Synthesis of 2-Aminomethyldipyrrylmethanes of Biosynthetic Interest

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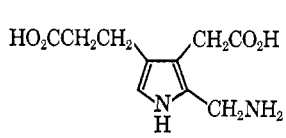
Abstract: A new method for the synthesis of 2-aminomethyl-3,3′-carboxymethyl-4,4′-(β-carboxyethyl)dipyrrylmethane and of 2-aminomethyl-3,4′-carboxymethyl-4,3′-(β-carboxyethyl)dipyrrylmethane is described. The reaction of 5-carboxyporphobilinogen and 5-carboxyisoporphobilinogen with benzyloxycarbonyl chloride and *tert*-butyloxycarbonyl azide afforded the protected 2-aminomethylpyrroles, which were then transformed into the 3-(or 4-)methoxycarbonylmethyl-4-(or -3-)β-methoxycarbonylethyl-5-benzyloxycarbonylpyrroles. Cleavage of the protecting groups and treatment with nitrous acid followed by condensation with porphobilinogen lactam esters gave the corresponding 5′-benzyloxycarbonyldipyrrylmethane lactams. Hydrogenolysis of the benzyl groups, decarboxylation, and hydrolysis of the esters and the lactam afforded the 2-aminomethyldipyrrylmethanes. The incorporation of these compounds into uroporphyrins by the enzymes involved in porphobilinogen polymerization is discussed in terms of the specificity of the system.

Few biochemical mechanisms have aroused as much interest as the enzymatic conversion of porphobilinogen (**1**) into uroporphyrinogen III (**2**), the basic skeleton of all the natural porphyrins and chlorins. More than 20 hypotheses have been put forward to explain this reaction,[1] but none has yet been proved. The multiplicity of hypotheses is understandable, since it is easy to visualize a repetitive head-to-tail attack of a porphobilinogen molecule on a second porphobilinogen unit (a Mannich base reacting with an α-pyrrolenine anion) with concomitant loss of 1 mol of ammonia giving rise, after four units have reacted, to the cyclic uroporphyrinogen I (**3**).

The sequence is not clear, however, for the isomeric uroporphyrinogen III (**2**) where an inversion takes place. The stoichiometry of the enzymatic reaction has been shown[2] to be porphobilinogen, ammonia,



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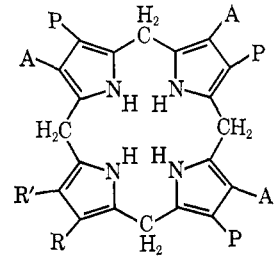


A = CH_2CO_2H
P = $CH_2CH_2CO_2H$
2, R = P; R′ = A
3, R = A; R′ = P

uroporphyrinogen, 4:4:1. It has also been proved that no other monopyrrolic substrate, apart from porphobilinogen, takes part in the process,[3] and it has been established[3] that the enzymic reaction takes place without formation of oxidized intermediates (pyrrylmethenes). The conclusion then is that we are dealing with an intramolecular rearrangement.

The enzymatic system that transforms porphobilinogen into uroporphyrin III was known to consist[3] of two proteins: uroporphyrinogen III cosynthetase, a heat labile protein, which when destroyed by heat left a second enzyme, porphobilinogen deaminase, or uroporphyrinogen I synthetase, which formed exclusively uroporphyrinogen I. Only porphobilinogen deaminase used porphobilinogen as a substrate. When the system was separated nondestructively and both enzymes were isolated and later recombined[4,5] it was found that the cosynthetase was present in at least a tenfold excess over the deaminase. However, during the course of enzymic formation of uroporphyrinogen III, the cosynthetase was inactivated[4,6] and uroporphyrinogen I was the main reaction product.

The enzymatic synthesis of uroporphyrinogens with the deaminase–cosynthetase complex could be steered to the formation of either isomer III or isomer I by the application of synthetic pyrroles analogous to porphobilinogen,[7] with the net result that the disappearance of one isomer resulted in an equivalent formation of the other. However, in no case were discrete intermediates (pyrrylmethanes) isolated.[8] The deaminase behaved like a single enzyme and could not be split

(1) For a brief evaluation of the hypotheses for enzymic polymerization of porphobilinogen prior to 1961, see E. Margoliash, *Annu. Rev. Biochem.*, **30**, 551 (1961). Recent hypotheses are (a) selective bilane cyclization: J. H. Mathewson and A. H. Corwin, *J. Amer. Chem. Soc.*, **83**, 135 (1961); (b) interconversion of uroporphyrinogens: E. Bullock, *Nature (London)*, **205**, 70 (1965); (c) condensation of porphobilinogen and a pyrrylmethyldipyrrylmethane: P. Conford, *Biochem. J.*, **91**, 64 (1964); J. Dalton and R. C. Dougherty, *Nature (London)*, **223**, 1151 (1969).

(2) L. Bogorad, *J. Biol. Chem.*, **233**, 501 (1958).

(3) L. Bogorad in "Comparative Biochemistry of Photoreactive Systems," M. B. Allen, Ed., Academic Press, New York, N. Y., 1960, p 227.

(4) E. Stevens and B. Frydman, *Biochim. Biophys. Acta*, **151**, 429 (1968).

(5) E. Stevens, R. B. Frydman, and B. Frydman, *ibid.*, **158**, 496 (1968).

(6) E. Y. Levin, *Biochemistry*, **7**, 3781 (1968).

(7) R. B. Frydman, E. Stevens, and B. Frydman, *An. Asoc. Quim. Argent.*, **55**, 287 (1967).

(8) A recent report [E. B. C. Llambias and A. M. del C. Battle, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **6**, 285 (1970)] claims that free dipyrrylmethanes accumulated in soybean callus extracts when porphobilinogen was consumed. However, the properties ascribed to the dipyrrylmethanes differ considerably from what we have observed (see Experimental Section).

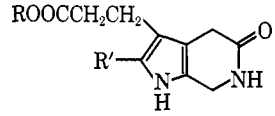
into different components, but two active sites could be recognized, one for porphobilinogen consumption and one for uroporphyrinogen formation.[9]

To explain the inversion of the side chains in uroporphyrinogen III and several of the properties of the described enzymatic system, the majority of the postulated hypotheses[1] interpolate a rearrangement step in the process. They can roughly be separated into four groups depending on at which stage of the process the proposed rearrangement takes place: (a) dipyrrylmethane formation, (b) pyrrylmethyldipyrrylmethane formation, (c) bilane formation, and (d) the cyclization step. While alternatives (a) and (d) propose for the cosynthetase the role of a proteic effector of the deaminase, alternatives (b) and (c) propose that the cosynthetase is an enzyme that uses as substrate a pyrrylmethane formed by the deaminase and porphobilinogen. Since the 2-aminomethylpyrrylmethanes which would result from the stepwise polymerization of porphobilinogen were unknown, there was no possibility of testing the above mentioned hypotheses. The systematic synthesis of these compounds was then undertaken with the purpose of clarifying whether (a) 2-aminomethylpyrrylmethanes are discrete intermediates (substrates) of the enzymatic process and (b) different isomeric pyrrylmethanes show different specificity toward the enzymatic system, so as to be able to examine the inversion problem.

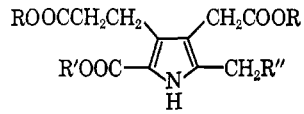
We shall describe first the general synthetic approach used for the synthesis of two aminomethyldipyrrylmethanes, one, **47**, resulting from the formal head-to-tail condensation of porphobilinogen and the other, **48**, a "nonsense dipyrrylmethane" from the biosynthetic standpoint, to be used as a control in the enzymatic studies. We shall then discuss the preliminary enzymatic results obtained with these dipyrrylmethanes.

Synthesis of Dipyrrylmethanes. The synthesis of aminomethyldipyrrylmethanes of general structures **47** and **48** had to be planned with the prior assumption that they would be extremely reactive and would condense rapidly and quantitatively to porphyrins if submitted to extensive manipulation. Thus, it was decided to keep the reactive aminomethyl group blocked until the last step, and then free it by a mild reaction. The dipyrrylmethane lactams of type **41** and **45** seemed suited for this purpose, since this type of lactam can be hydrolyzed by mild procedures,[10] and a good general synthesis is available for pyrrole lactams and 2-aminomethylpyrroles from azaindoles.[10] A dipyrrylmethane lactam is known,[11] but our objective was a general method of synthesis of these compounds which would also be useful for further propagation of the chain.

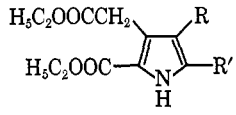
In a preliminary approach the direct reaction, with elimination of ammonia, of the protected 2-aminomethylpyrrole **14** with the porphobilinogen lactam esters **7** and **9**, was tested. 5-Carboxyporphobilinogen lactam (**4**)[10] was smoothly hydrolyzed to 5-carboxyporphobilinogen (**10**). Attempts to esterify **10** by treatment with ethanol and acid led only to esterification of the side chain carboxyls, but not the α-carboxyl group, and the hydrochloride **11** was obtained. This reaction was found to be general for different α-carboxypyrroles. Since treatment of **11** with diazoethane afforded only the diethyl ester of 5-carboxyporphobilinogen lactam (**5**) due to rapid lactamization it was necessary to block the free amino group. This was done by converting it in high yield into the benzyloxycarbonylamide **12** under mild reaction conditions. Further treatment of **12** with diazomethane afforded the triester **13** which was in turn transformed into the hydrochloride **14** by hydrogenolysis in an acidic medium. The reaction of **14** with the lactams **7** and **9** at low pH resulted in recovery of the starting materials, due undoubtedly to the strong deactivating effect of the 5-carbethoxy group on the aminomethyl bond.



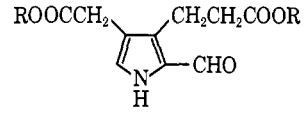
4, R = H; R′ = CO_2H
5, R = C_2H_5; R′ = $CO_2C_2H_5$
6, R = H; R′ = H
7, R = CH_3; R′ = H
8, R = $C_6H_5CH_2$; R′ = H
9, R = C_2H_5; R′ = H



10, R = H; R′ = H; R″ = NH_2
11, R = C_2H_5; R′ = H; R″ = NH_2·HCl
12, R = H; R′ = H; R″ = NHCO·$OCH_2C_6H_5$
13, R = CH_3; R′ = CH_3; R″ = NHCO·$OCH_2C_6H_5$
14, R = CH_3; R′ = CH_3; R″ = NH_2·HCl
15, R = $CH_2C_6H_5$; R′ = $CH_2C_6H_5$; R″ = NHCO·$OC(CH_3)_3$
16, R = $CH_2C_6H_5$; R′ = $CH_2C_6H_5$; R″ = NH_2·CF_3COOH
17, R = CH_3; R′ = $CH_2C_6H_5$; R″ = NH·CO·$OC(CH_3)_3$
18, R = CH_3; R′ = $CH_2C_6H_5$; R″ = NH_2·CF_3COOH



19, R = $COOC(CH_3)_3$; R′ = CH_3
20, R = COOH; R′ = CH_3
21, R = I; R′ = CH_3
22, R = H; R′ = CH_3
23, R = $CH_2CH_2COOC_2H_5$; R′ = CH_2NH_2·HCl



24, R = H
25, R = CH_3

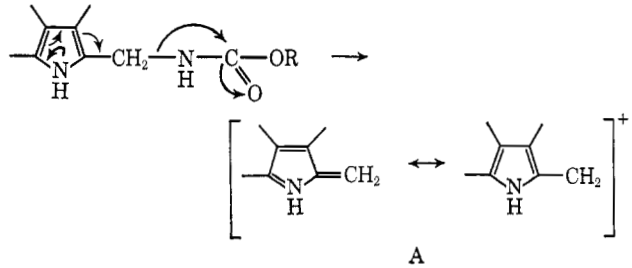
A second approach to the desired dipyrrylmethanes was explored by attempting the reaction of the aldehyde acid **24**[11] and its methyl ester **25** with the lactams **7** and **9**. Repeated attempts under diverse reaction conditions and employing different acidic catalysts ended in failure and this approach was abandoned. Finally, a preparative and mild synthetic procedure for dipyrrylmethanes was found in the treatment of 2-aminomethylpyrroles with nitrous acid and subsequent reaction of the resulting reactive intermediates with the corresponding lactam. The excess of nitrous acid had to be eliminated before the lactam was brought into the reaction mixture, since the latter was easily nitrosated.

(9) R. B. Frydman and B. Frydman, *Arch. Biochem. Biophys.*, **136**, 193 (1970).
(10) B. Frydman, S. Reil, M. E. Despuy, and H. Rapoport, *J. Amer. Chem. Soc.*, **91**, 2338 (1969).
(11) G. P. Arsenault and S. F. MacDonald, *Can. J. Chem.*, **39**, 2043 (1961). Also, the nmr has been reported [Y. C. Kim, *ibid.*, **47**, 3259 (1969)] for some of the dipyrrylmethanes we describe, but no methods of synthesis or other properties were presented.

Thus, the hydrochloride **14** was brought into reaction, *via* treatment with nitrous acid, with lactam ester **7** and dipyrrylmethane **36** was obtained in good yield. In the same manner the isomeric hydrochloride **23** produced the dipyrrylmethane **42** when allowed to react with the ethyl lactam **9**, and the tribenzyl trifluoroacetate **16** afforded the dipyrrylmethane tetrabenzyl ester **37** when treated with the benzyl ester lactam **8**. The hydrochloride **23** was obtained as described[11-13] with slight modifications, the most important of which was the transformation of pyrrole **19** to pyrrole **22**, where we had difficulty in reproducing the published results. The *tert*-butyl ester **19**,[14] when treated with trifluoroacetic acid, was transformed quantitatively into the acid **20**, which was in turn decarboxylated and iodinated to **21**; the iodine was removed by hydrogenolysis, and **22** was obtained in 39% overall yield. Trifluoroacetate **16** was obtained by converting 5-carboxyporphobilinogen (**10**) to its *tert*-butyloxycarbonyl derivative using Schnabel's procedure[15] (other methods gave negative results), followed by treatment of the BOC derivative with distilled diazotoluene. The BOC-benzyl ester thus obtained, **15**, was then transformed by mild treatment with trifluoroacetic acid into its trifluoroacetate **16**, which was directly used for dipyrrylmethane synthesis.

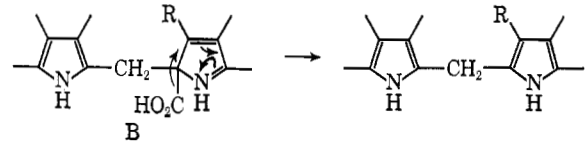
In order to complete the synthesis of dipyrrylmethanes **47** and **48**, selective cleavage of the 5'-carboxy ester groups of **36** and **42** was required. To explore a general selective esterification method for 2-aminomethyl-5-carboxypyrroles, we used as a model pyrrole **26** which could be easily prepared.[10] It was transformed into its benzyloxycarbonyl derivative **27**, which on esterification with methanol and acid afforded the monomethyl ester **28**. By treatment of **28** with phosphorus oxychloride and *tert*-butyl alcohol in pyridine the 5-*tert*-butyl ester **29** was obtained, and then transformed by the usual procedure into the hydrochloride **30**. But this protocol failed when applied to the benzyloxycarbonyl derivative **12** in the porphobilinogen series.

When **12** was submitted to acidic esterification conditions it was extensively polymerized to uroporphyrins. Two factors were found to govern this reaction. At low pH the carbamate residue of the Mannich base was cleaved, affording the reactive diene A which polymerized. The attack of A at the α position to



A

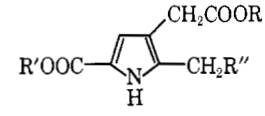
form intermediate B depended, however, on the electron-releasing properties of the β substituent R. Accordingly, the polymerization rate was highly increased



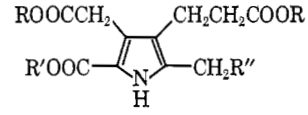
B

as a function of R in the series $CH_3 > CH_2CH_2CO_2H > CH_2CO_2H > H$. A detailed study of the polymerization of the corresponding 2-aminomethylpyrroles supports the outlined mechanism and will be published elsewhere. Since the alkylation of **12** in alkaline media[16] gave poor yields, the approach was changed in favor of controlled transesterification of the tribenzyl ester **15** with sodium methoxide, which afforded the expected dimethylmonobenzyl ester **17** in good yields. Transesterification of the dipyrrylmethanes **36** and **42** gave inseparable ester mixtures.

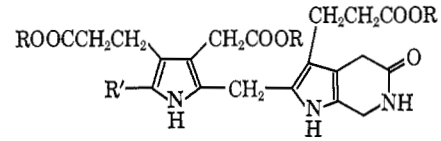
Synthesis of the isomeric BOC-monobenzyl ester **34** was straightforward. 5-Carboxyisoporphobilinogen (**31**) was esterified in acid medium to give the ester hydrochloride **32**, which was then converted to the BOC derivative **33** by the usual procedure, since no lactamization to the seven-membered lactam ring takes place at 25°. The acid **33** was then treated with diazo-



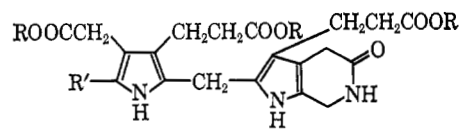
26, R = H; R' = H; R'' = NH_2
27, R = H; R' = H; R'' = NH·CO·$OCH_2C_6H_5$
28, R = CH_3; R' = H; R'' = NH·CO·$OCH_2C_6H_5$
29, R = CH_3; R' = $C(CH_3)_3$; R'' = NH·CO·$OCH_2C_6H_5$
30, R = CH_3; R' = $C(CH_3)_3$; R'' = NH_2·HCl



31, R = H; R' = H; R'' = NH_2
32, R = CH_3; R' = H; R'' = NH_2·HCl
33, R = CH_3; R' = H; R'' = NH·BOC
34, R = CH_3; R' = $CH_2C_6H_5$; R'' = NH·BOC
35, R = CH_3; R' = $CH_2C_6H_5$; R'' = NH_2·CF_3COOH



36, R = CH_3; R' = CO_2CH_3
37, R = $CH_2C_6H_5$; R' = $CO_2CH_2C_6H_5$
38, R = H; R' = CO_2H
39, R = CH_3; R' = $CO_2CH_2C_6H_5$
40, R = CH_3; R' = CO_2H
41, R = CH_3; R' = H

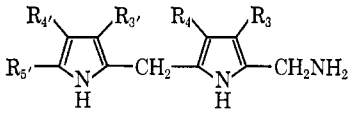


42, R = C_2H_5; R' = $CO_2C_2H_5$
43, R = CH_3; R' = $CO_2CH_2C_6H_5$
44, R = CH_3; R' = CO_2H
45, R = CH_3; R' = H

(12) S. F. MacDonald, *J. Chem. Soc.*, 4176 (1952).
(13) E. Bullock, T. S. Chen, and C. E. Loader, *Can. J. Chem.*, **44**, 1007 (1966).
(14) A. Treibs and F. Hintermeier, *Chem. Ber.*, **87**, 1178 (1954).
(15) E. Schnabel, *Justus Liebigs Ann. Chem.*, **702**, 188 (1967).
(16) F. Stodola, *J. Org. Chem.*, **29**, 2941 (1964).

toluene and the resulting ester **34**, as well as the isomeric ester **17**, were transformed into their trifluoroacetates **35** and **18**, and converted into the respective dipyrrylmethanes **39** and **43**. Hydrogenolysis of the benzyl esters afforded the dipyrrylmethanes **40** and **44** which were decarboxylated by heating briefly at 220° to the dipyrrylmethanes **41** and **45**. It is noteworthy that the C-2 methylenes of the dipyrrylmethanes **39** and **43** are strongly shifted in the nmr spectra from the usual position at 4.7–4.8 ppm downfield to 5.2–5.5 ppm, thus indicating a probable conformation in the dipyrrylmethane in which the 5′-benzyloxycarbonyl group is folded beneath the lactam ring.

Hydrolysis of the dipyrrylmethane lactams was easily monitored using the nmr. Hydrogenolysis of dipyrrylmethane **37** afforded dipyrrylmethane **38** which was used as a reference for the lactam group shifts. When **36** was hydrolyzed to **46** in the usual manner[10] the nmr spectrum indicated the disappearance of the C-2 lactam methylene signals and the appearance of the new aminomethyl and carboxymethyl signals. The same applied for hydrolysis of **41** and **45** to **47** and **48**.



46, $R_3 = CH_2CO_2H$; $R_4 = CH_2CH_2CO_2H$; $R_{3'} = CH_2CO_2H$; $R_{4'} = CH_2CH_2CO_2H$; $R_{5'} = CO_2H$

47, $R_3 = CH_2CO_2H$; $R_4 = CH_2CH_2CO_2H$; $R_{3'} = CH_2CO_2H$; $R_{4'} = CH_2CH_2CO_2H$; $R_{5'} = H$

48, $R_3 = CH_2CO_2H$; $R_4 = CH_2CH_2CO_2H$; $R_{3'} = CH_2CH_2CO_2H$; $R_{4'} = CH_2CO_2H$; $R_{5'} = H$

49, $R_3 = CH_2CH_2CO_2H$; $R_4 = CH_2CO_2H$; $R_{3'} = CH_2CO_2H$; $R_{4'} = CH_2CH_2CO_2H$; $R_{5'} = H$

When **46**, **47**, and **48** were isolated, either by precipitation at pH 3.5 or as mercuric salts, which were then decomposed by hydrogen sulfide, they behaved as amorphous solids which were transformed on handling to yield porphyrins. As expected, **47** and **48** were devoid of any uv or visible absorption. They were transformed into porphyrins when subjected to paper or tlc chromatography, and they were reasonably stable (several days) in solution at pH 7–8 in the cold, as indicated by Ehrlich's reaction and the nmr spectra. The very rapid exchange of the C-5′ hydrogen with deuterium at pH 7–8, in contrast to the stability of analogous protons in the monopyrroles, was striking. This susceptibility to electrophilic attack could explain the very rapid dimerization of the dipyrrylmethanes to porphyrins.

Enzymic Results. The dipyrrylmethanes **47** and **48** were not substrates for either porphobilinogen deaminase, uroporphyrinogen III cosynthetase, or the combined enzymic system. In the presence of porphobilinogen however, a difference between the dipyrrylmethanes is evident. While dipyrrylmethane **47** inhibited porphobilinogen consumption and increased the porphyrin yields, the dipyrrylmethane **48** had only a slight inhibitory effect on porphyrin formation (Table I).

It is thus evident that dipyrrylmethane **47** was incorporated in the presence of porphobilinogen into the enzymic system, and dipyrrylmethane **48** was not.

Table I. Effect of Dipyrrylmethanes on Porphyrin Formation

Dipyrrylmethane added[a]	Time, min	PBG consumed, nmol	Porphyrin formed, nmol	Yields, %
	30	2.8	0.69	100
47	30	2.0	0.55	110
48	30	2.7	0.58	85
	60	4.6	1.02	88
47	60	3.24	1.14	140
48	60	4.3	0.90	82
	90	4.4	1.00	90
47	90	3.24	1.00	125
48	90	4.2	0.88	84

[a] The complete system contained, in a final volume of 100 μl: 10 μmol of buffer Pi, pH 7.4, 6 nmol of porphobilinogen (PBG), 30 nmol of either dipyrrylmethane (where indicated), and deaminase cosynthetase system from wheat germ, prepared as described.[9] Blanks were run for deaminase activity, containing either PBG (enzyme added at the end of the incubation) or PBG added at the end of the incubation. When the incubation mixture contained dipyrrylmethane, blanks were run containing either enzyme plus dipyrrylmethane, or dipyrrylmethane plus PBG. The system was completed at the end of the incubation. Nonenzymically formed porphyrins from dipyrrylmethanes were deducted in each case.

Dipyrrylmethane **47** was a very poor substrate when compared with porphobilinogen, and even when the relative porphyrin yields increased by 10 to 40% at the expense of dipyrrylmethane **47**, the rise in the absolute amount of porphyrin formed was negligible. Dipyrrylmethane **47** was not a substrate of cosynthetase even in the presence of porphobilinogen. The enzymic incorporation of **47** into porphyrins was confirmed with the use of porphobilinogen-^{14}C (Table II). Dipyrrylmethane **47** was incorporated only into uroporphyrin I in an enzymic system forming both isomers (systems A and C). In a uroporphyrin III forming system (B) the addition of dipyrrylmethane **47** diverted the formation of uroporphyrin III to uroporphrin I, but it was incorporated only in the latter. The same results were obtained when the uroporphyrins were decarboxylated to coproporphyrins and analyzed as such (Table III). Dipyrrylmethane **48** was not incorporated into the enzymic system and was independently transformed by its chemical polymerization into coproporphyrin II (90% yield), thus indirectly confirming the correct order of its side chain.

From these data it is possible to extract two main conclusions. The first is that dipyrrylmethane **47**, although formally derived from the condensation of two units of porphobilinogen, is an intermediate but not a substrate of the enzymic system involved in porphobilinogen polymerization. When present in a fivefold excess over porphobilinogen (Table I) it is incorporated in a low proportion into uroporphyrins, and it also inhibited the porphobilinogen consumption. It is thus apparently competing with porphobilinogen for the first binding site of the deaminase. The second conclusion is that the dipyrrylmethane **47** is not incorporated at all into uroporphyrin III, although it inhibits formation of this isomer.

The only biosynthetic scheme that can accommodate both facts is one in which both isomers originate by different pathways from the start of the polymerization, with the process taking place at all stages on the enzymes' surface without liberation of free pyrrylmethanes. It is very unlikely, in view of the results obtained with

Table II. Effect of Dipyrrylmethanes on Isomer Formation

Enzymes	Dipyrrylmethane added[a]	Uroporphyrin I nmol	Uroporphyrin I cpm	Uroporphyrin I Sp act., cpm/nmol	Uroporphyrin III nmol	Uroporphyrin III cpm	Uroporphyrin III Sp act., cpm/nmol
A[b]		0.13	1,908	14,680	0.12	1,840	15,330
		0.144	2,174	15,970	0.10	1,566	15,600
	47	0.13	1,418	10,770	0.09	1,430	15,890
	47	0.165	1,669	10,115	0.08	1,330	16,650
B[c]					0.12	2,127	17,720
					0.145	2,218	15,330
	47	0.07	885	12,640	0.09	1,400	15,550
	47	0.07	830	11,860	0.09	1,600	17,780
C[d]		0.054	829	15,370	0.18	2,311	12,840
	47	0.15	1,770	11,800	0.08	1,260	15,750
	47	0.11	1,180	10,730	0.09	1,360	15,100

[a] The complete system contained in a final volume of 100 μl: 10 μmoles of buffer Tris HCl, pH 8.2, PBG-^{14}C (4 nmol, 15,000 cpm), and enzyme from wheat germ (deaminase–cosynthetase system). [b] A formed equivalent amounts of both isomers. [c] B formed only uroporphyrin III. [d] C formed an excess of uroporphyrin III. The porphyrins formed were esterified and separated by paper chromatography as described.[4] Blanks were prepared as described in Table I and deducted in each case.

Table III. Effect of Dipyrrylmethanes on Isomer Formation

Enzymes	Dipyrrylmethane added[a]	Coproporphyrin I nmol	Coproporphyrin I cpm	Coproporphyrin I Sp act. cpm/nmol	Coproporphyrin III nmol	Coproporphyrin III cpm	Coproporphyrin III Sp act., cpm/nmol
A[b]		0.35	3200	9000			
	48 (20 nmol)[d]	0.26	2400	9200			
	47 (10 nmol)	0.32	2400	7500			
B[c]		0.05	460	9200	0.30	2750	9160
	48 (20 nmol)[d]	0.03	290	9600	0.21	1880	9000
	47 (10 nmol)	0.28	2080	7400	0.02	180	9000

[a] The complete system contained in a final volume of 100 μl: buffer Tris HCl, pH 8.2, PBG-^{14}C, 4 nmol (15,000 cpm), and wheat germ enzyme. [b] A formed only coproporphyrin I. [c] B formed an excess of coproporphyrin III. The incubation was carried out at 37° for 60 min. The uroporphyrins formed were esterified, extracted as octamethyl esters, decarboxylated, and separated as described: L. Eriksen, *Scand. J. Clin. Lab. Invest.*, **10**, 319 (1958). [d] Dipyrrylmethane **48** formed chemically 90% coproporphyrin II.

the dipyrrylmethane **47**, that higher free polymers of porphobilinogen (pyrrylmethyldipyrrylmethanes or bilanes) serve as substrates for either enzyme. The different isomer formations can be explained with the two mechanisms by which porphobilinogen reacts chemically: a repetitive head-to-tail condensation will give bound dipyrrylmethane **47** first followed by uroporphyrin I, and a repetitive head-to-head condensation, followed by an intramolecular migration (as proposed in several hypotheses[1]), will afford bound dipyrrylmethane **49** first and then urophyrin III. Uroporphyrinogen III cosynthetase would thus be one of the so-called "specifier proteins," changing the mode of porphibilinogen polymerization on the porphobilinogen deaminase. Dipyrrylmethane **49** becomes an intermediate in uroporphyrin III formation but not in uroporphyrin I formation.

Experimental Section[17]

2-Aminomethyl-5-carboxy-3-carboxymethyl-4-pyrrolepropionic Acid (Carboxyporphobilinogen) (10). 5-Carboxyporphobilinogen lactam **4**[10] (10 g) was dissolved in 150 ml of 2 *N* potassium hydroxide and the mixture left at room temperature for 72 hr. The solution was then adjusted to pH 7 with concentrated acetic acid and cooled at 5° for 3 hr. The filtered solid was further purified by dissolution in dilute ammonium hydroxide and precipitation with acetic acid; 10.3 g (90%) of 5-carboxyporphobilinogen was obtained: mp 234–235° dec (lit.[18] mp 233–234°); R_f 0.40; nmr 2.65 (m, 4, CH_2CH_2-CO_2H), 3.35 (s, 2, CH_2CO_2H), 3.7 (s, 2, CH_2NH_2).

Anal. Calcd for $C_{11}H_{14}O_8N_2 \cdot H_2O$: C, 45.8; H, 5.6; N, 9.7. Found: C, 46.0; H, 5.5; N, 9.6.

Ethyl 2-Aminomethyl-3-ethoxycarbonylmethyl-5-carboxy-4-pyrrolepropionate Hydrochloride (11). 5-Carboxyporphobilinogen (**10**) (2 g) dissolved in 20 ml of 10% ethanolic hydrogen chloride was kept at 5° during 12 hr, after which the solution was evaporated to dryness *in vacuo* at 30°, and the residue dissolved in a small volume of absolute ethanol and crystallized by gradual addition of ether. Recrystallization from the same solvent mixture afforded 2.05 g (82%) of the hydrochloride **11**: mp 187–190° dec; uv λ_{max} 264 nm (ϵ 14,300); ir 1739 cm^{-1} ($COOC_2H_5$), 1681 (C=C-*C*OOH).

Anal. Calcd for $C_{15}H_{22}N_2O_6 \cdot HCl$: C, 49.9; H, 6.3; N, 7.7. Found: C, 49.8; H, 6.5; N, 7.7.

Ethyl 2-Ethoxycarbonyl-5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (5-Carboxyporphobilinogen Lactam Diethyl Ester) (5). Hydrochloride **11** (1 g) was treated in an ethanolic (20 ml) suspension with an excess of ethereal diazoethane at 5°, after which the solution was evaporated to dryness *in vacuo* and the residue crystallized from ethanol: 680 mg (80%); mp 235–239° (lit.[18] mp 235–236°); uv λ_{max} 277 nm (ϵ 16,900); identical (melting point, ir, uv) with a sample prepared by the action of ethereal diazoethane on 5-carboxyporphobilinogen lactam.

Anal. Calcd for $C_{15}H_{20}O_5N_2$: C, 58.4; H, 6.5; N, 9.1. Found: C, 58.6; H, 6.2; N, 9.3.

Methyl 5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (porphobilinogen lactam methyl ester) (7) was prepared following the same procedure used for the synthesis of the diethyl ester **5**. From 2.1 g of **6** was obtained, using ethereal diazomethane, 1.5 g (68%) of **7**: mp 240–242°; R_f 0.80 (tlc, 15% ethanol in chloroform); nmr (TFA) 3.1 (m, 4, $-CH_2CH_2-$), 4.0 (s, 2, $-CH_2CO$), 5.1 (s, 2, CH_2NH), 5.3 (broad, 2, $=NCH_2-$), 3.9 (s, 3, OCH_3); (0.1 *M* KOD) 6.5 (s, 1, NHCH=).[19]

(17) All melting points were taken on the Kofler block; ultraviolet absorptions were measured in ethanol, infrared spectra were obtained in potassium bromide wafers, and nmr spectra (δ) were taken in 0.1 *M* KOD unless otherwise noted. A Varian A-60D instrument was used with TMS or DSS as internal standard. R_f values are for paper chromatography on Whatman No. 1 paper with the upper layer of a butanol–acetic acid–water 4:1:4 mixture unless otherwise specified; tlc was carried out on silica gel G coated plates.

(18) A. H. Jackson and S. F. MacDonald, *Can. J. Chem.*, **35**, 715 (1957).

Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.5; H, 6.3; N, 12.6. Found: C, 59.4; H, 6.3; N, 12.5.

Benzyl 5-Oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (Porphobilinogen Lactam Benzyl Ester) (8). **7** (3 g) was added to a solution of 50 mg of sodium in 100 ml of benzyl alcohol and the mixture was heated at 100° for 2 hr. A 10-ml sample of the solution was distilled *in vacuo*, 90° (10 mm), after which 10 ml of fresh benzyl alcohol was added to the mixture and heating was resumed for another 2 hr; the operation was then repeated. After a total of 6-hr heating, the benzyl alcohol was evaporated *in vacuo* and the residue was crystallized twice from ethanol; 2.25 g (56%) of **8** was obtained: mp 207–208°; nmr (C_5D_5N) 3.15 (m, 4, $-CH_2CH_2-$), 3.75 (m, 2, $-CH_2CO-$), 4.65 (m, 2, $-CH_2NH$), 5.25 (s, 2, $CH_2C_6H_5$), 6.75 (s, 1, ≡CH), 7.45 (m, 5, C_6H_5).

Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.4; H, 6.0; N, 9.4. Found: C, 68.4; H, 6.1; N, 9.3.

2-Benzyloxycarbonylaminomethyl-5-carboxy-3-carboxymethyl-4-pyrrolepropionic Acid (12). 5-Carboxyporphobilinogen (**10**) (2.88 g, 0.01 mol) was dissolved in a mixture of 6.6 g of sodium bicarbonate in 50 ml of water and benzyloxycarbonyl chloride (3.1 ml, 0.022 mol) was added in five portions over a period of 30 min, with vigorous stirring. The stirring was continued 1 hr; the reaction mixture was extracted with two 10-ml portions of ether, then carefully acidified to congo red with 5 *N* hydrochloric acid and extracted with six 15-ml portions of ethyl acetate. The dried (Na_2SO_4) ethyl acetate extracts were evaporated to dryness at 30°, and the residue crystallized twice by dissolution in ethyl acetate and careful addition of chloroform; 2 g (46%) of **12** was obtained as pink crystals: mp 160–162°; R_f 0.80; nmr (TFA) 3.0 (m, 4, $-CH_2-CH_2-$), 3.7 (s, 2, $-CH_2CO_2H$), 4.5 (d, 2, CH_2NHCO), 5.3 (s, 2, $CH_2C_6H_5$), 7.4 (m, 5, C_6H_5).

Anal. Calcd for $C_{19}H_{20}N_2O_8$: C, 56.4; H, 4.9; N, 6.9. Found: C, 56.2; H, 4.7; N, 6.8.

Methyl 2-(Benzyloxycarbonylaminomethyl)-3-methoxycarbonylmethyl-5-methoxycarbonyl-4-pyrrolepropionate (13). A solution of 2 g of the benzyloxycarbonyl acid **12** in 10 ml of methanol was treated with an excess of diazomethane dissolved in ether until esterification was complete; the solution was then evaporated to dryness, and the residue was crystallized from benzene–cyclohexane: 1.8 g (73%); mp 110–113°; nmr ($CDCl_3$) 2.75 (m, 4, CH_2-CH_2CO), 3.5 (s, 2, CH_2CO), 3.65 (s, 6, $-CO_2CH_3$), 4.15 (d, 2, CH_2-NHCO), 5.1 (s, 2, $CH_2C_6H_5$), 7.3 (m, 5, C_6H_5).

Anal. Calcd for $C_{22}H_{26}N_2O_8$: C, 59.2; H, 5.8; N, 6.3. Found: C, 59.2; H, 5.7; N, 6.3.

Methyl 2-Aminomethyl-3-methoxycarbonylmethyl-5-methoxycarbonyl-4-pyrrolepropionate Hydrochloride (14). A 2-g sample of the triester was dissolved in 20 ml of methanol containing 3 drops of concentrated hydrochloric acid, and reduced with hydrogen at 40 psi over 600 mg of 10% palladium on charcoal during 3 hr. The catalyst was removed, the methanol evaporated to dryness at 30°, and the residue crystallized from methanol–ether, giving 1.1 g (73%) of **14**: mp 158–160°; nmr (TFA) 3.0 (m, 4, CH_2CH_2), 3.81 (s, 2, CH_2CO_2-), 3.85, 3.91 (s, 6, CO_2CH_3), 4.08 (s, 3, $C=C-CO_2CH_3$), 4.6 (broad, 2, $CH_2N^+H_3$), 7.5 (broad, 3, N^+H_3).

Anal. Calcd for $C_{14}H_{21}N_2O_6Cl$: C, 49.6; H, 6.2; N, 8.3. Found: C, 49.5; H, 6.1; N, 8.2.

2-Benzyloxycarbonylaminomethyl-5-carboxy-3-pyrroleacetic acid (27) was prepared following the same procedure used for the synthesis of benzyloxycarbonyl derivative **12**. From 5 g of **26** was obtained 6 g (68%) of **27**, mp 168–170°.

Anal. Calcd for $C_{16}H_{16}N_2O_6 \cdot H_2O$: C, 54.8; H, 5.1; N, 8.0. Found: C, 54.9; H, 5.2; N, 7.9.

Methyl 2-Benzyloxycarbonylaminomethyl-5-carboxy-2-pyrroleacetate (28). Acid **27** (4 g) was dissolved in 100 ml of 8% methanolic hydrogen chloride and kept at 5° during 5 hr. The solution was evaporated *in vacuo* (25°), the residue dissolved in 50 ml of ethyl acetate, the ethyl acetate washed with water, dried (Na_2SO_4), and evaporated to dryness, and the residue crystallized from ethyl acetate–cyclohexane: 3.5 g (84%); mp 138–140°; R_f 0.20 (tlc, 15% methanol in chloroform); nmr ($CDCl_3$) 3.5 (s, 2, CH_2CO-), 3.73 (s, 3, OCH_3), 4.4 (d, 2, CH_2NHCO), 5.17 (s, 2, $CH_2C_6H_5$), 6.7 (broad, 1, =CH–), 7.35 (m, 5, C_6H_5).

Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.9; H, 5.2; N, 8.1. Found: C, 58.8; H, 5.1; N, 8.0.

Methyl 2-Benzyloxycarbonylaminomethyl-5-*tert*-butyloxycarbonyl-3-pyrroleacetate (29). **28** (1 g, 23 mmol) was dissolved in a mixture of 10 ml of pyridine and 20 ml of *tert*-butyl alcohol, and 0.36 ml (4 mmol) of phosphorus oxychloride was added at 5°. The solution was then allowed to reach room temperature and after 14 hr it was diluted with an equal volume of water and extracted with three 15-ml portions of ethyl acetate and the extracts were washed successively with 1 *M* hydrochloric acid, 10% sodium carbonate, and water. The dried (Na_2SO_4) extracts were evaporated, and the oily resdue dissolved in a 5% methanol–benzene solution was filtered through a short (5 cm × 1 cm) column of silica gel previously washed with the same solvent. The eluted oily substance (650 mg, 59%) was pure by tlc and was used directly for the next step: R_f 0.90 (tlc, 5% methanol in benzene); nmr ($CDCl_3$) 1.6 (9, s, $(CH_3)_3C-$), 3.5 (s, 2, CH_2CO), 3.7 (s, 3, OCH_3), 4.4 (d, 2, CH_2NHCO), 5.2 (s, 2, $CH_2C_6H_5$), 6.75 (broad, 1, =CH–), 7.4 (m, 5, C_6H_5).

Methyl 2-Aminomethyl-5-*tert*-butyloxycarbonyl-3-pyrroleacetate Hydrochloride (30). **29** (1 g) dissolved in 20 ml of absolute methanol containing 0.1% dry hydrogen chloride was reduced following the same procedure used in the synthesis of **14**. The hydrochloride was crystallized from ethyl acetate–ether: 500 mg (67%); mp 170–172°; uv λ_{max} 275 mm (ε 19,700); R_f 0.30 (tlc, 15% methanol in chloroform); nmr (D_2O) 1.6 (s, 9, $(CH_3)_3C$), 3.73 (s, 2, CH_2CO), 3.81 (s, 3, OCH_3), 4.4 (broad, 2, CH_2NH_3), 6.88 (s, 1, =CH–).

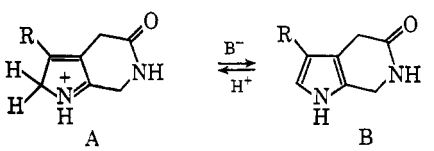
Anal. Calcd for $C_{13}H_{21}N_2O_4Cl$: C, 51.2; H, 6.9; N, 9.2. Found: C, 51.0; H, 6.7; N, 9.3.

Benzyl 2-*tert*-Butyloxycarbonylaminomethyl-3-benzyloxycarbonylmethyl-5-benzyloxycarbonyl-4-pyrrolepropionate (15). Carboxyporphobilinogen (**10**) (2.88 g, 0.01 mol) was suspended in 110 ml of 5% aqueous dioxane, 1.95 g (0.0135 mol) of *tert*-butyloxycarbonyl azide was added, and the mixture was titrated with constant stirring at pH 9.8, with 4 *M* sodium hydroxide in a pH-stat. The reaction came to an end after 3 hr; it was then diluted with 50 ml of water, extracted with two 25-ml portions of ether adjusted to pH 3 with citric acid, and extracted with six 26-ml portions of ethyl acetate. The extracts were washed with two 5-ml portions of cold water, dried (Na_2SO_4), and evaporated *in vacuo*. The residue was washed with three 25-ml portions of hot chloroform and centrifuged, and the remaining solid (2.6 g) was dissolved in methanol, and treated dropwise with distilled, 53° (0.2 mm), diazotoluene[20] until Ehrlich's reaction was negative on heating. The excess diazotoluene was destroyed with acetic acid, the solution evaporated to dryness *in vacuo*, and the residue crystallized from methanol, affording 3.6 g (56%) of **15**: mp 115–117°; uv λ_{max} 268 mm (ε 17,600); nmr ($CDCl_3$) 1.4 (s, 9, $C(CH_3)_3$), 2.75 (m, 4, $-CH_2CH_2-CO-$), 3.45 (s, 2, CH_2CO-), 4.5 (d, 2, CH_2NHCO), 5.05 (s, 4, $CH_2-C_6H_5$), 5.2 (s, 2, $C=CCO_2CH_2C_6H_5$), 7.2 (m, 15, C_6H_5).

Anal. Calcd for $C_{37}H_{40}N_2O_8$: C, 69.5; H, 6.4; N, 4.4. Found: C, 69.4; H, 6.2; N, 4.4.

Methyl 2-*tert*-Butyloxycarbonylaminomethyl-3-methoxycarbonylmethyl-5-benzyloxycarbonyl-4-pyrrolepropionate (17). The tribenzyl ester **15** (2.24 g, 3.5 mmol) was dissolved in 60 ml of boiling methanol and cooled to room temperature, and a solution of 23 mg (1 mmol) of sodium in 8 ml of methanol was added carefully avoiding crystallization in the saturated solution. The mixture was kept during 1 hr at 20°, then poured into a saturated sodium chloride solution (300 ml), and extracted with three 50-ml portions of ethyl acetate. The dried extracts (Na_2SO_4) were evaporated and the resulting solid, dissolved in a small volume of 3% methanol in benzene, was adsorbed on a column (33 cm × 3 cm) packed with tlc silica gel prewashed with the same solvent. The column was eluted by applying slight pressure and two fractions were collected. Fraction I (300 mg, R_f 0.80; tlc, 3% methanol in benzene) consisted mainly of the dibenzyl monomethyl ester. Fraction II (R_f 0.75; tlc, 3% methanol in benzene) was the dimethyl monobenzyl ester **17** and was crystallized from methanol–water: 980, mg (57%); mp 83–84°; nmr ($CDCl_3$) 1.4 (s, 9, $(CH_3)_3$), 2.75 (m, 4, CH_2CH_2), 3.45 (s, 2, CH_2CO-), 3.58, 3.62 (s, 6, OCH_3), 4.2 (d, 2 CH_2NHCO), 5.25 (s, 2, $CH_2C_6H_5$), 7.3 (s, 5, C_6H_5), 7.3 (s, 5, C_6H_5); uv λ_{max} 280 mm (ε 20,290).

(19) In trifluoroacetic acid solution the lactams exist entirely as the conjugate acids in the α-pyrrolenine form (A), which reverts to the pyrrole form (B) in alkaline solution



A B

(20) The diazotoluene had to be freshly distilled, since its decomposition products were inseparable from the reaction products.

Anal. Calcd for $C_{25}H_{32}N_2O_8$: C, 61.5; H, 6.5; N, 5.7. Found: C, 61.4; H, 6.4; N, 5.7.

Ethyl 2-Methyl-3-carboxy-5-ethoxycarbonyl-4-pyrroleacetate (20). A solution of 50 g of ethyl 2-methyl-3-*tert*-butyloxycarbonyl-5-ethoxycarbonyl-4-pyrroleacetate (**19**)[14] in 500 ml of trifluoroacetic acid was kept at room temperature during 12 hr. A tenfold volume of water was then added and the solid filtered and dried, affording 36 g (87%) of **20**, mp 237–239° (lit.[12] mp 238–239°).

Ethyl 2-Methyl-3-iodo-5-ethoxycarbonyl-4-pyrroleacetate (21). A solution of 28.3 g (0.1 mol) of the crude acid **20** and of 67 g of sodium bicarbonate in 40 ml of water was added with constant stirring to a second solution of 25.4 g (0.1 mol) of iodine and 67 g of potassium iodide in 40 ml of water. The mixture was heated at 90° during 1 hr, then cooled, and the solid filtered, dried, and crystallized from methanol, 21.9 g (60%) of **21**, mp 116–119°.

Anal. Calcd for $C_{12}H_{16}O_4NI$: N, 3.5. Found: N, 3.6.

Ethyl 2-Methyl-5-ethoxycarbonyl-4-pyrroleacetate (22). The iodopyrrole **21** (7 g) was dissolved in 200 ml of ethanol, 7 g of sodium acetate was added, and the solution was reduced at 3 psi with hydrogen during 2 hr over 3.5 g of 10% palladium on charcoal. The catalyst was removed and washed with ethanol. The combined filtrate and washings were evaporated *in vacuo*, and the residue dissolved in 20 ml of water and extracted with five 20-ml portions of chloroform. The dried (Na_2SO_4) extracts were evaporated *in vacuo* and the residue was crystallized from cyclohexane; 3.5 g mp 90–92° (lit.[12] mp 91–92°).

2-Aminomethyl-4-carboxymethyl-5-carboxy-3-pyrrolepropionic Acid (31). Triethyl ester hydrochloride **23**[11] (3 g) was dissolved in 27 ml of ethanol, 27 ml of a 1 *N* sodium hydroxide solution was added, and the mixture was heated under reflux for 1 hr. The solution was then evaporated to half-volume, adjusted to pH 5 with acetic acid, cooled, and filtered. The solid, 1.9 g (86%), turned reddish on standing and had no definite melting point. It was reprecipitated by dissolving in a small volume of 1 *M* ammonium hydroxide solution and adding acetic acid: R_f 0.27; nmr (D_2O) (m, 4, $-CH_2CH_2CO-$), 3.8 (s, 2, $-CH_2CO$), 4.2 (s, 2, $-CH_2NH_2$).

Anal. Calcd for $C_{11}H_{14}N_2O_6\cdot H_2O$: C, 45.8; H, 5.6; N, 9.7. Found: C, 45.6; H, 5.5; N, 9.4.

Methyl 2-aminomethyl-5-carboxy-4-methoxycarbonylmethyl-3-pyrrolepropionate hydrochloride (32) was prepared following the procedure described for the synthesis of hydrochloride **11**. From 3 g of **31** was obtained 2.2 g (61%) of **32**: mp 183–185°; nmr (TFA) 2.8 (m, 4, CH_2CH_2), 3.7, 3.8 (s, 6, OCH_3), 3.95 (s, 2, CH_2CO), 4.6 (m, 2, CH_2NH_2), 7.4 (m, 3, $^+NH_3$).

Anal. Calcd for $C_{13}H_{18}N_2O_6\cdot HCl\cdot H_2O$: C, 44.3; H, 5.9; N, 7.9. Found: C, 44.5; H, 5.7; N, 7.6.

Methyl 2-*tert*-butyloxycarbonylaminomethyl-4-methoxycarbonylmethyl-5-carboxy-4-pyrrolepropionate (33) was prepared by the action of *tert*-butyloxycarbonyl azide on the hydrochloride **32** as described for **15**. The reaction was complete in 1 hr. From 3 g of **32** was obtained 1.7 g (50%) of **33**: mp 176–180° (crystallized from methanol–water); nmr (TFA) 1.55 (s, 9, $(CH_3)_3$), 2.77 (m, 4, CH_2CH_2), 3.67, 3.7 (s, 6, OCH_3), 3.9 (s, 2, CH_2CO), 4.5 (d, 2, CH_2CH_3), 7.4 (m, 3, $^+NH_3$).

Anal. Calcd for $C_{18}H_{26}N_2O_8$: C, 54.3; H, 6.5; N, 7.0. Found: C, 54.2; H, 6.6; N, 7.1.

Methyl 2-*tert*-butyloxycarbonylaminomethyl-4-methoxycarbonylmethyl-5-benzyloxycarbonyl-5-pyrrolepropionate (34) was prepared by the action of diazotoluene on **33** as described for **15**. From 1 g of **33** was obtained 1 g (83%) of **34**: mp 108–110°; uv λ_{max} 281 mm (ϵ 20,000); nmr ($CDCl_3$) 1.45 (s, 9, $(CH_3)_3$), 2.67 (m, 4, CH_2CH_2), 3.65, 3.68 (s, 6, OCH_3), 3.87 (s, 2, CH_2CO), 4.28 (d, 2, CH_2NHCO), 5.34 (s, 2, $CH_2C_6H_5$), 7.45 (s, 5, C_6H_5).

Anal. Calcd for $C_{25}H_{32}N_2O_8$: C, 61.5; H, 6.5; N, 5.7. Found: C, 61.6; H, 6.5; N, 5.8.

Methyl 2-Formyl-4-methoxycarbonylmethyl-3-pyrrolepropionate (25). Acid **24**[11] (500 mg) was treated, in a methanolic solution, with an excess of ethereal diazomethane. The solution was evaporated to dryness and the oily residue purified by chromatography on a column of tlc silica using 2% methanol in chloroform as a solvent. The substance, which was pure by tlc, was not crystallized: 390 mg (70%); nmr ($CDCl_3$) 2.85 (m, 4, CH_2CH_2), 3.55 (s, 2, $-CH_2CO$), 3.72, 3.67 (s, 6, OCH_3), 7.1 (m, 1, H-5), 9.65 (s, 1, CHO).

Methyl 2-(3′-Methoxycarbonylmethyl-4′-β-methoxycarbonylethyl-5′-methoxycarbonyl-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (36). To a solution of 1.05 g (3 mmol) of the hydrochloride **14** in 25 ml of glacial acetic acid was added 414 mg (3 mmol) of sodium nitrite and the mixture stirred during 15 min. The precipitated salts were removed and nitrogen was passed through the solution for 10 min, after which 660 mg (3 mmol) of porphobilinogen lactam methyl ester was added and the solution heated at 90° during 30 min. The solution was then diluted with 100 ml of water and the precipitate filtered and crystallized from ethanol: 1.1 g (73%) of **36**; R_f 0.70 (tlc, 5% methanol in chloroform); mp 215–217°; uv λ_{max} 281 mμ (ϵ 17,300); ir 1740, 1710 cm^{-1} (R-*COO*CH_3), 1680 (α,β unsat. *COO*CH_3), 1650, 1630 (*CO*NH); nmr (C_5D_5N) 2.8 (m, 8, CH_2CH_2), 3.6, 3.65 (s, 14, OCH_3, $-CH_2$CONH–), 4.25 (s, 2, $-CH_2CO_2-$), 4.4 (s, 2, $-CH_2-$), 4.85 (m, 2, $-CH_2$NH).

Anal. Calcd for $C_{26}H_{31}N_3O_9$: C, 58.0; H, 6.0; N, 8.1. Found: C, 58.0; H, 5.9; N, 8.1.

Ethyl 2-(3′-β-ethoxycarbonylethyl-4′-ethoxycarbonylmethyl-5′-ethoxycarbonyl-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (42) was obtained following the same procedure used for the synthesis of the isomer **36**. From 390 mg (1 mmol) of hydrochloride **23** and 236 mg (1 mmol) of porphobilinogen lactam ethyl ester **9**[11] was obtained 401 mg (70%) of **42**: mp 178–180°; ir 1730 cm^{-1} (*COO*C_2H_5), 1680 (α,β unsat. *COO*C_2H_5), 1660, 1630 (*CO*NH).

Anal. Calcd for $C_{29}H_{39}N_3O_9$: C, 60.7; H, 6.8; N, 7.3. Found: C, 60.8; H, 6.7; N, 7.3.

Benzyl 2-(3′-Benzyloxycarbonylmethyl-4′-β-benzyloxycarbonylethyl-5′-benzyloxycarbonyl-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (37). BOC-tribenzylcarboxyporphobilinogen **15** (640 mg, 1 mmol) was dissolved in 3 ml of trifluoroacetic acid and the mixture kept at room temperature for 20 min, after which 6 ml of water was added and the precipitated solid centrifuged and washed twice with 1 ml of cold water. The dried trifluoroacetate **16** (603 mg, 0.9 mmol, 90%) was brought into reaction with the benzyl lactam **8** (268 mg, 0.9 mmol) following the procedure described for the synthesis of **36**. The acetic acid solution was poured, at the end of the reaction, into a saturated aqueous sodium chloride solution and extracted with chloroform. The chloroform extracts were washed with a 10% sodium bicarbonate solution, then with water, dried (Na_2SO_4), and evaporated, and the residue crystallized from methanol: 480 mg (65%) of **37**; mp 108–110°; nmr (C_5D_5N) 3.0 (m, 8, CH_2CH_2), 3.65 (m, 2, $-CH_2CO-$), 4.25 (s, 2, CH_2COO-), 4.4 (s, 2, $-CH_2-$), 4.9 (m, 2, CH_2NH), 5.27 (s, 8, $CH_2C_6H_5$), 7.4 (br, 20, C_6H_5).

Anal. Calcd for $C_{49}H_{47}N_3O_9$: C, 71.6; H, 5.7; N, 5.1. Found: C, 71.6; H, 5.6; N, 5.1.

Methyl 2-(3′-Methoxycarbonylmethyl-4′-β-methoxycarbonylethyl-5′-benzyloxycarbonyl-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (39). The BOC-aminopyrrole **17** (976 mg, 2 mmol) was dissolved in 4 ml of distilled trifluoroacetic acid and kept at room temperature for 20 min; the solution was then diluted with 8 ml of water and freeze-dried. The oily residue of the trifluoroacetate **18** (1 g, 100%) was brought into reaction with the methyl ester lactam **7** (444 mg, 2 mmol) following the procedure described for the synthesis of the dipyrrylmethane **36**. The dipyrrylmethane **39** obtained was crystallized from methanol–water: 659 mg (55%); mp 166–170°; uv λ_{max} 286 nm (ϵ 21,400); nmr (C_5D_5N) 3.0 (m, 8, CH_2CH_2), 3.67 (broad, 11, OCH_3, $-CH_2CO-$), 4.3 (s, 2, CH_2COO-), 4.45 (s, 2, $-CH_2-$), 5.2 (m, 2, CH_2NH-), 5.4 (s, 2 $CH_2C_6H_5$), 7.45 (m, 5, C_6H_5). The substance was homogeneous by tlc (5% methanol in chloroform).

Anal. Calcd for $C_{31}H_{35}N_3O_9$: C, 62.7; H, 5.9; N, 7.0. Found: C, 62.8; H, 6.0; N, 7.0.

Methyl 2-(3′-methoxycarbonylethyl-4′-methoxycarbonylmethyl-5′-benzyloxycarbonyl-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionate (43) was prepared following the same procedure described for **39**. From 488 mg (1 mmol) of crude trifluoroacetate **35** was obtained 356 mg (60%) of **43**: mp 150–153°; uv λ_{max} 287 nm (ϵ 21,000); nmr (C_5D_5N) 2.8 (m, 8, CH_2CH_2), 3.63 (s, 9, OCH_3), 4.15 (s, 2, $-CH_2CO-$), 4.3 (s, 2, $-CH_2COO-$), 4.5 (s, 2, $-CH_2-$), 5.37 (s, 2, $CH_2C_6H_5$), 5.6 (m, 2, CH_2NH-), 7.43 (m, 5, C_6H_5). The substance was homogeneous by tlc (5% methanol in chloroform).

Anal. Calcd for $C_{31}H_{35}N_3O_9$: C, 62.7; H, 5.9; N, 7.0. Found: C, 62.6; H, 6.0; N, 7.1.

2-(3′-Carboxymethyl-4′-β-carboxyethyl-5′-carboxy-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-3-propionic Acid (38). **37** (500 mg) was dissolved in a mixture of 20 ml of ethyl acetate and 5 ml of a 3% sodium bicarbonate solution, and reduced with hydrogen over 200 mg of 10% palladium on charcoal at 50 psi during 2 hr. The catalyst was removed and washed with a small volume of 3% sodium bicarbonate and the pooled aqueous layers were separated, adjusted to pH 4 with glacial acetic acid, and filtered. The precipitate was recrystallized by

dissolving in dilute ammonium hydroxide solution and precipitating with dilute acetic acid: 270 mg (97%) of **38**; mp 188–190°; ir 1710 cm^{-1} (*COO*H), 1640, 1620 (*CO*NH); nmr 2.3 (m, 8, CH_2CH_2), 3.18 (m, 2, CH_2CO–), 3.38 (s, 2, *CH_2*COO–), 3.67 (s, 2, –CH_2–), 4.12 (m, 2, *CH_2*NH–).

Anal. Calcd for $C_{21}H_{23}N_3O_9$: C, 54.7; H, 5.0; N, 9.1. Found: C, 54.5; H, 4.9; N, 9.2.

Methyl 2-(3′-Methoxycarbonylmethyl-4′-β-methoxycarbonylethyl-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-[2,3-*c*]pyrrolopyridine-3-propionate (41). The monobenzyldipyrrylmethane **39** (420 mg) was dissolved in 40 ml of glacial acetic acid and reduced with hydrogen over 200 mg of 10% palladium on charcoal at 50 psi during 90 min. The catalyst was removed, the solution was freeze-dried, and the residue of **40** (320 mg, 90%) was melted at 220° (0.2 mm) under nitrogen, and kept as a molten mass for 30 sec. The residue was dissolved in a small volume of 5% methanol in chloroform and filtered through a 30 cm × 3 cm column of tlc silica prewashed with the same solvent to afford, after evaporation and crystallization from methanol, 100 mg (34%) of **41**: mp 192–194°; nmr (C_5D_5N) 2.75 (m, 8, CH_2CH_2), 3.6 (s, 11, OCH_3, CH_2CO), 4.17 (s, 2, *CH_2*COO), 4.4 (s, 2, –*CH_2*–), 4.7 (m, 2, *CH_2*-NH–), 6.75 (s, 1, =CH–). The substance was homogeneous on tlc (R_f 0.60; 5% methanol in chloroform).

Anal. Calcd for $C_{23}H_{29}N_3O_7$: C, 60.1; H, 6.3; N, 9.5. Found: C, 60.1; H, 6.1; N, 9.3.

Methyl 2-(3′-β-methoxycarbonylethyl-4′-methoxycarbonylmethyl-2′-pyrrylmethyl)-5-oxo-4,5,6,7-tetrahydro-1*H*-[2,3-*c*]pyrrolopyridine-3-propionate (45) was obtained following the same procedure described for isomer **41**. From 296 mg of **43** was obtained 200 mg (79%) of crude **44**, which in turn was decarboxylated affording 100 mg (55%) of **45**: mp 163–165°; nmr (C_5D_5N) 2.8 (m, 8, CH_2CH_2), 3.6, 3.65 (s, 11, OCH_3, –*CH_2*CO), 4.2 (s, 2, CH_2COO), 4.5 (s, 2, –CH_2–), 4.87 (m, 2, *CH_2*NH). The substance was homogeneous on tlc (R_f 0.30; 5% methanol in chloroform)

Anal. Calcd for $C_{23}H_{29}N_3O_7$: C, 60.1; H, 6.3, N, 9.5. Found: C, 60.2; H, 6.2; N, 9.4.

2-Aminomethyl-3,3′-carboxymethyl-4,4′-β-carboxyethyl-5′-carboxydipyrrylmethane (46). Dipyrrylmethane **36** (100 mg) was suspended in 4 ml of 2 *N* potassium hydroxide, and nitrogen passed through the mixture at room temperature until it dissolved. The solution was kept at room temperature for 72 hr, it was then diluted to 10 ml with water and adjusted to pH 3.5 with 6 *M* hydrochloric acid. The pink precipitate was centrifuged, washed twice with water, and dried: 90 mg (97%) of **46**; ir 1695 cm^{-1} (COOH); nmr 2.5 (m, 8, CH_2CH_2), 3.35 (s, 4, –*CH_2*COO-), 3.6 (s, 2, –*CH_2*–), 3.8 (s, 2, –*CH_2*NH_2). No definite melting point could be determined. No analytical sample could be prepared due to very rapid porphyrin formation. Paper chromatography or tlc yielded only porphyrins.

2-Aminomethyl-3,3′-carboxymethyl-4,4′-β-carboxyethyldipyrrylmethane (47). Dipyrrylmethane **41** (60 mg) was dissolved in 1.8 ml of ethanol, 1.8 ml of 2 *M* potassium hydroxide was added, and the mixture was kept at room temperature under nitrogen for 24 hr. The ethanol was then removed with a stream of nitrogen, 1.8 ml of water was added, and the solution kept for an additional 48 hr at room temperature. The solution was then adjusted to pH 4 with acetic acid and 15% aqueous mercuric acetate was added until no more precipitate formed. The solid was centrifuged, the precipitate was suspended in water, and hydrogen sulfide was passed through the suspension. The HgS was centrifuged and washed with water, and the pooled supernatant and wash were freeze-dried. The residue (51 mg, 89%) of **47** was a slightly colored amorphous powder: nmr 2.5 (m, 8, –*CH_2CH_2*–), 3.38, 3.42 (s, 4, *CH_2*COO–), 3.6 (s, 2, –*CH_2*–), 3.88 (s, 2, *CH_2*NH_2), 6.65 (s, 1, =CH–, fading in 10 min). The substance was rapidly transformed into porphyrins by any manipulation.

2-Aminomethyl-3,4′-carboxymethyl-4,3′-β-carboxyethyldipyrrylmethane (48) was obtained as described above. From 60 mg of **45** was obtained 50 mg (87%) of **48** as an unstable amorphous solid: nmr 2.4 (m, 8, CH_2CH_2–), 3.4 (s, 4, *CH_2*COO–), 3.9 (s, 2, –CH_2–), 4.05 (s, 2, *CH_2*NH_2), 6.7 (s, 1, =CH–, fading rapidly in 5 min).

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