Synthesis of a New Decahydrohexapyrrin of Biosynthetic Interest

Marcelo J. Kogan

Facultad de Farmacia y Bioquímica, Cátedra de Química Biológica Vegetal,
Universidad de Buenos Aires, Junín 956 BuenosAires (1113) Argentina

Received January 20, 1998
Revised March 31, 1998

A new decahydrohexapyrrin containing a *tert*-butoxycarbonylbilane of type III was prepared applying a convergent type synthesis. The order of the side chains of the *tert*-butoxycarbonylbilane belonging to this oligopyrrole corresponds to that of a type III uroporphyrinogen. A symmetric hexapyrrol dilactam was formed as a by product of the synthesis.

J. Heterocyclic Chem., **35**, 907 (1998).

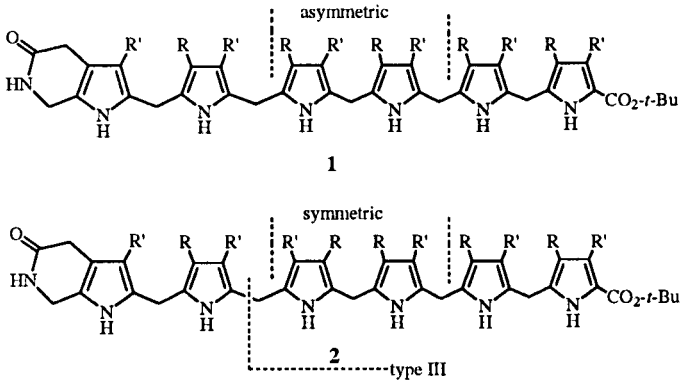
The enzyme porphobilinogen deaminase catalyses the stepwise polymerization of four molecules of porphobilinogen into the linear tetrapyrrole 1-hydroxymethylbilane, preuroporphyrinogen. It has been convincingly established that a linear hexapyrrylmethane that grows on the condensing enzyme, porphobilinogen deaminase, is enzymatically cleaved to afford a tetrapyrrole segment which rearranges and cyclizes to give uroporphyrinogen III [1-3]. The mechanism by which the enzyme manoeuvres the growing polypyrrole to allow repeated use of the same catalytic machinery, and structural factors that cause chain growth and cleavage to be terminated at a tetrapyrrole are intriguing problems [4,5].

Synthesis of **1**, a close model mimicking the natural hexapyrrole intermediate (Figure), has been recently carried out in our laboratory [6]. In this report we describe the preparation of decahydrohexapyrrin **2** (Figure) bearing a central symmetric dipyrrylmethane. Comparative study of cleavage in an acid medium of these decahydrohexapyrrins could serve as a model to understand the way chemical breakage of the hexapyrrol bound to the enzyme takes place. In the case of hexapyrrin **2** a cleavage on the level of the terminal *tert*-butoxycarbonylbilane would lead to a series III hydroxymethylbilane.

For the preparation of **2**, a convergent type path (Scheme, below), based on previous polypyrrol synthesis work, was employed [7-13]. One mole of the dipyrrylmethane lactam **3** was condensed with the dipyrrylmethane **4** [14] using a 1:1 ratio of **3** to **4** in appropriate dilutions (see Experimental) and hydrobromic acid to avoid the formation of the hexapyrrin **5**. Monoformylbilene-b **6** was obtained under these conditions and was made to react with dipyrrylmethane **7** [10] to give the hexapyrrol **8**. Attempts to crystallize **5** and **8** with different solvents were unsuccessful. After a reduction of **8** with sodium borohydride, **2** was obtained in adequate yields (9%, 3 steps).

The use of inappropriate dilutions of dipyrrylmethanes **3** and **4** for the preparation of **6** led to the formation of a mixture of the hexapyrrines **5** and **8**. The hexapyrrin **5** was identified in the form of its decahydrohexapyrrin **9**. The synthesis of this last compound was carried out by condensation of two moles of dipyrrylmethane lactam **3** with one mole of the dipyrrylmethane **4** and a further reduction with sodium borohydride.

Synthesis of these decahydrohexapyrrins points to another way to contribute to the interpretation of how the cleavage of the natural hexapyrrolic intermediate lead to the formation of hydroxymethylbilane **I**.



1

2

R = -$CH_2CO_2CH_3$, R' = -$CH_2CH_2CO_2CH_3$

Figure

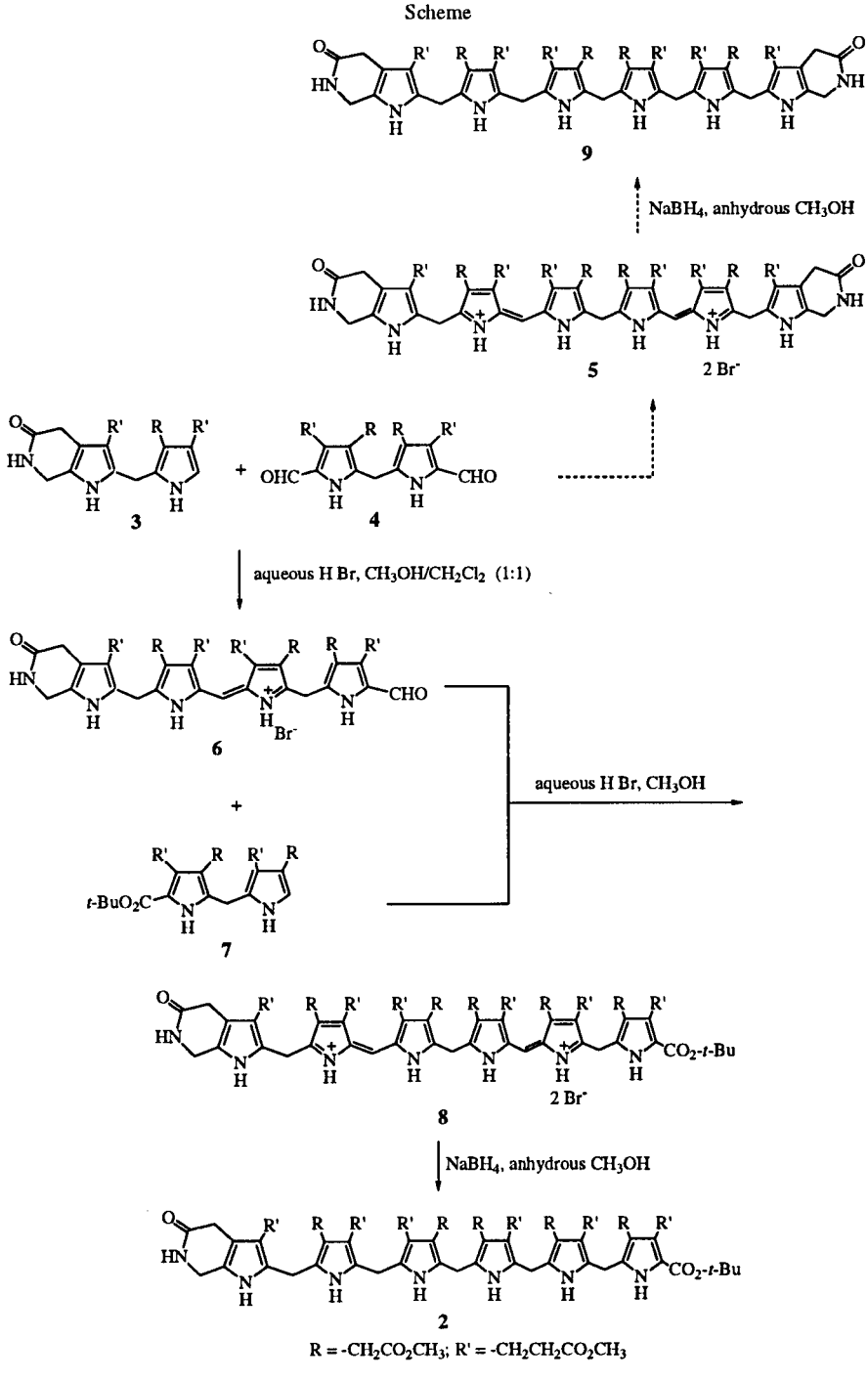
EXPERIMENTAL

Melting point was determined with a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H nmr spectrum was recorded in deuterated chloroform on a Bruker MSL 300 spectrometer in δ (ppm) and with tetramethylsilane as the internal standard. FAB-mass spectrums were determinated on a ZAB-SEQ (VG, Fisons) spectrometer. The silica gel used in column chromatography was tlc Kieselgel DG (Riedel de Haen) and neutral aluminum oxide (Merck) previously washed with methanol. The tlc were performed on precoated silica gel F-254 plates (Merck, 0.25-mm layer thickness). The tlc were spotted by spraying the plates with Ehrlich's reagent (2% *p*-(dimethylamino)benzaldehyde in 6*N* aqueous hydrochloric acid) or by treatment with bromine vapor. Microanalyses were performed using a Carlo Erba EA 1108 elemental analyzer.

tert-Butyl 2,7,12,18,22,27-Hexa-[(2-methoxycarbonyl)ethyl]-3,8,13,17,23-penta-[(methoxycarbonyl)methyl]-29-aminomethyl-28-carboxymethyl-5,10,15,20,25,30,32,33,34,35-decahydrohexapyrrin lactam-1-carboxylate (**2**).

A solution of dipyrrylmethane **3** (18 mg, 0.039 mmole) in 5 ml of anhydrous methylene chloride-anhydrous methanol (1:1) was added dropwise at 5° and protected from light to a stirred solution

Scheme



of dipyrrylmethane **4** (20 mg, 0039 mmole) in 5 ml of anhydrous methylene chloride-anhydrous methanol (1:1) containing 0.4 ml of 48% aqueous hydrobromic acid. After 20 minutes at 20°, it was filtered using a neutral aluminum oxide column employing chloroform as solvent.

Bilene-b **6** was collected as the hydrobromide. It was then evaporated *in vacuo* at 20° (in the dark). The red residue, FAB-mass spectrum 960 ($M+H^+$, 10%; using thioglycerol as a matrix), was dissolved in anhydrous methanol (5 ml) containing 0.1 ml of 48% aqueous hydrobromic acid and a solution of dipyrrylmethane **7** (22 mg, 0.039 mmole) in anhydrous methanol (5 ml) was added. The mixture was allowed to stand at 20° for 20 minutes (protected from light). It was then filtered using a neutral aluminum oxide column (2 x 5 cm) with chloroform as the solvent. Hexapyrrin **8**, FAB-mass spectrum: 1506 ($M+H^+$, 8%, using thioglycerol as a matrix) was collected as the hydrobromide. The fractions containing the product were evaporated *in vacuo* (in the dark). The residue was dissolved in anhydrous methanol and 60 mg of sodium borhydride

was added keeping the temperature at 5° in the dark. After 20 minutes at 20°, methanol was removed and the residue was suspended in water (20 ml), extracted with methylene chloride (2 x 10 ml). The combined organic layers were dried and evaporated. The residue was purified using a tlc on silica gel with 7% methanol in chloroform as the solvent. The eluted decahydrohexapyrrin (R_f: 0.60 in 7% methanol-chloroform, gave a red color with Ehrlich's reagent after heating and a brown color with bromine vapor) was filtered through a small column of silica gel (2 x 1 cm) using the above solvent. Fractions containing the product were evaporated *in vacuo* and the solid obtained was washed with cold methanol providing 5 mg (9%, 3 steps) of **2** as a yellowish solid, 220° dec. ^{1}H nmr, 300 MHz (deuteriochloroform): 1.25 (s, 9H), 1.80-2.70 (m, 24H), 3.20-3.45 (m, 22H), 3.45-3.90 (b, 33H), 4.30 (b, 2H), 8.36 (s, 1H), 8.57 (s, 1H), 8.75 (s, 1H), 8.90 (s, 1H), 9.05 (s, 1H), 9.30 (s, 1H). The FAB-mass spectrum was obtained with *m*-nitrobenzyl alcohol as a matrix: m/z (relative intensity) 1507 (M^+, 8), 946 (47), 812 (6); 709 (24). hrms Calcd. for $C_{76}H_{97}O_{25}N_7$ 1507.6531, Found: 1507.6535.

Anal. Calcd. for $C_{76}H_{97}O_{25}N_7$: C, 60.51; H, 6.48; N, 6.50. Found: C, 60.27; H, 6.50; N, 6.48.

3,8,12,18,22,27-Hexa-[(2-methoxycarbonyl)ethyl]-7,13,17,23-tetra-[(methoxycarbonyl)methyl]-1,29-diaminomethyl-2,28-dicarboxymethyl -5,10,15,20,25,30,32,33,34,35-decahydrohexapyrrin Dilactam (**9**).

A mixture of dipyrrylmethane **3** (40 mg, 0.087 mmole) and dipyrrilmethane **4** (22 mg, 0.042 mmole) dissolved in 10 ml of anhydrous methylene chloride-anhydrous methanol (1:1), and 0.4 ml of 48% aqueous hydrobromic acid was added. The solution was allowed to stand at 20° during 20 minutes and was then poured over a neutral aluminum oxide column (1.5 x 20 cm) employing chloroform as the solvent. The hexapyrrin **5** was eluted with chloroform and collected as the hydrobromide. It was evaporated *in vacuo* at 20° (in the dark). The red residue, FAB-mass spectrum: 1400 (M^+, 10%, using thioglycerol as a matrix), was dissolved in anhydrous methanol and 60 mg of sodium borhydride was added keeping the temperature at 5° and in the dark. After 20 minutes at 20°, methanol was removed and the residue was suspended in water (20 ml), extracted with methylene chloride (2 x 10 ml), and the combined organic layers were dried and evaporated. The residue was purified by tlc on silica gel with 7% methanol in chloroform as the solvent. The eluted decahydrohexapyrrin **9** (R_f: 0.30 in 7% methanol-chloroform, gave a red color with Ehrlich's reagent without heating and a brown color with bromine vapor) was filtered through a small column of silica gel (2 x 1 cm) using the above solvent. Fractions containing the product were evaporated *in vacuo* and the solid obtained was washed with cold methanol providing 5 mg (8.3%, 2 steps) of **9** as a yellowish solid, 190° dec; ^{1}H nmr, 300 MHz (deuteriochloroform): 2.18-2.70 (m, 24H), 3.30-3.40 (m, 8H), 3.42 (s, 4H), 3.50-3.78 (m, 40H), 4.30 (b, 4H), 8.63 (s, 2H), 8.83 (s, 2H), 9.23 (s, 2H), A FAB-mass spectrum was obtained with thioglycerol as a matrix, m/z (relative intensity) 1404 (M^+, 72), 1183 (10), 1169 (18), 946 (83), 932 (42), 709 (100).

Anal. Calcd. for $C_{71}H_{88}O_{22}N_8$: C, 60.67; H, 6.31; N, 7.97. Found: C, 60.50; H, 6.32; N, 7.95.

Acknowledgements.

The author is deeply indebted to Drs. Aldonia Valasinas and Benjamín Frydman for having initiated and developed this line of research. This work was supported by a grant from National Research Council (CONICET), Argentina.

REFERENCES AND NOTES

[1] R. B. Frydman; B. Frydman and A. Valasinas, The Porphyrins, Vol **VI**, D. Dolphin, ed, Academic Press, New York, 1979, Chaper I, p 1.

[2] A. R.Battersby and F. J. Leeper, *Chem. Rev.*, **90**, 1261 (1990) and references therein.

[3] A. I. Scott, *Angew. Chem., Int. Ed. Engl.*, **32**, 1223 (1993) and references therein.

[4] G. V. Louie, P. B. Brownlie, R. Lambert, J. Cooper, T. L. Blundell, S. P. Wood, M. J. Warren, S. C. Woodcock and P. M. Jordan, *Nature*, **359**, 33 (1992).

[5] G. V. Louie, P. B. Brownlie, R. Lambert, J. Cooper, T. L. Blundell, S. P. Wood, V. N. Malashkevich, A. Hadener, M. J. Warren and P. M. Shoolingin-Jordan, *Proteins: Structure, Function, and Genetics*, **25**, 48 (1996).

[6] M. Kogan, A. Valasinas and B. Frydman, *Tetrahedron Letters*, **37**, 6, 763 (1996).

[7] B. Frydman, S. Reil, A. Valasinas, R. B. Frydman and H. Rapoport, *J. Am. Chem. Soc.*, **93**, 2738 (1971).

[8] A. Valasinas, E. S. Levy and B. Frydman, *J. Org. Chem.*, **39**, 2872 (1974).

[9] A. Valasinas, E. S. Levy and B. Frydman, *Tetrahedron Letters*, **5**, 337 (1976).

[10] L. Diaz, R. B. Frydman, A. Valasinas and B. Frydman, *J. Am. Chem. Soc.*, **101**, 2710 (1979).

[11] L. Diaz; A. Valasinas and B. Frydman, *J. Org. Chem.*, **46**, 864 (1981).

[12] V. D. Rumyantseva, A. F. Mironov and R. P. Evstigneeva, *Zh. Org. Khim.*, 837 (1971).

[13] P. S. Clezy, A. J. Liepa and N. W. Webb, *Aust. J. Chem.*, **25**, 1991 (1972).

[14] B. Franck, D. Gantzy and F. Huper, *Angew. Chem., Int., Ed. Engl.*, **11**, 420 (1972).