Carbon-5 Regiospecific Synthesis of Deuteroporphyrin IX

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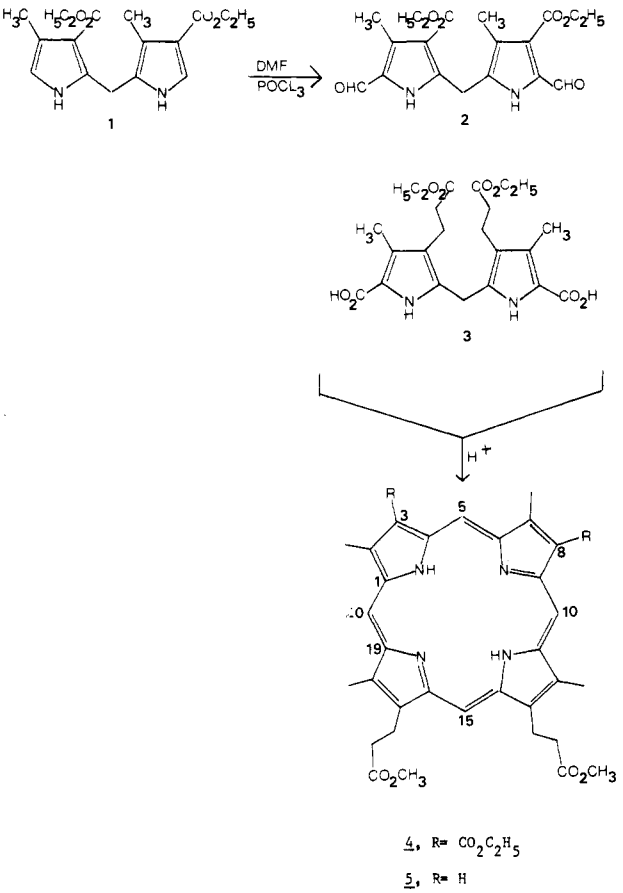
Benzyl 3-(ethoxycarbonyl)-4-methyl-2-pyrrolecarboxylate was formylated with dimethylformamide (DMF)–phosphorous oxychloride, and the 5-formylpyrrole was obtained in 50% yield. It was reduced with sodium borohydride to the alcohol, which was condensed with benzyl 3-methyl-4-(ethoxycarbonyl)-2-pyrrolecarboxylate to afford dibenzyldipyrrylmethane in good yield. The latter was transformed into its 5,5′-diformyl derivative which when condensed with bis[5-carboxy-3-[β-(ethoxycarbonyl)ethyl]-4-methylpyrryl]methane afforded the 3,8-bis(ethoxycarbonyl)deuteroporphyrin IX dimethyl ester in 40% yield. Decarboxylation of the latter in hot hydrochloric acid gave deuteroporphyrin IX (isolated as its dimethyl ester) in 50% yield.

Deuteroporphyrin IX dimethyl ester (**5**, Scheme I) is an important intermediate for the synthesis of porphyrins and hemins of the natural type IX series. Deuterohemin (iron(III)deuteroporphyrin chloride) could be transformed into its 3,8-diacetyl, 3,8-dibromo, and 3,8-dipropionyl derivatives,[1,2] while the acylation of copper(II) dueteroporphyrin dimethyl ester with valeric anhydride, isovaleric anhydride, and lauric anhydride gave the expected 3,8-diacyl derivatives.[3,4] The 3,8-diacyl substituents were easily transformed into alkyl side chains.[3,4] Vilsmaier formylations at the C_3 and C_8 positions of copper(II) deuteroporphyrin dimethyl ester and deuterohemin have also been successful.[5,6]

Work from this laboratory has provided evidence that the enzymatic[3,7] and in vivo[8] degradation of synthetic hemins proceeds with loss of the C-5 bridge to give α-biliverdins. To put these results on a firm basis, we need a novel C(5)-regiospecific synthesis of deuteroporphyrin IX dimethyl ester **5**, since the known syntheses of **5**[9–11] were not suitable for this purpose. The new synthesis of **5** was carried out by using MacDonald's approach,[12] which gave good results for the syntheses of uroporphyrin[13] and coproporphyrin[14] isomers as well as of type XIII and type III porphyrins.[15,16] The outline of the synthesis (Scheme I) required the preparation of the new 5,5′-diformyldipyrrylmethanes **1** and **2** and of the known[14] 5,5′-dicarboxydipyrrylmethane **3**. The dipyrrylmethane **2** was chosen in order to keep the β-free positions protected by the ethoxycarbonyl residues until the synthesis of the porphyrin ring **4** was achieved.

Scheme I



The synthesis of **1** was explored by first using the α-unprotected pyrrole **6** (Scheme II). There is a report[17] that the difference between the electron-releasing properties of the 4-methyl residue and the deactivating effect of the 3-ethoxycarbonyl residue in **6** is sufficient to achieve the asymmetric synthesis of an α-unsubstituted dipyrrylmethane. However, when we attempted a model synthesis similar to the reported one,[17] it was found that both possible dipyrrylmethanes **7** and **8** were formed (Scheme II). They could not be separated, but the ^1H NMR data (in $CDCl_3$: **7**, $H_{5'}$, δ 7.25; **8**, $H_{5'}$, δ 6.51) clearly indicated that **7** and **8** were present in a proportion of 2:1 in the mixture. The synthesis of an α-protected pyrrole was then designed.

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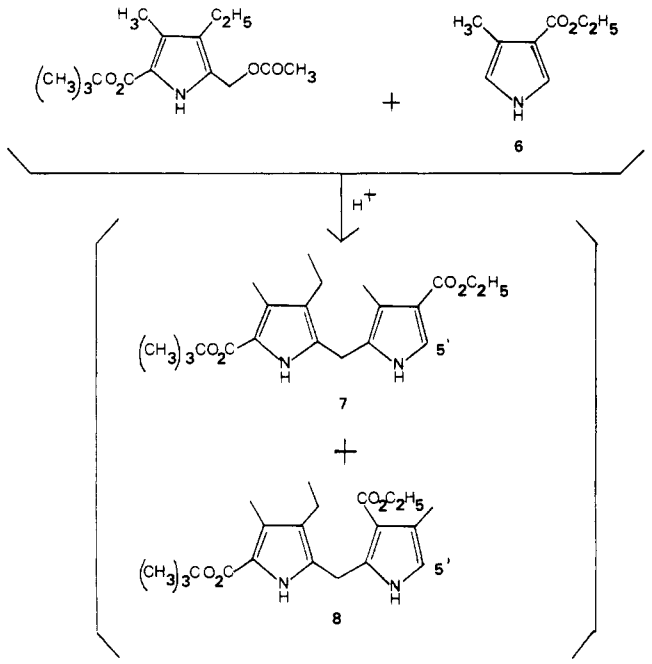
Scheme II



Scheme III

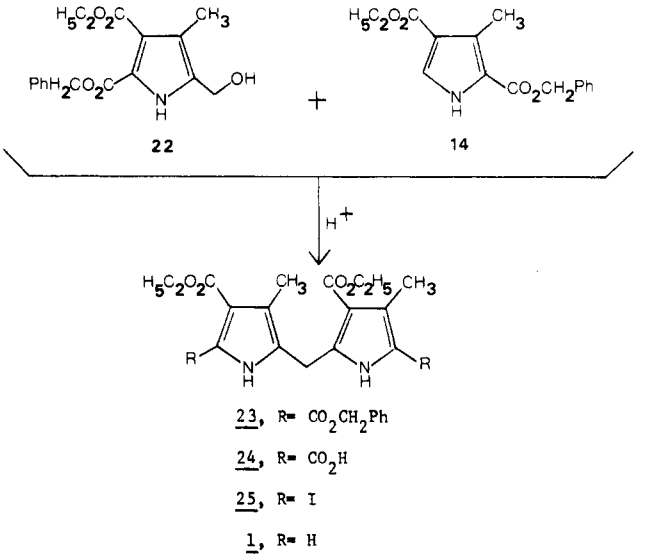
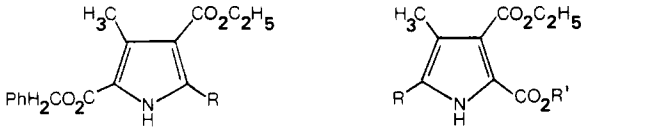


Chart I



9, R = CH_3
10, R = CO_2H
11, R = CHO
12, R = CH_2OH
13, R = I
14, R = H

15, R = CO_2CH_2Ph; R' = $C(CH_3)_3$
16, R = CO_2H; R' = $C(CH_3)_3$
17, R = I; R' = $C(CH_3)_3$
18, R = H; R' = $C(CH_3)_3$
19, R = H; R' = H
20, R = H; R' = CH_2Ph
21, R = CHO; R' = CH_2Ph
22, R = CH_2OH; R' = CH_2Ph

Synthesis of Pyrroles. Oxidation of the easily available pyrrole **9** to the acid **10** (Chart I) was achieved by first using bromine to activate the α-methyl group, followed by sulfuryl chloride treatment and a hydrolysis step. The acid was obtained in good yields and the contaminant aldehyde **11** was easily separated. Reduction of the aldehyde **11** with sodium borohydride gave the 2-(hydroxymethyl)pyrrole **12**. The acid **10** could be decarboxylated by treatment with iodine to yield the iodopyrrole **13**, followed by hydrogenolysis of the latter to **14** with zinc in boiling acetic acid.[14]

The acid **10** was esterified with *tert*-butyl alcohol and dicyclohexylcarbodiimide[18] to **15**, the benzyl group was cleaved by hydrogenolysis, and the acid **16** was decarboxylated by treatment with iodine and subsequent hydrogenolysis of the iodopyrrole **17** to the α-unsubstituted pyrrole diester **18**. All this synthetic sequence took place with good yields at every step. The *tert*-butyl ester **18** could be converted to the acid **19** by treatment with trifluoroacetic acid, and **19** was reesterified to the benzyl ester **20** by treatment with benzyl chloride in the presence of triethylamine.[14] A Vilsmeier reaction (using dimethylformamide–phosphorous oxychloride) on **20** gave the aldehyde **21** in 80% yield, notwithstanding the strong deactivating effect of the ester groups. Reduction of **21** with sodium borohydride gave the alcohol **22**.

Synthesis of Dipyrrylmethanes and Porphyrins. When the 2-(hydroxymethyl)pyrrole **12** (or its acetate) was brought into reaction with the α-unsubstituted pyrrole **18**, very little of the expected dipyrrylmethane was obtained. The slightly acidic conditions used for achieving the condensation (dry methylene chloride–*p*-toluensulfonic acid) were sufficient to cleave the *tert*-butyl ester, and the pyrrole acid **19** was the main reaction product.[19] When the condensation was attempted by using the benzyl ester **20** with the same 2-(hydroxymethyl)pyrrole **12** or its acetate, it again ended in failure, this time due to the almost complete recovery of the reactants. It was therefore decided to use the more reactive 2-(hydroxymethyl)pyrrole **22** which was brought into reaction with **14** to give the dipyrrylmethane **23** in good yields (Scheme III). The bis[[(benzyloxy)carbony]pyrryl]methane **23** was transformed into the dipyrrylmethane **1** by hydrogenolysis to the diacid **24** and decarboxylation of **24** by treatment with iodine in ethanol, followed by hydrogenolysis of the crude bis(iodopyrryl)methane **25** thus obtained to the 5,5′-unsubstituted dipyrrylmethane **1**. The Vilsmeier-type formylation of **1** to **2** was achieved with dimethylformamide–phosphorous oxychloride at 50 °C (Scheme I).

The condensation of **2** and the dipyrrylmethane diacid **3** was carried out by following established procedures,[13] and the porphyrin **4** was obtained to 40% yield (Scheme I). Decarboxylation of **4** by treatment with hydrochloric acid at 180 °C gave deuteroporphyrin IX, isolated as its dimethyl ester **5**.

The synthesis allows the simple and ambiguous labeling of **5** at C-5 by using enriched or radioactive dimethylformamide (see Experimental Section) for the synthesis of the 2-formylpyrrole **21**. Alternatively, the synthesis could be useful to label C-10 and C-20 by using labeled dimethylformamide in the preparation of **2** from **1**. [5-^{14}C]Deuteroporphyrin is the suitable starting material for the obtention of many 5-^{14}C hemins.

(18) Clezy, P. S.; Hai, T. T.; Gupta, P. C. *Aust. J. Chem.* **1976**, *29*, 393.

(19) The cleavage of the *tert*-butyl ester in **18** under the reaction conditions used for the synthesis of dipyrrylmethanes is unusual, since in similar syntheses this ester group is preserved.[20,21]

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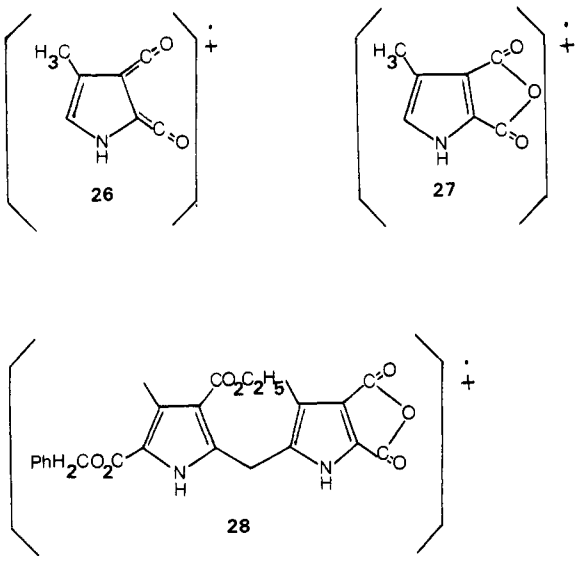
Chart II



Mass Spectra. The vicinal 2,3-pyrrole diesters **18** and **20** gave characteristic mass spectra with formation of a pyrrole anhydride ion, **27**, and a stabilized diacylium ion, **26** (Chart II). These ions are present whenever vicinal ester groups are present, whether in pyrroles or pyrrylmethanes. Thus, dipyrrylmethane **23** gave the pyrrylmethane anhydride ion **28**. The fragmentation pattern of the dipyrrylmethanes followed the outline expected for this type of compound.[22]

Experimental Section

General Procedures. Melting points were determined on a Kofler melting point apparatus and are uncorrected, and NMR spectra were recorded in $CDCl_3$ on a FT-80A spectrometer. Mass spectra were obtained with Varian CH-7 spectrometer at UMYMFOR (Buenos Aires). The silica gel used in column chromatography was TLC Kieselgel (Merck). TLC was performed on precoated silica gel F-254 plaques (Merck, 0.25-mm layer thickness). The substances were spotted by spraying the plaques with Ehrlich's reagent [2% *p*-(dimethylamino)benzaldehyde in 6 N HCl] or by treatment with bromine vapor which gave orange or red colors with the dipyrrylmethanes.

Benzyl 3,5-Dimethyl-4-(ethoxycarbonyl)-2-pyrrolecarboxylate (9). A solution of 7.2 g of sodium nitrite in 25 mL of water was slowly added to a stirred mixture of 19.2 g of benzyl acetoacetate in 30 mL of acetic acid kept at 5 °C. The mixture was further kept at 5 °C during 15 h and was then slowly added with mechanical stirring to a mixture of 13 g of ethyl acetoacetate in 70 mL of glacial acetic acid. Simultaneously, a mixture of 18.5 g of Zn and 18.5 g of anhydrous sodium acetate was added in small portions. When the additions were completed, the mixture was heated and stirred for a further 1.5 h at 75 °C. It was then poured over a fivefold volume of ice–water and filtered, and the residue was crystallized from methanol to give **9**: 21 g (70%); mp 119–120 °C; NMR δ 1.30 (t, 3, OCH_2CH_3), 2.48 (s, 3, 3-CH_3), 2.56 (s, 3, 5-CH_3) 4.28 (m, 2, OCH_2CH_3), 5.30 (s, 2, $CH_2C_6H_5$), 7.37 (br, 5, C_6H_5). Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.77; H, 6.32; N, 4.65. Found: C, 67.70; H, 6.31; N, 4.55.

Benzyl 3-Methyl-4-(ethoxycarbonyl)-5-carboxy-2-pyrrolecarboxylate (10). Pyrrole **9** (10 g) dissolved in 120 mL of glacial acetic acid was treated with 8 mL of acetic anhydride and 1.7 mL of bromine at 5° C, and the mixture was stirred at 30 °C during 1 h. A total of 8 mL of sulfuryl chloride was added in portions over a period of 2 h, while the stirred mixture was kept at 10–15 °C. After the addition was completed, the mixture was kept for 15 h at 20 °C, when 10 mL of water was added dropwise, and the mixture was further stirred for 5 h at 20 °C. The mixture was poured over a large excess of ice–water, the precipitate was filtered and redissolved in ethanol, and the solution was adjusted to pH 8 with solid sodium bicarbonate. Enough water was then added to start the precipitation of the contaminant aldehyde **11** and the precipitation was completed by keeping the solution at 5 °C during 15 h. The precipitate was then filtered off, and the filtrates were adjusted to pH 2 with concentrated hydrochloric acid. The precipitated acid **10** was filtered: 7 g (63%); mp 150–151 °C (ethanol–water); NMR δ 1.45 (t, 3, OCH_2CH_3), 2.63 (s, 3, 3-CH_3), 4.54 (q, 2, OCH_2CH_3), 5.38 (s, 2, $CH_2C_6H_5$), 7.43 (br, 5, C_6H_5). Anal. Calcd for $C_{17}H_{17}NO_6$: C, 61.63; H, 5.13; N, 4.22. Found: C, 61.53; H, 5.00; N, 4.12.

Benzyl 3-Methyl-4-(ethoxycarbonyl)-5-(*tert*-butoxycarbonyl)-2-pyrrolecarboxylate (15). A solution of 3 g of the pyrrole acid **10** in 40 mL of dry tetrahydrofuran was mixed with 46 mL of *tert*-butyl alcohol and 3.6 g of dicyclohexylcarbodiimide. The resulting solution was kept at 20 °C during 18 h, the formed precipitate was filtered, the filtrates were evaporated to dryness in vacuo, the residue was redissolved in 2 mL of benzene and filtered again, and the filtrates were evaporated to dryness. The residue was dissolved in a small volume of 1% methanol in benzene and applied to a TLC silica gel column (4 × 30 cm) prewashed with the same solvent under pressure. The pyrrole **15** was eluted under pressure by using the same solvent: 2.25 g (63%); mp 66–68 °C (methanol); NMR δ 1.43 (t, 3, OCH_2CH_3), 1.63 (s, 9, $C(CH_3)_3$), 2.49 (s, 3, 3-CH_3), 4.42 (q, 2, OCH_2CH_3), 5.43 (s, 2, $CH_2C_6H_5$), 7.35 (br, s, C_6H_5). Anal. Calcd for $C_{21}H_{25}NO_6$: C, 65.11; H, 6.46; N, 3.62; Found: C, 65.01; H, 6.35; N, 3.82.

***tert*-Butyl 3-(Ethoxycarbonyl)-4-methyl-5-carboxy-2-pyrrolecarboxylate (16).** Pyrrole **15** (3 g) dissolved in 100 mL of ethanol was reduced with hydrogen over 1.5 g of 10% Pd on charcoal at 50 psi of H_2 during 3 h. The catalyst was filtered, the filtrates were evaporated in vacuo, and the residue was crystallized from ethanol–water: 1.34 g (60%); mp 144–145 °C (ethanol–water); NMR δ 1.36 (t, 3, OCH_2CH_3), 1.55 (s, 9, $C(CH_3)_3$), 2.40 (s, 3, CH_3), 4.35 (q, 2, OCH_2CH_3). Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.56; H, 6.39; N, 4.71. Found: C, 56.50; H, 6.31; N, 4.61.

***tert*-Butyl 3-(Ethoxycarbonyl)-4-methyl-2-pyrrolecarboxylate (18).** A solution of 2.7 g of iodine in 100 mL of ethanol was slowly added to a solution of 3 g of the pyrrole acid **16** in 120 mL of water containing 9 g of sodium bicarbonate. The mixture was stirred at 20 °C during 1.5 h, when water was slowly added to produce the complete precipitation of the iodopyrrole **17**. The precipitate was filtered and dried, and the unstable iodopyrrole **17** thus obtained (3.75 g, 97%; mp 98–99 °C) was dissolved in 200 mL of ethanol containing 3 g of anhydrous sodium acetate and was reduced with hydrogen over 1 g of 10% Pd on charcoal at 45 psi of H_2 during 3 h. The catalyst was filtered off, the solution was evaporated to dryness, the residue was dissolved in chloroform, and the latter was washed twice with water, dried (Na_2SO_4), and evaporated to dryness in vacuo. The pyrrole **18** was obtained as an oily residue: 2.3 g (93%); NMR δ 1.34 (t, 3, OCH_2CH_3), 1.50 (s, 9, $(C(CH_3)_3$), 2.10 (s, 3, CH_3), 4.35 (q, 2, OCH_2CH_3), 6.66 (b, 1, H_5); mass spectrum, *m/e* (relative intensity) 253 (M^+, 7), 197 ($M^+ - C_4H_9$, 30), 151 (**26**, 100), 135 (**27**, 20).

Ethyl 2-Carboxy-4-methyl-3-pyrrolecarboxylate (19). A solution of 2 g of the *tert*-butyl ester **18** in 20 mL of trifluoroacetic acid was kept at 5° C during 15 min. It was then poured in 100 mL of ice–water, and the acid **19** was filtered off, dried, and recrystallized from ethanol–water: 1.1 g (80%); mp 156–158 °C; NMR δ 1.16 (t, 3, OCH_2CH_3), 2.04 (s, 3, CH_3), 4.27 (q, 2, OCH_2CH_3), 6.66 (br, 1, H_5). Anal. Calcd for $C_9H_{11}NO_4$: C, 54.82; H, 5.58; N, 7.10. Found: C, 54.70; H, 5.43; N, 7.00.

Benzyl 3-(Ethoxycarbonyl)-4-methyl-2-pyrrolecarboxylate (20). A mixture of 1.4 g of the acid **19** 13 mL of triethylamine and 13 mL of benzyl chloride in 22 mL of dry dimethylformamide was kept during 48 h at 20 °C. The mixture was then evaporated to dryness in vacuo at 100 °C, the residue was dissolved in 150 mL of chloroform, the organic layer was washed twice with water, dried (Na_2SO_4), and evaporated to dryness, and the residue dissolved in a small volume of 2% methanol in benzene was applied to a TLC silica gel column (3 × 15 cm) packed and washed with the same solvent. The pyrrole **20** was eluted by applying a slight pressure: 1.87 g (93%); NMR δ 1.23 (t, 3, OCH_2CH_3), 2.15 (s, 3, CH_3), 4.20 (q, 2, OCH_2CH_3), 5.30 (s, 2, $CH_2C_6H_5$), 6.50 (br; 1, H_5), 7.35 (br, 5, Ph); mass spectrum, *m/e* (relative intensity)

(22) Valasinas, A.; Levy, E. S.; Frydman, B. *Tetrahedron Lett.* **1976**, 337.

287 (M^+, 6), 151 (**26**, 30), 135 (**27**, 25).

Benzyl 3-(Ethoxycarbonyl)-4-methyl-5-formyl-2-pyrrolecarboxylate (21). Phosphorous oxychloride (1.77 mL, 19.2 mmol) was added to 1.6 mL (20.8 mmol) of dry dimethylformamide at 5 °C, the mixture was diluted with 7 mL of 1,2-dichloroethane, and the resulted solution was kept at 20 °C during 20 min. A solution of 1.85 g (3.2 mmol) of the pyrrole **20** in 20 mL of 1,2-dichloroethane was then added to the aforementioned mixture, and the solution was heated at 85 °C during 2 h with continuous stirring under moisture-exclusion conditions. The reaction mixture was then cooled, diluted with 30 mL of water, adjusted to pH 8 with a concentrated sodium hydroxide solution, and further heated at 70 °C during 15 min. The cooled mixture was diluted with 20 mL of chloroform, and the organic layer was separated, washed with water, dried (Na_2SO_4), and evaporated to dryness. The oily residue of **21** was used for the next step: 1.6 g (80%); NMR δ 1.15 (t, 3, OCH_2CH_3), 2.35 (s, 3, CH_3), 4.13 (q, 2, OCH_2CH_3), 5.25 (s, 2, CH_2Ph), 7.30 (br s, Ph), 9.77 (s, 1, CHO); mass spectrum, m/e (relative intensity) 315 (M^+, 7), 287 ($M^+ - CO$, 10), 151 (**26**, 25), 135 (**27**, 40). When [*formyl*-^{14}C]dimethylformamide was used in the synthesis (1.6 mL, 1 mCi), the formylpyrrole had 2.6×10^6 dpm/mg.

Benzyl 3-(Ethoxycarbonyl)-4-methyl-5-(hydroxymethyl)-2-pyrrolecarboxylate (22). Sodium borohydride (1.6 g) was added to a solution of 1.6 of the formylpyrrole **21** in 40 mL of dry methanol. The mixture was stirred at 20 °C during 30 min, it was then poured over ice–water and filtered, and the precipitated **22** was crystallized from methanol–water: 1.21 g (75); mp 95–96 °C; NMR δ 1.20 (t, 3, OCH_2CH_3), 2.06 (s, 3, CH_3), 4.20 (q, 2, OCH_2CH_3), 4.56 (s, 2, CH_2OH), 5.26 (s, 2, CH_2Ph), 7.36 (br, 5, Ph). Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.35; H, 5.99; N, 4.41. Found: C, 64.20; H, 6.01; N, 4.30. When [*formyl*-^{14}C]pyrrole **21** (2.6×10^6 dpm/mg) was reduced, the obtained $^{14}CH_2OH$-substituted pyrrole **22** had 9.7×10^5 dpm/mg.

Benzyl 5-Iodo-4-(ethoxycarbonyl)-3-methyl-2-pyrrolecarboxylate (13). A solution of 3 g of the pyrrole acid **10** in 50 mL of water containing 6 g of sodium bicarbonate was slowly added to a mixture of 3 g of iodine and 6 g of potassium iodide in 50 mL of water. The mixture was stirred during 1 h at 75 °C, it was then poured over an excess of ice–water, filtered, dried, and crystallized from ethanol–water: 3.2 g (90%); mp 132–133 °C; NMR δ 1.36 (t, 3, OCH_2CH_3), 2.60 (s, 3, CH_3), 4.35 (q, 2, OCH_2CH_3), 5.35 (s, 2, CH_2Ph), 7.40 (br, 5, C_6H_5). Anal. Calcd for $C_{16}H_{16}NO_4I$: C, 46.49; H, 3.87; N, 3.38. Found: C, 46.39; H, 3.76; N, 3.28.

Benzyl 3-Methyl-4-(ethoxycarbonyl)-2-pyrrolecarboxylate (14). To a solution of 2 g of the iodopyrrole **13** in 40 mL of acetic acid were added 2 g of zinc powder, and the mixture was heated at 130–140 °C during 3 h with continuous stirring. During the heating period additional zinc powder (2 g) was added in small portions. The mixture was filtered while hot, and the filtrates were adjusted to pH 2 with concentrated hydrochloric acid and poured over ice–water. The product was crystallized from ethanol–water: 1.26 g (90%); mp 115–116 °C; NMR δ 1.30 (t, 3, OCH_2CH_3), 2.6 (s, 3, CH_3), 4.28 (q, 2, OCH_2CH_3), 5.34 (s, 2, CH_2Ph), 7.40 (br, 5, Ph), 7.50 (br, 1, H_5). Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.90; H, 5.92; N, 4.88. Found: C, 66.88; H, 5.87; N, 4.80.

Dibenzyl 3,4′-Bis(ethoxycarbonyl)-3′,4-dimethyldipyrrylmethane-5,5′-dicarboxylate (23). A solution of 300 mg of the α-unsubstituted pyrrole **14** and 300 mg of 2-(hydroxymethyl)pyrrole **22** in 24 mL of glacial acetic acid containing 45 mg of *p*-toluensulfonic acid was heated under nitrogen at 40 °C during 3 h. The mixture was then poured over 120 mL of ice–water, it was extracted with chloroform (3 × 10 mL), and the organic layer was washed with a 5% solution of sodium bicarbonate and then with water, dried (Na_2SO_4), and evaporated to dryness in vacuo. The residue was purified through a TLC silica gel column (2 × 20 cm), which was packed and eluted with 2% methanol in benzene. The eluted dipirrylmethane **23** was crystallized from methylcyclohexane: 300 mg (50%); mp 120–122 °C; NMR ($CDCl_3$) δ 1.35 (m, 6, OCH_2CH_3), 2.20 (s, 3, 3′-CH_3), 2.65 (s, 3, 4′-CH_3), 4.25 (m, 6, OCH_2CH3 and CH_2), 5.35 (br, 4, CH_2PH), 7.40 (br, 10, C_6H_5); mass spectrum, m/e (relative intensity) 586 (M^+, 1), 494 ($M^+ - C_7H_7$, 13), 450 (**28**, 18), 287 (10), 225 (34), 209 (45), 151 (**26**, 26), 135 (**27**, 30). Anal. Calcd for $C_{33}H_{34}N_2O_8$: C, 67.57; H, 5.82; N, 4.78. Found: C, 67.47; H, 5.60; N, 4.58. When 2-(hydroxymethyl)pyrrole **22** was labeled (9.7×10^5 dpm/mg), the obtained dipyrrylmethane **23** had 9.9×10^5 dpm/mg.

Diethyl 3′,4-Dimethyldipyrrylmethane-3,4′-dicarboxylate (1). A solution of 350 mg of the bis[(benzyloxy)carbonyl]dipyrrylmethane **23** in 100 mL of glacial acetic acid was reduced with hydrogen over 300 mg of 10% Pd on charcoal at 50 psi of H_2 for 3 h. The catalyst was filtered off, the solvent was evaporated to dryness, and the obtained diacid **24** was dissolved in 16 mL of water containing 1 g of sodium bicarbonate. A solution of 240 mg of iodine in 16 mL of ethanol was then slowly added to the solution containing the dipyrrylmethane dicarboxylate, and the precipitated 5.5′-diiododipyrrylmethane **25** was filtered off after the mixture was cooled at 5 °C; 285 mg (84%). It was dissolved in 100 mL of ethanol containing 600 mg of anhydrous sodium acetate, and it was reduced with hydrogen at 45 psi during 3 h. The catalyst was filtered off, the filtrate was evaporated to dryness, the residue was dissolved in chloroform, and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from hexane: 129 mg (82%); mp 168–170 °C, NMR δ 1.38 (m, 6, OCH_2CH_3), 2.22 (s, 3, 3′-CH_3), 2.30 (s, 3, 4-CH_3), 4.25 (m, 6, OCH_2CH_3 and CH_2), 6.34 (b, 1, H-5), 7.28 (br, 1, H-5′): mass spectrum, m/e (relative intensity), 318 (M^+, 3), 166 (11), 153 (14). Anal. Calcd for $C_{17}H_{22}N_2O_4$: C, 64.15; H, 6.91; N, 8.80. Found: C, 64.05; H, 6.81; N, 8.90.

Diethyl 3′,4-Dimethyl-5,5′-diformyldipyrrylmethane-3,4′-dicarboxylate (2). Phosphorous oxychloride (0.72 mL, 8 mmol) was added to a solution of 560 mg (7.6 mmol) of dimethylformamide in 1 mL of dry methylene chloride kept at 5 °C. A second solution of 280 mg (0.88 mmol) of the dipyrrylmethane **1** in 4 mL of methylene chloride containing 0.2 mL (2.16 mmol) of phosphorous oxychloride was then added to the former one, and the mixture was stirred at 20 °C during 1 h and then heated at 50 °C for a further h. The solution was then evaporated to dryness in vacuo, the residue was dissolved in 400 mL of water, and the solution was adjusted to pH 7 with 10% sodium hydroxide and kept at 20 °C during 72 h. The precipitate was filtered, dried, and crystallized from benzene: 313 mg (96%); mp 98–100 °C; NMR δ 1.35 (m, 6, OCH_2CH_3), 2.25 (s, 3, 3′-CH_3), 2.45 (s, 3, 4-CH_3), 4.30 (m, 6, OCH_2CH_3 and CH_2), 9.56 (s, 1, 5-CHO), 10.01 (s, 1, 5-CHO); mass spectrum, m/e (relative intensity) 374 (M^+, 7). Anal. Calcd for $C_{19}H_{22}N_2O_6$: C, 60.96; H, 5.88; N, 7.48. Found: C, 60.76; H, 5.67; N, 7.34.

Diethyl 2,7,12,18-Tetramethyl-13,17-bis[β-(methoxycarbonyl)ethyl]porphyrin-3,8-dicarboxylate (4). A mixture of 270 mg (0.7 mmol) of the bis(formylpyrryl)methane **2** and 337.5 mg (0.73 mmol) of the diacid dipyrrylmethane **3** was dissolved in 135 mL of dichloromethane and 115 mL of anhydrous methanol containing 600 mg of *p*-toluenesulfonic acid, and the solution was kept at 20 °C in the dark during 24 h. Methanol saturated with zinc acetate (32 mL) was then added to the solution which was then kept for further 48 h. The mixture was then evaporated to dryness, and the residue was redissolved in 5% sulfuric acid in methanol (150 mL) and kept for 20 h at 20 °C in the dark. It was then poured into an excess of water, the aqueous solution was extracted with chloroform, and the extracts were washed with water, dried (Na_2SO_4), and evaporated to dryness in vacuo. The residue was filtered through a TLC silica gel column (2 × 20 cm), prewashed and developed with 0.5% methanol in chloroform. The eluted porphyrin was crystallized from chloroform–hexane: 200 mg (40%); mp 210 °C; NMR (0.025 M in $CDCl_3$) δ 1.78 (m, 6, OCH_2CH_3), 3.10 (t, 4, $CH_2CH_2CO_2R$), 3.38, 3.45, 3.57, 3.71, and 3.75 (5 s, 3, 3, 6, 3, 3, $C_{18}CH_3$, $C_{12}CH_3$, OCH_3, C_2CH_3, C_7 CH_3); 4.19 (t, 4, $CH_2CH_2CO_2R$), 4.85 (m, 4, OCH_2CH_3), 9.53 and 9.58 (2 s, 1, 1, H_{20} and H_{15}), 10.64 and 10.73 (2 s, 1, 1, H_{10} and H_5); mass spectrum, m/e (relative intensity) 682 (M^+, 100), 667 ($M^+ - CH_3$, 5), 651 ($M^+ - OCH_3$, 2.5), 609 ($M^+ - CH_2CO_2CH_3$, 20). When the preparation of **4** started from dipyrryl[^{14}C]methane **23** carrying 9.9×10^5 dp/mg, it afforded [5-^{14}C]porphyrin **4** having 1.2×10^5 dpm/mg.

Deuteroporphyrin IX Dimethyl Ester 5. A solution of 40 mg of **4** in 8 mL of 10% hydrochloric acid was heated at 200 °C during 8 h in a sealed glass vessel. The vessel was cooled and opened, and its content was poured over 30 mL of ice–water. The solution was adjusted to pH 5 with pyridine and extracted with

chloroform (3 × 10 mL). The organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness, and the residue was dissolved in 30 mL of 5% sulfuric acid in methanol. The solution was kept at 20 °C for 20 h. It was then diluted with 50 mL of water and extracted with chloroform (3 × 15 mL), and the extracts were washed with water, with a 5% sodium bicarbonate solution, and again with water. The chloroform solution was dried (Na_2SO_4) and evaporated to dryness, and the residue was recrystallized from chloroform–hexane: 17 mg (53%); mp 222 °C (lit.[10,11] mp 218–220, 222 °C[5]); NMR (0.005 M in $CDCl_3$) δ 3.31 (t, 4, CH_2CH_2COR), 3.65, 3.68, 3.77 (3 s, 6, 6, 6, C_2CH_3 and C_7CH_3, OCH_3, $C_{12}CH_3$, and $C_{18}CH_3$), 4.46 (t, 4, $CH_2CH_2CO_2R$), 9.11 (br, 2, H_3 and H_8), 10.05, 10.09, 10.14, 10.17 (4 s, 1, 1, 1, 1, H_{20}, H_{10}, H_5, and H_{15}); mass spectrum, *m/e* (relative intensity) 538 (M^+, 100). Anal. Calcd for $C_{32}H_{34}N_4O_2$: C, 71.37, H, 6.35; N, 10.40. Found: C, 71.20; H, 6.20; N, 10.31. When [5-^{14}C]-4 was used (1.2 × 10^5 dpm/mg), the [5-^{14}C]deuteroporphyrin IX dimethyl ester had 1.4 × 10^5 dpm/mg.

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Crystal and Molecular Structure of Bestatin and Its Implications Regarding Substrate Binding to the Active Site of Leucine Aminopeptidase[1,2]

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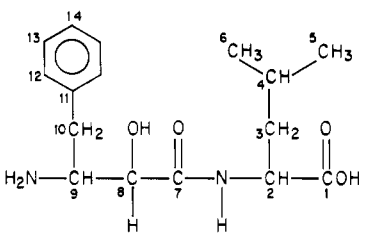
The X-ray crystal structure of bestatin, [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine ($C_{16}H_{24}N_2O_4$), has been determined. Four molecules of bestatin crystallize with four molecules of 2-methyl-2,4-pentanediol (MPD) and eight molecules of water in the space group $P2_12_12_1$. Unit cell dimensions are a = 6.653 (1), b = 15.150 (3), and c = 27.309 (4) Å. The final R was 8.5%, based on 2871 independent structure amplitudes. The MPD was found to be disordered. In addition to the usual functional groups needed for binding to leucine aminopeptidase, bestatin includes a tetrahedral carbon, C(8), as might be found in the putative transition-state intermediate. The structure indicates that the nonpolar side chains are oppositely disposed and separated by ~10 Å. The peptide bond is trans. There is no H bonding between OH on C(8) and the adjacent carbonyl. These data suggest possible modes of binding of this transition-state analogue to leucine aminopeptidase.

Bestatin, [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine[3] (1), is a tightly bound inhibitor of hog



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kidney leucine aminopeptidase (K_i = 2 × 10^{-8}) and aminopeptidase B.[4,5] This peptide analogue, originally isolated from actinomycetes, has three optical centers.[6] Kinetic tests of synthetically prepared isomers show that isomers with the 8*S*,9*S*,2*S* or 8*R*,9*R*,2*S* configurations are 7- and 750-fold less active, respectively, as inhibitors of LAP than (8*S*,9*R*,2*S*)-bestatin.

In bestatin the C(9) is derived from D-phenylalanine. The configuration about this carbon seems to be a less important determinant of the binding constant than is the configuration about C(8). C(8) is an analogue of the putative tetrahedral intermediate which is formed during the enzyme-catalyzed hydrolysis of L-aminoacyl amides. C(8) bears the hydroxyl which is thought to be ligated to the activation-site metal ion of leucine aminopeptidase.[4,7-9] The bestatin analogue derived from D-leucine is 340-fold less active as an inhibitor than bestatin.

Our previous work shows that K_m is a close approximation of K_I for peptide substrates.[10] Comparison of K_m values of peptide substrates and K_I of bestatin shows that K_m values of peptidyl substrates are 200–65 000-fold higher

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(3) In this paper the optical center in the leucyl moiety is C(2). The 2*S* and 3*R* centers of the phenylalanyl moiety are C(8) and C(9), respectively. 2-Methyl-2,4-pentanediol = MPD, and leucine aminopeptidase = LAP.

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