Biosynthesis of porphyrins and related macrocycles. Part 44.[1,2] Synthetic and stereochemical studies on the proposed spiro intermediate for biosynthesis of the natural porphyrins

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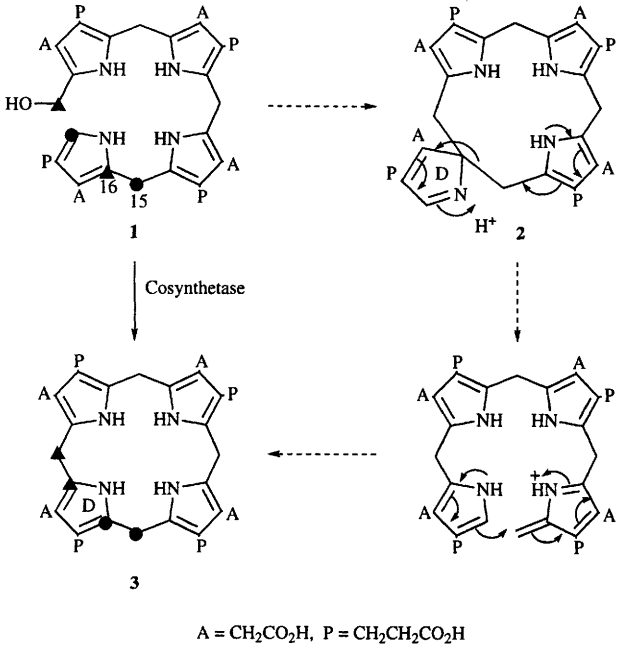
A route is devised for synthesis of both enantiomers of the spiro lactam 4. The enzyme uroporphyrinogen III synthase (cosynthetase), which converts hydroxymethylbilane 1 into uroporphyrinogen III 3, is competitively inhibited more than twenty times more strongly by one enantiomer of 4 than by the other. This finding adds further strong support to the view that cosynthetase acts by generating the spiro pyrrolenine 2 as an intermediate.

Haem, chlorophyll and vitamin B_{12} are all biosynthesised from the same parent macrocycle, uroporphyrinogen III **3**, shortened to uro'gen III. The formation of this macrocycle from the open-chain hydroxymethylbilane **1** is catalysed by the enzyme cosynthetase (systematically uroporphyrinogen III synthase, E.C. 4.2.1.75). Comparison of structures **1** and **3** shows why this process has attracted such strong mechanistic interest; intriguingly, ring D of uro'gen III **3** has been inverted relative to its position in the bilane **1**. Both the building of the hydroxymethylbilane **1** and the ring-closure catalysed by cosynthetase have been extensively studied and this work has been reviewed.[3] The key finding relevant to the present paper was that the inversion of ring D occurs by an intramolecular mechanism[4] which only affects ring D.[5]

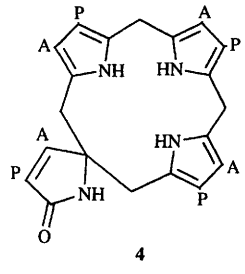
These results, based on a series of ^{13}C-labelling experiments as indicated by the black spots and triangles in Scheme 1,[4,5]



Scheme 1 Proposed mechanism for the formation of uro'gen III by cosynthetase

eliminated most of the 20 or so speculative mechanisms proposed for the ring D inversion and left just two. In one, ring D of the hydroxymethylbilane **1** is detached by cleavage of the bond between C-15 and C-16 but is held in the active site of the enzyme. Ring D is then inverted before rejoining to C-15 and the hydroxymethyl carbon to give **3**; there is no experimental support for this mechanism. The other, illustrated in Scheme 1, is fundamentally different in that the bond between the hydroxymethyl carbon and C-16 is suggested to be made first, to form the spiro pyrrolenine† **2**. This pyrrolenine could then undergo fragmentation–recombination as in Scheme 1 to form uro'gen III **3** and the chemistry involved has been shown to be both feasible and facile.[3] This second mechanism is based on a suggestion by Mathewson and Corwin[6] but it is drawn in a simplified form for reasons that have been outlined earlier.[7]

Strong support for the second mechanism has been provided by the synthesis[7] of the spiro lactam **4** which strongly inhibited



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cosynthetase from carrying out its normal conversion of hydroxymethylbilane **1** into uro'gen III **3**; the inhibition was competitive.[7] In addition, other synthetic macrocycles, lacking one or other parts of the five-membered lactam system, were not inhibitory.[7] This finding was very significant in that the inhibitory spiro lactam **4** is different in structure from both the substrate **1** for cosynthetase and the product **3** from the enzyme; the inhibitor only resembles the putative spiro pyrrolenine **2**. It should also be emphasised that the two molecules, **2** and **4**, only differ around the nitrogen atom of the five-membered spiro ring, otherwise they are identical.

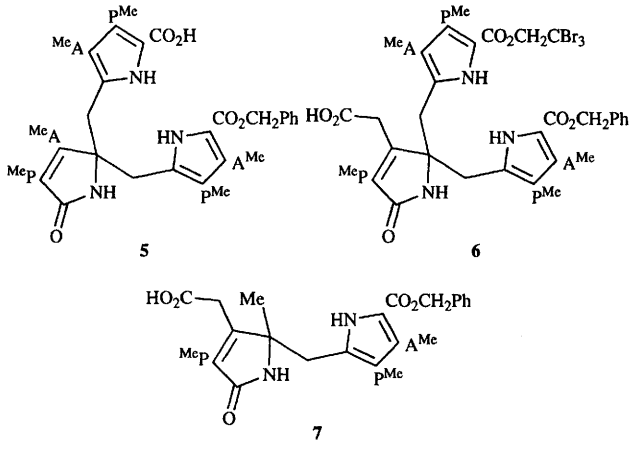
The spiro lactam **4** used for the foregoing studies was racemic, but only one enantiomer can accurately match the putative spiro pyrrolenine **2** held in the active site of cosynthetase. Our aim, therefore, was to synthesise both enantiomers of the spiro lactam **4** for testing as inhibitors of cosynthetase. Of the four substituents carried by the chiral centre of the lactam **4**, two are extremely similar so a difficult resolution was expected, best carried out early rather than late in the synthesis.

† IUPAC name: 2*H*-pyrrole.

Results and discussion

Trial experiments on the model system 7

Our initial studies were made on the acid **5**, which had been synthesised earlier for elaboration to the spiro lactam **4**.[7] Salts were prepared from **5** using brucine, cinchonine, cinchonidine, quinine and quinidine. Systematic attempts at crystallisation using seven different solvents gave few crystals and no resolution. A totally different approach was needed and we envisaged synthesising the acid **6** so that a variety of chiral auxiliaries could be covalently attached to the carboxy group close to the chiral centre. However, as acid **6** contains a vinylogous malonic acid residue there was concern about its stability. This was checked by synthesising the simpler lactam **7**.



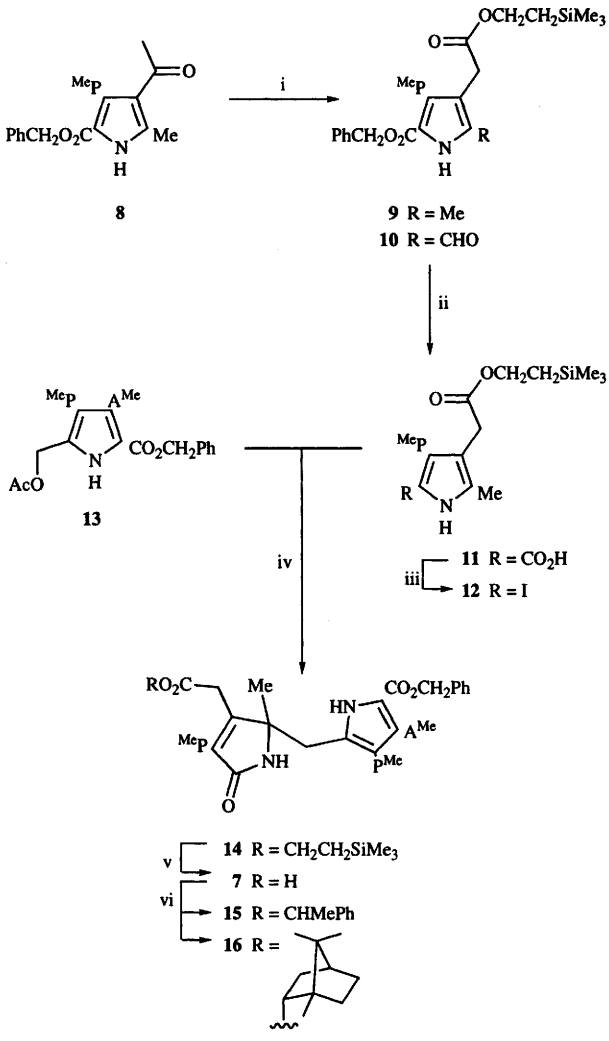
5 **6** **7**

A^{Me} = CH_2CO_2Me, P^{Me} = $CH_2CH_2CO_2Me$

The differentially protected pyrrole **9** was needed as starting material and this was initially prepared by having 2-trimethylsilylethanol present during the thallium-catalysed rearrangement[8] of the ketone **8** (Scheme 2). The aldehyde **10** was a by-product (a reaction previously observed in a related case[9]) and the yield of **9** was too poor for this route to be used for the synthesis of **6**; however, it yielded enough material for the preparation of the model **7**. Scheme 2 also shows the synthetic route from **9** to **7** *via* **11**, **12** and **14**, which was mainly based on similar earlier work,[7] though the iodination step required new conditions (see Experimental section). Removal of the trimethylsilylethyl group from **14** gave the acid **7** which could be handled without decomposition. The synthesis of the dipyrrolic lactam **6** could, therefore, be confidently undertaken but first the attachment of chiral auxiliaries was tested on the model **7**. The esters **15** and **16** were prepared from (*R*)-1-phenylethanol and (1*S*)-(−)-*endo*-borneol, respectively, using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). The former ester **15** was shown by NMR to contain two diastereoisomers in the ratio 1:1.25 and the ratio for the latter one **16** was 1:1.1. In neither case could the diastereoisomers be separated by TLC or HPLC. Attention therefore turned to the real system **6**.

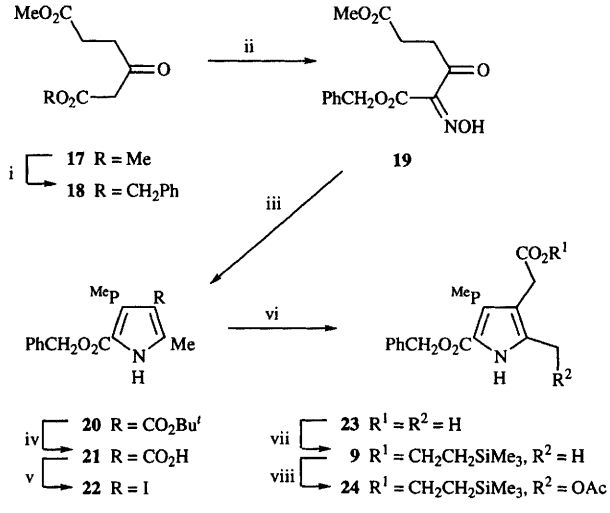
Synthesis of the enantiomeric lactams 40a and 40b

The first requirement was to develop a practical alternative synthesis of the starting pyrrole **9**. This was achieved in good yield (16% over seven steps) on a large scale by the sequence shown in Scheme 3, based mainly on chemistry developed earlier[4,10] for similar pyrroles. The key step is reductive alkylation[11,12] of the 4-iodopyrrole **22** using glyoxylic acid as the source of the acetate side-chain. Trial experiments involving some of the later chemistry required for construction of the required lactam **33** then showed that the benzyl ester of **9** caused problems. It was therefore conveniently changed at the stage of

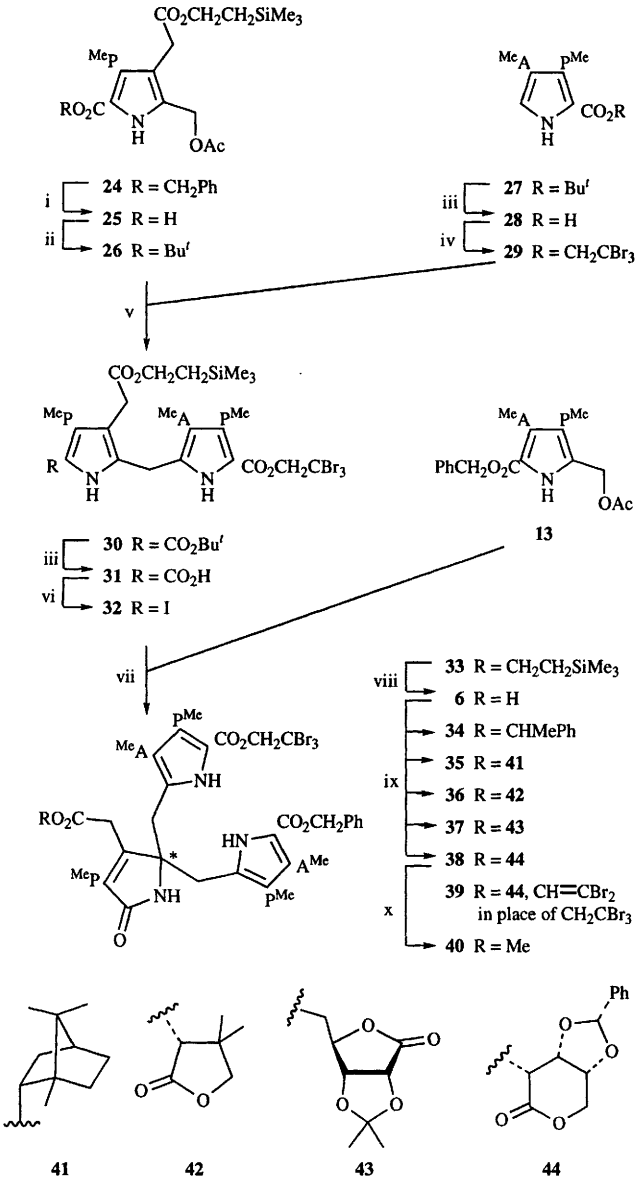


Scheme 2 *Reagents:* i, $Tl(NO_3)_3{\cdot}3H_2O$, HNO_3, $Me_3SiCH_2CH_2OH$; ii, H_2/Pd; iii, KI_3, $NaHCO_3$; iv, $SnCl_4$ then AgOAc, TsOH, H_2O; v, TBAF; vi, DCC, DMAP, ROH

the next intermediate **24** to the *tert*-butyl ester **26** by the steps shown in Scheme 4. This product was then coupled with the α-free pyrrole **29**, prepared from the available *tert*-butyl ester **27**, to yield the dipyrromethane **30**. The lactam **33** was synthesised



Scheme 3 *Reagents:* i, $PhCH_2OH$, heat; ii, $NaNO_2$, AcOH; iii, $MeCOCH_2CO_2Bu^t$, Zn, AcOH; iv, CF_3CO_2H; v, KI_3, $NaHCO_3$; vi, $OHCCO_2H$, HI, H_3PO_2, Ac_2O; vii, $(COCl)_2$ then $Me_3SiCH_2CH_2OH$; viii, SO_2Cl_2 then NaOAc, AcOH



Scheme 4 *Reagents:* i, H_2/Pd; ii, Bu^{t}OH, DCC; iii, $SnCl_4$; iv, $(COCl)_2$ then Br_3CCH_2OH; v, TsOH, H_2O; vi, KI_3, $NaHCO_3$; vii, $SnCl_4$ then AgOAc, TsOH, H_2O; viii, TBAF; ix, $Me_2C{=}C(Cl)NMe_2$ then the relevant alcohol; x, NaOMe, MeOH

by the steps **30** ⟶ **31** ⟶ **32** ⟶ **33** essentially as earlier for similar systems,[7] Scheme 4. Removal of the trimethylsilylethyl group by fluoride then afforded the acid **6** ready for esterification with a range of chiral alcohols.

The esters were prepared by reacting the acid **6** with 1-chloro-1-dimethylamino-2-methylpropene[13] to give the corresponding acid chloride, which smoothly acylated the various chiral alcohols. Other standard methods for these esterifications failed or gave unacceptable yields. The chosen alcohols were (*R*)-1-phenylethanol, (1*S*)-(−)-*endo*-borneol (as **41**), (*R*)-(−)-pantolactone‡ (as **42**), 2,3-*O*-isopropylidine-D-(+)-ribonic acid γ-lactone[14] (as **43**) and the commercially available 3,4-*O*-benzylidene-D-(+)-ribonic acid δ-lactone (as **44**). In no case could the diastereoisomeric esters **34** to **38** so formed be separated by TLC, but HPLC gave encouraging results for the esters **36** and **38**, derived from pantolactone and the ribonic acid δ-lactone, respectively. However, there were impurities in the former ester which were difficult to separate from the required materials, so the latter ester was selected for further study. HPLC

‡ IUPAC name: (*R*)-3-hydroxy-4,4-dimethyltetrahydrofuran-2-one.

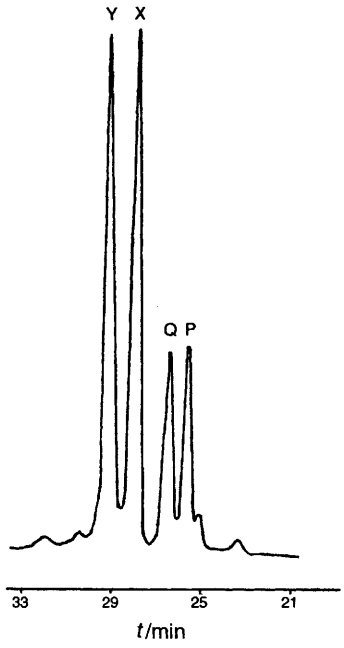


Fig. 1 HPLC trace for the separation of the diastereoisomers **38a** (peak X) and **38b** (peak Y) together with **39a** (peak P) and **39b** (peak Q)

analysis of this ribonolactone ester **38** initially showed three pairs of peaks, the four peaks that appear in Fig. 1 together with another pair, smaller than P and Q, having a longer retention time than X and Y. Mass spectrometry and NMR spectroscopy indicated that this slower running pair represented the separated diastereoisomers of material in which the CH_2CBr_3 group had been reduced to CH_2CHBr_2. Consideration of the earlier chemistry suggested that this reduction was probably due to attack by iodide ion on a bromine atom of the CBr_3 group during the preparation of the dipyrromethane **32**; a CBr_3 group can act as a source of 'Br^+'. Accordingly, the conditions for the iodination step **31** ⟶ **32** were made as mild as possible and when this product was carried forward to the ester **38**, the HPLC trace appeared as in Fig. 1, without the slowest running pair of peaks.

Similar analysis of the four remaining peaks in Fig. 1 indicated that peaks X and Y were the desired diastereoisomers **38a** and **38b**,§ respectively, whereas peaks P and Q were the corresponding diastereoisomers **39a** and **39b**, respectively, which had lost HBr from the tribromoethyl group at some stage during the synthetic transformations. Again efforts were made to avoid the production of the compounds in peaks P and Q; presumably they are formed by some base-catalysed elimination of hydrogen bromide. Suffice it to say that the various modifications tried either had little effect or they somewhat reduced the yield of the desired compounds in peaks X and Y. Since these by-products were readily separated, the large scale separation was carried out on the mixture as seen in Fig. 1. This effective separation of the diastereoisomers was only possible with very low loading of the column, so automatic HPLC equipment for injection and collection was needed to provide sufficient material for the rest of the synthesis. Analytical HPLC and 400 MHz ^1H NMR proved that complete separation of the diastereoisomers had been achieved.

The first task was to confirm that the peaks X and Y in Fig. 1 in fact contained the desired diastereoisomers **38a** and **38b**. Certainly their ^1H NMR, ^{13}C NMR and mass spectra

§ The two configurations at the starred chiral centre of **40**, Scheme 4, are not illustrated but are distinguished by **a** and **b** attached to the number. The same system with **a** and **b** is used for Scheme 5, where one configuration is illustrated but always the other separate enantiomer is being handled as well.

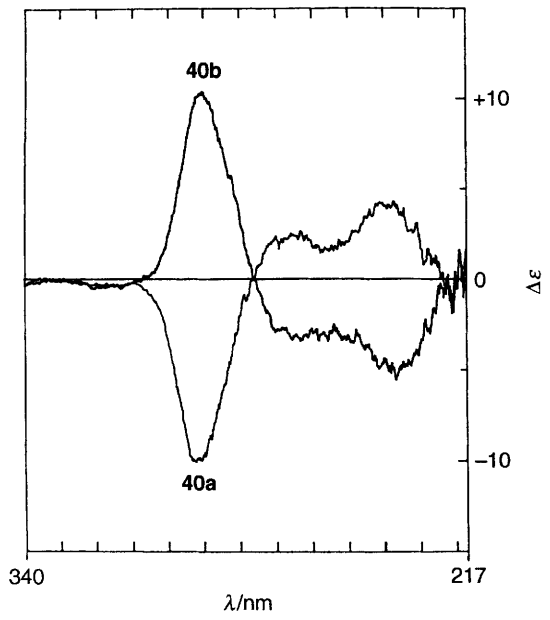
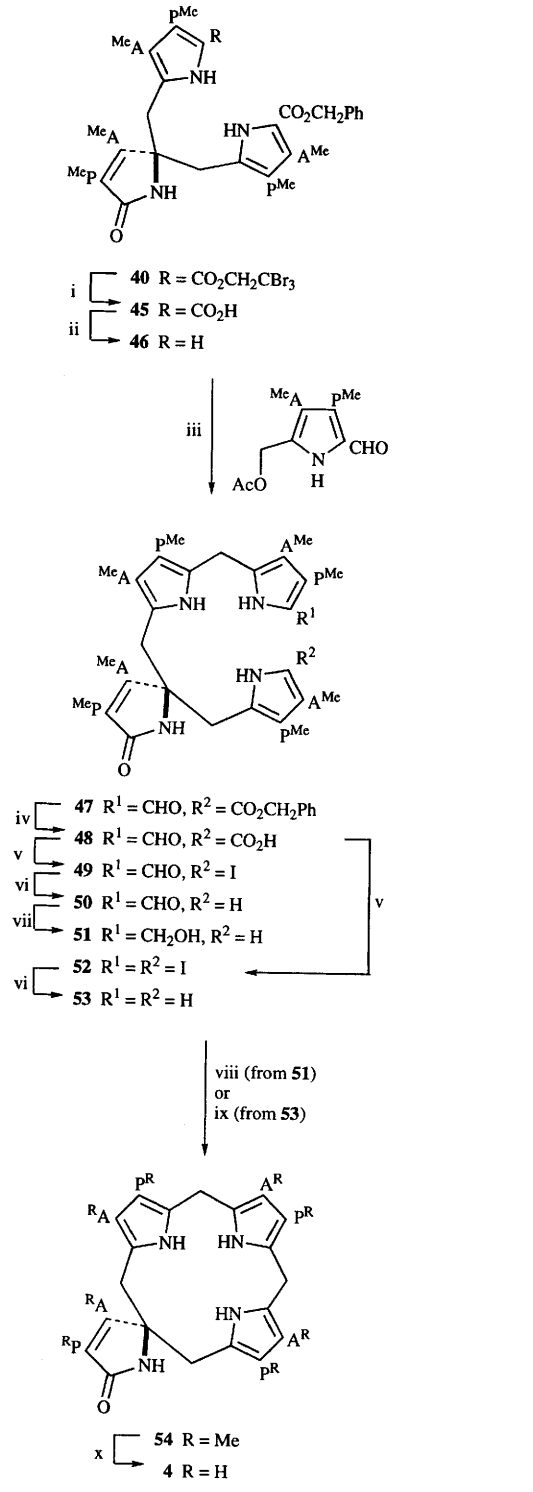


Fig. 2 Circular dichroism spectra of the enantiomers **40a** derived from peak X (Fig. 1) and **40b** derived from peak Y

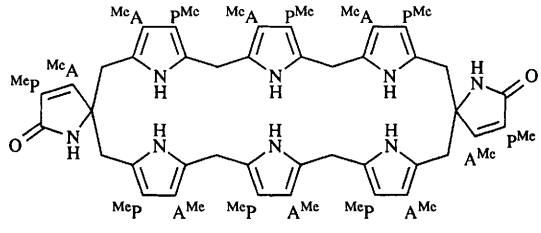
supported that view. Rigorous proof was obtained by treating each diastereoisomer with sodium methoxide in methanol to effect transesterification with loss of the ribonic lactone residue. As had been planned, the benzyl and tribromoethyl esters did not undergo transesterification under the mild conditions used, because they are vinylogous urethanes and thus much less reactive. That the two products, **40a** and **40b**, were enantiomers was shown by their identical HPLC retention times and ^{1}H NMR and mass spectra; importantly, their circular dichroism (CD) spectra were mirror images with a common crossover point on the zero line (Fig. 2).

Synthesis of the enantiomeric spiro lactams 54a and 54b

The racemic form **40** of the foregoing final products **40a** and **40b** was the key intermediate for the earlier synthesis[7] of the racemic spiro lactam **54a,b**. Now that the pure enantiomers of this intermediate were in hand, each could be carried through the same remaining steps of the synthesis, **40** ⟶ **45** ⟶ **46** ⟶ **47** ⟶ **48** ⟶ **49** ⟶ **50** ⟶ **51** ⟶ **54**, shown in Scheme 5. These conversions were carried out as for the racemic series[7] and were successfully completed for both enantiomers, but there was one surprise, probably due to small differences in reaction conditions used by different workers. This appeared when the first enantiomer of the acid to be used **48b** was decarboxylated by iodination and removal of the iodine by hydrogenation (expected steps **48b** ⟶ **49b** ⟶ **50b**). The major product was the bis(α-free pyrrole) **53b**, not **50b**. Evidently both decarboxylation and deformylation had occurred in the iodination step to yield the di-iodo derivative **52b**, which on hydrogenation afforded the observed product **53b**. Modification of the conditions for the iodination overcame this problem to allow the original steps *via* **50b** (Scheme 5) to be used to build the spiro lactam **54b**. In addition the bis(α-free) system **53b** was successfully converted in modest yield into the final spiro lactam **54b** by acid-catalysed condensation with formaldehyde. The sum of all this synthetic effort, starting from the two enantiomers of the dipyrrolic lactam **40a** and **40b**, afforded ample quantities of the enantiomeric spiro lactams **54a** and **54b** for the necessary enzymic experiments. The last ring-closure step, **51a** or **b** ⟶ **54a** or **b** (Scheme 5) again yielded small amounts of the macrocyclic dimer **55**, readily separable from the monomer, which had been fully characterised in the racemic series;[7] these enantiomeric dimers are not further examined here.



Scheme 5 *Reagents:* i, Zn, AcOH; ii, CF_3CO_2H; iii, $SnCl_4$; iv, H_2/Pd; v, KI_3, $NaHCO_3$; vi, H_2/Pt; vii, $NaBH_4$; viii, TsOH; ix, CH_2O, CF_3CO_2H; x, KOH, H_2O, MeOH



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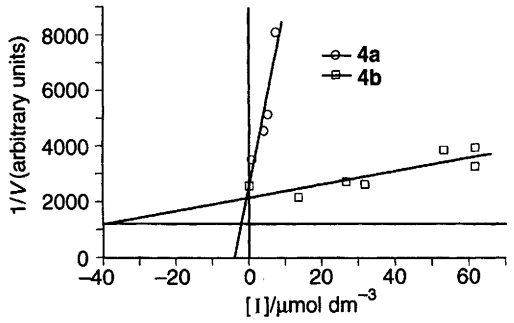

Fig. 3 Dixon plot for the inhibition of cosynthetase by the enantiomeric spiro lactams **4a** and **4b**. The horizontal line at $1/V = 1218$ is at $1/V_{max}$ and the straight lines through the experimental points intersect it at $[I] = -K_i$.

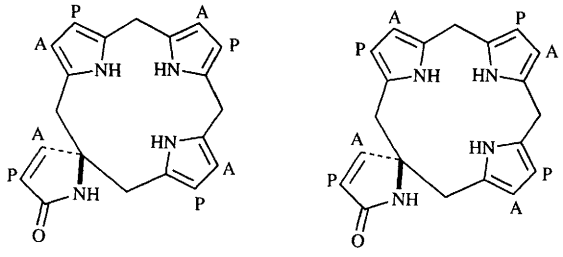

Fig. 4 The two enantiomers of the spiro lactam **4**

Table 1 Inhibition of cosynthetase by enantiomers of the spiro lactam **4**

Compound	Inhibition constant K_i (or K_M)/ μmol dm^{-3}
Enantiomer **4a** synthesised from peak X, Fig. 1	K_i 1.8
Racemic **4**	K_i 2.5
Enantiomer **4b** synthesised from peak Y, Fig. 1	K_i 38
Hydroxymethylbilane **1**	K_M 37

Enzymic studies: inhibition of cosynthetase

The two enantiomers of the spiro lactam **54a** and **54b** were each treated with aqueous methanolic potassium hydroxide under conditions known to hydrolyse only the ester groups [7] to afford the enantiomeric octa-acids **4a** and **4b**. Cosynthetase isolated from *Euglena gracilis*,[15] was used in standard assays of the enzyme's activity [15] for catalysing the conversion of hydroxymethylbilane **1** into uro'gen III **3**. These assays were carried out as for the earlier ones in the racemic series [7] and were run in the presence of varying amounts of each enantiomer of the spiro lactam octa-acids **4a** and **4b**; kinetic runs using the racemic octa-acid [7] **4** were also included to act as a standard. The K_M value for hydroxymethylbilane **1** was determined at the same time by standard assays [15] at a range of substrate concentrations in the absence of any inhibitor, the K_M value being determined from a Hanes plot.[16]

Dixon plots of the kinetic data from the experiments with the enantiomeric spiro lactams **4a** and **4b** are shown in Fig. 3, from which the inhibition constants K_i shown in Table 1 were derived. They show a striking difference in the effectiveness of the two enantiomers as inhibitors of cosynthetase, with the spiro lactam **4a** derived from peak X in Fig. 1 being *ca.* 20 times more inhibitory than its enantiomer **4b** derived from peak Y. Also, the K_i for the strongly inhibiting enantiomer is more than an order of magnitude lower than the K_M for the substrate (hydroxymethylbilane **1**) of cosynthetase.

The fact that one of the two enantiomers of the spiro lactam **4** is a weak inhibitor, rather than having no effect at all, can be understood by setting the lactam ring of these enantiomers in the same orientation, see Fig. 4. This is reasonable since it is the chemistry around ring D of hydroxymethylbilane **1**, corresponding to the pyrrolenine ring of the putative spiro intermediate **2**, that lies at the core of the action of cosynthetase. The three pyrrole rings of the macrocycle will have the same conformation in the two systems and inspection of Fig. 4 shows the only difference between the two systems is that the acetate (A) and propionate (P) side-chains on each pyrrole ring are interchanged. It follows that, whereas the side-chains on the pyrrolic rings of the strongly inhibitory enantiomer presumably fit well into the enzymic active site, the same side-chains of the other enantiomer, though all acidic, are of slightly the wrong size (A for P and P for A). The observed weak inhibition by this enantiomer, rather than a complete lack of inhibition, fits the foregoing analysis.

These experiments with the two enantiomers of the spiro lactam, **4a** and **4b**, add further powerful support to the view that the conversion of hydroxymethylbilane **1** into uro'gen III **3**, catalysed by cosynthetase, goes *via* the spiro pyrrolenine **2**. In addition, determination of the absolute configuration of the strongly inhibiting enantiomer will allow the absolute configuration of the spiro pyrrolenine **2** to be deduced. The following paper [17] describes the way in which that stereochemical problem has been solved.

Experimental

General directions

Most general directions are given in Part 34 of this series.[18] In addition, CD spectra were recorded on Jasco J-40CS or J-600 spectrometers, with a 1 cm pathlength in strain-free cuvettes. HPLC was carried out on Spherisorb S5W silica columns or Spherisorb S5CN nitrile columns with a Waters 6000A pump connected to a Cecil CE272 detector. Solvents for HPLC were filtered through a 0.5 μm sieve. Evaporation was carried out at *ca.* 15 mmHg on a Büchi rotatory evaporator and residual solvent was removed at high vacuum using an oil pump.

Methyl 2-benzyloxycarbonyl-5-methyl-4-(2-trimethylsilylethoxycarbonylmethyl)pyrrole-3-propionate 9 and methyl 2-benzyloxycarbonyl-5-formyl-4-(2-trimethylsilylethoxycarbonylmethyl)pyrrole-3-propionate 10

A solution of acetylpyrrole **8** [19] (1 g, 2.92 mmol) in dimethoxyethane (6 cm^3) and 2-trimethylsilylethanol (1.38 g, 11.67 mmol) was treated with a suspension of thallium(III) nitrate trihydrate (2.59 g, 5.83 mmol) in dimethoxyethane (11 cm^3) and concentrated nitric acid (0.2 cm^3). The resulting mixture was stirred at room temperature for 28 h and then filtered through Celite. The filtrate was neutralised with 10% aqueous sodium carbonate, mixed with water (10 cm^3) and extracted with dichloromethane (3 × 30 cm^3). The combined extracts were dried and evaporated under reduced pressure. The residual oil was purified by flash silica chromatography, eluting with dichloromethane–ethyl acetate (24:1), followed by preparative TLC, eluting with dichloromethane–ethyl acetate (4:1), to give the *pyrrole ester* **9** (88.6 mg, 7%), mp 56–57 °C (from hexane) (see later for spectroscopic data) and the *formyl pyrrole* **10** (117.5 mg, 11.7%), mp 63–64 °C (from dichloromethane–diethyl ether–hexane) (Found: M$^+$, 473.1867. $C_{24}H_{31}NO_7Si$ requires M, 473.1870); $\lambda_{max}(CH_2Cl_2)$/nm 302 and 232; $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3395, 2935, 1720, 1650, 1190, 1165 and 835; $\delta_H(CDCl_3, 400\ MHz)$ −0.01 (9 H, s, $SiMe_3$), 0.96 (2 H, t, J 9, CH_2Si), 2.54 and 3.01 (each 2 H, t, J 8, $CH_2CH_2CO_2$), 3.60 (3 H, s, OMe), 3.78 (2 H, s, CH_2CO_2), 4.15 (2 H, t, J 9, OCH_2CH_2Si), 5.31 (2 H, s, CH_2Ph), 7.30–7.40 (5 H, m, Ph), 9.73 (1 H, s, CHO) and 10.01 (1 H, br s, NH); $\delta_C(CDCl_3, 100\ MHz)$ 1.61, 17.25, 19.66, 29.51, 34.29, 51.53, 63.75, 67.02, 123.89, 125.54, 128.65, 130.60, 135.01, 159.92, 170.61, 173.21 and 179.60; m/z (FD) 473.

3-(2-Methoxycarbonylethyl)-5-methyl-4-(2-trimethylsilylethoxycarbonylmethyl)pyrrole-2-carboxylic acid 11

To a solution of the pyrrole ester **9** (100 mg, 0.22 mmol) in

tetrahydrofuran (2 cm^3) was added 10% palladium-on-charcoal (10 mg). The mixture was hydrogenated at room temperature for 2.5 h, filtered through Celite and evaporated. The residue was recrystallised from diethyl ether–hexane to give the *pyrrole acid* **11** (79 mg, 98%), mp 105.5–106.5 °C (Found: M^+, 369.1602. $C_{17}H_{27}NO_6Si$ requires *M*, 369.1608); δ_H($CDCl_3$, 400 MHz) 0.01 (9 H, s, Me_3Si), 0.92 (2 H, t, *J* 9, CH_2Si), 2.23 (3 H, s, CMe), 2.62 and 3.03 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.41 (2 H, s, CH_2CO_2), 3.65 (3 H, s, OMe), 4.15 (2 H, t, *J* 9, CH_2CH_2Si) and 9.22 (1 H, br s, NH); δ_C($CDCl_3$, 100 MHz) −1.55 (Me_3Si), 11.72 (*CMe*), 17.33 (CH_2Si), 20.56, 30.13 and 34.66 (3 × CH_2), 51.49 (OMe), 63.12 (OCH_2), 115.12, 116.08, 132.61 and 132.95 (4 × pyrrole-C), 165.65 (CO_2H) and 171.89 and 173.91 (2 × CO_2Me); *m*/*z* (FD) 369 (M^+, 100%).

9-Benzyloxycarbonyl-2,7-bis(2-methoxycarbonylethyl)-8-methoxycarbonylmethyl-4-methyl-3-(2-trimethylsilylethoxycarbonylmethyl)-4,5-dihydrodipyrrin-1(10*H*)-one 14

A solution of pyrrole acid **11** (2.0 g, 5.42 mmol) in dichloromethane (30 cm^3) was stirred vigorously with a solution of sodium hydrogen carbonate (1.35 g, 16.07 mmol) in water (25 cm^3) under argon. An aqueous solution (60 cm^3) of iodine (0.1 mol dm^{-3}) and potassium iodide (0.2 mol dm^{-3}) was added over 5 min and the resulting mixture was stirred for a further 2 min before addition of solid sodium metabisulfite to destroy the excess iodine. The organic layer was separated and the aqueous layer extracted with dichloromethane (3 × 30 cm^3). The combined organic layers were dried and evaporated. Flash chromatography on silica, eluting with diethyl ether–hexane (1:1), gave the α-iodopyrrole **12** as an oil which was used directly in the next step.

A stirred solution of the iodopyrrole **12** and acetoxymethylpyrrole **13**[8] (2.28 g, 5.41 mmol) in anhydrous dichloromethane (50 cm^3) was cooled to 0 °C under argon. Stannic chloride (698 mm^3, 5.96 mmol) was added dropwise. After 30 min saturated aqueous sodium hydrogen carbonate (25 cm^3) was added and the mixture was stirred for a further 10 min. The organic layer was separated and the aqueous layer extracted with dichloromethane (5 × 25 cm^3). The combined organic layers were dried and evaporated. The residual oil was dissolved in tetrahydrofuran (100 cm^3) and water (10 cm^3) and treated with toluene-*p*-sulfonic acid (1.5 g, 8.7 mmol) and silver acetate (250 mg, 1.5 mmol). The mixture was stirred under argon for 13 h, then mixed with water (400 cm^3) and extracted with dichloromethane (4 × 150 cm^3). The combined extracts were dried and evaporated and the residue was purified by flash chromatography on silica, eluting with diethyl ether followed by diethyl ether–ethyl acetate (1:1), to give the *lactam* **14** as an oil (1.43 g, 37%) (Found: M^+, 712.3028. $C_{36}H_{48}N_2O_{11}Si$ requires *M*, 712.3027); λ_{max}(CH_2Cl_2)/nm 280; ν_{max}(CH_2Cl_2)/cm^{-1} 3685, 2940, 1720, 1690, 1600 and 1165; δ_H($CDCl_3$, 400 MHz) 0.02 (9 H, s, Me_3Si), 0.99 (2 H, t, *J* 9, CH_2Si), 1.32 (3 H, s, CMe), 2.40–2.70 (8 H, m, 2 × CH_2CH_2), 2.74 and 3.04 (2 H, ABq, *J* 15, 5-H_2), 3.32 and 3.49 (2 H, ABq, *J* 17, CH_2CO_2), 3.51, 3.58 and 3.63 (each 3 H, s, OMe), 3.62 and 3.84 (2 H, ABq, *J* 17, CH_2CO_2), 4.18 (2 H, t, *J* 9, CH_2CH_2Si), 5.13 and 5.23 (2 H, ABq, *J* 12, CH_2Ph), 7.19 (1 H, s, lactam-NH), 7.23–7.34 (5 H, m, Ph), 10.18 (1 H, s, pyrrole-NH); δ_C($CDCl_3$, 100 MHz) −1.54 (Me_3Si), 17.36 (CH_2Si), 19.26 and 19.67 (2 × $CH_2CH_2CO_2$), 23.77 (CMe), 30.86, 31.15, 31.39, 33.36, 34.76 (2 × $CH_2CH_2CO_2$, 2 × CH_2CO_2, C-5), 51.48 (OMe), 51.77 (2 × OMe), 63.39 (CH_2CH_2Si), 64.31, 65.67 (C-4 and CH_2Ph), 119.16, 121.91, 122.22, 127.95, 128.19, 128.35, 128.70, 135.30, 136.29 and 151.53 (C=C), 160.67 (α-CO_2) and 170.22, 171.84, 171.04, 173.47 and 173.96 (4 × CO_2 and CONH); *m*/*z* (FD) 712 (M^+, 100%).

9-Benzyloxycarbonyl-3-carboxymethyl-2,7-bis(2-methoxycarbonylethyl)-8-methoxycarbonylmethyl-4-methyl-4,5-dihydrodipyrrin-1-(10*H*)-one 7

Tetrabutylammonium fluoride trihydrate (118 mg, 0.37 mmol) was added to a solution of the lactam **14** (88.7 mg, 0.12 mmol) in tetrahydrofuran (1 cm^3) and the solution was stirred under argon at room temperature for 40 min. Water (6 cm^3) was added and the pH of the solution adjusted to 3.0–3.5 with dilute sulfuric acid. The solution was extracted with dichloromethane (2 × 5 cm^3) and the combined organic extracts were dried and evaporated. The residue was recrystallised from dichloromethane to give the *acid* **7** (46.8 mg, 61%), mp 159–161 °C (Found: MH^+, 613.2361. $C_{31}H_{36}N_2O_{11}$ requires *MH*, 613.2397); δ_H($CDCl_3$, 400 MHz) 1.34 (3 H, s, CMe), 2.34–2.64 (8 H, m, 2 × CH_2CH_2), 2.83 and 3.13 (2 H, ABq, *J* 15, 5-H_2), 3.32 and 3.53 (2 H, ABq, *J* 17, CH_2CO_2), 3.49, 3.56, 3.57 (each 3 H, s, OMe), 3.60 and 3.80 (2 H, ABq, *J* 17, CH_2CO_2), 5.19 (2 H, s, CH_2Ph), 7.25–7.39 (5 H, m, Ph), 7.51 (1 H, br s, lactam-NH) and 10.20 (1 H, br s, pyrrole-NH); δ_C($CDCl_3$, 100 MHz) 19.16 and 19.74 (2 × $CH_2CH_2CO_2$), 23.66 (*CMe*), 30.91, 30.97, 31.11, 32.75 and 34.86 (2 × $CH_2CH_2CO_2$, 2 × CH_2CO_2, C-5), 51.53, 51.69 and 51.82 (3 × OMe), 64.44 and 65.84 (C-4 and CH_2Ph), 118.95, 121.95, 122.36, 127.94, 128.05, 128.49, 128.89, 134.93, 136.16 and 152.48 (C=C), 161.07 (α-CO_2) and 172.36, 172.68, 173.43 and 173.74 (4 × CO_2 and CONH); *m*/*z* (FD) 612 (M^+, 100%).

9-Benzyloxycarbonyl-2,7-bis(2-methoxycarbonylethyl)-8-methoxycarbonylmethyl-4-methyl-3-(1- phenylethoxycarbonylmethyl)-4,5-dihydrodipyrrin-1(10*H*)-one 15

N,*N*′-Dicyclohexylcarbodiimide (7.6 mg, 37 μmol) and 4-dimethylaminopyridine (0.7 mg, 6 μmol) were added to a solution of the acid **7** (15 mg, 25 μmol) in dichloromethane (1.5 cm^3) and (*S*)-(−)-1-phenylethanol (30 mg, 245 μmol) and the mixture was stirred under argon at room temperature for 20 min. The solvent was evaporated and the residue was purified by preparative TLC, eluting with diethyl ether–methanol (19:1), to give the *ester* **15** as a foam (10.3 mg, 57%) (Found: M^+, 716.2944. $C_{39}H_{44}N_2O_{11}$ requires *M*, 716.2945); λ_{max}(CH_2Cl_2)/nm 280; δ_H($CDCl_3$, 400 MHz, two diastereoisomers in a ratio of *ca.* 1:1.25) 1.28 and 1.31 (2 × 3 H, s, 5-Me), 1.54 and 1.55 (2 × 3 H, d, *J* 7, *Me*CH), 2.38–2.71 (18 H, m, 4 × CH_2CH_2, 2 × 5-H_A), 2.97 and 3.06 (each 1 H, d, *J* 15, 5-H_B), 3.34 and 3.89 (2 H, ABq, *J* 17, CH_2CO_2), 3.37 and 3.89 (2 H, ABq, *J* 17, CH_2CO_2), 3.56 (6 H, s, 2 × OMe), 3.57, 3.59, 3.61 and 3.62 (each 3 H, s, OMe), 3.52–3.66 (4 H, m, 2 × CH_2CO_2), 5.14 and 5.26 (2 H, ABq, *J* 12, CH_2Ph), 5.15 and 5.26 (2 H, ABq, *J* 12, CH_2Ph), 5.85 (1 H, q, *J* 6, CHMePh), 5.88 (1 H, q, *J* 7, CHMePh), 6.64 and 6.69 (2 × 1 H, br s, lactam-NH), 7.26–7.37 (20 H, m, 4 × Ph) and 9.88 and 9.89 (2 × 2 H, s, pyrrole-NH); δ_C($CDCl_3$, 100 MHz): 19.27, 19.71, 19.86, 21.84, 21.98, 24.01, 24.13, 30.89, 31.28, 33.04, 33.26, 34.65, 51.62, 51.97, 52.02, 63.62, 65.71, 74.38, 74.48, 119.24, 121.95, 122.03, 122.55, 126.34, 126.46, 128.08, 123.33, 128.42, 128.48, 127.70, 128.74, 135.44, 136.37, 140.47, 140.63, 151.98, 160.54, 169.71, 169.93, 171.67, 172.15, 173.43, 173.55, 174.30 and 174.39; *m*/*z* (FD) 716.

9-Benzyloxycarbonyl-2,7-bis(2-methoxycarbonylethyl)-8-methoxycarbonylmethyl-4-methyl-3-{(1*S*,2*R*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yloxycarbonylmethyl}-4,5-dihydrodipyrrin-1(10*H*)-one 16

A mixture of *N*,*N*′-dicyclohexylcarbodiimide (7.6 mg, 37 μmol), 4-dimethylaminopyridine (0.7 mg, 6 μmol) and a solution of the acid **7** (15 mg, 25 μmol) and (1*S*)-*endo*-borneol (75.6 mg, 0.49 mmol) in dichloromethane (1.5 cm^3) was stirred under argon at room temperature for 35 min. The solvent was evaporated and the residue was purified by preparative TLC, eluting with diethyl ether–methanol (95:5), to give the *ester* **16** (13.5 mg, 73%) as an oil (Found: M^+, 748.3572. $C_{41}H_{52}N_2O_{11}$ requires *M*, 748.3571); λ_{max}(CH_2Cl_2)/nm 280; δ_H($CDCl_3$, 400 MHz, 2 diastereoisomers, ratio 1:1.1) 0.80 and 0.83 (each 3 H, s, bornyl Me), 0.86 (12 H, s, 4 × bornyl Me), 0.90–0.98, 1.18–1.21, 1.66–1.90, 2.30–2.33 (14 H, m, 6 × bornyl CH_2 and

2 × CH), 1.35 (6 H, s, 2 × 4-Me), 2.46–2.75 (18 H, m, 4 × $CH_2CH_2CO_2$ and 2 × 5-H_AH_B), 3.06 (2 H, d, *J* 15, 2 × 5-H_AH_B), 3.38–3.66 (4 H, m, 2 × CH_2CO_2), 3.54, 3.62 and 3.65 (each 6 H, s, 2 × OMe), 3.86 and 3.87 (4 H, 2 × ABq, *J* 17, CH_2CO_2), 4.84–4.96 (2 H, m, 2 × bornyl OCH) 5.14 and 5.26 (2 H, ABq, *J* 12, CH_2Ph), 5.15 and 5.27 (2 H, ABq, *J* 12, CH_2Ph), 6.84 and 6.87 (each 1 H, br s, lactam-NH), 7.26–7.36 (10 H, m, 2 × Ph) and 10.01 (2 H, br s, 2 × pyrrole-NH); δ_C($CDCl_3$, 100 MHz) 13.54, 13.59, 15.26, 18.81, 19.20, 19.65, 23.95, 27.06, 27.98, 30.80, 31.19, 31.28, 33.32, 34.62, 36.63, 36.70, 44.74, 47.88, 48.80, 48.86, 51.51, 51.81, 63.26, 65.59, 65.84, 81.85, 81.97, 119.14, 121.86, 122.34, 127.95, 128.16, 128.35, 135.22, 135.28, 136.28, 151.63, 151.68, 160.53, 170.51, 170.63, 171.66, 172.04, 173.45, 174.06 and 174.10; *m*/*z* (FD) 748.

Methyl 2-(benzyloxycarbonyl)-4-(*tert*-butoxycarbonyl)-5-methylpyrrole-3-propionate 20

A solution of sodium nitrite (17.46 g, 253 mmol) in water (31 cm^3) was added dropwise to a stirred mixture of 1-benzyl 6-methyl 3-oxohexanedioate **18** (64.25 g, 243 mmol) (made from **17** by the method in ref. 19) in acetic acid (100 cm^3) at such a rate that the temperature did not exceed 25 °C. After being stirred overnight at room temperature, the mixture, containing the oxime **19**, was added dropwise to a stirred mixture of *tert*-butyl acetoacetate (45.37 g, 287 mmol) in acetic acid (100 cm^3). At the same time a mixture of zinc dust (50 g) and ammonium acetate (50 g) was added at such a rate that it was always in excess and the temperature remained between 50 and 70 °C. The resulting mixture was stirred for 30 min, then diluted with ice–water (750 cm^3), stirred for a further 30 min and filtered. The residue was washed with water (200 cm^3) and dissolved in dichloromethane (250 cm^3). The organic solution was washed with water (250 cm^3) and then with 5% aqueous sodium carbonate, dried and evaporated. The residue was recrystallised from diethyl ether to give the *pyrrole ester* **20** (35.3 g, 36%), mp 95–98 °C (Found: M^+, 401.1829. $C_{22}H_{27}NO_6$ requires *M*, 401.1838); δ_H($CDCl_3$, 400 MHz) 1.54 (9 H, s, Bu^t), 2.46 (3 H, s, 5-Me), 2.53 and 3.35 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.61 (3 H, s, OMe), 5.29 (2 H, s, CH_2Ph), 7.30–7.40 (5 H, m, Ph) and 9.15 (1 H, br s, NH); *m*/*z* (FD) 401 (M^+, 100%).

Methyl 2-benzyloxycarbonyl-4-iodo-5-methylpyrrole-3-propionate 22

A solution of pyrrole ester **20** (19.8 g, 49.3 mmol) in 1,2-dichloroethane (65 cm^3) was treated with trifluoroacetic acid (4.94 cm^3), heated under reflux with stirring for 75 min and then cooled to room temperature. Water (42 cm^3) was added and after 10 min the acid **21** was collected by filtration, washed with dichloromethane then water and dried *in vacuo* for use directly in the next step.

The acid **21** was added to a stirred solution of sodium hydrogen carbonate (12.4 g, 148 mmol) in water (82 cm^3). When foaming began, 1,2-dichloroethane (82 cm^3) was added and the mixture was heated gently at reflux to dissolve the pyrrole. A solution of iodine (13.8 g, 109 mmol) and potassium iodide (16.4 g, 99 mmol) in water (82 cm^3) was added over 5 min and the resulting mixture was heated under reflux with stirring for 30 min. Solid sodium metabisulfite was added to destroy excess iodine and the organic layer was separated. The aqueous layer was extracted with dichloromethane (60 cm^3) and the combined organic layers were dried and evaporated to give the pale yellow *iodopyrrole* **22** (16.65 g, 79%), mp 130–131 °C (from diethyl ether) (Found: M^+, 427.0241. $C_{17}H_{18}NO_4I$ requires *M*, 427.0239); δ_H($CDCl_3$, 400 MHz) 2.26 (3 H, s, 5-Me), 2.47 and 3.02 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.63 (3 H, s, OMe), 5.28 (2 H, s, CH_2Ph), 7.30–7.39 (5 H, m, Ph) and 9.35 (1 H, br s, NH); *m*/*z* (FD) 427 (M^+, 100%).

5-Benzyloxycarbonyl-4-(2-methoxycarbonylethyl)-2-methylpyrrole-3-acetic acid 23

To a stirred solution of 57% aqueous hydriodic acid (125 cm^3) at 0 °C was *slowly* added acetic anhydride (125 cm^3) followed by 50% aqueous hypophosphorous acid (24.5 cm^3). To this cooled solution was added finely powdered iodopyrrole **22** (21.4 g, 50 mmol). The resulting mixture was stirred for 5 min and then glyoxylic acid monohydrate (13.81 g, 150 mmol) was added in 3 portions over 15 min. After a further 20 min at 0 °C, dichloromethane (425 cm^3) was added and the solution was washed with water (500 cm^3). The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 × 330 cm^3). The combined organic layers were washed with 5% aqueous sodium hydrogen sulfite (250 cm^3) and then water (250 cm^3), dried and evaporated. Crystallisation from dichloromethane–hexane gave the *acid* **23** (17.53 g, 97%), mp 145–146 °C (Found: MH^+, 360.1454. $C_{19}H_{21}NO_6$ requires *M* + *H*, 360.1447); λ_{max}(CH_2Cl_2)/nm 278; ν_{max}(Nujol)/cm^{-1} 3700–3000, 3300, 2993, 1735, 1705, 1665 and 1465; δ_H($CDCl_3$, 400 MHz) 2.20 (3 H, s, CMe), 2.52 and 2.99 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.45 (2 H, s, CH_2CO_2), 3.58 (3 H, s, OMe), 5.27 (2 H, s, CH_2Ph), 7.33 (5 H, m, Ph) and 9.26 (1 H, br s, NH); δ_C($CDCl_3$, 100 MHz) 11.60 (*CMe*), 20.64, 29.66, 34.82 (3 × CH_2), 51.48 (OMe), 60.06 (CH_2Ph), 113.94, 116.72, 128.25, 128.37 (2 C), 128.59 (2 C), 130.94, 132.06 and 135.05 (C=C) and 161.04, 173.74 and 177.01 (3 × C=O); *m*/*z* (FD) 359 (M^+, 100%).

Methyl 2-benzyloxycarbonyl-5-methyl-4-(2-trimethylsilylethoxycarbonylmethyl)pyrrole-3-propionate 9

To a solution of the *acid* **23** (3.25 g, 9.05 mmol) in anhydrous dichloromethane (30 cm^3) was added oxalyl chloride (3.16 cm^3, 4.60 g, 36.24 mmol) dropwise, followed by 3 drops of anhydrous *N*,*N*-dimethylformamide. Vigorous effervescence ensued and the red solution was stirred at room temperature under argon for 30 min and then evaporated. A solution of the residue in anhydrous dichloromethane (10 cm^3) was stirred with 2-(trimethylsilyl)ethanol (2.68 g, 22.66 mmol) and 4-dimethylaminopyridine (1.33 g, 10.89 mmol) under argon for 20 min at room temperature and then evaporated. The residue was purified by flash chromatography on silica, eluting with hexane–diethyl ether (9:1) followed by dichloromethane–ethyl acetate (95:5), and crystallisation from hexane to give pyrrole ester **9** (3.85 g, 93%), mp 56–57 °C (Found: M^+, 459.2076. $C_{24}H_{33}NO_6Si$ requires *M*, 459.2077); λ_{max}(CH_2Cl_2)/nm 278; ν_{max}(CH_2Cl_2)/cm^{-1} 3425, 2925, 1760–1640s, 1165, 1065 and 835; δ_H($CDCl_3$, 400 MHz) 0.01 (9 H, s, Me_3Si), 0.96 (2 H, t, *J* 9, CH_2Si), 2.20 (3 H, s, CMe), 2.53 and 3.00 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.39 (2 H, s, CH_2CO_2), 3.60 (3 H, s, OMe), 4.13 (2 H, t, *J* 9, CH_2CH_2Si), 5.27 (2 H, s, CH_2Ph), 7.36 (5 H, m, Ph) and 8.99 (1 H, br s, NH); δ_C($CDCl_3$, 100 MHz) −1.54 (Me_3Si), 11.66 (*CMe*), 17.34 (CH_2Si), 20.68 ($CH_2CH_2CO_2$), 30.12 (CH_2CO_2), 34.86 ($CH_2CH_2CO_2$), 51.39 (OMe), 63.05 and 65.83 (CH_2CH_2Si and CH_2Ph), 114.59, 116.68, 128.18, 128.30 (2 C), 128.57 (2 C), 130.84, 131.54, 136.23 (C=C), 160.76 (CO_2) and 171.87 and 173.62 (2 × CO_2Me); *m*/*z* (FD) 459 (M^+, 100%).

Methyl 5-acetoxymethyl-2-benzyloxycarbonyl-4-(2-trimethylsilylethoxycarbonylmethyl)pyrrole-3-propionate 24

A solution of sulfuryl chloride (559 mg, 335 mm^3, 4.14 mmol) in anhydrous dichloromethane (4 cm^3) was added dropwise over 1 min to a stirred solution of the methyl pyrrole **9** (2 g, 4.36 mmol) in anhydrous dichloromethane (16 cm^3) under argon at 0 °C. After 1 h at 0 °C, the solution was evaporated and a solution of the residue in acetic acid (20 cm^3) was stirred with sodium acetate (1.18 g, 14.38 mmol) under argon at 60 °C for 90 min, then diluted with water (80 cm^3) and extracted with dichloromethane (3 × 20 cm^3). The combined organic extracts were washed with saturated aqueous sodium hydrogen

carbonate (2 × 50 cm^3) and then with water (30 cm^3), dried and evaporated to give the *acetoxymethylpyrrole* **24** as fine needles (2.12 g, 94%), mp 64.5–65.5 °C (from diethyl ether–hexane) (Found: M^+, 517.2121. $C_{26}H_{35}NO_8Si$ requires *M*, 517.2132); $\lambda_{max}(CH_2Cl_2)$/nm 269; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3420, 2940, 1760–1650s, 1200, 1170, 1070 and 835; $\delta_H(CDCl_3$, 400 MHz) 0.00 (9 H, s, Me_3Si), 0.95 (2 H, t, *J* 9, CH_2Si), 2.04 (3 H, s, Ac), 2.52 and 2.99 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.49 (2 H, s, CH_2CO_2), 3.60 (3 H, s, OMe), 4.12 (2 H, t, *J* 9, CH_2CH_2Si), 5.03 (2 H, s, CH_2OAc), 5.28 (2 H, s, CH_2Ph), 7.35 (5 H, m, Ph) and 9.18 (1 H, br s, NH); $\delta_C(CDCl_3$, 100 MHz) −1.56 (Me_3Si), 17.33 (CH_2Si), 20.40 ($CH_2CH_2CO_2$), 20.85 (CO*Me*), 29.81 (CH_2CO_2), 34.70 ($CH_2CH_2CO_2$), 51.44 (OMe), 56.91 (CH_2OAc), 63.28 and 66.14 (CH_2CH_2Si and CH_2Ph), 117.17, 119.08, 128.30, 128.39 (2 C), 128.61 (2 C), 128.79, 129.97 and 135.93 (C=C) and 160.43, 171.45, 171.51 and 173.49 (4 × C=O); *m*/*z* (FD) 517 (M^+, 100%).

5-Acetoxymethyl-3-(2-methoxycarbonylethyl)-4-(2-trimethylsilylethoxycarbonylmethyl)pyrrole-2-carboxylic acid 25

A solution of the pyrrole ester **24** (1 g, 1.93 mmol) in tetrahydrofuran (7 cm^3) was stirred with 10% palladium-on-charcoal (0.1 g) under an atmosphere of hydrogen at room temperature for 90 min and then filtered through Celite. The residue was washed with tetrahydrofuran (3 cm^3) and the combined filtrate and washings were evaporated and the residue was recrystallised from methyl acetate–hexane to give the *acid* **25** (0.81 g, 98%), mp 108–110 °C (decomp.) (Found: M^+, 427.1660. $C_{19}H_{29}NO_8Si$ requires *M*, 427.1662); $\lambda_{max}(CH_2Cl_2)$/nm 276; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3420, 3300–2500, 2940, 1725 br, 1660, 1170 and 835; $\delta_H(CDCl_3$, 400 MHz) 0.02 (9 H, s, Me_3Si), 0.98 (2 H, t, *J* 8, CH_2Si), 2.07 (3 H, s, Ac), 2.61 and 3.03 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.52 (2 H, s, CH_2CO_2), 3.65 (3 H, s, OMe), 4.15 (2 H, t, *J* 8, CH_2CH_2Si), 5.07 (2 H, s, CH_2OAc) and 9.30 (1 H, br s, NH); $\delta_C(CDCl_3$, 100 MHz) −1.56 (Me_3Si), 17.33 (CH_2Si), 20.27 ($CH_2CH_2CO_2$), 20.85 (CO*Me*), 29.81 (CH_2CO_2), 34.54 ($CH_2CH_2CO_2$), 51.53 (OMe), 56.97 (CH_2OAc), 63.36 (CH_2CH_2Si), 117.41, 118.42, 129.98 and 131.59 (C=C) and 165.43, 171.55 (2 C) and 173.71 (4 × C=O); *m*/*z* (FD) 427 (M^+, 100%).

Methyl 5-acetoxymethyl-2-*tert*-butoxycarbonyl-4-(2-trimethylsilylethoxycarbonylmethyl)pyrrole-3-propionate 26

A solution of the acid **25** (1 g, 2.34 mmol) in *tert*-butyl alcohol (8 cm^3) and dichloromethane (8 cm^3) was stirred with a solution of *N*,*N*′-dicyclohexylcarbodiimide (0.58 g, 2.81 mmol) in *tert*-butyl alcohol (2 cm^3) under argon for 90 min and then evaporated. The residue was purified by flash chromatography on silica, eluting with diethyl ether–hexane (1:1), and crystallisation from diethyl ether–hexane to give the *pyrrole ester* **26** (1.09 g, 96%) as needles, mp 69–71 °C (Found: M^+, 483.2297. $C_{23}H_{37}NO_8Si$ requires *M*, 483.2288); $\lambda_{max}(CH_2Cl_2)$/nm 269; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3430, 1723s br, 1690, 1170 and 835; $\delta_H(CDCl_3$, 400 MHz) 0.01 (9 H, s, Me_3Si), 0.97 (2 H, t, *J* 9, CH_2Si), 1.54 (9 H, s, Bu^t), 2.05 (3 H, s, Ac), 2.55 and 2.97 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.49 (2 H, s, CH_2CO_2), 3.64 (3 H, s, OMe), 4.13 (2 H, t, *J* 9, CH_2CH_2Si), 5.04 (2 H, s, CH_2OAc) and 9.13 (1 H, br s, NH); $\delta_C(CDCl_3$, 100 MHz) −1.55 (Me_3Si), 17.34 (CH_2Si), 20.50 ($CH_2CH_2CO_2$), 20.87 (CO*Me*), 28.38 (CMe_3), 29.85 (CH_2CO_2), 34.95 ($CH_2CH_2CO_2$), 51.44 (OMe), 56.95 (CH_2OAc), 63.24 (CH_2CH_2Si), 81.28 (CMe_3), 116.84, 120.81, 127.81 and 128.30 (C=C) and 160.36, 171.46, 171.63, 173.60 (4 × C=O); *m*/*z* (FD) 483 (M^+, 100%).

3-(2-Methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-pyrrole-2-carboxylic acid 28

A solution of the pyrrole ester **27**[20] (360 mg, 1.11 mmol) in dry dichloromethane (20 cm^3) was cooled to 0 °C and treated with stannic chloride (140 mm^3, 1.20 mmol) dropwise. The mixture was stirred for 2 h, then treated with 10% aqueous sodium acetate (20 cm^3) and stirred for a further 10 min. The layers were separated and the aqueous layer extracted with dichloromethane (2 × 50 cm^3). The combined organic layers were extracted with 10% aqueous sodium carbonate (3 × 50 cm^3) and these aqueous extracts were acidified with concentrate hydrochloric acid to pH 1. The precipitated solid was collected by filtration and dissolved in dichloromethane (4 × 50 cm^3). The solution was dried and evaporated and the residue was crystallised from dichloromethane–diethyl ether–hexane to give the *acid* **28** (233 mg, 78%), mp 140–142 °C (Found: MH^+, 270.0963. $C_{12}H_{15}NO_6$ requires $M + H$, 270.0978); $\lambda_{max}(CH_2Cl_2)$/nm 268; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3685, 1735, 1660, 1605, 1200 and 1175; $\delta_H(CDCl_3$, 400 MHz) 2.62 and 3.04 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.52 (2 H, s, CH_2CO_2), 3.65 and 3.69 (each 3 H, s, OMe), 6.91 (1 H, d, *J* 3, 5-H) and 9.27 (1 H, br s, NH); $\delta_C(CDCl_3$, 100 MHz) 20.21 ($CH_2CH_2CO_2$), 30.48 (CH_2CO_2), 34.56 ($CH_2CH_2CO_2$), 51.56 and 52.06 (OMe), 117.69, 118.54 and 131.31 (C=C), 122.95 (C-5) and 165.72, 172.38 and 173.84 (3 × C=O).

Methyl 4-methoxycarbonylmethyl-2-(2,2,2-tribromoethoxycarbonyl)pyrrole-3-propionate 29

A solution of acid **28** (150 mg, 0.558 mmol) in dry dichloromethane (4 cm^3) was cooled to 0 °C, treated with oxalyl chloride (146 mm^3, 1.67 mmol) and *N*,*N*-dimethylformamide (3 drops) (vigorous effervescence), stirred under argon at 0 °C for 30 min and evaporated. The residue was dissolved in dichloromethane (2 cm^3), stirred with 2,2,2-tribromoethanol (1.18 g, 4.18 mmol) and 4-dimethylaminopyridine (81.7 mg, 0.669 mmol) under argon for 30 min and then evaporated under reduced pressure. The residue was purified by flash chromatography on silica, eluting with diethyl ether–hexane (1:1), to give the *tribromoethyl ester* **29** as an oil (198 mg, 67%) (Found: MH^+, 531.8643. $C_{14}H_{16}{}^{79}Br_3NO_6$ requires $M + H$, 531.8608); $\lambda_{max}(CH_2Cl_2)$/nm 274; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3450, 1740, 1570, 1510, 1410, 1120 and 940; $\delta_H(CDCl_3$, 400 MHz) 2.64 and 3.09 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.53 (2 H, s, CH_2CO_2), 3.63 and 3.69 (each 3 H, s, OMe), 5.10 (2 H, s, CH_2CBr_3), 6.96 (1 H, d, *J* 3, 5-H) and 9.03 (1 H, br s, NH); $\delta_C(CDCl_3$, 100 MHz) 20.24 ($CH_2CH_2CO_2$), 30.44 (CH_2CO_2), 34.98 ($CH_2CH_2CO_2$), 35.83 (CH_2CBr_3), 51.52 and 52.07 (OMe), 76.87 (CH_2CBr_3), 117.78, 117.85 and 130.97 (C=C), 123.29 (C-5) and 159.00, 172.28 and 173.55 (3 × C=O).

Dimethyl 1-(*tert*-butoxycarbonyl)-7-methoxycarbonylmethyl-9-(2,2,2-tribromoethoxycarbonyl)3-(2-trimethylsilylethoxycarbonylmethyl)-5,10-dihydrodipyrrin-2,8-dipropionate 30

A solution of the α-free pyrrole **29** (599 mg, 1.12 mmol) and the acetoxymethylpyrrole **26** (542 mg, 1.12 mmol) in dichloromethane (7 cm^3) was stirred with toluene-*p*-sulfonic acid (21 mg, 0.11 mmol) under argon at room temperature for 2 h, then washed with 5% aqueous sodium carbonate (2 × 15 cm^3) followed by water (1 × 15 cm^3), dried and evaporated. The residue was purified by flash chromatography on silica, eluting with diethyl ether–hexane (1:1), to give the *dihydrodipyrrin* **30** as a foam (893 mg, 84%) (Found: M^+, 954.0590. $C_{35}H_{49}Br_3N_2O_{12}Si$ requires *M*, 954.0605); $\delta_H(CDCl_3$, 400 MHz) 0.05 (9 H, s, Me_3Si), 1.02 (2 H, t, *J* 8, CH_2Si), 1.51 (9 H, s, Bu^t), 2.52, 2.58, 2.98 and 3.10 (each 2 H, t, *J* 8, 2 × $CH_2CH_2CO_2$), 3.52 and 3.59 (each 2 H, s, CH_2CO_2), 3.62, 3.63 and 3.77 (each 3 H, s, OMe), 3.82 (2 H, s, 5-H_2), 4.26 (2 H, t, *J* 8, CH_2CH_2Si), 5.06 (2 H, s, CH_2CBr_3) and 10.11 and 10.47 (each 1 H, br s, NH); $\delta_C(CDCl_3$, 100 MHz) −1.44 (Me_3Si), 17.27 (CH_2Si), 20.40 and 22.58 (2 × $CH_2CH_2CO_2$), 28.39 (CMe_3), 29.24 and 29.76 (2 × CH_2CO_2), 35.00 (2 C) and 35.19 (2 × $CH_2CH_2CO_2$ and CH_2CBr_3), 51.41 (2 C) and 52.65 (3 × OMe), 64.02 (CH_2CH_2Si), 77.00 (CH_2CBr_3), 80.49 (CMe_3), 113.67, 114.22,

117.04, 119.90, 128.43, 130.63, 130.78 and 134.09 (C=C) and 158.84, 160.20, 173.56, 173.66 (2 C) and 173.74 (6 × C=O); *m/z* (FD) 954, 956, 958 and 960 (1:3:3:1, M^+, 100%).

2,8-Bis(2-methoxycarbonylethyl)-7-methoxycarbonylmethyl-9-(2,2,2-tribromoethoxycarbonyl)-3-(2-trimethylsilylethoxycarbonylmethyl)-5,10-dihydrodipyrrin-1-carboxylic acid 31

A solution of the dipyrromethane **30** (4.71 g, 4.92 mmol) in dry dichloromethane (33 cm^3) was stirred under argon at between −10 and −15 °C and stannic chloride (432 mm^3, 3.69 mmol) was added dropwise over 1 min. The solution was allowed to warm to 5 °C over 40 min and then treated with 5% aqueous sodium acetate (47 cm^3). After a further 5 min, the organic layer was separated and the aqueous layer was extracted with dichloromethane (5 × 50 cm^3). The combined organic layers were washed with brine (2 × 50 cm^3), dried and evaporated. The residue was purified by flash chromatography on silica, eluting with dichloromethane–methanol (19:1), to give the acid **31** (2.78 g, 63%), mp 154–155 °C (from methyl acetate–hexane); $\lambda_{max}(CH_2Cl_2)$/nm 287; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3400–2800, 3310, 2950, 1750–1630s, 1230, 1165 and 890; $\delta_H(CDCl_3$, 400 MHz) 0.05 (9 H, s, Me_3Si), 1.01 (2 H, t, *J* 8, CH_2Si), 2.56, 2.60, 3.02 and 3.10 (each 2 H, t, *J* 8, $CH_2CH_2CO_2$), 3.54 and 3.61 (each 2 H, s, CH_2CO_2), 3.62, 3.63 and 3.77 (each 3 H, s, OMe), 3.88 (2 H, s, 5-H_2), 4.24 (2 H, t, *J* 8, CH_2CH_2Si), 5.05 (2 H, s, CH_2CBr_3) and 10.34 and 10.66 (each 1 H, br s, NH); *m/z* (FD) 898, 900, 902 and 904 (1:3:3:1, M^+, 100%).

4-[5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-ylmethyl]-2,8-bis(2-methoxycarbonylethyl)-7-methoxycarbonylmethyl-9-(2,2,2-tribromoethoxycarbonyl)-3-(2-trimethylsilylethoxycarbonylmethyl)-4,5-dihydrodipyrrin-1(10*H*)-one 33

A solution of the acid **31** (250 mg, 0.278 mmol) in dichloromethane (1.7 cm^3) was stirred vigorously with a solution of sodium hydrogen carbonate (69.9 mg, 0.832 mmol) in water (1.3 cm^3) and an aqueous solution (2.78 cm^3) of iodine (0.1 mol dm^{-3}) and potassium iodide (0.2 mol dm^{-3}) was added over 5 min. After a further 35 min, solid sodium metabisulfite was added to destroy the excess iodine, the organic layer was separated and the aqueous layer was extracted with dichloromethane (3 × 1 cm^3). The combined organic layers were dried and evaporated under reduced pressure. The residual oil was purified by flash silica chromatography on silica, eluting with diethyl ether–hexane (3:2), to give the iododihydrodipyrrin **32** as a foam (241 mg) which was used directly in the next step.

A stirred solution of the iododihydrodipyrrin **32** (239 mg, 0.243 mmol) and the acetoxymethylpyrrole[8] **13** (105 mg, 0.243 mmol) in dry dichloromethane (2.5 cm^3) under argon at 0 °C and was treated with a solution of stannic chloride (28.4 mm^3, 0.243 mmol) in dichloromethane (1 cm^{-3}) and then, after 30 min, with saturated aqueous sodium hydrogen carbonate (2.6 cm^3). After a further 10 min, the organic layer was separated and the aqueous layer extracted with dichloromethane (3 × 5 cm^3). The combined organic layers were dried and evaporated under reduced pressure. The residual oil was dissolved in tetrahydrofuran (3.4 cm^3) and water (0.34 cm^3) and toluene-*p*-sulfonic acid monohydrate (66.2 mg, 0.35 mmol) and silver acetate (21.9 mg, 0.13 mmol) were added. The mixture was stirred under argon for 17 h, then diluted with water (18 cm^3) and extracted with dichloromethane (4 × 18 cm^3). The combined organic extracts were dried and evaporated and the residual oil was purified by flash chromatography on silica, eluting with diethyl ether–hexane (97:3) followed by diethyl ether, to give the *dipyrrolic lactam* **33** as a foam (104 mg, 30%); $\lambda_{max}(CH_2Cl_2)$/nm 284; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3410, 3290, 2940, 1710s, br, 1190, 1170 and 1075; $\delta_H(CDCl_3$, 400 MHz) 0.05 (9 H, s, Me_3Si), 1.05 (2 H, t, *J* 8, CH_2Si), 2.43–2.55 and 2.69–2.71 (each 6 H, m, 3 × $CH_2CH_2CO_2$), 2.74 and 3.04 (2 H, ABq, *J* 15) and 2.85 and 3.13 (2 H, ABq, *J* 15, CH_2CCH_2), 3.14 and 3.49 (2 H, ABq, *J* 16), 3.38 and 3.66 (2 H, ABq, *J* 18) and 3.73 and 3.83 (2 H, ABq, *J* 17, 3 × CH_2CO_2), 3.54, 3.59, 3.59, 3.60 and 3.63 (each 3 H, s, OMe), 4.29 (2 H, t, *J* 8, CH_2CH_2Si), 5.01 and 5.11 (2 H, ABq, *J* 12) and 5.18 and 5.27 (2 H, ABq, *J* 12, CH_2CBr_3 and CH_2Ph), 7.29–7.39 (5 H, m, Ph), 7.54 (1 H, br s, lactam-NH) and 9.22 and 10.36 (each 1 H, br s, pyrrole-NH); $\delta_C(CDCl_3$, 100 MHz) −1.47 (Me_3Si), 17.34 (CH_2Si), 19.28, 19.92 and 20.39 (3 × $CH_2CH_2CO_2$), 29.02, 30.46 (2 C), 30.58, 30.93, 32.95, 34.73, 34.98 and 36.07 (3 × CH_2CO_2, 3 × $CH_2CH_2CO_2$, CH_2CCH_2 and CH_2CBr_3), 51.47, 51.58, 51.80, 51.88 and 52.34 (5 × OMe), 65.17 and 65.73 (2 C) (CH_2CH_2Si, CH_2Ph and C-4), 76.88 (CH_2CBr_3), 115.99, 116.83, 119.44, 122.18, 122.51, 127.60, 128.18, 128.42, 128.49, 130.09, 136.00, 138.51 and 149.10 (C=C), 158.54 and 160.11 (pyrrole-CO_2) and 171.60, 171.75, 172.02, 173.35, 173.42 and 173.59 (6 × CO_2 and CONH); *m/z* (FD) 1241, 1243, 1245 and 1247 (1:3:3:1, M^+, 100%).

4-[5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-ylmethyl]-7-carboxymethyl-2,8-bis(2-methoxycarbonylethyl)-9-(2,2,2-tribromoethoxycarbonyl)-3-(2-trimethylsilylethoxycarbonylmethyl)-4,5-dihydrodipyrrin-1(10*H*)-one 6

A solution of ester **33** (419 mg, 0.33 mmol) in tetrahydrofuran (12 cm^3) was stirred with tetrabutylammonium fluoride trihydrate (316 mg, 1.00 mmol) under argon at room temperature for 1 h, then mixed with dichloromethane (15 cm^3) and washed with dilute sulfuric acid (pH 3.0–3.5; 2 × 10 cm^3) followed by water (10 cm^3), dried and evaporated. The residue was purified by flash chromatography on silica, eluting with dichloromethane–methanol (9:1), to give the acid **6** as a glass (271 mg, 71%) which was used without further purification to make the following series of esters; $\delta_H(CDCl_3$, 400 MHz) 2.21–3.93 (37 H, series of br m, 3 × CH_2CO_2, 3 × $CH_2CH_2CO_2$, CH_2CCH_2 and 5 × OMe), 4.95–5.33 (4 H, br m, CH_2Ph and CH_2CBr_3), 7.20–7.46 (5 H, m, Ph), 8.00 (1 H, br s, lactam-NH) and 9.45 (2 H, br s, 2 × pyrrole-NH); *m/z* (FD) 1141, 1143, 1145 and 1147 (1:3:3:1, M^+, 50%) and 1097, 1099, 1101 and 1103 (1:3:3:1, M − CO_2 100%).

4-[5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-ylmethyl]-2,8-bis(2-methoxycarbonylethyl)-7-methoxycarbonylmethyl-3-[(1*S*)-1-phenylethoxycarbonylmethyl]-9-(2,2,2-tribromoethoxycarbonyl)-4,5-dihydrodipyrrin-1(10*H*)-one 34

A solution of acid **6** (14.6 mg, 13 μmol) in dry dichloromethane (0.5 cm^3) was stirred with 1-chloro-1-dimethylamino-2-methylprop-1-ene[13] (3.42 mg, 20 μmol) under argon at room temperature for 20 min and then treated with (*S*)-(−)-1-phenylethanol (18.0 mg, 147 μmol). After a further 2.5 h, the solvent was evaporated under reduced pressure and the residue was purified by preparative TLC, eluting with dichloromethane–methanol (19:1), to give recovered acid **6** (3.2 mg, 22%) and ester **34** (6.0 mg, 36%) as an oil, shown by ^1H NMR to be a mixture of two diastereoisomers (1:1), contaminated with byproducts giving NMR signals indicating that they had a 2,2-dibromoethenyl group and a 2,2-dibromoethyl group, respectively, replacing the 2,2,2-tribromoethyl residue of the main products. This interpretation is supported by the isolation and characterisation of analogous byproducts in a later experiment; $\lambda_{max}(CH_2Cl_2)$/nm 279 and 219; $\delta_H(CDCl_3$, 400 MHz) 1.59–1.63 (6 H, m, 2 × PhCH*Me*), 2.33–3.14 and 3.32–3.85 (44 H, series of m, 6 × $CH_2CH_2CO_2$, 2 × CH_2CCH_2 and 6 × CH_2CO_2), 3.47–3.64 (30 H, m, 10 × OMe), 5.00–5.12 (4 H, m, 2 × CH_2CBr_3) and 5.17 and 5.27 (each 2 H, ABq, *J* 13, 2 × CH_2Ph), 5.98–6.05 (2 H, m, 2 × PhC*H*Me), 7.29–7.37 (20 H, m, 4 × Ph), 7.48 and 7.53

(each 1 H, br s, lactam-NH), 9.17 and 9.27 (each 1 H, br s, pyrrole-NH) and 10.16 (2 H, br s, 2 × pyrrole-NH) [byproducts: 4.63–4.74 (m, 2 × CH_2CHBr_2) and 5.83 (m, 2 × $CHBr_2$) (*ca.* 22%); 8.01 and 8.06 (each s, 2 × $CH{=}CBr_2$) (*ca.* 22%)]; *m/z* (FD) 1245, 1247, 1249 and 1251 (1:3:3:1, M^+, 100%).

4-[5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-ylmethyl]-2,8-bis(2-methoxycarbonylethyl)-7-methoxycarbonylmethyl-9-(2,2,2-tribromoethoxycarbonyl)-3-{(1*S*,2*R*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yloxycarbonylmethyl}-4,5-dihydrodipyrrin-1(10*H*)-one 35

A solution of acid **6** (11 mg, 10 μmol) in dry dichloromethane (0.5 cm^3) was stirred with 1-chloro-1-dimethylamino-2-methylprop-1-ene[13] (2.6 mg, 19 μmol) under argon at room temperature for 30 min and then treated with (1*S*)-*endo*-borneol (27 mg, 220 μmol). After a further 2 h, the solvent was evaporated under reduced pressure and the residue was purified by preparative TLC, eluting with dichloromethane–methanol (24:1), to give recovered acid **6** (3.2 mg, 29%) and ester **35** (4.1 mg, 32%) as an oil, shown by ^{1}H NMR to be a mixture of two diastereoisomers (1:1) contaminated with byproducts analogous to those reported in the foregoing experiment; $\lambda_{max}(CH_2Cl_2)$/nm 285 and 218; $\delta_H(CDCl_3$, 400 MHz) 0.84 and 0.86 (each 3 H, s, 2 × bornyl-Me), 0.87 (12 H, s, 4 × bornyl-Me), 0.98–1.07, 1.24–1.43, 1.58–1.83, 1.93–2.03 and 2.31–2.42 (14 H, m, 6 × bornyl-CH_2 and 2 × bornyl-CH), 2.42–2.57, 2.70–2.74 and 2.82–3.05 (28 H, m, 6 × $CH_2CH_2CO_2$ and 2 × 5-CH_2), 2.85 and 3.17 (each 1 H, ABq, *J* 15, 5-CH_2), 2.85 and 3.17 (each 1 H, ABq, *J* 15, 5-CH_2), 3.16 and 3.43 (each 1 H, ABq, *J* 15, CH_2CO_2), 3.16 and 3.44 (each 1 H, ABq, *J* 15, CH_2CO_2), 3.48–3.75 (6 H, m, 3 × CH_2CO_2), 3.54–3.64 (30 H, m, 10 × OMe), 3.65 and 3.66 (each 1 H, ABq, *J* 15, CH_2CO_2), 4.97–5.16 (6 H, m, 2 × CH_2CBr_3 and 2 × bornyl-CHO), 5.18–5.27 (4 H, 2 × ABq, *J* 12, 2 × CH_2Ph), 7.26–7.39 (10 H, m, 2 × Ph), 7.54 (2 H, s, 2 × lactam-NH), 9.28 and 10.28 (each 2 H, br s, 4 × pyrrole-NH) [impurities: 4.60–4.76 (ABq of d, *J* 11 and 7, 2 × CH_2CHBr_2) and 5.79 and 5.80 (each t, *J* 7, 2 × $CHBr_2$) (*ca.* 21%); 8.01 (s, 2 × $CH{=}CBr_2$) (*ca.* 22%)]; *m/z* (FD) 1277, 1279, 1281 and 1283 (1:3:3:1, M^+, 100%).

4-[5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-ylmethyl]-3-[(3*R*)-4,4-dimethyl-2-oxotetrahydrofuran-3-yloxycarbonylmethyl]-2,8-bis(2-methoxycarbonylethyl)-7-methoxycarbonylmethyl-9-(2,2,2-tribromoethoxycarbonyl)-4,5-dihydrodipyrrin-1(10*H*)-one 36

A solution of acid **6** (21.1 mg, 22 μmol) in dry dichloromethane (1 cm^3) was stirred with 1-chloro-1-dimethylamino-2-methylprop-1-ene[13] (12.1 mg, 90 μmol) under argon at room temperature for 20 min, then treated with (*R*)-(−)-pantolactone‡ (80 mg, 0.61 mmol). After a further 30 min, the solvent was evaporated and the residue was purified by preparative TLC, eluting with dichloromethane–methanol (9:1), to give recovered acid **6** (7 mg, 33%) and the ester **36** (6 mg, 25%) as an oil, shown by ^{1}H NMR to be a mixture of two diastereoisomers (1:1) contaminated with small amounts of dibromo byproducts analogous to those seen earlier; $\lambda_{max}(CH_2Cl_2)$/nm 284 and 218. This mixture was purified by HPLC on a Spherisorb S5CN semi-preparative column, eluting with diethyl ether–ethyl acetate (3:1), to give the pure separate diastereoisomers of *ester* **36** as oils.

First eluted diastereoisomer: $\delta_H(CDCl_3$, 400 MHz) 1.19 and 1.22 (each 3 H, s, CMe_2), 2.49–2.57 and 2.68–3.23 (17 H, series of m, 3 × $CH_2CH_2CO_2$, CH_2CCH_2 and $CH_AH_BCO_2$), 3.50 (1 H, d, *J* 17, $CH_AH_BCO_2$), 3.57, 3.58, 3.59, 3.61 and 3.64 (each 3 H, s, OMe), 3.64–3.88 (4 H, m, 2 × CH_2CO_2), 4.04–4.09 (2 H, ABq, *J* 9, lactone-CH_2), 5.06 (2 H, s, CH_2CBr_3) and 5.18–5.28 (2 H, ABq, *J* 12, CH_2Ph), 5.46 (1 H, s, lactone-CH), 7.28–7.39 (5 H, m, Ph), 7.43 (1 H, br s, lactam-NH) and 9.35 and 9.92 (each 1 H, br s, pyrrole-NH); *m/z* (FD) 1253, 1255, 1257 and 1259 (1:3:3:1, M^+, 100%).

Second eluted diastereoisomer: $\delta_H(CDCl_3$, 400 MHz) 1.22 and 1.27 (each 3 H, s, CMe_2), 2.45–2.56, 2.71–2.78 and 2.95–3.21 (17 H, series of m, 3 × $CH_2CH_2CO_2$, CH_2CCH_2 and $CH_AH_BCO_2$), 3.50 (1 H, d, *J* 16, $CH_AH_BCO_2$), 3.56, 3.58, 3.60, 3.61 and 3.63 (each 3 H, s, OMe), 3.69–3.96 (4 H, m, 2 × CH_2CO_2), 4.05–4.10 (2 H, ABq, *J* 9, lactone-CH_2), 5.05 (2 H, s, CH_2CBr_3) and 5.19–5.28 (2 H, ABq, *J* 12, CH_2Ph), 5.50 (1 H, s, lactone-CH), 7.30–7.42 (5 H, m, Ph), 7.67 (1 H, br s, lactam-NH) and 9.09 and 10.14 (each 1 H, br s, pyrrole-NH); *m/z* (FD) 1253, 1255, 1257 and 1259 (1:3:3:1, M^+, 100%).

4-[5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-ylmethyl]-3-[(2*R*,3*R*,4*R*)-3,4-isopropylidenedioxy-5-oxotetrahydrofuran-2-ylmethoxycarbonylmethyl]-2,8-bis(2-methoxycarbonylethyl)-7-methoxycarbonylmethyl-9-(2,2,2-tribromoethoxycarbonyl)-4,5-dihydrodipyrrin-1(10*H*)-one 37

A solution of acid **6** (8.39 mg, 8 μmol) in dry dichloromethane (0.5 cm^3) was stirred with 1-chloro-1-dimethylamino-2-methylprop-1-ene[13] (2.95 mg, 22 μmol) under argon at room temperature for 10 min, then treated with 2,3-*O*-isopropylidene-D-(+)-ribono-1,4-lactone[21] (24.4 mg, 0.10 mmol). After a further 1 h, the solvent was evaporated and the residue purified by preparative TLC, eluting with dichloromethane–methanol (19:1), to give the ester **37** (4.3 mg, 43%) as an oil, shown by ^{1}H NMR to be a mixture of two diastereoisomers (1:1) contaminated with small amounts of dibromo byproducts analogous to those seen earlier; $\lambda_{max}(CH_2Cl_2)$/nm 284 and 218 nm; $\delta_H(CDCl_3$, 400 MHz) 1.40 and 1.49 (12 H, 2 × s, 2 × CMe_2), 2.33–2.56, 2.68–2.72, 2.83–2.86, 2.98–3.06, 3.14–3.19 and 3.41–3.79 (78 H, series of m, 6 × $CH_2CH_2CO_2$, 2 × CH_2CCH_2, 6 × CH_2CO_2 and 10 × OMe), 4.22–4.40 (4 H, m, 2 × lactone-CH_2), 4.76–4.83 (4 H, m, 2 × lactone-3- and -4-H), 4.99 and 5.01 (2 H, ABq, *J* 9, CH_2CBr_3), 5.04–5.06 (4 H, m, CH_2CBr_3 + 2 × lactone-2-H), 5.17 and 5.26 (2 H, ABq, *J* 12, CH_2Ph), 5.18 and 5.26 (2 H, ABq, *J* 12, CH_2Ph), 7.27–7.38 (10 H, m, 2 × Ph), 7.51 and 7.67 (each 1 H, br s, lactam-NH) and 9.18, 9.41, 9.96 and 10.17 (each 1 H, br s, pyrrole-NH) [impurities: 4.65–4.71 (m, CH_2CHBr_2) and 5.04–5.06 (m, $CHBr_2$) (*ca.* 15%); 8.01 and 8.02 (each s, $CH{=}CBr_2$) (*ca.* 25%)]; *m/z* (FD) 1311, 1313, 1315 and 1317 (1:3:3:1, M^+, 100%).

3-{(3*R*,4*R*,5*R*)-4,5-[(*R*)-Benzylidenedioxy]-2-oxotetrahydropyran-3-yloxycarbonylmethyl}-4-[5-benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-ylmethyl]-2,8-bis(2-methoxycarbonylethyl)-7-methoxycarbonylmethyl-9-(2,2,2-tribromoethoxycarbonyl)-4,5-dihydrodipyrrin-1(10*H*)-one 38

A solution of acid **6** (500 mg, 0.438 mmol) in dry dichloromethane (20 cm^3) was stirred with 1-chloro-1-dimethylamino-2-methylprop-1-ene[13] (221 mg, 1.581 mmol) under argon at room temperature for 9 min, then treated with 3,4-*O*-benzylidene-D-ribonic δ-lactone (550 mg, 2.328 mmol). After a further 3 h, the solvent was evaporated and the residue purified by flash chromatography on silica, eluting with dichloromethane–methanol (19:1), to give a mixture of the diastereoisomeric esters **38** as an oil (537 mg, 90%). An aliquot (106 mg) was purified by HPLC on a Spherisorb S5CN semi-preparative column, eluting with diethyl ether–ethyl acetate (3:1), to give four fractions, as follows.

(i) The *ester* **38b** (peak Y in Fig. 1) as an oil (27.6 mg, 23%) (Found: MH^+, 1360.1447. $C_{57}H_{60}Br_3N_3O_{21}$ requires *M* + *H*, 1360.1349); λ_{max}(MeCN)/nm 283; $\delta_H(CDCl_3$, 400 MHz) 2.35–2.67 (10 H, m, $CH_2CH_2CO_2$), 2.82 and 3.07 (2 H, ABq, *J* 15) and 2.86 and 3.10 (2 H, ABq, *J* 15, CH_2CCH_2), 3.00 (2 H, m, $CH_2CH_2CO_2$), 3.32 and 3.42 (2 H, ABq, *J* 17, CH_2CO_2), 3.51,

3.54, 3.55, 3.58, 3.60 (each 3 H, s, OMe), 3.68 and 3.79 (2 H, ABq, J 18, CH_2CO_2), 3.69 (2 H, s, CH_2CO_2), 4.33 and 4.53 (2 H, ABq, J 13, lactone-6-H_2), 4.55 (1 H, d, J 8, lactone-5-H), 4.81 (1 H, dd, J 8 and 3, lactone-4-H), 5.00 and 5.06 (2 H, ABq, J 12, CH_2CBr_3), 5.12 and 5.19 (2 H, ABq, J 12, CH_2Ph), 5.61 (1 H, d, J 3, lactone-3-H), 5.74 (1 H, s, C*H*Ph), 7.25–7.43 (10 H, m, 2 × Ph) 7.48 (1 H, br s, lactam-NH) and 9.69 and 10.04 (each 1 H, br s, pyrrole-NH); δ_C($CDCl_3$, 100 MHz) 19.18, 19.79 and 20.53 (3 × $CH_2CH_2CO_2$), 29.28, 29.70, 30.28, 30.69, 31.02, 31.44, 34.64, 34.90 and 35.97 (3 × $CH_2CH_2CO_2$, 3 × CH_2CO_2, CH_2CCH_2, CBr_3), 51.50, 51.63, 51.80, 51.85 and 52.31 (5 × OMe), 65.81, 66.18, 67.62, 70.16, 73.38 and 74.10 (ribonic lactone, C-4 and CH_2Ph), 76.75 (CH_2CBr_3), 104.82 (CHPh), 116.53, 117.00, 119.25, 122.40 (2 C), 127.32 (2 C), 128.14 (2 C), 128.48 (2 C), 128.61 (2 C), 130.46 (2 C), 130.57, 131.41, 135.54, 138.08, 138.41 and 148.56 (C=C) and 158.81, 160.63, 165.59, 170.66, 171.83, 172.02, 173.00, 173.34, 173.55 and 173.94 (10 × C=O); m/z (FD) 1359, 1361, 1363 and 1365 (1:3:3:1, M^+, 100%).

(ii) The *ester* **38a** (peak X in Fig. 1) as an oil (25.4 mg, 22%) (Found: MH^+, 1360.1229); λ_{max}(MeCN)/nm 283; δ_H($CDCl_3$, 400 MHz) 2.39–2.54 (8 H, m) and 2.69 (2 H, t, 7, $CH_2CH_2CO_2$), 2.78 and 3.15 (2 H, ABq, J 16, CH_2CCH_2), 2.95–3.06 (4 H, m, $CH_2CH_2CO_2$ and CH_2CCH_2), 3.15 and 3.48 (2 H, ABq, J 16, CH_2CO_2), 3.55, 3.56, 3.56, 3.57, 3.58 (each 3 H, s, OMe), 3.68 and 3.87 (2 H, ABq, J 18, CH_2CO_2), 3.71 and 3.81 (2 H, ABq, J 18, CH_2CO_2), 4.40 and 4.61 (2 H, ABq, J 13, lactone-6-H_2), 4.73 (1 H, d, J 8, lactone-5-H), 4.94 (1 H, dd, J 8 and 3, lactone-4-H), 4.99 and 5.08 (2 H, ABq, J 12, CH_2CBr_3), 5.17 and 5.25 (2 H, ABq, J 12, CH_2Ph), 5.64 (1 H, d, J 3, lactone-3-H), 5.83 (1 H, s, C*H*Ph), 7.28–7.47 (10 H, m, 2 × Ph), 7.69 (1 H, br s, lactam-NH) and 9.24 and 9.89 (each 1 H, br s, pyrrole-NH); δ_C($CDCl_3$, 100 MHz) 19.28, 20.00 and 20.45 (3 × $CH_2CH_2CO_2$), 29.10, 30.12, 30.49, 30.61, 31.03, 32.94, 34.80, 35.05 and 36.05 (3 × $CH_2CH_2CO_2$, 3 × CH_2CO_2, CH_2CCH_2, CBr_3), 51.49, 51.64, 51.70, 51.85 and 52.37 (5 × OMe), 65.82, 66.02, 67.74, 70.22, 73.34 and 74.12 (ribonic lactone, C-4 and CH_2Ph), 76.71 (CH_2CBr_3), 105.07 (CHPh), 116.19, 116.72, 119.37, 122.52, 122.63, 127.32 (2 C), 127.88, 128.20, 128.39 (2 C), 128.53 (2 C), 128.63 (2 C), 130.48, 130.57, 131.10, 134.40, 136.03, 138.83 and 148.31 (C=C) and 158.82, 160.30, 165.22, 171.15, 171.75, 171.83, 173.30, 173.47 and 173.53 (2 C) (10 × C=O); m/z (FD) 1359, 1361, 1363 and 1365 (1:3:3:1, M^+, 100%).

(iii) The 2,2-*dibromoethenyl ester* **39a** (peak P in Fig. 1) as an oil (7.6 mg, 7%); λ_{max}(CH_2Cl_2)/nm 289; δ_H($CDCl_3$, 400 MHz) 2.41–2.69 and 2.96–3.01 (12 H, m, 3 × $CH_2CH_2CO_2$), 2.82 and 3.11 (2 H, ABq, J 15) and 2.85 and 3.14 (2 H, ABq, J 15, CH_2CCH_2), 3.23 and 3.48 (2 H, ABq, J 17, CH_2CO_2), 3.54, 3.56, 3.57, 3.61 and 3.62 (each 3 H, s, OMe), 3.68 and 3.87 (2 H, ABq, J 18, CH_2CO_2), 3.75 (2 H, s, CH_2CO_2), 4.37 and 4.61 (2 H, ABq, J 13, lactone-6-H_2), 4.70 (1 H, d, J 8, lactone-5-H), 4.89 (1 H, dd, J 8 and 3, lactone-4-H), 5.18 and 5.24 (2 H, ABq, J 12, CH_2Ph), 5.57 (1 H, d, J 3, lactone-3-H), 5.80 (1 H, s, C*H*Ph), 7.28–7.45 (11 H, m, 2 × Ph and lactam-NH), 8.02 (1 H, s, CH=CBr_2) and 9.38 and 9.80 (each 1 H, br s, pyrrole-NH); m/z (FD) 1279, 1281 and 1283 (1:2:1, M^+, 100%).

(iv) The 2,2-*dibromoethenyl ester* **39b** (peak Q in Fig. 1) as an oil (4.7 mg, 4%); λ_{max}(CH_2Cl_2)/nm 289; δ_H($CDCl_3$, 400 MHz) 2.39–2.58, 2.67–2.73 and 2.95–3.05 (15 H, m, 3 × $CH_2CH_2CO_2$ and $CH_2CCH_AH_B$), 3.12 (1 H, d, J 17, $CH_AH_BCO_2$), 3.16 (1 H, d, J 15, $CH_2CCH_AH_B$), 3.46–3.98 (5 H, m, $CH_AH_BCO_2$ and 2 × CH_2CO_2), 3.53, 3.55, 3.56, 3.58 and 3.59 (each 3 H, s, OMe), 4.39 and 4.63 (2 H, ABq, J 13, lactone-6-H_2), 4.77 (1 H, d, J 7, lactone-5-H), 4.98 (1 H, dd, J 8 and 3, lactone-4-H), 5.18 and 5.27 (2 H, ABq, J 12, CH_2Ph), 5.60 (1 H, d, J 3, lactone-3-H), 5.85 (1 H, s, C*H*Ph), 7.27–7.65 (10 H, m, 2 × Ph), 7.65 (1 H, br s, lactam-NH), 8.01 (1 H, s, CH=CBr_2) and 8.99 and 10.11 (each 1 H, br s, pyrrole-NH); m/z (FD) 1279, 1281 and 1283 (1:2:1, 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(10*H*)-one 40

A solution of the resolved lactam **38b** (75.4 mg, 55 μmol) in methanol (6.5 cm^3) and tetrahydrofuran (1.2 cm^3) was stirred with a solution of sodium methoxide (1.19 mg, 22 μmol) in methanol (0.1 cm^3) under argon at room temperature for 25 min. Water (5 cm^3) was added, the pH was adjusted to *ca.* 4 with glacial acetic acid and the solution was extracted with dichloromethane (4 × 4 cm^3). The combined extracts were washed with water (1 × 2.5 cm^3), dried and evaporated. The residue was purified by preparative TLC, eluting with diethyl ether–methanol (19:1), to give the *lactam* **40b** (61.9 mg, 97%) as a glass (Found: M^+, 1155.0782. $C_{46}H_{52}Br_3N_3O_{17}$ requires M, 1155.0847).

The resolved *lactam* **38a** (94.0 mg, 69 μmol) was similarly converted into the enantiomeric *lactam* **40a** (70.4 mg, 88%) (Found: M^+, 1155.0782). Both enantiomers **40** were identical (apart from optical properties) to authentic racemic material[7] by TLC [run separately and in admixture, R_f 0.32, diethyl ether–methanol (19:1)], ^1H and ^{13}C NMR and UV spectroscopy.

4,19-Methylene-2,8,13,18-tetrakis(2-methoxycarbonylethyl)-3,7,12,17-tetrakis(methoxycarbonylmethyl)bilan-1(4*H*)-one 54

The conversion of the resolved *lactams* **40** into the respective enantiomers of the spiro lactam **54** was accomplished using the chemistry and procedures developed for the racemic series.[7] At each stage of the sequence **47** ⟶ **48** ⟶ **49** ⟶ **50** ⟶ **51**, the pure enantiomers were shown by TLC, ^1H NMR spectroscopy and mass spectrometry to match authentic samples of their racemic analogues which had been rigorously characterised.[7] Finally, the spiro lactams **54b** (Found: M^+, 964.3916. $C_{48}H_{60}N_4O_{17}$ requires M, 964.3954) and **54a** (Found: M^+, 964.3905) were both identical to authentic racemic material by TLC [run separately and in admixture, R_f 0.37, diethyl ether–methanol (19:1)], UV and ^1H and ^{13}C NMR spectroscopy.[7]

As described in the text, the first time the conversion of **48b** into **50b** was attempted under the published conditions,[7] the main product after the hydrogenation step was the *bis(α-free) compound* **53b** (Found: M^+, 952.3946. $C_{47}H_{60}N_4O_{17}$ requires M, 952.3954); δ_H($CDCl_3$, 400 MHz) 2.24–2.40 and 2.51–2.72 (18 H, m, 4 × $CH_2CH_2CO_2$ and $CH_AH_BCCH_AH_B$), 2.91 and 2.92 (each 1 H, d, J 15, $CH_AH_BCCH_AH_B$), 3.25 (1 H, d, J 16, $CH_AH_BCO_2$), 3.34–3.77 (9 H, m, $CH_AH_BCO_2$, 3 × CH_2CO_2 and 10-H_2), 3.62 (6 H, s, 2 × OMe), 3.65 (9 H, s, 3 × OMe), 3.68, 3.71 and 3.74 (each 3 H, s, OMe), 6.43 (2 H, m, 2 × α-H), 7.12 (s, lactam-NH) and 8.55, 8.68 and 9.22 (each 1 H, br s, pyrrole-NH); m/z (FD) 952 (100%).

This undesired process could be almost completely avoided simply by carrying out the iodination in the normal way[7] except running the reaction at room temperature for just 20 min, followed by work-up and hydrogenation. This gave the desired product **50b** (86%) along with a very small amount of bis(α-free) product **53b** (trace to 7% in different runs).

For the conversion of the bis(α-free) product **53b** into the spiro lactam **54b**, paraformaldehyde (5.6 mg) was first stirred with trifluoroacetic acid (10 cm^3) at room temperature for 30 min. An aliquot of this solution (0.2 cm^3) and trifluoroacetic acid (0.88 cm^3) were stirred with a solution of the bis(α-free) tripyrrolic lactam **53b** (1.8 mg, 1.9 μmol) in methanol (83 mm^3) under argon at room temperature. After 20 min, a further aliquot (0.2 cm^3) of the paraformaldehyde–trifluoroacetic acid solution was added. After a further 35 min, the solution was evaporated and the residue was dissolved in dichloromethane (2 cm^3), washed with water (1 cm^3) followed by 5% aqueous sodium hydrogen carbonate (1 cm^3), dried and evaporated.

The residue was purified by preparative TLC, eluting with dichloromethane–methanol (19:1), to give the spiro lactam **54b** (0.5 mg, 27%) identified by direct chromatographic and spectroscopic comparison with authentic material.

Enzymic studies

Each enantiomer, **54a** and **54b**, of the spiro lactam was hydrolysed with aqueous methanolic potassium hydroxide exactly as described for racemic **54**.[7] These conditions had been shown to hydrolyse only the eight methyl ester groups. The solutions of the potassium salts of the enantiomeric octa-acids, **4a** and **4b**, after adjustment of the pH as earlier, were used for inhibition experiments on cosynthetase with synthetic hydroxymethylbilane **1** as substrate. The methods and controls developed for the study of racemic **4**, which have been fully described,[7] were followed. The data so obtained are presented as Dixon plots in Fig. 3, from which the K_i values in Table 1 are derived.

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