Total Synthesis of a ^{15}N-Labelled Macrocyclic Tetrapyrrole of the Preuroporphyrinogen Type

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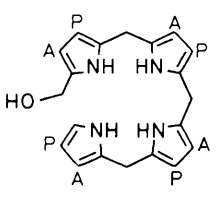
A fifteen-membered tetrapyrrole macrocycle closed through an N–CH_2 bond has been synthesized for the first time; chemical shifts and ^{15}N–^{13}C coupling constants of the ^{15}N-enriched macrocycle as well as those of several 1,2-dipyrrylmethanes have been compared with those of 'preuroporphyrinogen'.

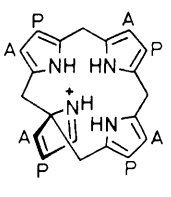
The mechanism of the biological formation of naturally occurring type III porphyrins has been a matter of controversy for many years. It has been demonstrated unequivocally that, during the transformation of four molecules of porphobilinogen into uroporphyrinogen III (uro'gen III), rotation of only *one* ring occurs, and that this process takes place *after* the formation of a bilane whose substitution pattern corresponds to that of type I porphyrins.[1] However, the nature of the intermediates involved in the transformation of type I bilanes [as (1)] into type III uroporphyrin is still unknown. The various possibilities for the reactive species connecting the bilane (1) (which has been isolated and used as a substrate of cosynthetase[2,3]) with uro'gen III have been discussed recently[4,5] and include the 'Methewson–Corwin' spiro compound (2) and the *N*-alkyl macrocycle (3) (named preuro'gen). The structure of the latter was deduced from a series of low-temperature direct n.m.r. experiments, which revealed unequivocally for the first time the presence of a specific substrate for cosynthetase, the second enzyme of the porphobilinogen → uro'gen III conversion.[5,6]

The most striking argument for the formation of (3) from isotopically enriched substrates is the presence of a doublet (J 6 Hz) centred at δ 54.78 and 155.7 p.p.m. in the ^{13}C and ^{15}N n.m.r. spectra, respectively.[5] Consequently, it was desirable to synthesize for the first time a tetrapyrrole of type (3) to obtain reliable values for the ^{13}C–^{15}N coupling in such an unusual macrocyclic system and to examine its chemistry in relation to the spiro compound (2) and the type III porphyrins.

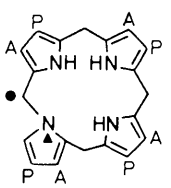
The hitherto reported values for the ^{13}C–^{15}N coupling constants and ^{13}C chemical shifts for the methylene bridge in two 1,2-dipyrrylmethane derivatives[3,7] do not agree with the figures given for the above.[5] For a more extensive inves-



(1)



(2)



(3)

A = CH_2CO_2H, P = $[CH_2]_2CO_2H$; ● = ^{13}C, ▲ = ^{15}N.

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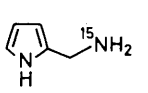

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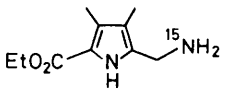

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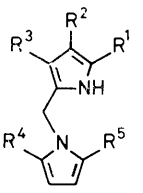
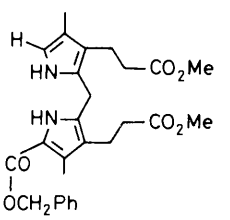
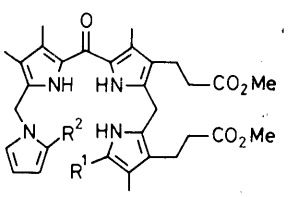


(6)

a; $R^1 = R^2 = R^3 = R^4 = R^5 = H$
b; $R^1 = R^2 = R^3 = H$, $R^4 = R^5 = Me$
c; $R^1 = CO_2Et$, $R^2 = R^3 = Me$, $R^4 = R^5 = H$
d; $R^1 = CO_2Et$, $R^2 = R^3 = Me$, $R^4 = H$, $R^5 = CHO$
e; $R^1 = CONMe_2$, $R^2 = R^3 = Me$, $R^4 = R^5 = H$

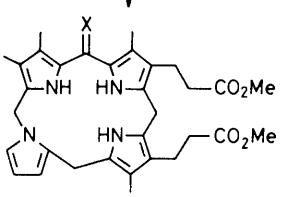


(7)



(8)

a; $R^1 = CO_2CH_2Ph$, $R^2 = H$
b; $R^1 = CO_2CH_2Ph$, $R^2 = CHO$
c; $R^1 = CO_2H$, $R^2 = CHO$
d; $R^1 = H$, $R^2 = CHO$
e; $R^1 = H$, $R^2 = CH_2OH$



(9)

a; X = O
b; X = H_2

Table 1. ^{13}C Chemical shifts and ^{13}C–^{15}N coupling constants for some ^{15}N-labelled α-aminomethylpyrrole derivatives (n.m.r. 20.0 MHz in $CDCl_3$).

Compound	δ($^{13}CH_2$)/p.p.m.	J(^{13}C–^{15}N)/Hz
(4)	39.01	3.9
(6a)	46.24	11.0
(6b)	40.91	11.2
(6c)	44.47	11.3
(6d)	43.17	9.6
(9a)	41.0	10.4
(9b)	41.52	10.7

tigation of the effect of substituents at the pyrrole rings on the ^{13}C–^{15}N coupling, a series of 1,2-dipyrrylmethane derivatives (**6a**—**d**) were synthesized starting from the ^{15}N enriched pyrroles (**4**) and (**5**).‡§ The observed chemical shifts for the methylene bridge C-atom and the ^{13}C–^{15}N coupling constants are in the range δ 41—46 p.p.m. and 9.6—11.3 Hz respectively, and agree, therefore, with the literature values[3,7] as well as with those of the synthetic macrocycle (**9b**) (see Table 1). The macrocycle (**9b**) was synthesized using (**6c**) as the starting material. Thus, the amide (**6e**) prepared by treating (**6c**) with lithium dimethylamide reacted with the dipyrrylmethane (**7**)[13] in the presence of phosphorous oxychloride to give the oxo-tetrapyrrole (**8a**) (*cf.* ref. 14). Formylation of (**8a**) with triethyl orthoformate in trifluoroacetic acid (*cf.* ref. 9) afforded the aldehyde (**8b**) which was hydrogenolysed (Pd–C) to the corresponding carboxylic acid (**8c**) which was subsequently decarboxylated to (**8d**). Sodium borohydride reduction of the formyl group of (**8d**) gave the carbinol (**8e**) which cyclized in the presence of toluene-*p*-sulphonic acid to the oxo-macrocycle (**9a**). Diborane reduction of (**9a**) afforded the desired compound (**9b**) whose structure was confirmed by analytical data. The crucial cyclization step (**8e**) → (**9a**) was unequivocally demonstrated by high-resolution ^{1}H n.m.r. spectroscopy. Thus, whereas the two methylene bridges of the linear tetrapyrrole (**8e**) show two singlets at δ 4.9 and 3.8, the 400 MHz ^{1}H n.m.r. spectrum of the macrocycle (**9a**) shows three AB quartets corresponding to the three methylene bridges present in the molecule with resonances of the individual protons at δ 4.91, 4.85; 3.99, 3.87; and 3.89, 3.83 (*J* 16, 18, and 18 Hz, respectively). Evidently, each pair of methylene protons in the conformationally more rigid (**9a**) becomes diastereotopic and gives rise to an AB spin system which is not observable in the flexible linear structure (**8e**).

‡ Compounds (**6a**) and (**6c**) were synthesized by the reaction of 1,5-dimethoxytetrahydrofuran with (**4**) and (**5**), respectively (*cf.* ref. 8). Formylation of (**6c**) with triethyl orthoformate gave (**6d**) (*cf.* ref. 9). Compound (**6b**) was synthesized by treating (**4**) with hexane-2,5-dione (*cf.* ref. 10).

§ The pyrroles (**4**) and (**5**) were synthesized according to refs. 11 and 12, respectively, using 95% ^{15}N-hydroxylamine (KOR Isotopes, Cambridge, Massachusetts).

Our work demonstrates that, in principle, a fifteen-membered tetrapyrrole macrocycle of the preuro'gen type can be formed from linear tetrapyrrole precursors. The observed chemical shifts for the methylene bridge C-atom and the ^{13}C–^{15}N coupling constant of (**9b**) do not support, however, structure (**3**) as a biogenetic precursor of uroporphyrinogen III. Experiments designed to elucidate the origin of the 6 Hz doublet observed by direct n.m.r. experiments with living cells are in progress.

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