Biosynthesis of Porphyrins and Related Macrocycles. Part 40.[1,2] Synthesis of a Spiro-lactam Related to the Proposed Spiro-intermediate for Porphyrin Biosynthesis: Inhibition of Cosynthetase

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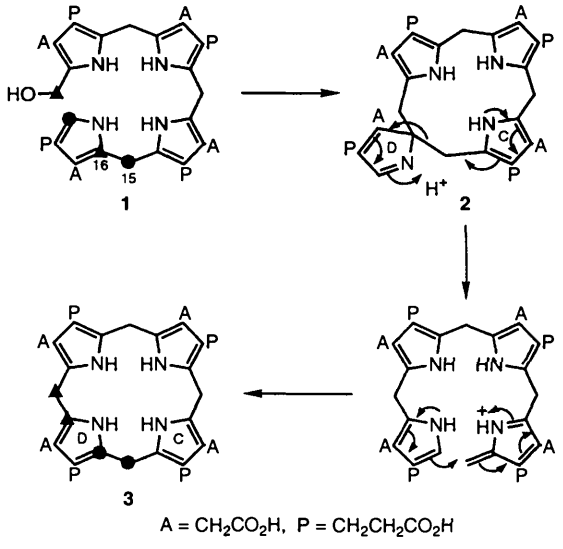
Routes are developed for synthesis of the tripyrrolic macrocyclic spiro-lactam **39**. A minor product from the synthesis, thought earlier to be an atropisomer, has been shown by molecular mechanics calculations and re-investigation to be a dimer.

The octa-acid derived from **39** closely resembles the spiro-pyrrolenine **2** proposed as a biosynthetic intermediate for uroporphyrinogen III. This octa-acid acts as a strong inhibitor of cosynthetase (uroporphyrinogen III synthase) whilst other similar systems which lack some of its functionality do not. These results strongly support the view that the spiro system **2** is indeed the biosynthetic intermediate for formation of uroporphyrinogen III **3** from hydroxymethylbilane **1**.

Uroporphyrinogen III **3**, shortened to uro'gen III, acts as the first macrocyclic precursor of all the tetrapyrrolic pigments of life (*e.g.* haem, chlorophyll and vitamin B_{12}). The enzymic formation of uro'gen III **3** from the linear hydroxymethylbilane **1**, Scheme 1, has attracted intense interest because of the rearrangement that occurs during the cyclisation process which is catalysed by the enzyme cosynthetase (systematically uroporphyrinogen III synthase, E.C. 4.2.1.75). This process[3] and also the formation of hydroxymethylbilane **1** have been extensively studied and the findings have been reviewed.[4] The outcome of two key experiments is illustrated by the symbols ● and ▲ on structures **1** and **3**, which show the results of ^{13}C-labelling experiments, one study with ^{13}C at ● and the other with ^{13}C at ▲. It was thus established that the inversion of ring D takes place by an intramolecular mechanism.[5]

The combined results from these various ^{13}C-labelling experiments eliminated almost all of the many mechanisms proposed for the phenomenon of ring D inversion but two mechanisms remained compatible.[6] In one, ring D of the hydroxymethylbilane **1** is detached by cleavage of the bond between C-15 and C-16 but is postulated not to escape from the enzymic active site. It is then turned over before reattachment to C-15 and the hydroxymethyl carbon. The other mechanism differs fundamentally in that the bond between C-16 and the hydroxymethyl carbon is suggested to be made first, to generate the spiro-pyrrolenine **2**; this system could then undergo fragmentation–recombination as illustrated in Scheme 1 to form uro'gen III **3**. The chemistry involved in this second mechanism has been shown to be both feasible and facile by the synthesis of several pyrrolylmethylpyrrolenines corresponding to rings C and D of structure **2**. These readily undergo the rearrangement required for the conversion of the spiro system **2** into uro'gen III .[7,8]

The principle of the second mechanism was suggested in 1961 by Mathewson and Corwin[9] but they did not postulate structure **2** for the spiro-intermediate because of doubts as to whether such a macrocycle with just three pyrrole rings was sterically possible. They proposed a triply protonated form of **2** which, by having additional sp^3 carbons, would be more flexible and so more readily formed. However, this was shown to be unnecessary by the synthesis[10,11] of two molecules having exactly the macrocycle present in the spiro-system **2**. X-ray analysis of one of these revealed that the steric compression in the macrocycle is relieved by substantial puckering, two pyrrole rings being tilted up relative to the third which tilts downwards.[10]

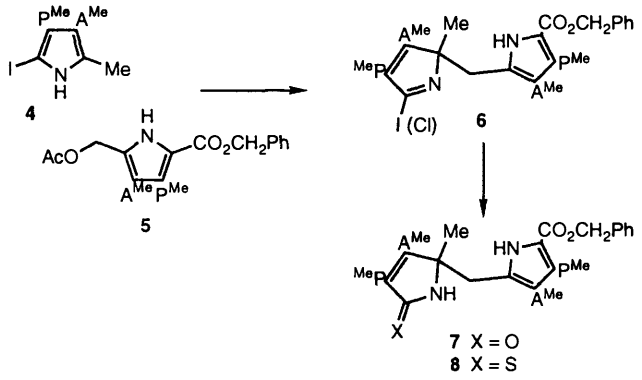


Scheme 1

It was clear from our experience with the synthetic pyrrolylmethylpyrrolenines mentioned above that the spiro-pyrrolenine **2** is almost certain to be highly labile. So, the aim of the work described in this paper was to synthesise a close analogue of **2**, carrying all the acetate and propionate side-chains, which should be stable. Our efforts finally focussed on synthesis of the spiro-lactam **39**, Scheme 4. Success in this venture would open the way to important experiments on the interaction of the synthetic product with the enzyme cosynthetase.

Results and Discussion

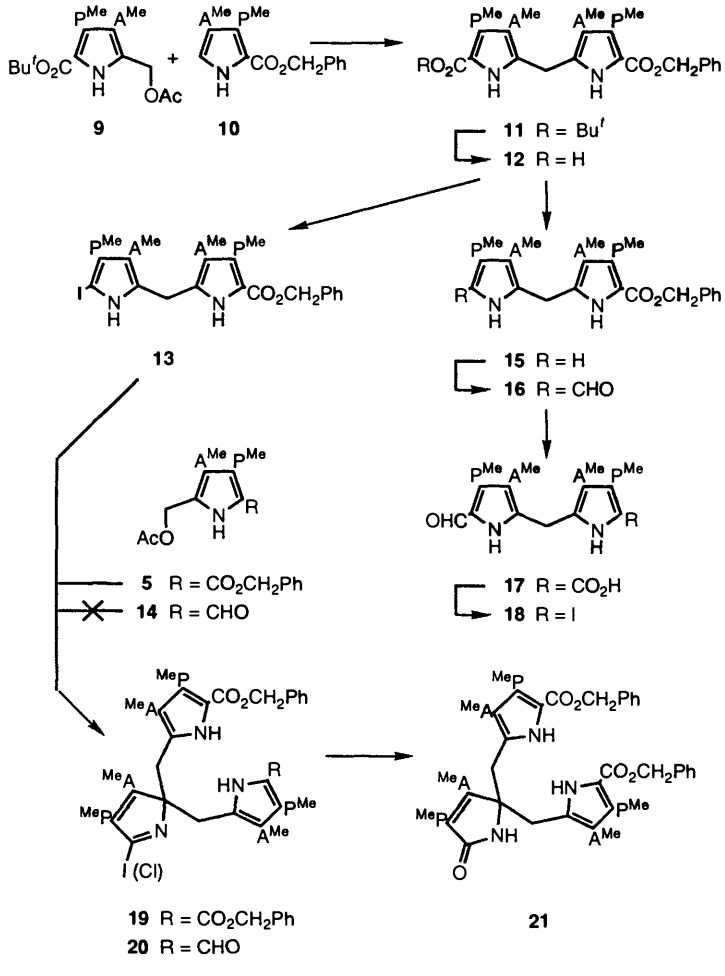
Synthesis of 5,5-Disubstituted Pyrrolin-2-ones.—None of our earlier synthetic routes[7] nor methods in the literature were applicable in any reasonably direct way to synthesis of the spiro-lactam **39**. A new approach was needed. We hoped that the lactam ring of **39** might be generated from a pyrrole which could be readily synthesised carrying the required acetate and propionate side-chains. It was envisaged that alkylation of an



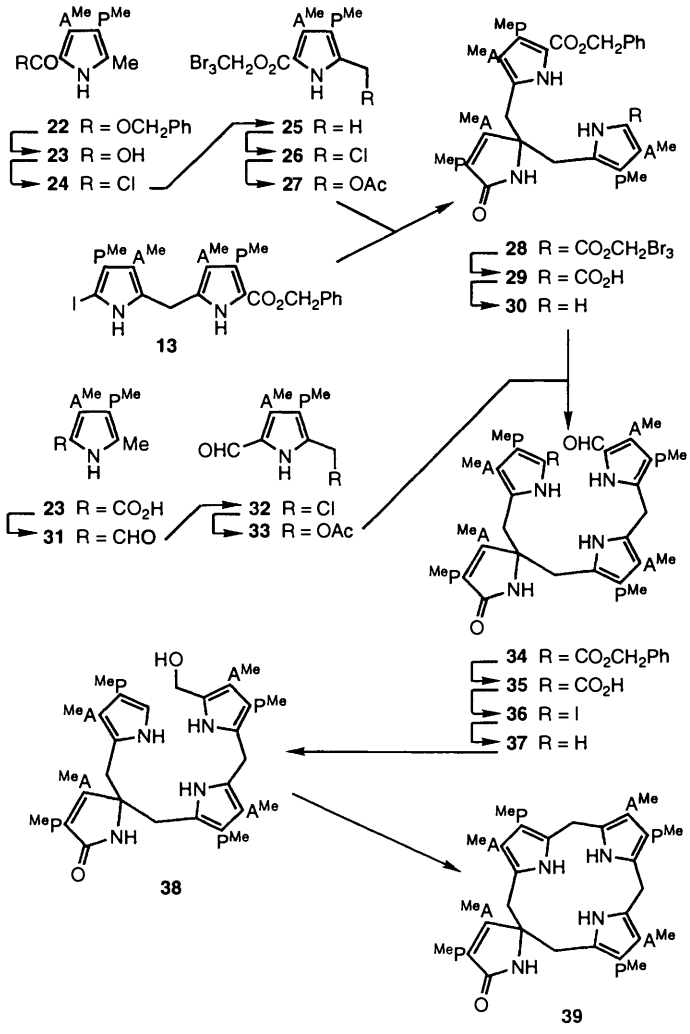
Scheme 2

iodopyrrole, *e.g.* **4** with an acetoxymethylpyrrole, *e.g.* **5**, using acid catalysis might generate an iodopyrrolenine **6** (Scheme 2). This iodo imine was expected to hydrolyse readily in aqueous acid to give the required type of substituted lactam **7**. When this alkylation reaction was carried out in aqueous tetrahydrofuran catalysed by toluene-*p*-sulfonic acid, the major product was the iodopyrrolenine **6**, which was remarkably stable and could be purified by normal chromatographic methods. A more reliable procedure for the alkylation used stannic chloride as Lewis acid in non-aqueous medium. Then the pyrrolenine **6** was produced consistently in *ca.* 30% yield; this is not a high value in absolute terms but it far outstrips the likely overall yield from any multi-stage route which would otherwise be needed. Mass spectrometry indicated that the major product under the non-aqueous conditions was indeed the iodopyrrolenine **6** but a smaller amount of the corresponding chloropyrrolenine was also present. Separation was not necessary since both halogenopyrrolenines were smoothly hydrolysed in acidic aqueous methanol containing silver acetate to afford the lactam **7**. The corresponding thiolactam **8** was readily obtained by treating the halogenopyrrolenines **6** with hydrogen sulfide. Also the iodopyrrolenine **6** from the aqueous route was found to hydrolyse during *ca.* 24 h just in the presence of traces of acidic water to yield the lactam **7**. The discovery of this new route to 5,5-disubstituted pyrrolin-2-ones and -2-thiones was the key to our later successes.

Synthesis of the Spiro-lactam **39**.—The synthesis of the spiro-lactam **39**, which was our real target, requires attachment of two pyrrolylmethyl groups to the 5-position of an unsaturated lactam. This involves extension of the foregoing approach to alkylation of an iododipyrromethane, *e.g.* **13**, with the aim of forming first the halogenopyrrolenines **19** followed by hydrolysis to yield the lactam **21**. The required halide **13** was synthesised from **9** and **10** *via* **11** and **12** by chemistry familiar to us, as shown in Scheme 3. It turned out that the alkylation step **13**→**19** using the acetoxymethylpyrrole **5** worked smoothly as did the hydrolysis **19**→**21**, so a route to the required type of lactam was available. However, it was necessary for the synthesis of the actual target molecule **39** (a) to set the correct substitution pattern on the two pyrrolic rings and (b) to protect the α-positions of these rings by groups which can be differentiated. The initial plan was to protect one ring with a formyl group and the other with a benzyloxycarbonyl group.



Scheme 3



Scheme 4

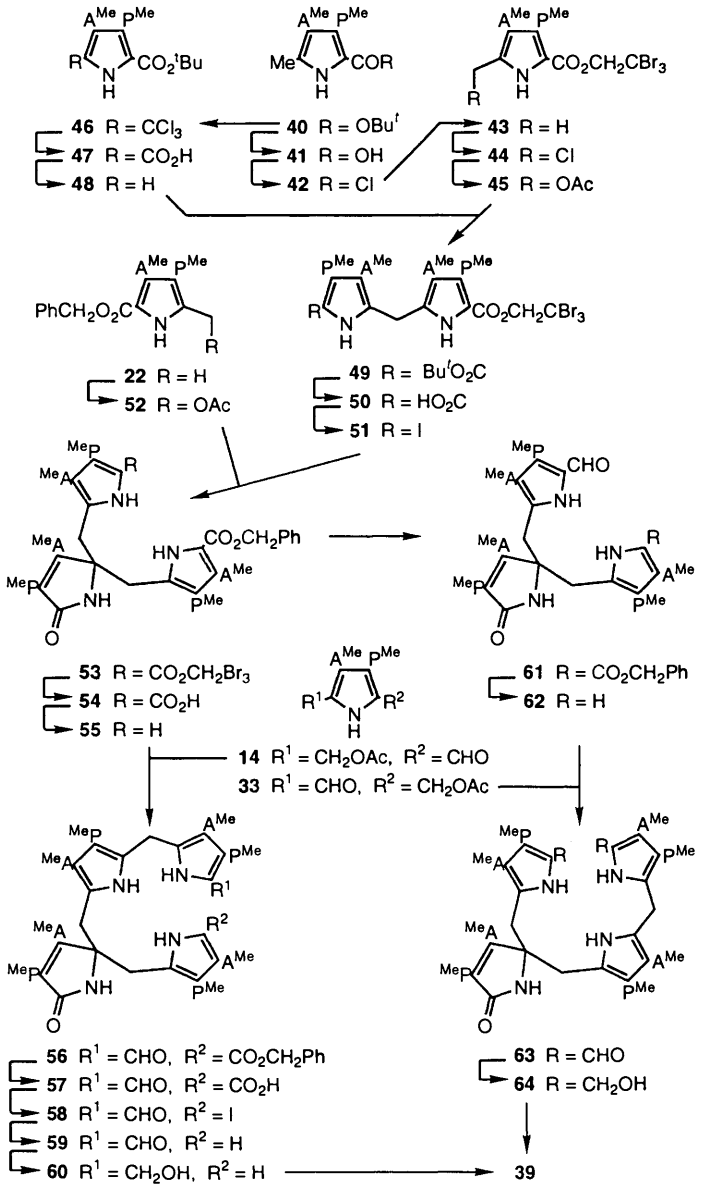
However, no trace of the halogenopyrrolenine **20** could be detected when the aldehyde **14** was treated with the iododipyrromethane **13** under the standard conditions. (Scheme 3). No better results were obtained when the protecting groups were exchanged. Scheme 3 shows the synthesis *via* **12**→**15**→**16**→**17** of the appropriate α-formyl-α′-iododipyrromethane **18** but this reacted only very slowly with the acetoxymethylpyrrole **5** and no satisfactory amount of product was formed.

We therefore decided to modify the successful synthesis of lactam **21** in Scheme 3 by adjusting the acetate–propionate substitution pattern and by using a tribromoethyl ester in place of one of the benzyl esters. Scheme 4 shows the construction of the required 5,5-disubstituted lactam **28**. The benzyl group was reductively cleaved from **22** and the resultant acid **23** was converted into its acid chloride **24** using oxalyl chloride. The crude product was treated with 2,2,2-tribromoethanol and dimethylaniline to yield the ester **25** in 91% overall yield. Monochlorination of the α-methyl group with sulfuryl chloride followed by displacement of chloride from **26** by acetate gave the acetoxymethylpyrrole **27** in 92% yield. The required lactam **28** was then prepared. (Scheme 4) from **13** and **27** by the alkylation–hydrolysis approach already developed (Scheme 3).

The stage was now set for attachment of the final pyrrole ring needed for the spiro-lactam **39**. Removal of the tribromoethyl group from **28** was achieved by reduction with zinc and acetic acid and the product **29** was decarboxylated in neat trifluoroacetic acid (TFA) to form the α-free pyrrole **30**, 76% from the ester **28**. The required pyrrolic aldehyde **33** was prepared from the acid **23** by the steps **23**→**31**→**32**→**33** analogous to those already described for the acetoxymethylpyrrole **27** at the top of Scheme 4. The reaction between these two building blocks **30** and **33**, importantly with strict exclusion of oxygen, was catalysed by stannic chloride to give a 73% yield of the tripyrrolic lactam **34**. Then a standard deprotection sequence, **34**→**35**→**36**→**37**, involving iodinative decarboxylation and reduction, afforded the α-free α-formyl tripyrrole **37** in 70% overall yield from the benzyl ester **34**. For preparative purposes, the deprotection sequence was run without isolation of intermediates.

The foregoing formylpyrrole **37** was reasonably stable but the corresponding hydroxymethyl system **38** prepared from it by borohydride reduction was not. This product was immediately cyclised by treatment with a catalytic amount of toluene-*p*-sulfonic acid in dichloromethane. Two products were isolated in a ratio of *ca.* 7:3. The major product, formed in these experiments in 2–3% yield, was the required spiro-lactam **39**; it has remained non-crystalline but its ^{1}H NMR spectrum was sharp and has been fully assigned using NOE difference and two-dimensional COSY spectra. The yield of the spiro-lactam **39** was eventually raised to almost 60% but these developments will be discussed after description of a modified route to this substance.

The ^{1}H NMR spectrum of the minor, crystalline product had a number of very broad signals, though these could be sharpened by heating the solution to 55 °C. Initially, this material was thought to be an isomer of the major product

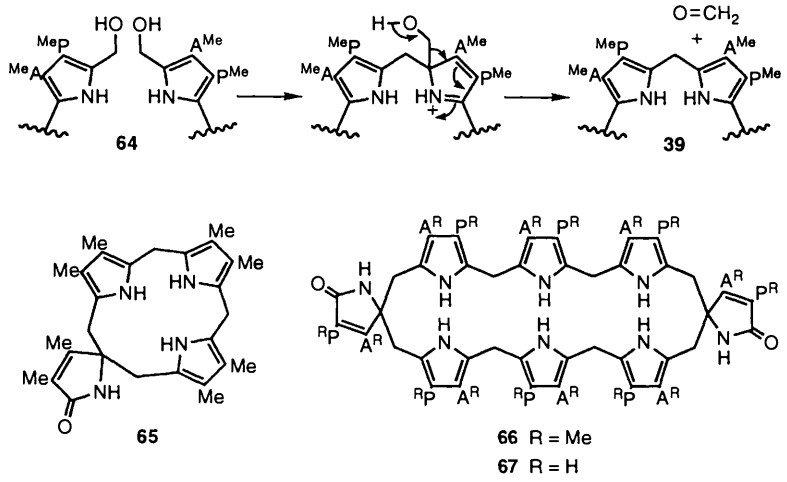


Scheme 5

because field desorption mass spectrometry gave a peak at the same mass (*m/z* 964.4) for both substances. Subsequently, these materials were found not to be isomeric and a later section will deal with this aspect of the work.

An Improved Synthesis of the Spiro-lactam **39**.—Further experiments allowed two substantial improvements to be made in our synthesis of the spiro-lactam **39**, the first coming from a study of the final cyclisation step **38**→**39**. Initially, the duration of this acid-catalysed step had been kept short because of our concern about the stability to acid of a molecule having three pyrrole rings all lacking electron-withdrawing groups. But it became clear that **39** is moderately robust to acid and the optimum conditions for the cyclisation were with 1.35 equiv. of toluene-*p*-sulfonic acid for 20 min, the yield of the lactam then being 34%. Substantially more acid or longer reaction times resulted in lower yields.

Altering the way in which the various building blocks are assembled led to a second improvement by allowing a considerable increase in scale of the synthesis. Two types of starting pyrrole were used in the original synthesis of the spiro-lactam **39** (Scheme 4). One pyrrole **40**, having the acetate adjacent to the 5-methyl group and the propionate adjacent to the 2-carboxylate, is readily prepared on a large scale. However the pyrrole **22**, having the acetate and propionate groups reversed, requires much more effort. We therefore explored a different sequence in which the required iododipyrromethane **51** is built entirely from the readily available pyrrole **40** (Scheme 5). One building block was prepared by the sequence **40**→**41**→**42**→**43**→**44**→**45** and the other came from the transformations **40**→**46**→**47**→**48**. Joining these two blocks gave the dipyrromethane **49** and the corresponding acid **50** was iodinated. Only at this stage, when the scale of the work was lower than at the outset, was the less readily available unit **22** used to yield acetoxymethylpyrrole **52** which gave the lactam **53** by the now familiar *ipso*-alkylation followed by hydrolysis. Reductive removal of the tribromoethyl group from **53** and decarboxylation of the acid **54** by TFA gave the α-free pyrrole **55** ready for alkylation with the more readily available acetoxymethylpyrrole aldehyde **14**. The benzyloxycarbonyl group was then removed from the tripyrrolic product **56** by the steps **56**→**57**→**58**→**59**, analogous to those used in Scheme 4, to afford the α-free aldehyde **59**. Borohydride reduction of the formyl group gave the corresponding unstable alcohol **60**, which was immediately ring-closed under the conditions developed above to give yields of the spiro-lactam **39** which were consistently in the 29–36% range. Many hundreds of milligrams of the lactam **39** have been synthesised by the now preferred route of Scheme 5.



Scheme 6

Throughout the foregoing discussion, attention focussed on the desired major product **39** but in all cases some of the minor product was also formed. On a small scale the two were separable chromatographically but this was not practical for larger amounts. However, conditions were devised for efficient removal of the minor product by crystallisation; the non-crystalline spiro-lactam **39** remained esentially pure in solution.

Brief mention will be made of a further variation, attractive in principle but less so in practice. The acid **54** could be formylated using trimethyl orthoformate and TFA and the product **61** was deprotected without isolation of intermediates by the same sequence as used for **56**→**59** in Scheme 5. Alkylation of the α-free lactam **62** by the acetoxymethylpyrrole **33** then yielded the dialdehyde **63** which was reduced to the corresponding diol **64** by borohydride. This diol was treated with toluene-*p*-sulfonic acid to bring about cyclisation. Either hydroxymethyl group could provide the methylene bridge between the two pyrrole rings and we expected that the other would be lost as formaldehyde; one of the two possibilities is shown in Scheme 6. Though the desired spiro-lactam **39** was formed, the yield was less than 3% and more forcing conditions or longer reaction times simply caused decomposition.

Nature of the Minor Product.—Field desorption mass spectrometry on the spiro-lactam **39** and on the minor product which had been formed with it showed *m/z* 964.4 for both samples and both gave satisfactory high-resolution accurate mass data. The two products were, therefore, considered to be isomeric and a reasonable explanation could be offerred. Mention has already been made of the X-ray crystal structure of a related substance [10] having the same tripyrrolic macrocycle as the spiro-lactam **39**. This showed a puckered large ring with two pyrrole rings pointing up relative to the third, central one which pointed down. Space-filling models indicated that there was insufficient space in the middle of the macrocycle for any of the pyrroles to invert. Hence it was concluded that the two conformations of **39**, corresponding to **65a** and **65d** in Scheme 7, would not interconvert and that these atropisomers represented the structures of the major and minor products obtained in the synthesis.[2,11] Serious doubt was cast on this interpretation by molecular mechanics calculations on the energy barrier to the interconversion process.

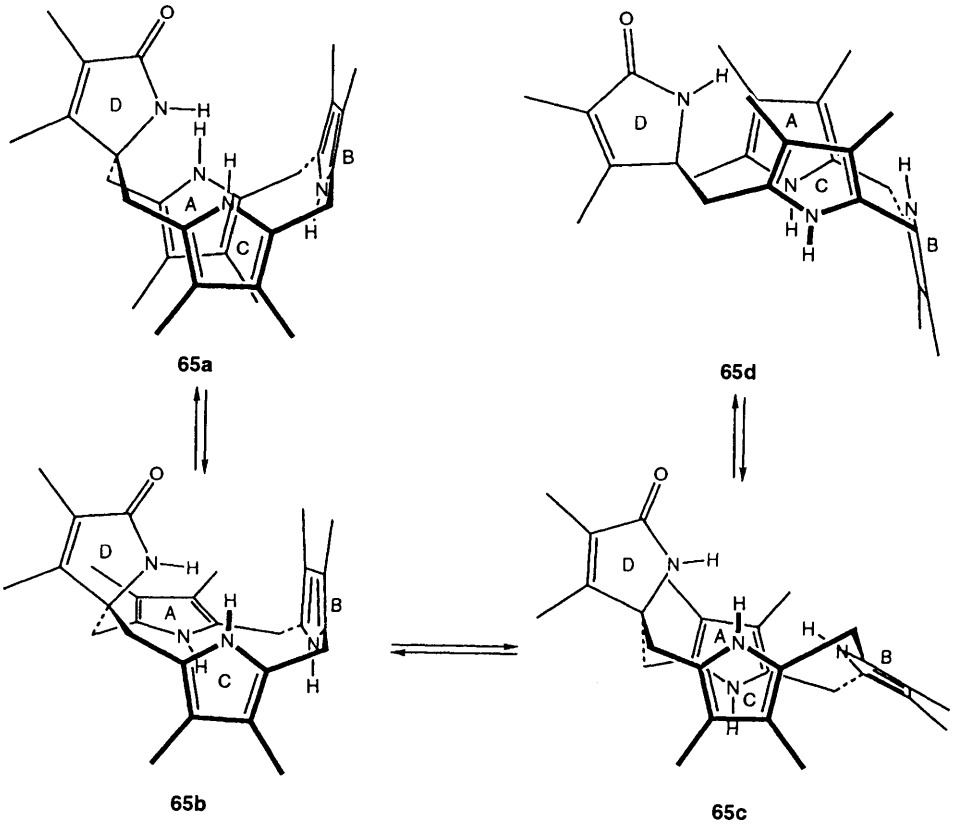
Initially the molecular mechanics program MacroModel [12] was used to calculate likely conformations for the two possible atropisomers. In order to avoid a multitude of conformations differing only in the orientation of the acetate and propionate side-chains, the calculations were performed on the spiro-lactam **65** (Scheme 6), having methyl groups in their place. Minimisation of a large number of starting conformations led to a limited number of minimum energy conformations, of which **65a** was the lowest, with **65d** higher in energy by 12.6 kJ mol^{-1}. There were also, however, two further minimum energy conformations, **65b** and **65c**, of similar energy to **65d** (Scheme 7). Furthermore, it seemed that **65b** and **65c** could be intermediates in a pathway for the interconversion of **65a** and **65d**. Thus, twisting first of ring A would convert **65a** into **65b**, then of ring B would give **65c** and finally of ring C would generate **65d**. The energy barriers to each of these ring-inversions was, therefore, investigated in order to determine whether there was a barrier high enough to allow separation of the atropisomers.

In order to calculate the energy barriers for each ring-inversion, the dihedral angle about a chosen bond was constrained to values intermediate between those for the starting and finishing conformations.* For example, in the interconversion of **65a** and **65b** the dihedral angle N_A–C(4)–C(5)–C(6) was varied from −60 to +55° in small incremental steps. At each chosen angle the structure was minimised and then the angle-constraint was removed and the energy recalculated. This procedure was also performed in the reverse direction, *i.e.* starting with **65b** and varying the torsion angle to get back to **65a**; the results are plotted in Fig. 1. As can be seen from the Figure, the largest of the three barriers, that between **65c** and **65d**,† was found to be no more than 32 kJ mol^{-1}. This is less than the barrier between chair and twist-boat conformations of cyclohexane and would imply an interconversion rate of at least 10^7 s^{-1} at room temperature. As a check, the same procedure was used to calculate the barrier to flipping of cyclohexane and the result, 44.0 kJ mol^{-1}, compared extremely well with the experimental value,[14] 44.8 ± 0.2 kJ mol^{-1}.

Even allowing for the simplifications introduced into the foregoing calculations, the results strongly indicated that the two products obtained in the synthesis are unlikely to be atropisomers and alternative explanations were sought. The

* A similar approach has recently been used to study the energy of interconversion of conformations of calix[4]arenes.[13]

† For the interconversion of **65c** and **65d**, two stable conformations were found at intermediate dihedral angles, one accessed from **65c** and the other from **65d**. Hence the curves in Fig. 1 cross each other rather than meeting at a common transition state. Each conformation becomes unstable beyond a certain angle and at that point minimises to the corresponding position on the other curve. The lower of the two energies at which this occurs has been taken to be an upper limit on the energy of the true transition state for this interconversion.



Scheme 7 The four lowest energy conformations of spiro-lactam **65** as calculated by molecular mechanics. The structures shown are direct projections of the three-dimensional ones.

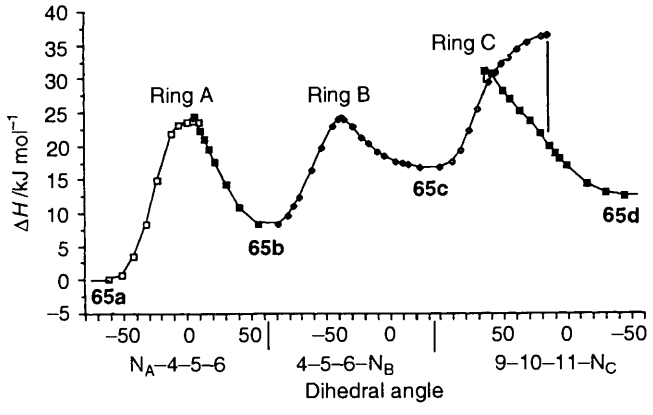


Fig. 1 The energy profile calculated by molecular mechanics for inversion of each of the three rings of spiro-lactam **65** in turn

possibility that one was the dimer **66** (Scheme 6) had not been considered previously because field desorption mass spectrometry (which generally gives molecular ions with little, if any, fragmentation) had given the same mass (*m*/*z* 964.4) for both compounds. On reinvestigation, however, it was found that the higher R_f compound also shows a peak at *m*/*z* 1928.8. Furthermore, the presence of a very small isotope peak at 0.5 mass units higher than the peak at 964.4 indicated that the latter is due, at least in part, to the M^{2+} ion.

It is unusual that a dimer is found at a higher R_f value than the corresponding monomer and so, in order to confirm this result further, the yields of the two compounds were measured at different dilutions of the reaction mixture. As can be seen from Table 1, the yields of the spiro-lactam **39** rose and those of the dimer **66** fell with increasing dilution as expected for monomer *vs.* dimer but not for two different monomeric atropisomers. The

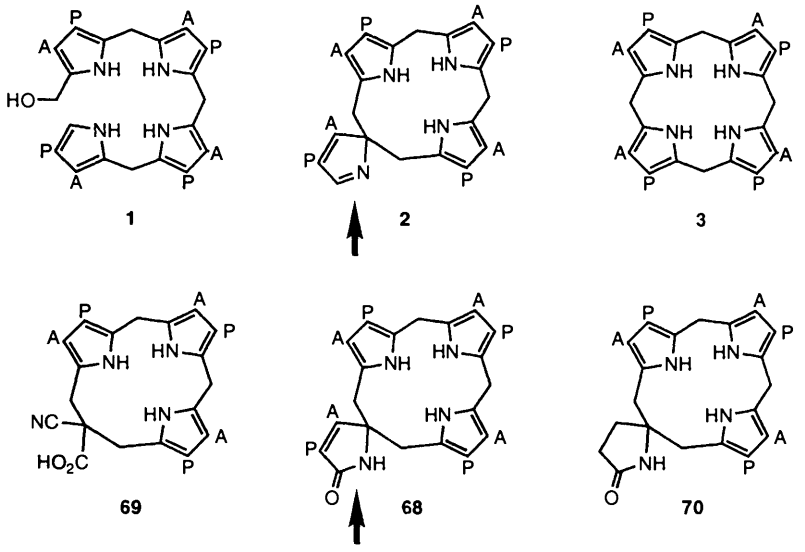
Table 1 Yields of the spiro-lactam **39** and dimer **66** from acid-catalysed cyclisation of **60** at different levels of dilution.

Conc. (*c*/mmol dm^{-3})	Spiro-lactam **39** (%)	Dimer **66** (%)
5.4	31	26
1.8	32	17
0.6	59	12

conditions yielding almost 60% of the spiro-lactam **39** were then used for all subsequent preparative runs.

Two further points are of interest. (*a*) The molecular mechanics calculations suggest that the conformation of the spiro-lactam **39** equivalent to **65a** in Scheme 7 is considerably more stable than any other. This is in agreement with the NOE experiments on **39** mentioned earlier, which showed a strong enhancement of the NH signals of rings A and C when the lactam NH was irradiated but only a weak enhancement of these signals when the NH of ring B was irradiated. (*b*) Efforts to carry out an X-ray structure determination on the crystalline dimer **66** have so far been frustrated by the instability of the crystals. An X-ray study would be of value to give evidence as to whether the synthesis yields just one or both of the two possible diastereoisomers of **66**, one with the two lactam NH groups on the same side of the molecule and the other having them directed to opposite faces.

Enzymic Studies: Inhibition of Cosynthetase.—With the spiro-lactam **39** in hand, it was possible to carry out the final important phase of the work, involving the enzyme cosynthetase. The proposal considered at the outset of this paper was that the conversion of the hydroxymethylbilane **1** into uro'gen III **3** catalysed by cosynthetase involves the spiro-pyrrolenine **2** as an intermediate. This spiro-system differs from the octa-acid **68** derived from the synthetic spiro-lactam **39** only around the nitrogen atom of the five-membered spiro-ring (see solid arrows



Scheme 8

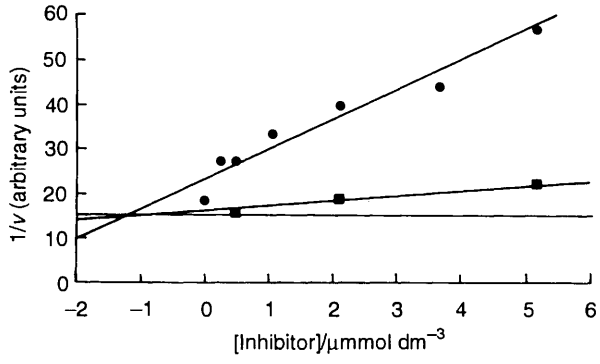


Fig. 2 Dixon plot of the kinetic data for inhibition of cosynthetase by spiro-lactam **68**. Rates were measured at the indicated concentrations of spiro-lactam using the substrate, hydroxymethylbilane **1**, at 33.2 μmol dm^{-3} (●) and 62.2 μmol dm^{-3} (■). The horizontal line at $1/v = 15$ indicates the $1/V_{max}$ value.

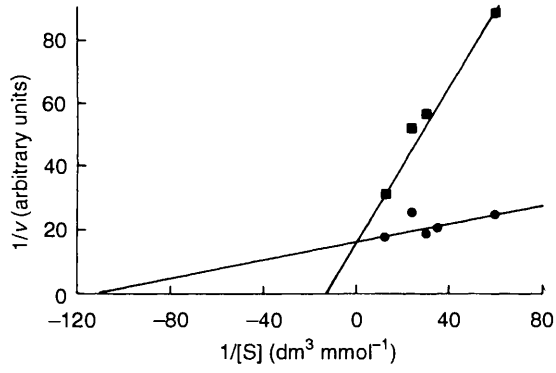


Fig. 3 Double reciprocal (Lineweaver–Burke) plot of the kinetic data for inhibition of cosynthetase by spiro-lactam **68**. Rates were measured at the indicated concentrations of the substrate, hydroxymethylbilane **1**, with (■) and without (●) the spiro-lactam at 5.2 μmol dm^{-3}.

in Scheme 8). So if the spiro-system **2** is, in fact, an intermediate, then because the spiro-lactam octa-acid **68** matches it in shape and by having almost all the likely binding groups, **68** should bind into the active site but, being unable to rearrange, should inhibit the enzyme.

Such an approach can be strengthened by examining a family of related substances rather than just one. The set included the sodium salts of (i) the hepta-acid **69** derived[11] from the corresponding dinitrile hexamethyl ester, (ii) the hexa-acid **70** from the corresponding hexamethyl ester,[11] (iii) the octa-acid **68** from the spiro-lactam **39** and (iv) the dimer **67** having 16 carboxylate residues. The structures of the acids under (i) and (ii) had been confirmed earlier[11] and those under (iii) and (iv) were prepared similarly by mild alkaline hydrolysis of the corresponding methyl esters. The ^1H NMR spectrum (in D_2O) of the hydrolysis product from the key spiro-lactam **39** confirmed complete hydrolysis of the esters and was consistent with both the lactam and macrocyclic parts of the molecule having remained intact. Four separate assays for cosynthetase activity[15] were run, using synthetic hydroxymethylbilane[6] **1** as substrate, in the absence and presence of increasing amounts of each of the four acids from (i)–(iv). Those from (i), (ii) and (iv), even at relatively high concentrations, had essentially no effect on the rate at which cosynthetase converted hydroxymethylbilane **1** into uro'gen III **3** but the spiro-lactam octa-acid **68** was a strong inhibitor. Dixon plots of the kinetic data (Fig. 2), were consistent with the inhibition by **68** being competitive with respect to hydroxymethylbilane **1** with a K_i in the 1–2 μmol dm^{-3} range. This result was confirmed by a second series of similar experiments, the concentration of substrate being changed and the concentration of inhibitor being fixed. The double-reciprocal plot of the data is given in Fig. 3.

The strong inhibition of cosynthetase by the spiro-lactam **68** is very significant and this assessment is strengthened first by the failure of the simpler systems **69** and **70** to act as inhibitors; these are based on the complete tripyrrolic macrocycle but lack part or all of the five-membered substituted spiro-system present in the spiro-lactam **68** itself. It seems that an effective inhibitor must carry essentially all the correct functions in the right orientation for there to be tight binding. Secondly the inhibitor **68** is quite different in structure from both the substrate **1** for cosynthetase and the product **3** from the enzyme; the inhibitor only resembles the putative spiro-intermediate **2** and the match is very close.

These results give very strong support to the view that the spiro-pyrrolenine **2** is indeed the biosynthetic intermediate between **1** and **3**. Since completion of this work, further evidence has been added by synthesis of the two enantiomers of the spiro-lactam **39**. These results have been reported briefly[16] and will be presented with full details in a subsequent paper.

Experimental

General Directions.—General directions are as given in Part 34 of this series.[17]

Methyl 2-Iodo-4-methoxycarbonylmethyl-5-methylpyrrole-3-propionate **4**.—The pyrrole carboxylic acid[18] **41** (566 mg, 2 mmol) was dissolved in dichloromethane (15 cm^3) and the solution stirred vigorously with water (15 cm^3) containing sodium hydrogen carbonate (400 mg). A solution of iodine (0.1 mol dm^{-3}) and potassium iodide (0.2 mol dm^{-3}) in water (24 cm^3) was added over 3 min to the mixture which was then stirred for a further 15 min; after this aqueous sodium hydrogen sulfite was added to it to destroy excess of iodine. The organic layer was decanted and the aqueous phase was extracted with dichloromethane (2 × 10 cm^3). The combined organic layers were dried and evaporated. The residue was purified on a silica gel column (5 × 2.5 cm), eluting with ether to give the *iodopyrrole* **4** as an oil (680 mg, 94%) (Found: *m/z* 365.0119. $C_{12}H_{16}INO_4$ requires *M*, 365.0123); λ_{max}(EtOH)/nm 224; ν_{max}(thin film)cm^{-1} 3350, 2950, 1740s, 1440 and 1170; δ_H($CDCl_3$, 90 MHz) 2.17 (3 H, s, C-Me), 2.35–2.90 (4 H, m, CH_2CH_2), 3.43 (2 H, s, CH_2CO_2), 3.70 (6 H, s, 2 × OMe) and 8.11 (1 H, br s, NH); *m/z* (FD) 365 (M^+, 100%).

Dimethyl 9-Benzyloxycarbonyl-1-iodo-3,7-bis(methoxycarbonylmethyl)-4-methyl-4,5-dihydrodipyrrin-2,8-dipropionate **6**.—The iodopyrrole **4** (1.32 g, 3.61 mmol) and acetoxymethylpyrrole benzyl ester **5**[19] (1.556 g, 3.61 mmol) were dissolved in dry dichloromethane (40 cm^3) and the solution stirred at 0 °C while stannic chloride (0.42 cm^3, 3.61 mmol) was added to it. The mixture was stirred at 0 °C for 30 min, after which saturated aqueous sodium hydrogen carbonate (40 cm^3) was added to it and stirring was continued for 10 min. The organic layer was decanted and the aqueous layer was diluted with water and extracted with dichloromethane (3 × 10 cm^3). The combined organic layers were dried and evaporated. The residue was purified on a silica gel column (8 cm × 2.5 cm), eluting with ether–hexane (8:2) and then ether. The higher R_f product was repurified on a similar column, using ether as eluent, to give the mixture of halogenopyrrolenines **6** as an oil (802 mg, 30% calculated for iodopyrrolenine); λ_{max}(EtOH)/nm 279; ν_{max}(thin film)/cm^{-1} 3350, 2950, 1745s, 1705s, 1440, 1245 and 1170; δ_H($CDCl_3$, 400 MHz) 1.16 (3 H, s, C-Me), 2.39 and 3.10 (each 1 H, ABq, *J* 15, 5-CH_2), 2.44 and 2.99 (each 2 H, t, *J* 7, CH_2CH_2), 2.52 (4 H, m, CH_2CH_2), 3.45 and 3.48 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.46 (2 H, s, CH_2CO_2), 3.60, 3.63, 3.63 and 3.70 (each 3 H, s, OMe), 5.23 and 5.32 (each 1 H, ABq, *J* 12, CH_2Ph), 7.25–7.50 (5 H, m, Ph) and 9.85 (1 H, br s, NH); δ_C($CDCl_3$, 100.57 MHz, DEPT) 19.85 (C-CH_3), 20.22, 20.63, 29.77, 31.81, 32.12, 32.39 and 34.89 (7 × CH_2), 51.42, 51.78, 51.96 and 52.62 (4 × OCH_3), 65.72 (PhCH_2), 128.07, 128.16 and 128.57 (phenyl CH), 81.07 (*C*-Me), 115.74, 117.59, 130.05, 130.18, 136.27 and 136.42 (6 × *C*=C) and 160.25, 160.39, 160.85, 169.72, 172.23, 172.74 and 173.72 (C=N and C=O); *m/z* (FD) 736 (M^+ for iodopyrrolenine, 70%) and 644 (M^+ for chloropyrrolenine, 30%).

Also isolated was a lower R_f product (~15%) identified as the pyrrolenine, methyl 2,2-bis[5-benzyloxycarbonyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrol-2-ylmethyl]-4-methoxycarbonylmethyl-5-methyl-2*H*-pyrrole-3-propionate (Found: *m/z* 981.3827. $C_{52}H_{59}N_3O_{16}$ requires *M*, 981.3895); λ_{max}(EtOH)/nm 282; ν_{max}(thin film)/cm^{-1} 3340, 2950, 1745s, 1705s, 1440, 1250, 1170 and 1070; δ_H($CDCl_3$, 400 MHz) 2.08 (3 H, s, C-Me), 2.44, 2.46, 2.64 and 2.91 (12 H, m, CH_2CH_2), 2.44 and 3.10 (each 2 H, ABq, *J* 15, 2-CH_2), 3.19 (2 H, s, CH_2CO_2), 3.36 and 3.41 (each 2 H, ABq, *J* 16, CH_2CO_2), 3.53, 3.59, 3.62 and 3.68 (18 H, s, OMe), 5.21 and 5.27 (each 2 H, ABq, *J* 12, CH_2Ph), 7.32–7.41 (10 H, m, Ph) and 9.67 (2 H, br s, NH); δ_C($CDCl_3$, 100.57 MHz, DEPT) 17.25 (C-CH_3), 20.60, 21.40, 29.65, 30.89, 31.14, 32.05 and 34.86 (CH_2), 52.02, 51.42, 52.02 and 52.26 (OCH_3), 65.78 (PhCH_2), 83.86 (*C*-Me), 128.32, 128.43 and 128.60 (phenyl CH), 114.99, 117.44, 129.85, 130.64, 131.98 and 136.41 (C=C) and 160.32, 166.47, 170.03, 172.38, 172.72, 173.69 and 174.96 (C=N and C=O); *m/z* (FD) 981 (M^+, 100%).

9-Benzyloxycarbonyl-2,8-bis(2-methoxycarbonylethyl)-3,7-bis(methoxycarbonylmethyl)-4-methyl-4,5-dihydrodipyrrin-1-(10H)-one **7**.—The mixture of iodo- and chloro-pyrrolenines **6** (26 mg, 0.035 mmol) was dissolved in tetrahydrofuran (2 cm^3) containing water (2 drops); silver acetate (40 mg) was added to the solution with stirring, and this was followed by toluene-*p*-sulfonic acid monohydrate (50 mg). The mixture was then stirred at room temperature for 3 h. 5% Aqueous sodium hydrogen carbonate (5 cm^3) was then added to the mixture which after being stirred for 3 min was diluted with water (10 cm^3). The mixture was extracted with dichloromethane (3 × 5 cm^3) and the combined extracts were washed with water (10 cm^3), dried, filtered through Celite and evaporated. The residue was purified on a silica gel column (5 cm × 2.5 cm), eluting with ether, then ether–methanol (19:1) to give the *lactam* **7** as a gum (19 mg, 86%) (Found: *m/z* 626.2462. $C_{32}H_{38}N_2O_{11}$ requires *M*, 626.2476); λ_{max}(EtOH)/nm 280; ν_{max}(thin film)/cm^{-1} 3320, 2960, 1745s, 1695s, 1435, 1420, 1270, 1200, 1175 and 1080; δ_H($CDCl_3$, 400 MHz), 1.34 (3 H, s, C-Me), 2.47 and 2.64 (each 3 H, m, 2-CH_2CH_2 and 8-CH_2CH_2), 2.75 and 2.94 (each 1 H, ABq, *J* 15, 5-CH_2), 2.96 (2 H, m, 8-CH_2CH_2), 3.29 and 3.66 (each 1 H, ABq, *J* 17, CH_2CO_2), 3.32 and 3.55 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.58 and 3.61 (each 2 H, s, OMe), 3.72 (6 H, s, 2 × OMe), 5.18 and 5.31 (each 1 H, ABq, *J* 12, CH_2Ph), 6.94 (1 H, br s, lactam NH), 7.25–7.40 (5 H, m, Ph) and 10.05 (1 H, br s, pyrrole NH); δ_C($CDCl_3$, 100.57 MHz, DEPT) 23.68 (C-CH_3), 19.56, 20.46, 29.55, 30.34, 31.19, 33.36 and 34.60 (7 × CH_2), 51.20, 51.33, 52.15 and 52.61 (4 × OCH_3), 65.62 (PhCH_2), 63.13 (*C*-Me), 127.84, 128.05 and 128.25 (phenyl CH), 115.33, 117.99, 129.48, 129.76, 135.59, 136.06 and 150.68 (7 × *C*=C), 160.46, 170.85, 171.34, 173.06, 173.31 and 173.46 (6 × C=O); *m/z* (FD) 626 (M^+, 100%).

9-Benzyloxycarbonyl-2,8-bis(2-methoxycarbonylethyl)-3,7-bis(methoxycarbonylmethyl)-4-methyl-4,5-dihydrodipyrrin-1(10H)-thione **8**.—Hydrogen sulfide was bubbled into dichloromethane (3 cm^3), cooled in ice, until saturation was reached. A solution of the mixture of iodo- and chloro-pyrrolenines **6** (36 mg, 0.05 mmol) in dichloromethane (2 cm^3) was then added to it and the mixture was stirred at room temperature for 1 h. After this, the solvent was evaporated in a stream of nitrogen in the fume cupboard and the residue was purified on a silica gel column (8 cm × 1 cm), eluting with ether–hexane (8:2) and then ether, to give the *thiolactam* **8** as a gum (25 mg, 80%) (Found: *m/z* 642.2251. $C_{32}H_{38}N_2O_{10}S$ requires *M*, 642.2247); λ_{max}(EtOH)/nm 304sh, 279 and 237; ν_{max}(thin film)/cm^{-1} 3300, 2950, 1740s, 1710, 1680, 1580, 1490, 1455, 1440, 1265, 1200, 1185, 1065 and 1000; δ_H($CDCl_3$, 400 MHz) 1.38 (3 H, s, C-Me), 2.45–2.60 (4 H, m, 2- and 8-CH_2CH_2), 2.68 and 2.80 (each 1 H, m, 2-CH_2CH_2), 2.81 and 2.99 (each 1 H, ABq, *J* 15, 5-CH_2), 2.98 (m, 8-CH_2CH_2), 3.32 and 3.70 (each 1 H, ABq, *J* 17, CH_2CO_2), 3.35 and 3.57 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.59, 3.62, 3.71 and 3.77 (each 3 H, s, OMe), 5.19 and 5.30 (each 1 H, ABq, *J* 12, $PhCH_2$), 7.25–7.45 (5 H, m, Ph) and 9.10 and 10.14 (each 1 H, br s, NH); *m/z* (FD) 642 (M^+, 100%).

Dimethyl 1-Benzyloxycarbonyl-9-carboxy-3,7-bis(methoxycarbonylmethyl)-5,10-dihydrodipyrrin-2,8-dipropionate **12**.—A solution of the dihydrodipyrrin[20] **11** (11.88 g, 17.07 mmol) in dichloromethane (200 cm^3) was stirred and cooled in ice while stannic chloride (2.20 cm^3, 1.1 equiv.) was added to it. The

mixture was stirred at 0 °C for 90 min after which 10% aqueous sodium acetate (200 cm^3) was added to it. The two-phase mixture was stirred vigorously for 5 min and then filtered through a thick pad of Celite, washing through with dichloromethane and water. The filtrate was extracted with dichloromethane (3 × 80 cm^3) and the combined extracts were washed with brine (100 cm^3), dried and evaporated. The residue was crystallised from dichloromethane–ether to give the *dipyrrincarboxylic acid* **12** as powdery crystals (9.586 g, 88%), m.p. 178–180 °C (decomp.) (Found: C, 60.0; H, 5.6; N, 4.4. $C_{32}H_{36}N_2O_{12}$ requires C, 59.85; H, 5.57; N, 4.31%); λ_{max}(EtOH)/nm 284; ν_{max}($CHCl_3$)/cm^{-1} 3300, 3500–2450br, 1720s, 1690, 1660, 1445 and 1175; δ_H($CDCl_3$, 400 MHz), 2.48 and 2.55 (each 2 H, t, *J* 8, 2 × $CH_2CH_2CO_2$), 2.99 (4 H, m, 2 × $CH_2CH_2CO_2$), 3.54 and 3.57 (each 2 H, s, CH_2CO_2), 3.59 and 3.74 (each 3 H, s, OMe), 3.61 (6 H, s, 2 × OMe), 3.85 (2 H, s, 5-CH_2), 5.24 (2 H, s, CH_2Ph), 7.25–7.40 (5 H, m, Ph) and 10.34 and 10.56 (each 1 H, br s, NH).

Dimethyl 1-*Benzyloxycarbonyl*-9-*iodo*-3,7-*bis*(*methoxycarbonylmethyl*)-5,10-*dihydrodipyrrin*-2,8-*dipropionate* **13**.—The carboxylic acid **12** (14.39 g, 22.5 mmol) was stirred vigorously in dichloromethane (200 cm^3) and water (200 cm^3) with sodium hydrogen carbonate (10 g) while a solution of iodine (0.1 mol dm^{-3}) in aqueous potassium iodide (0.1 mol dm^{-3}; 240 cm^3) was added to it over 5 min. The mixture was stirred for a further 20 min after which sodium metabisulfite was added to it until excess of iodine was destroyed; the organic layer was then decanted. The aqueous phase was extracted with dichloromethane (2 × 40 cm^3) and the combined organic phases were dried and evaporated to give the *iododihydrodipyrrin* **13** as an oil (16.4 g, 100%), which was used immediately in the next reaction. (After purification by column chromatography, the iododipyrromethane could be crystallised from ether–hexane; however, this was not normally done since it was believed that the compound was unstable.) (Found: *m*/*z* 722.1337. $C_{31}H_{35}IN_2O_{10}$ requires *M*, 722.1335); λ_{max}(EtOH)/nm 282; ν_{max}(thin film)/cm^{-1} 3330, 2950, 1740s, 1710, 1585, 1440, 1260, 1175 and 1065; δ_H($CDCl_3$, 250 MHz) 2.40, 2.50, 2.65 and 3.01 (each 2 H, t, *J* 7, CH_2CH_2), 3.52 (2 H, s, CH_2CO_2), 3.57 (5 H, s, CH_2CO_2 and OMe), 3.61 and 3.65 (each 3 H, s, OMe), 3.78 (5 H, s, OMe and 5-CH_2), 5.24 (2 H, s, CH_2Ph), 7.25–7.42 (5 H, m, Ph) and 9.64 and 10.20 (each 1 H, br s, NH); *m*/*z* (FD) 722 (M^+, 100%).

5,5-*Bis*[5-*benzyloxycarbonyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-3-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethyl*-1H-*pyrrol*-2(5H)-one **21**.—A solution of iododipyrromethane **13** (180 mg, 0.25 mmol) and acetoxymethylpyrrole **5**[19] (108 mg, 0.25 mmol) in dichloromethane (5 cm^3) was stirred in an ice-bath while stannic chloride (35 mm^3, 0.3 mmol) was added to it. The mixture was stirred for 20 min and then saturated aqueous sodium hydrogen carbonate (10 cm^3) was added to it and stirring was continued for 10 min. The organic layer was decanted and the aqueous layer was extracted with dichloromethane (2 × 5 cm^3). The combined organic phases were dried and evaporated. The residue was purified on a silica gel column (5 cm × 2.5 cm), eluting with ether–hexane (8:2) then ether, to give a mixture of the iodo- and chloro-pyrrolenines **19** as an oil (94 mg, 34%); δ_H($CDCl_3$, 100 MHz) 2.37–2.67 (8 H, m, 3-CH_2CH_2 and 2 × pyrrole-CH_2CH_2), 2.90 (4 H, m, 2 × pyrrole-CH_2CH_2), 2.60 and 3.20 (each 2 H, ABq, *J* 15, CH_2–C–CH_2), 3.43 (4 H, close ABq, 2 × pyrrole CH_2CO_2), 3.57 (2 H, s, 4-CH_2CO_2), 3.67 (6 H, s, 2 × OMe), 3.69 (9 H, s, 3 × OMe), 3.79 (3 H, s, OMe), 5.30 (4 H, close ABq, 2 × CH_2Ph), 7.25–7.45 (10 H, m, Ph) and 9.71 (2 H, br s, NH).

The oil, on storage overnight in the fridge at 0 °C, spontaneously hydrolysed to a much more polar compound, which was purified on a silica gel column (5 cm × 2.5 cm), eluting with ether then ether–methanol (9:1), to give the *lactam* **21** as an oil (65 mg, 78%) (Found: *m*/*z* 983.3678. $C_{51}H_{57}N_3O_{17}$ requires *M*, 983.3688); λ_{max}(EtOH)/nm 281; ν_{max}($CHCl_3$)/cm^{-1} 3420, 3280, 2950, 1720s, 1695s, 1445 and 1080; δ_H($CDCl_3$, 250 MHz) 2.37 (2 H, m, 3-$CH_2CH_2CO_2$), 2.46 (6 H, m, 3 × $CH_2CH_2CO_2$), 2.72 and 2.99 (each 2 H, ABq, *J* 16, CH_2–C–CH_2), 2.91 (4 H, m, pyrrole $CH_2CH_2CO_2$), 3.30 and 3.43 (each 2 H, ABq, *J* 17, pyrrole CH_2CO_2), 3.49 (2 H, s, 4-CH_2CO_2), 3.55 and 3.71 (each 3 H, s, OMe), 3.58 (12 H, s, 4 × OMe), 5.16 and 5.26 (each 2 H, ABq, *J* 12, CH_2Ph), 7.25–7.38 (5 H, m, Ph), 7.64 (1 H, br s, lactam NH) and 9.82 (2 H, br s, pyrrole NH); δ_C($CDCl_3$, 100.57 MHz, DEPT) 19.53 (3-$CH_2CH_2CO_2$), 20.30 (pyrrole $CH_2CH_2CO_2$), 29.21 (pyrrole CH_2CO_2), 30.09 and 30.72 (3-$CH_2CH_2CO_2$ and 4-CH_2CO_2), 31.58 (CH_2–C–CH_2), 34.47 (pyrrole $CH_2CH_2CO_2$), 51.25 and 52.08 (pyrrole OCH_3), 51.40 and 53.00 (lactam OCH_3), 65.68 ($PhCH_2$), 65.79 (C-4), 115.76, 118.39, 128.62 and 130.00 (pyrrole C=C), 128.10, 128.35 and 128.48 (phenyl CH), 136.18 (phenyl C), 138.38 (C-2), 149.13 (C-3), 160.33 (CO_2Bn), 171.94, 172.16 and 173.51 (lactam CONH and CO_2Me) and 173.05 and 173.74 (pyrrole CO_2Me); *m*/*z* (FD) 983 (M^+, 100%).

The lactam **21** was also prepared in a more controlled manner as follows. The mixture of iodo- and chloro-pyrrolenines **19** was prepared as above from iododipyrromethane **13** (3.20 g) and acetoxymethylpyrrole **5** (2.16 g). The orange oil was dissolved in tetrahydrofuran (100 cm^3) and water (10 cm^3) and toluene-*p*-sulfonic acid hydrate (2.0 g) and silver acetate (0.5 g) were added to the solution. The mixture was stirred in the dark at room temperature for 10 h and then added to water (400 cm^3) and extracted with dichloromethane (4 × 80 cm^3). The combined extracts were washed with water (3 × 100 cm^3), dried and evaporated. Purification of the residue on a silica gel column (6 cm × 8 cm diam.), eluting first with ether and then ether–methanol (19:1), gave the lactam **21** (1.60 g, 33% from **13**).

Dimethyl 1-*Benzyloxycarbonyl*-9-*formyl*-3,7-*bis*(*methoxycarbonylmethyl*)-5,10-*dihydrodipyrrin*-2,8-*dipropionate* **16**.—The diester **11**[20] (1.39 g, 2 mmol) was stirred in trifluoroacetic acid (10 cm^3) at room temperature in the dark under argon for 1 h when TLC showed that there was no remaining diester. The solution was cooled in ice and trimethyl orthoformate (10 cm^3) was added to it. After 10 min, the solution was poured into 10% aqueous sodium carbonate (100 cm^3) and extracted with dichloromethane (3 × 25 cm^3). The combined extracts were dried and evaporated. Purification of the residue on a silica gel column (6 cm × 2.5 cm diam.), eluting with methyl acetate–hexane (2:1), gave the *aldehyde* **16**, which crystallised from dichloromethane–ether as needles (1.14 g, 91%), m.p. 115–116 °C (Found: C, 61.4; H, 5.8; N, 4.4. $C_{32}H_{36}N_2O_{11}$ requires C, 61.53; H, 5.81; N, 4.48%); λ_{max}(EtOH)/nm 307 and 278; ν_{max}($CHCl_3$)/cm^{-1} 3320, 2950, 1720s, 1690, 1640, 1440 and 1075; δ_H($CDCl_3$, 400 MHz) 2.49 (2 H, t, *J* 7, $CH_2CH_2CO_2$), 2.52 (2 H, t *J* 8, $CH_2CH_2CO_2$), 2.99 (4 H, t, *J* 7, 2 × $CH_2CH_2CO_2$), 3.53 and 3.57 (each 2 H, s, CH_2CO_2), 3.59, 3.60, 3.61 and 3.77 (each 3 H, s, OMe), 3.85 (2 H, s, 5-CH_2), 5.22 (2 H, s, CH_2Ph), 7.25–7.37 (5 H, m, Ph), 9.53 (1 H, s, CHO) and 10.25 and 10.52 (each 1 H, br s, NH); *m*/*z* (FD) 624 (M^+, 100%).

Dimethyl 1-*Carboxy*-9-*formyl*-3,7-*bis*(*methoxycarbonylmethyl*)-5,10-*dihydrodipyrrin*-2,8-*dipropionate* **17**.—To a solution of the benzyl ester **16** (624 mg, 1 mmol) in methanol (25 cm^3) was added 10% palladium-on-charcoal (50 mg). The mixture was hydrogenated at room temperature until 28 cm^3 of hydrogen had been absorbed (30 min) and was then filtered through Celite. The filtrate was evaporated to give the carboxylic acid **17** as a solid (530 mg, ~100%), which was

not purified further; δ_H($CDCl_3$, 400 MHz) 2.57 (4 H, m, 2 × $CH_2CH_2CO_2$), 3.02 (4 H, m, 2 × $CH_2CH_2CO_2$), 3.52 and 3.59 (each 2 H, s, CH_2CO_2), 3.63, 3.65, 3.69 and 3.73 (each 3 H, s, OMe), 3.96 (2 H, s, 5-CH_2), 9.37 (1 H, s, CHO) and 10.68 and 11.58 (each 1 H, br s, NH); *m/z* (FD) 534 (M^+, 100%).

Dimethyl 9-Formyl-1-iodo-3,7-bis(methoxycarbonylmethyl)-5,10-dihydrodipyrrin-2,8-dipropionate **18**.—Water (10 cm^3) and sodium hydrogen carbonate (300 mg) were added to the carboxylic acid **17** (275 mg, 0.51 mmol) dissolved in dichloromethane (10 cm^3) and the mixture was stirred vigorously while a solution of iodine (0.1 mol dm^{-3}) in aqueous potassium iodide (0.2 mol dm^{-3}; 7 cm^3) was added to it over 1 min. After the mixture had been stirred for a further 15 min, sodium metabisulfite was added to it to destroy excess of iodine. The organic layer was decanted and the aqueous phase was extracted with dichloromethane (2 × 5 cm^3). The combined organic phases were dried and evaporated. Purification of the residue on a column of silica gel (3 cm × 2.5 cm diam.), eluting with ether, gave the *iododipyrromethane* **18**, which crystallised from dichloromethane–ether–hexane as needles (212 mg, 67%), m.p. 117–118 °C (decomp.) (Found: C, 46.9; H, 4.7; N, 4.5. $C_{24}H_{29}IN_2O_9$ requires C, 46.75; H, 4.75; N, 4.5%); λ_{max}(EtOH)/nm 308; ν_{max}($CHCl_3$)/cm^{-1} 3300, 2950, 1720s, 1640, 1440, 1175 and 1005; δ_H($CDCl_3$, 400 MHz) 2.42, 2.54, 2.66 and 3.02 (each 2 H, t, *J* 7, $CH_2CH_2CO_2$), 3.55 and 3.56 (each 2 H, s, CH_2CO_2), 3.64, 3.66, 3.80 and 3.81 (each 3 H, s, OMe), 3.82 (2 H, s, 5-CH_2), 9.49 (1 H, br s, NH), 9.57 (1 H, s, CHO) and 10.35 (1 H, br s, NH); *m/z* (FD) 616 (M^+, 100%).

Methyl 5-Carboxy-4-methoxycarbonylmethyl-2-methylpyrrole-3-propionate **23**.—To a solution of the pyrrole benzyl ester[18] **22** (14.92 g, 40 mmol) in methanol (240 cm^3) was added 10% palladium-on-charcoal (800 mg). The mixture was hydrogenated at room temperature for 4 h and then filtered through Celite and evaporated. Recrystallisation from methanol–water gave the acid **23** as prisms (10.15 g, 90%), m.p. 140–142 °C (decomp., lit.[21] 133–138 °C) (Found: C, 55.3; H, 6.0; N, 4.9. $C_{13}H_{17}NO_6$ requires C, 55.1; H, 6.05; N, 4.95%); λ_{max}(EtOH)/nm 280; ν_{max}($CHCl_3$)/cm^{-1} 3430, 3500–2300, 1725s, 1655, 1455 and 1435; δ_H($CDCl_3$, 90 MHz) 2.22 (3 H, s, C-Me), 2.42 and 2.72 (each 2 H, m, CH_2CH_2), 3.67 and 3.71 (each 3 H, s, OMe), 3.86 (2 H, s, CH_2CO_2), 7.55 (1 H, br s, CO_2H) and 9.05 (1 H, br s, NH); *m/z* (M^+, 30%), 239, 180, 166 (100), 120 and 108.

Methyl 4-Methoxycarbonylmethyl-2-methyl-5-(2,2,2-tribromoethoxycarbonyl)pyrrole-3-propionate **25**.—The carboxylic acid **23** (9.89 g, 34.95 mmol) was suspended in dichloromethane (80 cm^3) and oxalyl chloride (10 cm^3) was added dropwise to it with stirring over 10 min. Vigorous effervescence occurred and after 30 min the solution was evaporated. The residue was dissolved in dichloromethane (40 cm^3) and tribromoethanol (10.88 g, 38.45 mmol) was added to the solution, followed by *N,N*-dimethylaniline (12 cm^3). The mixture was stirred at room temperature for 30 min and then heated at reflux for 10 min. Dichloromethane (400 cm^3) was added to the solution which was then washed successively with hydrochloric acid (2 mol dm^{-3}; 2 × 200 cm^3), saturated aqueous sodium hydrogen carbonate (2 × 200 cm^3) and water (200 cm^3) and then dried and evaporated. Purification of the residue on a silica gel column (6 cm × 10 cm diam.), eluting with ether–hexane (1 : 1), gave the *tribromoethyl ester* **25**, which recrystallised from dichloromethane–ether–hexane as needles (17.49 g, 91%), m.p. 111–112 °C (Found: C, 33.1; H, 3.3; N, 2.6. $C_{15}H_{18}Br_3NO_6$ requires C, 32.87; H, 3.31; N, 2.56%); λ_{max}(EtOH)/nm 289 and 241; ν_{max}($CHCl_3$)/cm^{-1} 3430, 2950, 1725s, 1690s, 1435, 1175, 1145, 1100 and 645; δ_H($CDCl_3$, 90 MHz) 2.28 (3 H, s, C-Me), 2.50 and 2.77 (each 2 H, m, $CH_2CH_2CO_2$), 3.66 and 3.69 (each 3 H, s, OMe), 3.98 (2 H, s, CH_2CO_2), 5.09 (2 H, s, CH_2CBr_3) and 9.03 (1 H, br s, NH); *m/z* (FD) 545, 547, 549 and 551 (1 : 3 : 3 : 1, M^+, 100%).

Methyl 2-Acetoxymethyl-4-methoxycarbonylmethyl-5-(2,2,2-tribromoethoxycarbonyl)pyrrole-3-propionate **27**.—A solution of the α-methyl tribromoethyl ester **25** (17.4 g, 31.75 mmol) in dry dichloromethane (200 cm^3) was cooled in ice and redistilled sulfuryl chloride (2.55 cm^3, 31.75 mmol) was added to it over 1 min with stirring. After 15 min the solution was evaporated at below room temperature. The residual oil was dissolved in acetic acid (200 cm^3) and sodium acetate (12 g) was added to it. The mixture was stirred at 75 °C for 1 h and was then added to water (1 dm^3) and extracted with dichloromethane (3 × 150 cm^3). The combined extracts were washed with water (2 × 200 cm^3), dried and evaporated. Purification of the residue on a silica gel column (6 cm × 10 cm diam.), eluting with ether–hexane (6 : 4), and crystallisation from ether–hexane gave the *acetoxymethylpyrrole* **27** as needles (17.8 g, 92%), m.p. 131–132 °C (Found: C, 33.5; H, 3.3; N, 2.35. $C_{17}H_{20}Br_3NO_8$ requires C, 33.69; H, 3.33; N, 2.31%); λ_{max}(EtOH)/nm 280 and 240; ν_{max}($CHCl_3$)/cm^{-1} 3410, 2950, 1725s, 1435, 1365, 1175, 1100 and 650; δ_H($CDCl_3$,90 MHz) 2.09 (3 H, s, MeCO), 2.50 and 2.83 (each 2 H, m, $CH_2CH_2CO_2$), 3.69 and 3.71 (each 3 H, s, OMe), 3.99 (2 H, s, CH_2CO_2), 5.12 (4 H, s, CH_2CO_2 and CH_2CBr_3) and 9.43 (1 H, br s, NH); *m/z* (FD) 603, 605, 607 and 609 (1 : 3 : 3 : 1, M^+, 100%).

4-[*5-Benzyloxycarbonyl-4-(2-methoxycarbonylethyl)-3-(methoxycarbonylmethyl)pyrrol-2-ylmethyl*]-*2,7-bis(2-methoxycarbonylethyl)-3,8-bis(methoxycarbonylmethyl)-9-(2,2,2-tribromoethoxycarbonyl)-4,5-dihydrodipyrrin-1(10H)-one* **28**.—The iododihydrodipyrrin **13** (*ca.* 22.5 mmol, from 14.39 g of acid **12**) and the tribromoethyl ester **27** (12.98 g, 21.4 mmol) were dissolved in dry dichloromethane (200 cm^3) and the solution was cooled in ice. Stannic chloride (2.63 cm^3, 22.5 mmol) was then added to it with stirring and the mixture was stirred at 0 °C for 20 min. 10% Aqueous sodium carbonate (100 cm^3) was added to the mixture which was then stirred vigorously for a further 5 min; the organic phase was then decanted and the aqueous phase was extracted with dichloromethane (4 × 30 cm^3). The combined organic phases were washed with water (3 × 100 cm^3), dried and evaporated. Purification of the residue on a column of silica gel PF_{254} (6 cm × 10 cm diam.), eluting with ether–hexane (2 : 1) and then ether, gave the crude halogenopyrrolenine (19.8 g), which was used directly for the next step; λ_{max}(EtOH)/nm 285 and 245sh; ν_{max}($CHCl_3$)/cm^{-1} 3320, 2950, 1725s, 1690, 1435, 1175 and 1090; δ_H($CDCl_3$, 400 MHz) 2.33, 2.41, 2.50, 2.50, 2.63 and 2.95 (each m, 3 × CH_2CH_2) 2.48 and 2.51 (each 1 H, d, *J* 15 Hz, pyrrole α-CH_AH_B), 3.14 and 3.24 (each 1 H, d, *J* 15 Hz, pyrrole α-CH_AH_B), 3.40 and 3.46 (2 H, ABq, *J* 16 Hz, CH_2CO_2), 3.53 and 3.57 (ABq, *J* 16, CH_2CO_2), 3.60, 3.60, 3.61, 3.61, 3.65 and 3.78 (each 3 H, s, OMe), 3.81 and 3.93 (each 1 H, ABq, *J* 17 Hz, CH_2CO_2), 5.00 and 5.12 (each 1 H, ABq, *J* 12, CH_2CBr_3), 5.22 and 5.30 (each 1 H, ABq, *J* 13, CH_2Ph), 7.25–7.42 (5 H, m, Ph) and 9.68 and 10.17 (each 1 H, br s, NH); *m/z* (FD) 1265, 1267, 1269 and 1271 (*ca.* 1 : 3 : 3 : 1, M^+ for iodopyrrolenine, 20% of ions), 1173, 1175, 1177, 1179 and 1181 (M^+ for chloropyrrolenine, 80% of ions).

The crude halogenopyrrolenines (19.8 g) were dissolved in tetrahydrofuran (300 cm^3) and water (30 cm^3) and toluene-*p*-sulfonic acid hydrate (6 g) and silver acetate (2 g) were added to the solution. The mixture was stirred under nitrogen for 12 h at room temperature and then added to water (1.5 dm^3) and extracted with dichloromethane (4 × 300 cm^3). The combined extracts were washed with water (2 × 1 dm^3) and 5% aqueous sodium hydrogen carbonate (500 cm^3) and then dried, filtered

through Celite and evaporated. Purification of the residue on a column of silica gel PF_{254} (8 cm × 10 cm diam.), eluting with ether and then ether–methanol (19:1), gave the *lactam* **28**, which crystallised from dichloromethane–ether as powdery crystals (5.84 g, 24% from the iododipyrromethane **13**), m.p. 156–158 °C (Found: C, 47.95; H, 4.4; N, 3.7. $C_{46}H_{52}Br_3N_3O_{17}$ requires C, 47.7; H, 4.5; N, 3.6%); λ_{max}(EtOH)/nm 285; ν_{max}($CHCl_3$)/cm^{-1} 3420, 3300, 2950, 1720s, 1695, 1680, 1575 and 1435; δ_H($CDCl_3$, 400 MHz) 2.35–2.51 (m, 4 × CH_2CH_2), 2.69 and 2.93 (each 2 H, m, CH_2CH_2), 2.81 and 3.02 (each 1 H, ABq, *J* 15, pyrrole α-CH_2), 2.83 and 3.19 (each 1 H, ABq, *J* 15, pyrrole α-CH_2), 3.16 and 3.52 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.40 and 3.62 (each 1 H, ABq, *J* 18, CH_2CO_2), 3.58, 3.58, 3.63, 3.66, 3.67 and 3.76 (each 3 H, s, OMe), 3.90 (2 H, s, CH_2CO_2), 5.01 and 5.04 (each 1 H, ABq, *J* 12, CH_2CBr_3), 5.18 and 5.29 (each 1 H, ABq, *J* 12, CH_2Ph), 7.25–7.40 (5 H, m, Ph), 7.38 (1 H, br s, lactam NH) and 9.37 and 10.05 (each 1 H, br s, NH); *m/z* (FD) 1155, 1157, 1159 and 1161 (*ca.* 1:3:3:1, M^+, 100%).

4-[5-*Benzyloxycarbonyl*-4-(2-*methoxycarbonylethyl*)-3-(*methoxycarbonylmethyl*)*pyrrol*-2-*ylmethyl*]-2,7-*bis*(2-*methoxycarbonylethyl*)-3,8-*bis*(*methoxycarbonylmethyl*)-4,5-*dihydrodipyrrin*-1(10H)-*one* **30**.—The lactam tribromoethyl ester **28** (5.84 g, 5.04 mmol) was dissolved in acetic acid and zinc dust (12 g) was added to the solution with stirring. After 20 min, the zinc was filtered off through Celite. The filtrate was diluted with water (300 cm^3) and extracted with dichloromethane (4 × 50 cm^3). The combined extracts were washed with water, dried and evaporated. The crude acid **29** was decarboxylated without further purification: δ_H($CDCl_3$, 400 MHz) 2.38–2.60 (8 H, 4 × CH_2CH_2), 2.74–2.98 (8 H, m, 2 × CH_2CH_2 and 2 × pyrrole α-CH_2), 3.08 and 3.20 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.28 (1 H, d, *J* 15, $CH_AH_BCO_2$), 3.50, 3.55, 3.61, 3.62, 3.66 and 3.76 (each 3 H, s, OMe and $CH_AH_BCO_2$, obscured), 3.80 and 3.88 (each 1 H, ABq, *J* 17, CH_2CO_2), 5.16 and 5.30 (each 1 H, ABq, *J* 12, CH_2Ph), 7.25–7.40 (6 H, m, Ph and lactam NH), 8.72 and 10.03 (each 1 H, br s, pyrrole NH) and 10.63 (1 H, br s, CO_2H).

A solution of the acid in redistilled trifluoroacetic acid (40 cm^3) was stirred for 7 h under argon at room temperature and then evaporated under reduced pressure at room temperature. The residue was dissolved in dichloromethane (200 cm^3) and the solution was washed with saturated aq. sodium hydrogen carbonate (2 × 50 cm^3) and then dried and evaporated. Purification of the residue on a column of silica gel PF_{254} (6 cm × 8 cm diam.), eluting with ether and then ether–methanol (19:1), gave the α-free pyrrole lactam **30** as blocks (3.26 g, 76% from the ester **28**), m.p. 125–127 °C (from methanol) (Found: C, 60.25; H, 5.85; N, 4.8. $C_{43}H_{51}N_3O_{15}$ requires C, 60.75; H, 6.05; N, 4.95%); λ_{max}(EtOH)/nm 281; ν_{max}($CHCl_3$)/cm^{-1} 3440, 3300, 2950, 1720, 1685, 1435 and 1175; δ_H($CDCl_3$, 400 MHz) 2.30–2.58 (m, 4 × CH_2CH_2), 2.70 and 2.72 (each 2 H, m, CH_2CH_2), 2.72 and 3.04 (each 1 H, ABq, *J* 16, pyrrole α-CH_2) 2.79 and 3.00 (each 1 H, ABq, *J* 15, pyrrole α-CH_2), 3.11 and 3.50 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.40 (s, CH_2CO_2), 3.35 and 3.65 (each 1 H, *J* 18, CH_2CO_2), 3.58, 3.60, 3.64, 3.67, 3.68 and 3.74 (each 3 H, s, OMe), 5.18–5.29 (each 1 H, ABq, *J* 12, CH_2Ph), 6.48 (1 H, d, *J* 3, α-H), 7.25–7.40 (5 H, m, Ph), 7.54 (1 H, br s, lactam NH) and 8.29 and 10.20 (each 1 H, br s, pyrrole NH); δ_C($CDCl_3$, 100 MHz, DEPT) 19.78 (2 C) and 20.61 (3 × $CH_2CH_2CO_2$), 29.34, 30.56, 30.63, 31.05, 31.46, 32.81, 34.77 and 35.28 (3 × CH_2CO_2, 3 × $CH_2CH_2CO_2$, 2 × pyrrole α-CH_2), 51.42, 51.63 (2 C), 51.84, 52.35 and 53.06 (6 × OCH_3), 65.85 ($PhCH_2$), 66.83 (C-4), 113.93, 115.51, 118.14, 118.68, 122.42, 129.25, 129.86, 136.26, 137.43, 149.96 (7 × pyrrole C, C-2, C-3, phenyl C), 116.85 (α-CH), 128.03, 128.25 and 128.45 (phenyl CH), 160.60 (α-CO_2), 171.59, 172.45, 172.80, 173.32, 173.48, 173.68 and 173.96 (6 × CO_2Me, CONH); *m/z* (FD) 849 (M^+, 100%).

Methyl 5-*Formyl*-4-*methoxycarbonylmethyl*-2-*methylpyrrole*-3-*propionate* **31**.—A solution of the carboxylic acid **23** (8.49 g, 30 mmol) in trifluoroacetic acid (50 cm^3) was stirred at room temperature for 1 h after which trimethyl orthoformate (30 cm^3) was added to it. After a further 20 min the mixture was diluted with water (300 cm^3) and extracted with dichloromethane (3 × 50 cm^3). The combined extracts were washed with water (2 × 100 cm^3) and then 5% aqueous sodium hydrogen carbonate (100 cm^3), dried and evaporated. Purification of the residue on a column of silica gel PF_{254} (6 cm × 8 cm diam.), eluting with dichloromethane–ether (1:1), gave the pyrrole aldehyde **31** as needles, (7.36 g, 92%), m.p. 78–79 °C (from dichloromethane–ether) (Found: C, 58.5; H, 6.2; N, 5.2. $C_{13}H_{17}NO_5$ requires C, 58.4; H, 6.4; N, 5.2%); λ_{max}(EtOH)/nm 311; ν_{max}($CHCl_3$)/cm^{-1} 3420, 3240, 2950, 1725s, 1635, 1435 and 1170; δ_H($CDCl_3$, 90 MHz) 2.30 (3 H, s, α-Me), 2.49 and 2.79 (each 2 H, m, CH_2CH_2), 3.70 and 3.74 (each 3 H, s, OMe), 3.77 (2 H, s, CH_2CO_2), 9.45 (1 H, s, CHO) and 10.30 (1 H, br s, NH); *m/z* 267 (M^+, 65%), 239, 194, 136 (100) and 120.

Methyl 2-*Acetoxymethyl*-5-*formyl*-4-(*methoxycarbonylmethyl*)*pyrrole*-3-*propionate* **33**.—A solution of α-methyl-α-formylpyrrole **31** (5.34 g, 20 mmol) in dry tetrahydrofuran (200 cm^3) and dry ether (200 cm^3) was cooled in ice. Freshly made *tert*-butyl hypochlorite (2.7 cm^3, 24 mmol) was then added to it over 0.5 min with stirring. After 15 min the solution was evaporated and the residual oil was dissolved in acetic acid (60 cm^3). Sodium acetate (10 g) was added to the solution which was stirred at 60 °C for 1.5 h and then diluted with water (400 cm^3) and extracted with dichloromethane (4 × 60 cm^3). The combined extracts were washed with water (2 × 200 cm^3), dried and evaporated. Purification of the residue on a silica gel column (2 cm × 8 cm diam.), eluting with ether, gave the *acetoxymethylpyrrole* **33**, which crystallised from dichloromethane–ether as needles (3.13 g, 48%), m.p. 93–94 °C (Found: C, 55.2; H, 6.0; N, 4.4. $C_{15}H_{19}NO_7$ requires C, 55.4; H, 5.9; N, 4.3%); λ_{max}(EtOH)/nm 302; ν_{max}($CHCl_3$)/cm^{-1} 3420, 3250, 2950, 2850, 1735s, 1720s, 1650, 1440, 1375 and 1175; δ_H($CDCl_3$, 90 MHz) 2.10 (3 H, s, MeCO), 2.54 and 2.89 (each 2 H, m, $CH_2CH_2CO_2$), 3.71 and 3.76 (each 3 H, s, OMe), 3.82 (2 H, s, CH_2CO_2), 5.16 (2 H, s, CH_2OAc), 9.64 (1 H, s, CHO) and 10.13 (1 H, br s, NH); *m/z* 325 (M^+, 3%), 283, 222, 194 and 60 (100).

4-[5-*Benzyloxycarbonyl*-4-(2-*methoxycarbonylethyl*)-3-(*methoxycarbonylmethyl*)*pyrrol*-2-*ylmethyl*]-14-*formyl*-2,7,12-*tris*(2-*methoxycarbonylethyl*)-3,8,13-*tris*(*methoxycarbonylmethyl*)-4,5,10,16-*tetrahydrotripyrrin*-2(15H)-*one* **34**.—The lactam **30** (2.03 g, 2.39 mmol) and aldehyde **33** (777 mg, 2.39 mmol) were dissolved in dry dichloromethane (50 cm^3) and dry tetrahydrofuran (0.7 cm^3) and stannic chloride (0.56 cm^3, 4.78 mmol) was added to the solution. The mixture was then stirred in the dark under argon for 18 h. After this methanol (10 cm^3) was added to it followed by 10% aqueous sodium carbonate (50 cm^3). After the mixture had been stirred vigorously for 5 min, the organic layer was decanted and the aqueous layer was diluted with water (200 cm^3) and extracted with dichloromethane (3 × 60 cm^3). The combined organic layers were dried and evaporated. Purification of the residue on a silica gel column (6 cm × 8 cm diam.), eluting with ether then ether–methanol (19:1), gave the title compound **34** as a gum (1.95 g, 73%) which was nearly pure by TLC and was used without further purification.

A sample purified by preparative TLC was also a gum (Found: *m/z* 1114.4251. $C_{56}H_{66}N_4O_{20}$ requires *M*, 1114.4270); λ_{max}(EtOH)/nm 310sh and 279; ν_{max}($CHCl_3$)/cm^{-1} 3300, 2950, 1720s, 1690, 1640, 1440, 1180 and 1075; δ_H($CDCl_3$, 400 MHz) 2.27 (2 H, m) and 2.32–2.94 (16 H, m, 4 × $CH_2CH_2CO_2$ and 2 × pyrrole-CH_AH_B-lactam), 2.99 and 3.01 (each 1 H, d, *J* 15,

pyrrole-CH_AH_B-lactam), 3.10 and 3.44 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.33 and 3.55 (each 1 H, ABq, *J* 17, CH_2CO_2), 3.42 (2 H, s, 3-CH_2CO_2), 3.58 (6 H, s, 2 × OMe), 3.62, 3.63, 3.64, 3.67, 3.72 and 3.75 (each 3 H, s, OMe and 2 H, obscured, CH_2CO_2), 3.80 and 3.86 (each 1 H, ABq, *J* 16, pyrrole-CH_2-pyrrole) 5.15 and 5.30 (each 1 H, ABq, *J* 12, CH_2Ph), 7.19 (1 H, br s, lactam NH), 7.28–7.39 (5 H, m, Ph), 8.86 (1 H, br s, NH), 9.51 (1 H, s, CHO) and 10.10 and 10.15 (each 1 H, br s, NH); *m/z* (FD) 1114 (M^+, 100%).

14-*Formyl*-4-[4-(2-*methoxycarbonylethyl*)-3-(*methoxycarbonylmethyl*)*pyrrol*-2-*ylmethyl*]-2,7,12-*tris*(2-*methoxycarbonylethyl*)-3,8,13-*tris*(*methoxycarbonylmethyl*)-4,5,10,16-*tetrahydrotripyrrin*-2(15H)-*one* **37**.—Sodium carbonate (700 mg) and 10% palladium-on-charcoal (150 mg) were added to a solution of the crude benzyl ester **34** (1.9 g, 1.71 mmol) in methanol (50 cm^3). The mixture was hydrogenated at room temperature and atmospheric pressure for 40 min, until 59 cm^3 of hydrogen had been absorbed. The mixture was then filtered and the filtrate was diluted with water (500 cm^3), acidified with acetic acid and extracted with dichloromethane (3 × 50 cm^3). The combined extracts were washed with water (200 cm^3), dried and evaporated to give the crude carboxylic acid **35**.

A solution of the carboxylic acid **35** in dichloromethane (50 cm^3) was stirred vigorously with 5% aqueous sodium hydrogen carbonate (50 cm^3) at ice-bath temperature whilst a solution of iodine (0.1 mol dm^{-3}) in aqueous potassium iodide (0.2 mol dm^{-3}; 18.8 cm^3) was added to it over 2 min. The mixture was stirred for 40 min at 0 °C after which sodium metabisulfite was added to it until excess of iodine was destroyed. The organic layer was decanted and the aqueous layer was extracted with dichloromethane (2 × 30 cm^3). The combined organic phases were dried and evaporated to give the crude iodopyrrole **36** as a brown gum which showed only one non-baseline spot on TLC (ether–methanol, 19:1).

The iodopyrrole was immediately dissolved in methanol (30 cm^3) and sodium acetate (1 g) and Adams' catalyst (50 mg) were added to the solution. The mixture was hydrogenated for 45 min at room temperature and then filtered through Celite. The filtrate was diluted with water (300 cm^3) and extracted with dichloromethane (3 × 50 cm^3) and the combined extracts were dried and evaporated. Purification of the residue on a silica gel column (4 cm × 2.5 cm diam.), eluting with ether–methanol–triethylamine (95:5:0.1), gave the crude α-free pyrrole **37** as a yellowish gum (1.18 g, 70%), which was used directly for the cyclisation.

A sample was further purified by preparative TLC to give the *α-free pyrrole* **37** as a gum (Found: *m/z* 980.3940. $C_{48}H_{60}N_4O_{18}$ requires *M*, 980.3903); λ_{max}(EtOH)/nm 311; ν_{max} ($CHCl_3$)/cm^{-1} 3340, 2950, 1720s, 1680, 1640, 1440, 1170 and 1005; δ_H($CDCl_3$, 400 MHz) 2.29–2.55 (12 H, m, 2 × $CH_2CH_2CO_2$ and 2 × $CH_2CH_2CO_2$), 2.63–2.79 (6 H, m, 2 × $CH_2CH_2CO_2$ and 2 × pyrrole-CH_AH_B-lactam), 2.97 and 2.98 (each 1 H, d, *J* 15, pyrrole-CH_AH_B-lactam), 3.12 and 3.33 (each 1 H, ABq, *J* 15, CH_2CO_2), 3.40 (2 H, s, CH_2CO_2), 3.40 and 3.51 (each 1 H, ABq, *J* 17, CH_2CO_2), 3.62, 3.64, 3.65, 3.67, 3.73 and 3.78 (total 26 H, 8 × OMe and CH_2CO_2), 3.83 (2 H, ABq, pyrrole-CH_2-pyrrole), 6.31 (1 H, d, *J* 2, α-H), 7.14 (1 H, br s, lactam NH), 8.88, 8.96 and 10.07 (each 1 H, br s, NH) and 9.51 (1 H, s, CHO); *m/z* (FD) 980 (M^+, 100%).

4,19-*Methylene*-2,7,12,17-*tetrakis*(2-*methoxycarbonylethyl*)-3,8,13,18-*tetrakis*(*methoxycarbonylmethyl*)*bilan*-1(4H)-*one* **39** *and* 4,19′; 4′,19-*Bismethylenebis*[2,7,12,17-*tetrakis*(2-*methoxycarbonylethyl*)-3,8,13,18-*tetrakis*(*methoxycarbonylmethyl*)-*bilan*-1(4H)-*one*] **66**.—(Note: Since all the compounds in the following experiment are very sensitive to light and air, precautions to avoid their exposure should be taken at all times.) The crude tripyrrole lactam **37** (1.03 g, 1.05 mmol) was dissolved in dichloromethane (15 cm^3) and methanol (30 cm^3). Sodium borohydride (400 mg) was added in portions with stirring to the solution over 5 min. The mixture was stirred for a further 10 min and then added to water (200 cm^3) and extracted with dichloromethane (3 × 50 cm^3). The combined extracts were washed with brine (3 × 100 cm^3), dried and evaporated to give the crude α-free α-hydroxymethyl tripyrrole lactam **38**, which showed one major spot by TLC.

This material was immediately dissolved in dry acid-free dichloromethane (600 cm^3) and a solution of toluene-*p*-sulfonic acid hydrate (100 mg) in methanol (10 cm^3) was added dropwise with stirring to the solution until its colour changed from yellow to reddish (~0.2 cm^3 of the solution was required). After 1 min, triethylamine (3 drops) was added to the solution, whereupon the yellow colour returned. The solution was evaporated and the residue was purified on a silica gel column (5 cm × 2.5 cm diam.), eluting with ether–methanol–triethylamine (95:5:0.1) to give a mixture of the monomeric and dimeric macrocycles **39** and **66** and by-products (205 mg). This material was separated further by preparative TLC on Merck 0.25 mm thick silica gel GF_{254} plates (20 mg per 20 cm × 20 cm plate), eluting under argon with ether–methanol (19:1). The higher R_f band was the dimeric macrocycle **66** (11 mg, 1.1%). The lower R_f band was still a mixture and this was separated again on the same type of plates, eluting with methyl acetate–hexane (6:4). The main impurity (which gave a bright pink coloration with ceric sulfate) ran at higher R_f than the monomeric macrocycle **39** (which gave an orange coloration with ceric sulfate), obtained as a gum (23 mg, 2.3%). Later experiments showed that the use of 1.35 equiv. of toluene-*p*-sulfonic acid and a reaction time of 20 min improved the combined yield of **39** and **66** to 49%.

The dimeric macrocycle **66** crystallised from benzene–hexane, m.p. 169–172 °C [Found: *m/z* (FAB +ve ion) 1929.7997. $C_{96}H_{120}N_8O_{34}$ requires $M + H^+$, 1929.7985]; λ_{max}(EtOH)/nm end absorption only; δ_H($CDCl_3$, 400 MHz, 25 °C) 2.00–2.80 (36 H, v br m, 8 × $CH_2CH_2CO_2$ and 4 × pyrrole-CH_AH_B-lactam), 2.87 and 2.97 (each 2 H, d, *J* 15, 4 × pyrrole-CH_AH_B-lactam), 3.0–3.5 (12 H, v br) and 3.36 (4 H, s, 8 × CH_2CO_2), 3.57 (6 H, br s, 2 × OMe), 3.58, 3.59, 3.62, 3.65 and 3.80 (each 6 H, s, 2 × OMe), 3.60 and 3.89 (each 6 H, br s, 2 × OMe), 7.78 (2 H, br s, 2 × lactam NH), 8.8 (2 H, v br s, 2 × NH), 9.06 (2 H, br s, 2 × NH) and 9.45 (2 H, v br s, 2 × NH); δ_H($CDCl_3$, 400 MHz, 55 °C) 2.03–2.48 (24 H, m) and 2.71 (8 H, m, 8 × $CH_2CH_2CO_2$), 2.51 and 2.93 (each 2 H, ABq, *J* 15, pyrrole-CH_2-lactam), 2.60 and 3.00 (each 2 H, ABq, *J* 15, pyrrole-CH_2-lactam), 3.23, 3.26, 3.33 and 3.38 (each 4 H, s, 8 × CH_2CO_2), 3.45 and 3.74 (each 2 H, ABq, *J* 17, pyrrole-CH_2-pyrrole), 3.57, 3.59, 3.62, 3.65 and 3.82 (each 6 H, s, 10 × OMe), 3.60 (6 H, s, 2 × OMe), 3.87 (6 H, br s, 2 × OMe), 3.67 (4 H, close ABq, pyrrole-CH_2-pyrrole), 7.70 (2 H, br s, 2 × lactam NH) and 8.60, 9.01 and 9.19 (each 2 H, br s, 2 × NH); *m/z* (FD) 1928 (M^+, 100%) and 964 (9).

For the lower R_f, monomeric macrocycle **39** (Found: *m/z* 964.3946. $C_{48}H_{60}N_4O_{17}$ requires *M*, 964.3953); λ_{max}(EtOH)/nm end absorption only; ν_{max}($CHCl_3$)/cm^{-1} 3400, 3300, 2950, 1720s, 1670, 1595, 1435 and 1170; δ_H($CDCl_3$, 400 MHz, 25 °C, assignments by NOE and COSY) 2.23–2.42 (2 H, m, 7-$CH_2CH_2CO_2$), 2.45–2.55 (m, 2- and 12-$CH_2CH_2CO_2$, 7-$CH_2CH_2CO_2$, 17-$CH_2CH_2CO_2$), 2.57 and 2.78 (each 1 H, ABq, *J* 14, 4,19-CH_2), 2.70 (4 H, m, 2- and 12-$CH_2CH_2CO_2$), 2.73 and 2.77 (each 1 H, ABq, *J* 14, 5-CH_2), 3.20 (2 H, s, 3-CH_2CO_2), 3.42 (2 H, close ABq, 13-CH_2CO_2), 3.47 (2 H, close ABq, 8-CH_2CO_2), 3.52 (2 H, close ABq, 18-CH_2CO_2), 3.57, 3.58, 3.61, 3.61, 3.62, 3.66, 3.69 and 3.74 (each 3 H, s, OMe), 3.61 and 3.85 (each 1 H, ABq, *J* 16, 15-CH_2), 3.65 and 3.73 (each 1 H, ABq, *J* 16, 10-CH_2), 5.42 (1 H, br s, lactam NH), 7.95 (2 H, br s, 22- and 24-NH) and 9.13 (1 H, br s, 23-NH); δ_C($CDCl_3$,

100.57 MHz) 19.61, 19.74 and 19.86 (2 C) (4 × $CH_2CH_2CO_2$), 22.45 and 22.62 (10- and 15-CH_2), 29.95, 30.09, 30.30, 31.49, 31.55, 31.87, 32.24, 35.14, 35.24 and 35.65 (4 × $CH_2CH_2CO_2$, 4 × CH_2CO_2, 2 × pyrrole-CH_2-lactam), 51.54, 51.61, 51.65, 51.80, 51.99, 52.35, 52.48 and 52.62 (8 × OCH_3), 65.46 (spiro-C), 110.65, 110.81, 113.26, 116.29, 116.53, 119.46, 122.25, 123.36, 126.78, 127.98 (2 C) and 129.57 (12 × pyrrole C=C), 132.96 (lactam C-3), 153.72 (lactam C-4) and 169.91, 171.92, 172.64, 173.48, 173.52, 173.96, 174.20, 174.35 and 174.63 (8 × CO_2Me and CONH); *m/z* (FD) 964 (M^+, 100%).

Methyl 4-*Methoxycarbonylmethyl*-5-*methyl*-2-(2,2,2-*tribromoethoxycarbonyl*)*pyrrole*-3-*propionate* **43**.—The carboxylic acid **41**[18] (4.61 g, 16.3 mmol) was suspended in dry dichloromethane (40 cm^3) and oxalyl chloride (7 g, 4.7 cm^3, 55 mmol) was added dropwise to it, followed by dry dimethylformamide (3 drops). Vigorous effervesence ensued and the dark solution was stirred at room temperature under argon for 30 min and then evaporated to dryness. The residue was redissolved in dry dichloromethane (20 cm^3) and 2,2,2-tribromoethanol (5.1 g, 18 mmol) was added to the solution, followed by *N*,*N*-dimethylaniline (6 cm^3). The solution was stirred under argon for 30 min and then heated at reflux for 15 min. On cooling, the solution was diluted with dichloromethane (200 cm^3) and washed successively with hydrochloric acid (2 mol dm^{-3}; 3 × 100 cm^3), saturated aq. sodium hydrogen carbonate (3 × 100 cm^3) and water (100 cm^3). Flash chromatography (5 cm × 5 cm), eluting with ether–hexane (1:1), gave the *tribromoethyl ester* **43** as needles (7.86 g, 88%), m.p. 100–102 °C (from dichloromethane–hexane) (Found: C, 32.9; H, 3.25; N, 2.5. $C_{15}H_{18}Br_3NO_6$ requires C, 33.1; H, 3.3; N, 2.6); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3400, 2950, 1730s, 1690, 1435, 1160 and 1100; $\delta_H(CDCl_3$, 400 MHz) 2.20 (3 H, s, C-Me), 2.55 and 2.90 (each 2 H, m, CH_2CH_2), 3.42 (2 H, s, CH_2CO_2), 3.62 and 3.67 (each 3 H, s, OMe), 5.07 (2 H, s, CH_2CBr_3) and 9.00 (1 H, br s, NH); *m/z* (FD) 545, 547, 549 and 551 (1:3:3:1, M^+, 100%).

Methyl 5-*Acetoxymethyl*-4-*methoxycarbonylmethyl*-2-(2,2,2-*tribromoethyloxycarbonyl*)*pyrrole*-3-*propionate* **45**.—The tribromoethyl ester **43** (2.4 g, 4.38 mmol) was dissolved in dry dichloromethane (30 cm^3) and the solution cooled to 0 °C. Redistilled sulfuryl chloride (590 mg, 0.36 cm^3, 4.4 mmol) was added over 1 min with stirring to the solution which, after 15 min, was evaporated at room temperature. The residue was dissolved in acetic acid (30 cm^3) and sodium acetate (1.66 g, 20.2 mmol) was added to the solution. The mixture was stirred at 75 °C for 1 h and then added to water (150 cm^3) and extracted with dichloromethane (5 × 50 cm^3). The combined extracts were washed with water (2 × 100 cm^3), dried and evaporated to dryness. Flash chromatography (50 cm × 2 cm) of the residue, eluting with hexane–ether (1:3), gave the *acetoxymethyl pyrrole* **45** as an oil (2.39 g, 90%) (Found: M^+, 602.8718. $C_{17}H_{20}{}^{79}Br_3NO_8$ requires *M*, 602.8739); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3420, 2960, 1720s, 1690, 1250, 1160 and 1080; $\delta_H(CDCl_3$, 400 MHz) 2.04 (3 H, s, C-Me), 2.59 and 3.00 (each 2 H, m, CH_2CH_2), 3.55 (2 H, s, CH_2CO_2), 3.64 and 3.69 (each 3 H, s, OMe), 5.07 and 5.11 (each 2 H, s, CH_2CBr_3 and CH_2OAc) and 9.37 (1 H, br s, NH); *m/z* (FD) 603, 605, 607 and 609 (1:3:3:1, M^+,100%).

Methyl 2-tert-*Butoxycarbonyl*-5-*carboxy*-4-*methoxycarbonylmethylpyrrole*-3-*propionate* **47**.—A solution of methylpyrrole **40**[22] (22.5 g, 66.4 mmol) in dry tetrahydrofuran (150 cm^3) and dry ether (150 cm^3) was cooled in ice and *tert*-butyl hypochlorite (23.3 cm^3, 206 mmol) was added dropwise to it over 5 min. The mixture was stirred at room temperature under argon for 3 h and then added over 10 min to acetone–water (2:1; 1.2 dm^3), gently boiling in an open flask. After 15 min, the solution was cooled and the organic solvents evaporated under reduced pressure. The residual aqueous layer was extracted with chloroform (4 × 250 cm^3) and the combined extracts were evaporated to dryness. The residue was redissolved in ether (400 cm^3) and extracted with 10% aqueous sodium carbonate (4 × 150 cm^3). The combined extracts were then acidified with conc. hydrochloric acid to pH 1 and the precipitated solid was extracted with chloroform (4 × 200 cm^3). The combined extracts were dried and evaporated to dryness to give the *pyrrole acid* **47** (21.8 g, 89%), m.p. 161–162 °C (from dichloromethane–hexane) (Found: C, 54.8; H, 6.3; N, 3.6. $C_{17}H_{23}NO_8$ requires C, 55.2; H, 6.3; N, 3.8%); ν_{max}-$(CH_2Cl_2)/cm^{-1}$ 3550–2400br, 2950, 1720s, 1690s, 1360, 1170 and 1040; $\delta_H(CDCl_3$, 400 MHz) 1.57 (9 H, s, Bu^t), 2.56 and 2.98 (each 2 H, t, *J* 8, CH_2CH_2), 3.65 and 3.70 (each 3 H, s, OMe), 3.89 (2 H, s, CH_2CO_2) and 9.60 (1 H, br s, pyrrole NH); *m/z* (FD) 369 (M^+, 100%).

Dimethyl 1-tert-*Butoxycarbonyl*-3,7-*bis*(*methoxycarbonylmethyl*)-9-(2,2,2-*tribromoethoxycarbonyl*)-5,10-*dihydrodipyrrin*-2,8-*dipropionate* **49**.—The α-free pyrrole **48**[22] (1.24 g, 3.81 mmol) and the acetoxymethyl pyrrole **45** (2.3 g, 3.81 mmol) were dissolved in dry dichloromethane (30 cm^3) and toluene-*p*-sulfonic acid (100 mg, 0.53 mmol) was added to the solution. The mixture was stirred under argon for 2 h and then washed with 10% aqueous sodium carbonate (2 × 20 cm^3), dried and evaporated to dryness. Flash chromatography of the residue, eluting with ether–hexane (9:1), gave the dihydrodipyrrin **49** as a foam (2.82 g, 85.5%) (Found: C, 42.6; H, 4.45; N, 3.05. $C_{31}H_{39}Br_3N_2O_{12}$ requires C, 42.7; H, 4.5; N, 3.2%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3300, 2960, 1710s, 1680, 1330, 1120 and 1050; $\delta_H(CDCl_3$, 250 MHz), 1.52 (9 H, s, Bu^t), 2.52, 2.59, 2.99 and 3.11 (each 2 H, t, *J* 8, 4 × CH_2CH_2), 3.58 and 3.60 (each 2 H, s, 2 × acetate CH_2), 3.63, 3.63, 3.78 and 3.79 (each 3 H, s, 4 × OMe), 3.83 (2 H, s, pyrr-CH_2-pyrr), 5.08 (2 H, s, CH_2CBr_3) and 10.13 and 10.42 (each 1 H, br s, NH); *m/z* (FD) 868, 870, 872 and 874 (1:3:3:1, M^+, 100%).

Dimethyl 1-*Carboxy*-3,7-*bis*(*methoxycarbonylmethyl*)-9-(2,2,2-*tribromoethoxycarbonyl*)-5,10-*dihydrodipyrrin*-2,8-*dipropionate* **50**.—The diester **49** (1.43 g, 1.65 mmol) was dissolved in dry dichloromethane (30 cm^3) and the solution stirred at 0 °C under argon while stannic chloride (220 mm^3, 1.81 mmol) was added to it. The mixture was stirred at 0 °C for 90 min after which 10% aqueous sodium acetate (20 cm^3) was added to it; the two-phase mixture so formed was then stirred vigorously for 5 min. The organic layer was removed and the aqueous layer extracted with chloroform (5 × 20 cm^3). The combined organic layers were washed with brine (50 cm^3), dried and evaporated to give the crystalline *pyrrole acid* **50** (1.14 g, 86%), m.p. 116–118 °C (from dichloromethane–ether–hexane) (Found: C, 39.7; H, 3.9; N, 3.4; $C_{27}H_{31}Br_3N_2O_{12}$ requires C, 39.8; H, 3.8; N, 3.45%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3300, 2950, 1720s, 1700, 1660, 1430, 1250 and 1170; $\delta_H(CDCl_3$, 250 MHz) 2.56, 2.60, 3.01 and 3.11 (each 2 H, t, *J* 8, 2 × CH_2CH_2), 3.58 and 3.61 (each 2 H, s, CH_2CO_2), 3.62, 3.62, 3.77 and 3.77 (each 3 H, s, OMe), 3.88 (2 H, s, pyrr-CH_2-pyrr), 5.07 (2 H, s, CH_2CBr_3) and 10.34 and 10.59 (each 1 H, br s, NH); *m/z* (FD) 812, 814, 816 and 818 (1:3:3:1, M^+, 100%).

Dimethyl 1-*Iodo*-3,7-*bis*(*methoxycarbonylmethyl*)-9-(2,2,2-*tribromoethoxycarbonyl*)-5,10-*dihydrodipyrrin*-2,8-*dipropionate* **51**.—The acid **50** (1.08 g, 1.33 mmol) was stirred vigorously with dichloromethane (15 cm^3) and water (15 cm^3) containing sodium hydrogen carbonate (0.7 g) whilst a solution of iodine (0.1 mol dm^{-3}) in aqueous potassium iodide (0.2 mol dm^{-3}; 15 cm^3) was added to it over 3 min. The resultant mixture was stirred for a further 20 min after which sodium metabisulfite was added until excess of iodine was destroyed. The organic layer

was separated and the aqueous layer was extracted with dichloromethane (3 × 10 cm^3). The combined organic layers were dried and evaporated to dryness. Flash chromatography of the residue, eluting with hexane–ether (1:4), gave the *iododipyrromethane* **51** as a gum (0.99 g, 83%) (Found C, 35.0; H, 3.4; N, 3.0. $C_{26}H_{30}Br_3IN_2O_{10}$ requires C, 34.8; H, 3.4; N, 3.1%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3320, 2920, 1730s, 1330, 1160 and 1075; $\delta_H(CDCl_3$, 250 MHz) 2.41, 2.59, 2.67 and 3.11 (each 2 H, t, *J* 8, 2 × CH_2CH_2), 3.55 and 3.60 (each 2 H, s, CH_2CO_2), 3.63, 3.66, 3.78 and 3.79 (each 3 H, s, OMe), 3.82 (2 H, s, pyrr-CH_2-pyrr), 5.08 (2 H, s, CH_2CBr_3) and 9.62 and 10.32 (each 1 H, br s, NH); *m/z* (FD) 894, 896, 898 and 900 (1:3:3:1, M^+, 100%).

4-[5-*Benzyloxycarbonyl*-3-(2-*methoxycarbonylethyl*)-4-(*methoxycarbonylmethyl*)*pyrrol*-2-*ylmethyl*]-2,8-*bis*(2-*methoxycarbonylethyl*)-3,7-*bis*(*methoxycarbonylmethyl*)-9-(2,2,2-*tribromoethoxycarbonyl*)-4,5-*dihydrodipyrrin*-1(10H)-*one* **53**.—The iododihydrodipyrrin **51** (1 g, 1.11 mmol) and the acetoxymethylpyrrole[20] **52** (0.5 g, 1.16 mmol) were dissolved in dry dichloromethane (10 cm^3) and the solution was stirred at 0 °C under argon whilst stannic chloride (120 mm^3, 1 mmol) was added to it. After 20 min, 10% aqueous sodium carbonate (20 cm^3) was added to the mixture which was then stirred for a further 20 min. The organic layer was decanted and the aqueous phase was extracted with chloroform (4 × 20 cm^3). The combined organic phases were washed with water, dried and evaporated. The dark oily residue was usually hydrolysed directly but, for characterisation, the halogenopyrrolenine could be purified on a column of silica gel PF_{254}, eluting with ether–hexane (1:1) and then ether; $\delta_H(CDCl_3$, 400 MHz) 2.31 and 2.83 (m, 5 × propionate-CH_2 and 2 × pyrrole-α-CH_AH_B), 3.03 (t, *J* 8 Hz, propionate CH_2), 3.17–3.20 (m, 2 × pyrrole-α-CH_AH_B), 3.05 and 3.45 (ABq, *J* 16 Hz, CH_2CO_2), 3.57, 3.59, 3.60, 3.61, 3.62 and 3.78 (each s, 6 × OMe, 2 × CH_2CO_2), 5.12 and 5.01 (ABq, *J* 12, CH_2Br_3), 5.21 and 5.28 (ABq, *J* 12, CH_2Ph) 7.28–7.40 (m, Ph), 9.80 (s, pyrrole NH) and 10.09 (s, pyrrole NH); $\delta_C(CDCl_3$, 100 MHz); 19.06, 20.09 and 20.30 (3 × $CH_2CH_2CO_2$), 29.32, 29.70, 30.26, 30.51, 31.44, 31.57, 34.72, 34.87 (3 × $CH_2CH_2CO_2$, 3 × CH_2CO_2, 2 × pyrrole-α-CH_2), 51.43, 51.55, 51.78, 51.94 and 53.12 (CO_2CH_3), 65.62 (CH_2Ph), 76.54 and 83.42 (quat. C), 116.13, 116.39, 116.35, 122.08, 122.37, 127.67, 130.51, 131.24, 136.17 and 138.85 (8 × pyrrole C, phenyl C, C-2), 128.04, 128.18 and 128.42 (phenyl CH) 158.14, 158.27, 160.14 and 161.67 (2 × pyrrole-CO_2, C-1 and C-3), 170.65, 171.64, 172.14, 172.48, 173.41 and 173.50 (CO_2CH_3); *m/z* (FD) 1173, 1175, 1177, 1179 and 1181 (M^+ for chloropyrrolenine, 80% of total ions).

Toluene-*p*-sulfonic acid hydrate (360 mg, 1.89 mmol) and silver acetate (124 mg, 0.75 mmol) were added to a solution of the crude halogenopyrrolenine in tetrahydrofuran (18 cm^3) and water (1.8 cm^3). The mixture was stirred under argon for 10 h and then diluted with water (100 cm^3) and extracted with dichloromethane (4 × 20 cm^3). The combined extracts were washed with 5% aqueous sodium hydrogen carbonate (20 cm^3) followed by water (20 cm^3) and then dried and evaporated. The residual oil was purified by flash chromatography, eluting with ether and then 1% methanol in ether, to give the *lactam* **53** as a gum (321 mg, 25%) (Found: C, 48.0; H, 4.5; N, 3.7; $C_{46}H_{52}Br_3N_3O_{17}$ requires C, 47.7; H, 4.5; N, 3.6%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3300, 2930, 1710, 1430 and 900; $\delta_H(CDCl_3$, 400 MHz) 2.40–2.55 and 2.67–2.71 (12 H, m, 3 × CH_2CH_2), 2.74 and 3.04 (2 H, ABq, *J* 15, pyrrole-α-CH_2), 2.85 and 3.14 (2 H, ABq, *J* 15, pyrrole-α-CH_2), 3.16 and 3.49 (2 H, ABq, *J* 16, CH_2CO_2), 3.42 and 3.78 (2 H, ABq, *J* 17, CH_2CO_2), 3.49 and 3.81 (2 H, ABq, *J* 16, CH_2CO_2), 3.55, 3.57, 3.58, 3.60, 3.62 and 3.80 (each 3 H, s, 6 × OMe), 5.02 and 5.08 (2 H, ABq, *J* 17, CH_2CBr_3), 5.15 and 5.25 (2 H, ABq, *J* 12, CH_2Ph), 7.30–7.37 (5 H, m, Ph), 7.61 (1 H, br s, lactam NH), 9.43 and 10.32 (each 1 H, br s, 2 × NH); $\delta_C(CDCl_3$, 100 MHz) 19.31, 19.82, 20.43 (3 × $CH_2CH_2CO_2$), 29.25, 30.44, 30.61, 30.95, 32.74, 34.71, 34.97, 35.95 (3 × $CH_2CH_2CO_2$, 3 × CH_2CO_2, 2 × pyrrole-α-CH_2), 51.30, 51.44, 51.63, 52.20, 53.15 (CO_2CH_3), 65.76 (C-4), 65.99 (CH_2Ph), 116.19, 117.02, 119.49, 122.30, 122.48, 127.70, 128.06, 128.18, 128.26, 128.41, 130.21, 131.04, 136.09, 138.28 and 148.98 (pyrrole and lactam C=C), 158.66 and 160.28 (pyrrole-CO_2) and 171.57, 171.63, 171.86, 173.01, 173.24, 173.40 and 173.52 (6 × CO_2 and CONH); *m/z* (FD): 1155, 1157, 1159 and 1161 (*ca.* 1:3:3:1, M^+ 100%).

4-[5-*Benzyloxycarbonyl*-3-(2-*methoxycarbonylethyl*)-4-(*methoxycarbonylmethyl*)*pyrrol*-2-*ylmethyl*]-2,8-*bis*(2-*methoxycarbonylethyl*)-3,7-*bis*(*methoxycarbonylmethyl*)-4,5-*dihydrodipyrrin*-1(10H)-*one* **55**.—The lactam tribromoethyl ester **53** (3 g, 2.57 mmol) was dissolved in acetic acid (30 cm^3) and zinc dust (6 g) was added to the solution. After the mixture had been stirred for 20 min, the zinc was filtered off through Celite and the filtrate was added to water (150 cm^3) and extracted with dichloromethane (4 × 50 cm^3). The combined extracts were washed with water, dried and evaporated to give the crude acid **54**, which was decarboxylated without further purification.

A solution of the acid in redistilled trifluoroacetic acid (30 cm^3) was stirred for 7 h under argon at room temperature. After this the trifluoroacetic acid was evaporated under reduced pressure at near room temperature and the residue was dissolved in dichloromethane (200 cm^3) and the solution washed with saturated aqueous sodium hydrogen carbonate (2 × 50 cm^3), dried and evaporated. Purification of the residue on a column of silica gel PF_{254}, eluting with ether and then ether–methanol (19:1), and, finally, recrystallisation from methanol gave the α-*free pyrrole lactam* **55** (1.42 g, 67.6% from the ester **53**) (Found: C, 60.8, H, 6.0; N, 4.8. $C_{43}H_{51}N_3O_{15}$ requires C, 60.8; H, 6.05; N, 4.9%); $\delta_H(CDCl_3$, 400 MHz) 2.39–2.50 and 2.63–2.68 (12 H, m, 3 × CH_2CH_2), 2.74 and 3.11 (each 1 H, ABq, *J* 15, pyrrole α-CH_2), 2.81 and 2.99 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.47 and 3.55 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.57, 3.59, 3.60, 3.63, 3.64 and 3.77 (each 3 H, s, 6 × OMe), 5.17 and 5.27 (each 1 H, ABq, *J* 12, CH_2Ph), 6.34 (1 H, d, *J* 3, α-CH), 7.28–7.38 (5 H, m, Ph), 7.40 (1 H, br s, lactam NH) and 8.91 and 9.54 (each 1 H, br s, NH), signals from 2 × CH_2 were obscured; $\delta_C(CDCl_3)$ 19.14, 19.62 and 20.61 (3 × $CH_2CH_2CO_2$), 29.80, 30.43, 30.59, 30.74, 31.62, 34.69 and 35.68 (3 × CH_2CO_2, 2 × pyrrole α-CH_2, 3 × $CH_2CH_2CO_2$), 51.45, 51.54, 51.75, 52.04, 52.95 (CO_2CH_3), 65.52 and 65.87 (C-4 and CH_2Ph), 112.25, 114.61, 118.99, 120.87, 121.87, 122.28, 123.25, 127.98, 128.19, 128.33, 136.00, 137.21 and 149.83 (pyrrole, lactam and phenyl C=C), 160.48 (α-CO_2), 171.86, 172.38, 173.29, 173.40, 173.51 and 173.62 (CO_2Me and CONH); *m/z* (FD) 849 (M^+, 100%).

4-[5-*Benzyloxycarbonyl*-3-(2-*methoxycarbonylethyl*)-4-(*methoxycarbonylmethyl*)*pyrrol*-2-*ylmethyl*]-14-*formyl*-2,8,13-*tris*(2-*methoxycarbonylethyl*)-3,7,12-*tris*(*methoxycarbonylmethyl*)-4,5,10,16-*tetrahydrotripyrrin*-2(15H)-*one* **56**.—The α-free pyrrole lactam **55** (577 mg, 0.68 mmol) and acetoxymethyl aldehyde **14**[3,11] (300 mg, 1.1 equiv.) were dissolved in dry dichloromethane (30 cm^3) and dry tetrahydrofuran (400 cm^3). Stannic chloride (170 cm^3, 2 equiv.) was added to the solution which was then stirred in the dark under argon for 18 h. Methanol (5 cm^3) was then added to it followed by 10% aqueous sodium carbonate (25 cm^3). After the mixture had been gently stirred for 5 min, the organic layer was decanted and the aqueous layer was diluted with water (10 cm^3) and extracted with dichloromethane (3 × 60 cm^3). The combined organic layers were dried and evaporated. Purification on a column of silica gel PF_{254}, eluting with ether then ether–methanol (19:1), gave the *aldehyde* **56** as a foam (586 mg, 76%) (Found: M^+,

1114.4243. $C_{56}H_{66}N_4O_{20}$ requires M, 1114.4270); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3300, 2950, 1720, 1640, 1430 and 1250; $\delta_H(CDCl_3$, 400 MHz) 2.16–2.57 (13 H, m, 6 × CH_2CH_2 and pyrrole α-CH_AH_B), 2.66 and 2.97 (each 1 H, ABq, J 15, pyrrole α-CH_2), 2.70 (2 H, t, J 7, CH_2CH_2), 2.96 (1 H, d, J 15, pyrrole α-CH_AH_B), 3.32 and 3.38 (each 1 H, ABq, J 16, CH_2CO_2), 3.42 (1 H, d, J 17, $CH_AH_BCO_2$), 3.48, 3.55, 3.58, 3.62, 3.63, 3.65, 3.71 and 3.75 (each 3 H, s, 8 × OMe and $CH_AH_BCO_2$), 3.74 (2 H, s, CH_2CO_2), 3.79–3.89 (4 H, m, CH_2CO_2 and pyrr-CH_2-pyrr), 5.16 and 5.26 (each 1 H, ABq, J 12, CH_2Ph), 7.02 (1 H, br s, lactam NH), 7.26–7.38 (5 H, m, Ph), 9.43, 9.73 and 10.08 (each 1 H, br s, 3 × NH and 9.55 (1 H, s, CHO); $\delta_C(CDCl_3$, 100 MHz) 18.96, 19.78 and 22.11 ($CH_2CH_2CO_2$), 29.17, 29.75, 30.39, 30.99, 33.16, 34.37, 34.52 and 35.88 (CH_2CO_2, pyrrole α-CH_2, $CH_2CH_2CO_2$), 51.70, 52.19, 52.50, 52.53 and 53.02 (CO_2CH_3), 63.58 and 65.53 (C-4, CH_2Ph), 112.83, 114.48, 116.79, 118.85, 121.72, 122.31, 122.84, 124.95, 127.76, 128.08, 128.23, 128.30, 128.51, 130.03, 131.21, 136.29, 137.05 and 149.68 (pyrrole, lactam and phenyl C=C), 160.25 (α-CO_2) and 171.49, 172.04, 173.28, 173.51, 174.55, 174.86, 175.28 and 175.85 (CO_2Me, CHO and CONH); m/z (FD) 1114 (M^+, 100%).

4,19-*Methylene*-2,7,12,17-*tetrakis*(2-*methoxycarbonylethyl*)-3,8,13,18-*tetrakis*(*methoxycarbonylmethyl*)*bilan*-1(4H)-*one* **39** *and* 4,19′; 4′,19-*Bismethylenebis*[2,7,12,17-*tetrakis*(2-*methoxycarbonylethyl*)-3,8,13,18-*tetrakis*(*methoxycarbonylmethyl*)-*bilan*-1(4H)-*one*] **66**: *Second method.*—A solution of the benzyl ester **56** (422 mg, 0.37 mmol) in methanol (25 cm^3) was mixed with sodium carbonate (180 mg) and 10% palladium-on-charcoal (70 mg) and hydrogenated at room temperature and atmospheric pressure for 40 min. The catalyst was filtered off and the filtrate was diluted with water (20 cm^3), acidified with glacial acetic acid and extracted with dichloromethane (3 × 20 cm^3). The combined extracts were washed with water (30 cm^3), dried and evaporated to dryness to give the crude carboxylic acid **57**.

A solution of the carboxylic acid in dichloromethane (25 cm^3) was stirred vigorously with 5% aqueous sodium hydrogen carbonate (25 cm^3) at 0 °C whilst a solution of iodine (0.1 mol dm^{-3}) in aqueous potassium iodide (0.2 mol dm^{-3}; 4.5 cm^3) was added dropwise over 2 min. After the mixture had been stirred for 40 min at 0 °C, sodium metabisulfite was added to it until excess of iodine was destroyed. The organic layer was decanted and the aqueous layer extracted with dichloromethane (2 × 30 cm^3). The combined organic phases were dried and evaporated to give the crude iodopyrrole **58** as a gum.

A solution of the iodopyrrole in methanol (20 cm^3) was mixed with sodium acetate (230 mg) and Adams' catalyst (43 mg) and hydrogenated for 45 min at room temperature. The catalyst was filtered off and the filtrate was diluted with water (20 cm^3) and extracted with dichloromethane (3 × 10 cm^3). The combined extracts were dried and evaporated to dryness. Flash chromatography of the residue, eluting with ether–methanol-triethylamine (95:1:0.1), gave the α-free pyrrole **59** as a gum (301 mg, 81%). It was usually used immediately for the cyclisation, although it could be stored for a few days under argon at −20 °C.

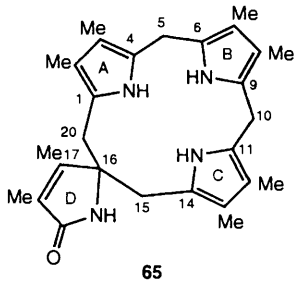
Note: Since all the compounds in the following experiment are very sensitive to light and air, precautions to avoid their exposure should be taken at all times. A solution of the α-free aldehyde (300 mg, 0.31 mmol) in dry, acid-free dichloromethane (4.5 cm^3) and methanol (10 cm^3) under argon was treated with sodium borohydride (120 mg) in portions over 2 min and the mixture stirred for a further 10 min. Water (10 cm^3) was added to the mixture which was then extracted with dichloromethane (3 × 10 cm^3), dried and evaporated to dryness to give the crude hydroxymethyl pyrrole **60**.

The hydroxymethyl pyrrole was immediately dissolved in dry, acid-free dichloromethane (160 cm^3) and a solution of toluene-*p*-sulfonic acid hydrate (80 mg) in methanol (2 cm^3) was added dropwise to it over 1 min. The solution was stirred at room temperature under argon for 20 min, after which triethylamine (3 drops) was added to it until the red colour turned yellow; the solution was then evaporated to dryness. Flash chromatography of the residue, eluting with ether–methanol–triethylamine (97:3:0.1) gave a mixture of the macrocycles **39** and **66** as a foam (140 mg, 47%). This mixture was dissolved in dry degassed benzene (0.7 cm^3) and filtered. A layer of dry degassed hexane (1.4 cm^3) was carefully added on top of the filtrate in a 5 cm^3 Craig tube. The tube was flushed with argon, stoppered and kept at 4 °C until the two layers had mixed and a precipitate formed (typically 48 h). The mixture was further cooled to −20 °C for 12 h and then filtered. The precipitate was essentially pure dimer **66**, whereas the filtrate generally gave essentially pure monomer **39**. Both compounds had the same spectral characteristics as reported above.

In a later experiment a solution of the hydroxymethyl pyrrole **60** from α-free pyrrole **59** (33 mg, 0.034 mmol) in dry acid-free dichloromethane (54 cm^3) was treated with a solution of toluene-*p*-sulfonic acid hydrate (8.9 mg) in methanol (2.6 cm^3) under argon for 20 min. Work-up as above and separation by PLC, eluting with dichloromethane–methanol (95:5), gave the monomeric macrocycle **39** (19.2 mg, 59%) and the dimeric macrocycle **66** (4 mg, 12%).

4-[5-*Benzyloxycarbonyl*-3-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-9-*formyl*-2,8-*bis*(2-*methoxycarbonylethyl*)-3,7-*bis*(*methoxycarbonylmethyl*)-4,5-*dihydrodipyrrin*-1(10H)-*one* **61**.—Zinc dust (1.7 g) was added to a solution of the tribromoethyl ester **53** (747 mg, 0.645 mmol) in glacial acetic acid (10 cm^3) and the mixture was stirred for 20 min; the zinc was then filtered off. The filtrate was diluted with water (30 cm^3) and extracted with dichloromethane (4 × 30 cm^3) and the combined extracts were dried and evaporated to dryness to give the crude carboxylic acid **54**.

The crude acid was dissolved in redistilled trifluoroacetic acid and stirred under argon for 90 min. The solution was then cooled in ice for 10 min and distilled trimethyl orthoformate (15 cm^3) was added to it. After a further 40 min, the solution was poured into saturated aqueous sodium carbonate (120 cm^3) and extracted with dichloromethane (4 × 50 cm^3). The combined extracts were dried and evaporated to dryness. Flash chromatography (30 × 2 cm) of the residue, eluting with ether–methanol (93:7), gave the *formyl lactam* **61** as a foam which crystallised from methanol–water (275 mg, 49%), m.p. 151–153 °C (Found: C, 60.0; H, 5.9; N, 4.8; $C_{44}H_{51}N_3O_{16}$ requires C, 60.2; H, 5.9; N, 4.8%); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3300, 2950, 1720, 1640, 1430, 1250 and 1160; $\delta_H(CDCl_3$, 400 MHz) 2.38–2.46 (6 H, m, 3 × CH_2CH_2), 2.50 and 2.65 (each 2 H, t, J 8, CH_2CH_2), 2.85 and 3.19 (each 1 H, ABq, J 15, pyrrole α-CH_2), 2.92 and 3.16 (each 1 H, ABq, J 15, pyrrole α-CH_2), 2.93 (2 H, m, CH_2CH_2), 3.28 and 3.46 (each 1 H, ABq, J 16, CH_2CO_2), 3.53, 3.58, 3.58, 3.60, 3.60 and 3.78 (each 3 H, s, 6 × OMe and CH_2CO_2), 3.74 (2 H, s, CH_2CO_2), 5.12 and 5.22 (each 1 H, ABq, J 12, CH_2Ph), 7.27–7.34 (5 H, m, Ph), 8.17 (1 H, br s, lactam NH), 9.41 (1 H, s, CHO) and 9.85 and 10.70 (each 1 H, br s, pyrrole NH); $\delta_C(CDCl_3$, 100 MHz) 19.23, 19.29, 19.72 (3 × $CH_2CH_2CO_2$), 29.38, 30.66, 30.72, 30.95, 31.55, 32.10, 34.73, 35.71 (3 × CH_2CO_2, 2 × pyrr–CH_2–pyrr, 3 × $CH_2CH_2CO_2$), 51.44, 51.46, 51.58, 51.60, 52.16 and 52.98 (6 × OCH_3), 65.69 (C-4), 66.28 (CH_2Ph), 127.77, 127.98 and 128.18 (phenyl CH) 116.22, 119.50, 122.32, 122.40, 132.76, 132.86, 136.17, 137.77 and 149.26 (pyrrole C=C and lactam C=C), 160.45 (pyrrole α-CO_2), 170.99, 171.69, 171.97, 172.40, 172.76, 173.32 and 173.51 (6 × CO_2Me and CONH) and 177.43 (CHO); m/z (FD) 877 (M^+, 100%).



65

Fig. 4 Numbering of the atoms of the spiro-lactam structure **65** used for molecular mechanics calculations

9-*Formyl*-4-[3-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-2,8-*bis*(2-*methoxycarbonylethyl*)-3,7-*bis*(*methoxycarbonylmethyl*)-4,5-*dihydrodipyrrin*-1(10H)-*one* **62**.—A solution of the benzyl ester **61** (90 mg, 0.103 mmol) in methanol (10 cm^3) was mixed with sodium carbonate (50 mg) and 10% palladium-on-charcoal (10 mg) and hydrogenated for 40 min. It was then filtered, diluted with water (20 cm^3), acidified with glacial acetic acid and extracted with dichloromethane (4 × 15 cm^3). The combined extracts were washed with water (20 cm^3), dried and evaporated to dryness to give the crude carboxylic acid.

A solution of the crude acid in dichloromethane (5 cm^3) was stirred vigorously with 5% aqueous sodium carbonate (5 cm^3) in an ice-bath while a solution of iodine (0.1 mol dm^{-3}) in aqueous potassium iodide (0.2 mol dm^{-3}; 1.13 cm^3) was added dropwise to it over 2 min. After 40 min at 0 °C, sodium metabisulfite was added to the mixture until excess of iodine was destroyed. The organic layer was decanted and the aqueous layer was extracted with dichloromethane (3 × 10 cm^3). The combined organic layers were washed with brine (20 cm^3), dried and evaporated to dryness to give the crude iodopyrrole.

The crude iodopyrrole was dissolved in methanol (10 cm^3) and sodium acetate (60 mg) and Adams catalyst (10 mg) were added to the mixture which was then hydrogenated for 45 min. The catalyst was filtered off and the filtrate diluted with water (15 cm^3) and extracted with dichloromethane (4 × 15 cm^3). The combined extracts were dried and evaporated to dryness. Flash chromatography (15 × 2 cm), eluting with ether–methanol (95:5), gave the α-*free lactam* **62** as a gum (62 mg, 81%) (Found: *m/z* 743.2896. $C_{36}H_{45}N_3O_{14}$ requires 743.2902); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3330, 2950, 1720s, 1680, 1640, 1440, 1170 and 1000; $\delta_H(CDCl_3$, 400 MHz) 2.41–2.51 (8 H, m, 4 × CH_2CH_2), 2.69 (2 H, t, *J* 7, CH_2CH_2), 2.72 and 3.05 (each 1 H, ABq, *J* 15, pyrrole α-CH_2), 2.83 and 3.06 (each 1 H, ABq, *J* 15, pyrrole α-CH_2), 2.94 (2 H, t, *J* 7, CH_2CH_2), 3.18 and 3.45 (each 1 H, ABq, *J* 16, CH_2CO_2), 3.40 (2 H, s, CH_2CO_2), 3.43 and 3.63 (2 H, ABq, *J* 17, CH_2CO_2), 3.62, 3.63, 3.64, 3.66, 3.69 and 3.84 (each 3 H, s, 6 × OMe), 6.49 (1 H, d, *J* 2, α-H), 7.52 (1 H, br s, lactam NH), 8.51 (1 H, br s, pyrrole NH), 9.50 (1 H, s, CHO) and 10.37 (1 H, br s, pyrrole NH); $\delta_C(CDCl_3$, 100 MHz) 19.18, 19.57 and 19.87 (3 × $CH_2CH_2CO_2$), 29.06, 29.68, 30.51, 30.72, 30.83, 31.34, 32.88 and 35.04 (3 × CH_2CO_2, 2 × pyrr-CH_2-pyrr, 3 × $CH_2CH_2CO_2$), 51.68, 51.85, 52.53 and 53.31 (OCH_3), 66.30 (C-4), 112.36, 114.02, 115.80, 116.73, 118.67, 122.34, 128.58 and 149.57 (pyrrole C=C and lactam C=C), 171.79, 172.17, 172.69, 173.14, 173.74 and 174.03 (CO_2Me and CONH) and 177.24 (CHO); *m/z* (FD) 743 (M^+, 100%).

4-[5-*Formyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-14-*formyl*-2,7,12-*tris*(2-*methoxycarbonylmethyl*)-3,8,13-*tris*(*methoxycarbonylmethyl*)-4,5,10,17-*tetrahydrotripyrrin*-1(15H)-*one* **63**.—The α-free lactam **62** (180 mg, 0.24 mmol) and the acetoxymethyl aldehyde **33** (79 mg, 0.24 mmol) were dissolved in dry dichloromethane (5 cm^3) and dry THF (70 mm^3). Stannic chloride (56 mm^3, 0.24 mmol) was added to the solution which was then stirred in the dark under argon for 18 h. Methanol (1 cm^3) was then added to the mixture followed by 10% aqueous sodium carbonate (5 cm^3) and stirring was continued at room temperature for 10 min. The mixture was diluted with dichloromethane (5 cm^3) and water (5 cm^3) and the organic layer was separated; the aqueous layer was then extracted with dichloromethane (3 × 10 cm^3). The combined organic layers were washed with water (20 cm^3), dried and evaporated to dryness. PLC of the residue, eluting with 20% methanol in ether, gave the *dialdehyde* **63** as a gum (168 mg, 70%) (Found: *m/z* 1008.3825. $C_{49}H_{60}N_4O_{19}$ requires 1008.3852); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3350, 2980, 1725s, 1690, 1640, 1430, 1220 and 1040; $\delta_H(CDCl_3$, 400 MHz) 2.30–2.35 and 2.47–2.50 (8 H, m, 4 × CH_2CH_2), 2.40, 2.65, 2.74 and 2.92 (each 2 H, t, *J* 7, CH_2CH_2), 2.67 and 3.00 (each 1 H, ABq, *J* 15, pyrr-CH_2-lactam), 2.80 and 3.17 (each 1 H, ABq, *J* 15, pyrr-CH_2-lactam), 3.17 (1 H, d, *J* 16, acetate $CH_AH_BCO_2$), 3.40 (2 H, s, CH_2CO_2), 3.59, 3.60, 3.61, 3.63, 3.64, 3.66, 3.71 and 3.80 (each 3 H, s, 8 × OMe, $CH_AH_BCO_2$ and 2 × CH_2CO_2), 3.80 and 3.85 (2 H, ABq, *J* 16, pyrr-CH_2-pyrr), 7.30 (1 H, br s, lactam NH), 9.08, 10.21 and 10.35 (each 1 H, br s, pyrrole NH) and 9.45 and 9.46 (each 1 H, s, CHO); $\delta_C(CDCl_3$, 100 MHz) 18.79, 19.23 and 19.85 ($CH_2CH_2CO_2$), 29.45, 29.85, 30.27, 30.70, 31.00, 34.43, 35.07 and 35.81 (CH_2CO_2, pyrrole α-CH_2 and $CH_2CH_2CO_2$), 51.55, 51.60, 51.72, 52.33, 52.72 and 53.20 (OCH_3), 66.34 (C-4), 120.64, 121.66, 128.71, 129.05, 132.35, 132.65, 136.40, 137.04, 137.76, 149.29, 156.9, 157.2 (pyrrole and lactam C=C), 171.23, 171.63, 171.77, 172.77, 172.81, 173.38, 173.47 and 173.84 (CO_2Me and CONH) and 177.23 and 177.45 (CHO); *m/z* (FD) 1008 (M^+, 100%).

Molecular Mechanics Calculations.—Molecular mechanics calculations were carried out using the program MacroModel [12] version 2.5 running on a MicroVax III computer with an Evans and Sutherland PS390 display. Energy minimisations were performed using the MM2 force-field with the dielectric constant set at 5. Initially the minimal tripyrrolic macrocycle was constructed, *i.e.* structure **69** with all side-chains removed. Starting conformations were generated by varying the dihedral angles about 5 of the single bonds from 0 to 180° (or −180° as appropriate) in 30° steps. 66 Conformations met the standard ring-closure conditions. Minimisation of these gave 6 different minimum-energy conformations. The methyl groups and the spiro-lactam ring (with the ring both possible ways round) were added to each of these 6 conformations to give 12 conformations of structure **65**, which were all minimised. In all cases it was found that the conformations having the spiro-atom (C-16) puckered in such a way as to make the NH of the lactam point inwards (as in structures **65a–d**) were considerably more stable (by up to 40 kJ mol^{-1}) than the corresponding conformations having the spiro-atom puckered in the opposite direction, in which the 17-methyl group on the lactam points inwards and experiences steric compression with pyrrole rings A and C (see Fig. 4 for the numbering of atoms). In all the 12 conformations, the pyrrole rings A–C could each be classified as having their NH bonds pointing up or down (as viewed in Scheme 7). All possible combinations were represented except conformations having all three rings pointing up or all three pointing down. These conformations were both constructed but found to be unstable, each minimising to one of the 12 previously found conformations. The four lowest minimum-energy conformations were **65a** (166.36 kJ mol^{-1}), **65b** (174.83 kJ mol^{-1}), **65d** (178.99 kJ mol^{-1}) and **65c** (183.10 kJ mol^{-1}), shown in Scheme 7.

To determine the energies of the transition states for the rotations of the pyrrole rings that interconvert conformations **65a–d**, the dihedral angle about certain bonds were constrained

to adopt angles intermediate between that found in one conformation and that found in the next conformation. For example, for the rotation of ring A which interconverts **65a** and **65b**, the dihedral angle N_A–4–5–6 (*i.e.* about the C-4 to C-5 bond) was chosen. In **65a** this angle is −60.4° and in **65b** it is +55.8°. Therefore, starting with structure **65a**, the C-20 to C-1 bond was broken, the dihedral angle N_A–4–5–6 was rotated to an angle of −50° and constrained at this angle, the C-20 to C-1 bond was remade and the whole structure was minimised. Finally, the constraint was removed, the energy recalculated and the exact dihedral angle measured. This procedure gave the first point on the graph shown in Fig. 1. For each successive point the dihedral angle was further increased and the structure minimised again in the same way. Exactly the same procedure was followed starting with structure **65b** and decreasing the same dihedral angle so as to rotate ring A to get back towards structure **65a**. For rotation of ring B, the dihedral angle 4–5–6–N_B was chosen and was varied from −89.0° in **65b** to +23.6° in **65c**. For rotation of ring C, the dihedral angle 9–10–11–N_C was chosen and was varied from +100.4° in **65c** to −43.9° in **65d**. In this latter case two different conformations were found for each dihedral angle between *ca.* 65° and *ca.* 15°, one conformation being obtained by varying **65c** and the other by varying **65d**. Outside this range of angles, the higher energy conformation became unstable and minimisation gave only the lower energy conformation (see Fig. 1). The same effect was observed to a much smaller extent in the rotation of ring A but for rotation of ring B exactly the same transition state was approached from either **65b** or **65c**. Where two different transition states were found the one of lower energy can be taken as being an upper limit to the true transition state energy. On this basis, the calculated transition state energies were 190.1 kJ mol^{-1} (ring A), 189.5 kJ mol^{-1} (ring B) and 197.8 kJ mol^{-1} (ring C). The difference in calculated energy between the most stable conformation **65a** (166.36 kJ mol^{-1}) and the highest of the three transition states (197.8 kJ mol^{-1}) is 31.4 kJ mol^{-1}.

Hydrolysis of the Macrocycles **39** and **66**.—The macrocyclic octaester **39** (3.22 mg) was dissolved in methanol (161 mm^3) in a vial and aqueous potassium hydroxide (4 mol dm^{-3}; 161 mm^3) was added to it. The mixture was shaken for 15 h in the dark under argon. This solution was then used for the enzyme inhibition studies (see below). Afterwards, an NMR spectrum of the hydrolysate was obtained by evaporating the solution under reduced pressure, dissolving the residue in D_2O (1 cm^3), evaporating again and repeating this procedure three times. Finally, the residue was dissolved in D_2O for NMR spectroscopy; $\delta_H(D_2O$, 400 MHz, chemical shifts relative to HOD signal at δ 5.02) 2.16–2.77 (m, 8 × $CH_2CH_2CO_2$), 2.87 and 3.01 (each 1 H, ABq, *J* 15, pyrr–CH_2–lactam), 2.94 and 3.01 (each 1 H, ABq, *J* 15, pyrr–CH_2–lactam), 3.15 (2 H, s), 3.35 and 3.50 (each 1 H, ABq, *J* 17) and 3.41 (4 H, s, 4 × CH_2CO_2) and 3.81 (4 H, 2 × ABq's, 2 × pyrr–CH_2–pyrr); λ_{max}(EtOH)/nm end absorption only below 220.

The same hydrolysis method was used for the dimeric macrocyclic lactam **66** (1.62 mg).

Inhibition of Cosynthetase.—The assays of cosynthetase activity using hydroxymethylbilane **1** as substrate were performed as described previously.[23] The hydrolysates containing the putative inhibitors were diluted with water to give a concentration of potassium hydroxide equal to 0.8 mol dm^{-3}. Appropriate volumes of these solutions were added to the buffered assay mixture which had already been treated with an equal volume of hydrochloric acid (0.8 mol dm^{-3}) in order to neutralise the alkali. The uroporphyrinogen produced was oxidised to the porphyrin using iodine and the concentration of porphyrin determined from the absorbance at 406 nm.[23] A simultaneous blank, without enzyme, was run to determine the rate of non-enzymatic cyclisation and this rate was subtracted from the rate observed in the presence of enzyme to give the true enzymic rate.

To obtain a K_i value for spiro-lactam **68**, assays were performed with hydroxymethylbilane **1** at concentrations of 33.2 × 10^{-6} and 62.2 × 10^{-6} mol dm^{-3} (*cf.* K_M value for **1**, 26 × 10^{-6} mol dm^{-3} in these experiments) and concentrations of spiro-lactam **68** varying from 0 to 5.2 × 10^{-6} mol dm^{-3}. The data, presented as a Dixon plot in Fig. 2, gave K_i = 1.3 × 10^{-6} mol dm^{-3}. The dimeric spiro-lactam **67**, hepta-acid **69** and hexa-acid **70** showed no significant inhibition of cosynthetase at concentrations of 208 × 10^{-6}, 400 × 10^{-6} and 500 × 10^{-6} mol dm^{-3}, respectively. A further series of assays was performed at least in duplicate with hydroxymethylbilane **1** at concentrations over the range 16–83 × 10^{-6} mol dm^{-3} with and without the spiro-lactam **68** at 5.2 × 10^{-6} mol dm^{-3}. Double reciprocal plots (one set is shown in Fig. 3) gave the K_i value for **68** in the range 1–2 × 10^{-6} mol dm^{-3}.

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