Biosynthesis of porphyrins and related macrocycles. Part 52.[1,2] Synthesis of (11-*S*)-[11-$^2H_1$]porphobilinogen and the (11*R*)-enantiomer for stereochemical studies on hydroxymethylbilane synthase (porphobilinogen deaminase)

Werner L. Neidhart, Paul C. Anderson, Graham J. Hart and Alan R. Battersby *

University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW

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A synthetic route is devised for the synthesis of (11*S*)-[11-$^2H_1$]porphobilinogen **1a** and of the (11*R*)-enantiomer **1b**. Their absolute configurations and enantiomeric purity are established by degradation to a derivative of [2-$^2H_1$]glycine of known stereochemistry. Methods are then developed, based on the synthesis of chiral imidate esters, for determination of the configuration of [$^2H_1$]-labelled aminomethylpyrroles by converting them into [$^2H_1$]-labelled amidines followed by analysis using ^{1}H-NMR. The labelled samples of PBG **1a** and **1b** serve as substrates for hydroxymethylbilane synthase and the products are trapped as [$^2H_1$]-labelled aminomethylbilanes **7c** and **7d**. Their configurations are determined by the NMR assay to demonstrate that as PBG **1** is enzymically converted into the aminomethylbilane **7**, there is overall retention of configuration at the aminomethyl carbon.

All the natural porphyrins, chlorins, corrins and their relatives are biosynthesised[3] from the same parent, uroporphyrinogen III **9**, shortened to uro'gen III, Scheme 1. This is built from porphobilinogen **1**, PBG, by the sequential action of two enzymes. The first, hydroxymethylbilane synthase, E.C.4.3.1.8 (formerly called PBG deaminase) assembles four PBG units **1** head-to-tail to generate hydroxymethylbilane **6**. This is then converted into uro'gen III **9** by a remarkable ring-closure and rearrangement process catalysed by the second enzyme, uroporphyrinogen III synthase, usually called cosynthetase.[3]

Earlier studies[3] showed that hydroxymethylbilane synthase is an unusual enzyme in that it binds its substrate covalently and, surprisingly, that the first PBG unit becomes attached to a novel dipyrrolic cofactor **2** present in the enzyme and covalently bound to it. Thus is formed the ES_1 complex **3**. Three further molecules of PBG are then added stepwise to generate the ES_2, ES_3 and finally the ES_4 complex **4** from which hydroxymethylbilane **6** is released so regenerating the unloaded enzyme **2** for a further cycle. There is strong evidence[4] that release of the bilane **6** from the ES_4 complex **4** occurs *via* the azafulvene **5**. This then reacts with water to form the hydroxymethylbilane **6** but it can also be trapped as **7** or **8** by including the better nucleophiles ammonia or hydroxylamine, respectively, in the incubation medium.[4,5] In addition, there is support for the view that the four interpyrrolic methylene groups of uro'gen III **9** are generated in a stereospecific way[6,7] but at none of them is it known whether the building process from PBG **1** involves overall retention or inversion of configuration.

The aim of the work described in the present paper was to determine the overall stereochemistry of the steps by which hydroxymethylbilane synthase generates the aminomethyl group of the bilane **7** formed by trapping the azafulvene **5** with ammonia. The amine **7** was chosen for this initial foray because it cyclises non-enzymically to uro'gen I **10** much less rapidly than does the hydroxymethylbilane **6** thus allowing sufficient quantities of material to be accumulated for study by NMR. The first requirement was the development of syntheses of PBG (as **1**) having C-11 labelled stereospecifically, or at least stereoselectively, with an appropriate isotope.

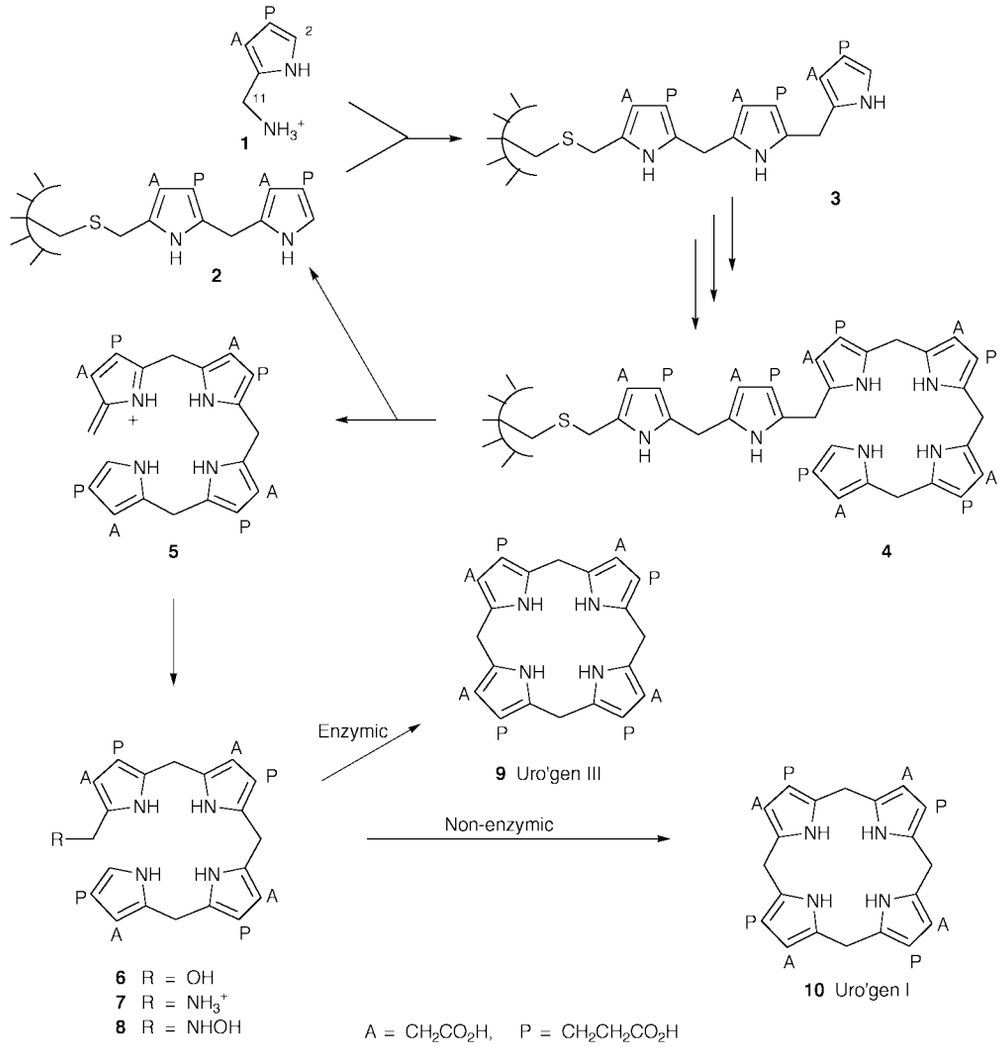
Results and discussion

Synthesis of (11*S*)-[11-$^2H_1$]PBG 1a and its (11*R*)-enantiomer 1b

The synthetic route to the labelled samples of PBG **1a** and **1b** is shown in Scheme 2. The first approach was subsequently superseded but it will be briefly described, without experimental detail, as it led to helpful findings. It involved condensation of the hydrazine **11a**, derived[8] from commercially available (−)-(1*R*,2*S*)-ephedrine, with the deuteriated aldehyde[9] **12a**; the unlabelled aldehyde **12** had been prepared earlier.[10] The hydrazone **13c** was reduced by cyanoborohydride with toluene-*p*-sulfonic acid as the proton source to yield the hydrazine containing **14a** and **14b**. An unlabelled sample **14** prepared in the same way from the aldehyde **12** gave well separated signals in its ^{1}H-NMR spectrum from the two diastereotopic protons of the $RNHCH_2$-group. The method for analysis of the products in the ^{2}H-labelled series was thus available and the foregoing labelled hydrazine was shown in this way to consist of the two diastereoisomers **14b** and its epimer at C-11 **14a** in the ratio 4:1. Proof that the major one was **14b** will be given later.

The improved synthetic route started with unlabelled hydrazone **13a** (97% yield from **11a** and **12**) which was reduced with cyanoborodeuteride, importantly in the presence of deuteriated toluene-*p*-sulfonic acid[11] ($ArSO_3D{\cdot}D_2O$). When $ArSO_3H{\cdot}H_2O$ was used, the product contained 40–50% of unlabelled material, no doubt due to the reducing agent undergoing rapid exchange of H for D.[12] Analysis of the product from the former conditions showed surprisingly that it contained equal amounts of the two diastereoisomers **14a** and **14b**. It turned out that this was a result of the larger quantities and so higher concentrations used initially for the improved route as compared with those for the first approach. Study of this concentration effect led to optimum conditions being selected for the reduction which afforded a 92% yield of product shown by ^{1}H-NMR to contain only 5% of undeuteriated molecules and of the deuteriated material, 83% was shown later to be **14a** and 17% was the isomer **14b**.

A strictly complementary synthesis starting with the aldehyde **12** and the hydrazine **11b**, prepared from (+)-(1*S*,2*R*)-ephedrine, yielded a mixture of the diastereoisomers **14c** and



Scheme 1

14d. The former will be shown below to make up 83% of the deuteriated materials which again formed 95% of the total product. The diastereoselectivity achieved in the two foregoing reductions was thus amply high enough to allow the problem outlined in the introduction to be solved. From now on, in order to avoid constant repetition, it will be taken as understood that in all cases the major enantiomer or diastereoisomer present is being discussed.

The stereochemical control realised in the foregoing syntheses seems to depend on two factors. a) One stereoisomer of the hydrazone **13a** is formed as shown by chromatographic homogeneity and by NMR; steric arguments suggest that it is the illustrated *E*-isomer and b) good stereocontrol in the reduction probably involves some form of complex between the reducing agent and the hydrazone, in keeping with the striking dilution effect, which for **13a** directs the transfer of deuteride to the *si*-face of the C=N bond.

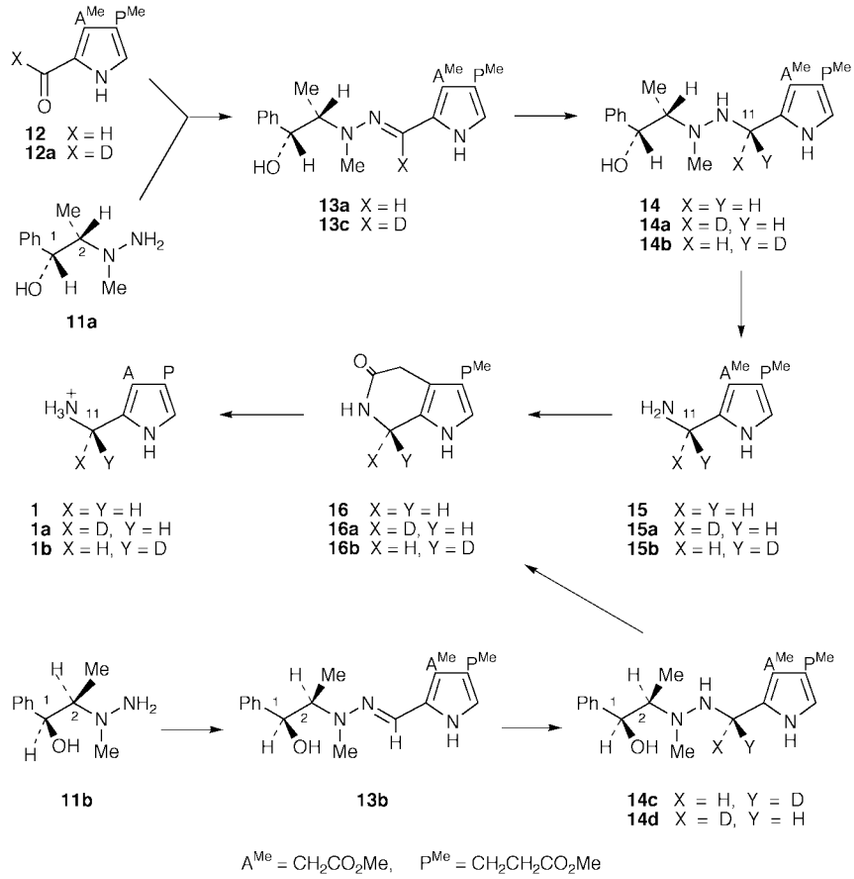
Advantage could now be taken of the susceptibility of N–N bonds to reductive cleavage. When the hydrazone **14a** was hydrogenated over a substantial amount of platinum in acetic acid–methanol, it was rapidly cleaved to afford the dimethyl ester of PBG **15a** as its acetate salt. Heating this product in methanol with sodium carbonate caused cyclisation to the crystalline (11*S*)-[11-$^2H_1$]PBG lactam ester **16a** containing *ca.* 17% of the (11*R*)-enantiomer **16b**. The overall yield of the lactam esters was 65% from the aldehyde **12**.

Similarly, the foregoing hydrazine **14c** afforded (11*R*)-[11-$^2H_1$]PBG lactam ester **16b** which made up 83% of the deuteriated product, the rest being the (11*S*)-enantiomer **16a**.

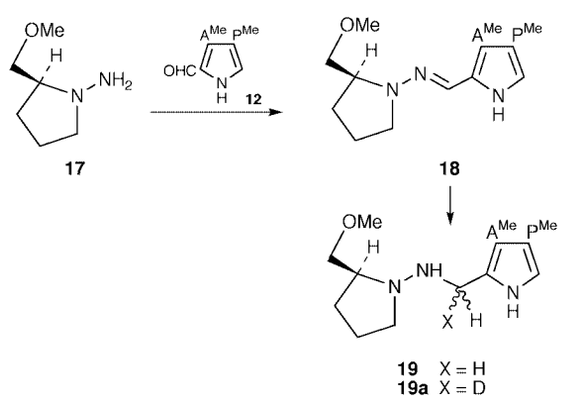
In parallel with the foregoing experiments, we also studied the use of (*S*)-1-amino-2-(methoxymethyl)pyrrolidine[13] (SAMP) **17** as the chiral auxiliary. The chemistry followed closely that described above and the reaction sequence *via* the hydrazone **18** is shown in Scheme 3. ^{1}H-NMR showed that the hydrazine **19a** contained 95% deuteriated material of which one diastereoisomer made up 78% and the other 22%. The previous approach gave a higher stereoselectivity and the hydrazine **19a** proved to be unstable so the effort focused on the route using ephedrine.

Determination of the absolute configuration of the ^{2}H-labelled samples of PBG

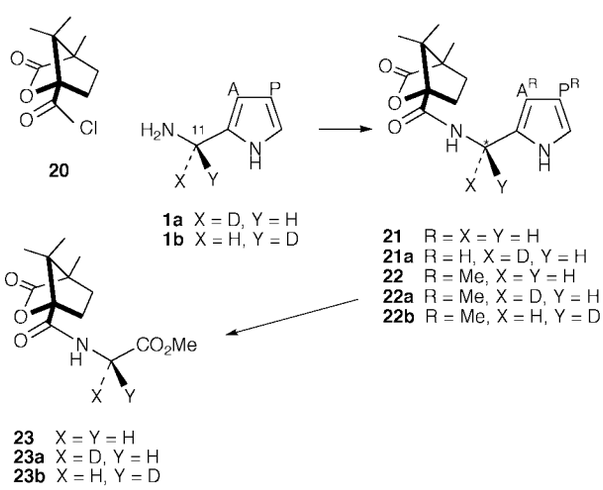
The plan was to degrade the PBG lactam ester **16a** to a derivative of glycine. Alkaline hydrolysis of **16a** yielded (11*S*)-[11-$^2H_1$]PBG **1a**, Scheme 4, which without isolation was converted by two-phase acylation with (−)-(1*S*,4*R*)-camphanyl chloride **20** into the amide **21a**. After treatment of the products with diazomethane, the diester **22a** was spread on silica[14] and oxidised with ozone to break down the pyrrole ring. The products were esterified with diazomethane and *N*-camphanyl glycine methyl ester was isolated from the mixture by chromatography. Amarego *et al.*[15] had assigned the ^{1}H-NMR signals from the diastereotopic protons of the unlabelled amide **23**



Scheme 2



Scheme 3



Scheme 4

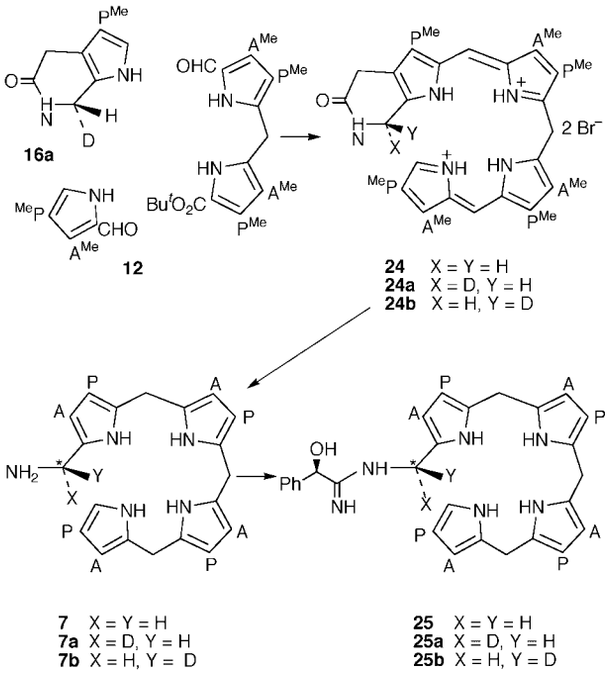
showing that H_R gives the higher field signal. Analysis of our product in this way proved it was *N*-camphanyl-(2*S*)-[2-$^2H_1$]-glycine methyl ester **23a** and therefore the original lactam had the (11*S*)-configuration **16a**.

The same degradative sequence was carried out on the lactam **16b** generated from the enantiomeric ephedrine derivative **11b**. NMR analysis of the glycine ester derived from the amide **22b** gave interlocking strength by proving it was *N*-camphanyl-(2*R*)-[2-$^2H_1$]glycine methyl ester **23b**. Thus the original PBG lactam ester was the [11*R*]-enantiomer **16b**.

The foregoing degradative methods (and several unsuccessful ones) were studied first using unlabelled PBG **1** which was thus required in substantial amounts. It was best prepared from the oxime of pyrrole **12** by formation of the oxime hydrochloride followed by hydrogenation over palladium to yield PBG dimethyl ester **15** as its hydrochloride salt, Scheme 2. Similar hydrogenation of the oxime free base gave much lower yields. The hydrochloride of **15** was then treated with methanolic sodium methoxide to afford PBG lactam methyl ester **16**, the precursor of PBG **1**, in 63% overall yield from **12**.

Synthesis of the (*S-aminomethyl*)bilane 7a and the (*R-aminomethyl*)-isomer 7b; development of the stereochemical assay

The aminomethylbilane **7** had been synthesised previously[10,16] in unlabelled form, the building blocks and a key intermediate **24** being shown in Scheme 5. This route was followed here and the experimental details now reported include some improvements and also fuller characterisations. Replacing unlabelled PBG lactam ester **16** used in the earlier work by (11*S*)-[11-$^2H_1$]PBG lactam ester **16a** from above led to the (*S-aminomethyl*)bilane **7a**. Similarly, (11*R*)-[11-$^2H_1$]PBG lactam ester **16b** afforded the (*R-aminomethyl*)-isomer **7b**.



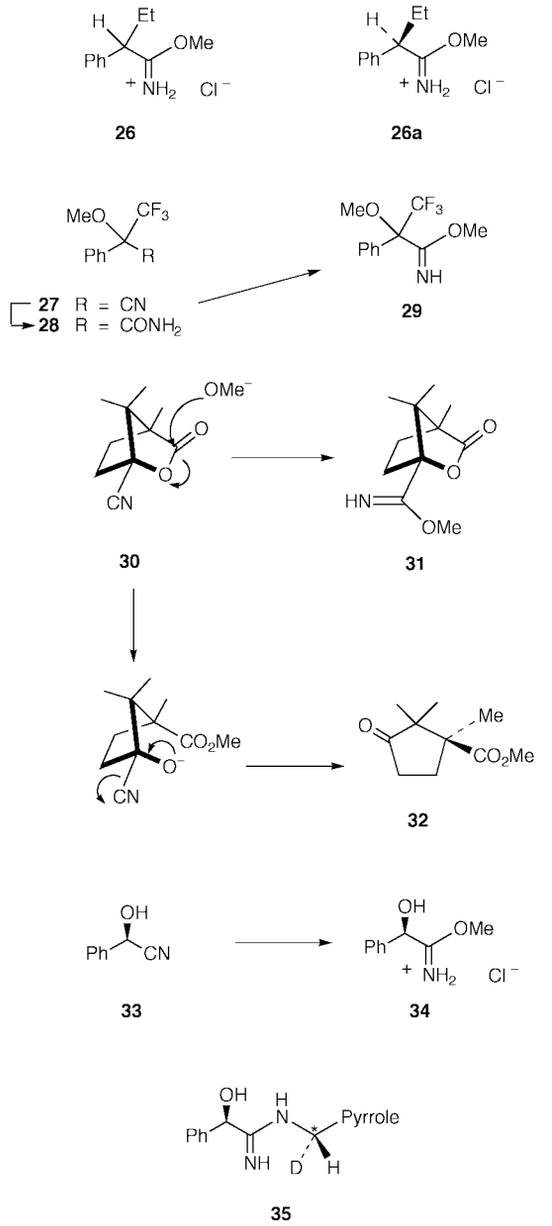
Scheme 5

It was now necessary to attach a chiral residue to the aminomethyl group of each of the foregoing standard bilanes of known absolute configuration to set up diastereoisomers for assay by ^{1}H-NMR. Though simple in principle, this proved to be extremely difficult in practice because a) the bilanes readily undergo non-enzymic ring closure to uro'gen I **10** and b) the derivatisation must be done in water to reproduce the conditions to be met subsequently with the bilanes produced enzymically. It is not necessary to report the many unsuccessful conditions and reagents tested. Success came by taking advantage of the smooth reaction between imidate esters and primary amines at *ca*. pH 10 in water to generate amidines; this reaction has been used to modify amino groups of proteins.[17]

Synthesis and reactions of chiral imidate esters

The key starting material for synthesis of an imidate ester is the corresponding nitrile and for the four cases studied, Scheme 6, this was either available or it was prepared from the acid *via* the amide which was dehydrated. For synthesis of **26** and its (2*R*)-enantiomer **26a**, treatment of the nitrile with hydrogen chloride in dry ethereal methanol afforded the required imidate ester hydrochloride in good yields. When these conditions were applied to the nitrile **27**, a high yield of the amide **28** was obtained. However, treatment of this nitrile **27** with methoxide in dry methanol smoothly yielded the imidate ester **29** as the free base. These conditions were also successful for preparation of the ester **31** from the nitrile **30** but a substantial by-product, $C_{10}H_{16}O_3$, was isolated showing spectroscopic data in agreement with structure **32**. A rationalisation of its formation is shown in Scheme 6.

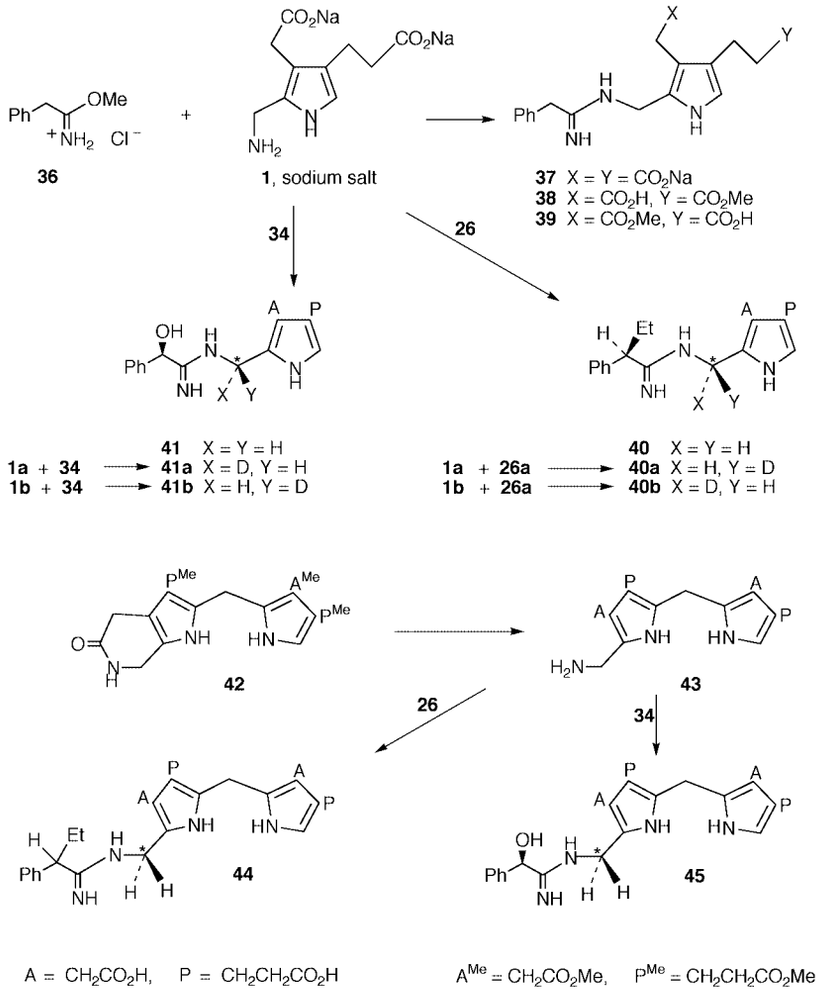
The fourth imidate ester we required was **34**, the corresponding nitrile being (+)-(2*R*)-mandelonitrile **33**. This has been prepared by acidic hydrolysis of natural (+)-amygdalin[18] but unfortunately the experimental section of the paper does not report either the solvent used or the concentration of acid. It was therefore important to demonstrate that the conditions now described had yielded enantiomerically pure mandelonitrile **33** and that no significant racemisation had occurred during the formation of the imidate ester **34** or in our use of the latter for the preparation of amidines. Our concern was reinforced by the reported racemisation of some chiral amidines.[19] The necessary proof came from reacting the imidate ester **34** with unlabelled



Scheme 6

PBG **1** and with the (11*S*)-, and (11*R*)-labelled forms, **1a** and **1b** respectively, followed by analysis by NMR; this preparative work is described more fully below. The argument is best presented using structure **35**, Scheme 6, making a simplifying assumption that the starred centre and the hydroxy bearing centre are both enantiomerically pure. The ^{1}H-NMR signal from the hydrogen at the starred centre (after hydrogens at exchangeable positions have been replaced by deuterium) will appear as a singlet. However, if the hydroxy bearing centre has been fully racemised, two separate singlets of equal size will be seen and the position where the second signal would appear is known from the spectrum shown by the amidine derived from unlabelled PBG **1**. Partial racemisation would give a result in between the foregoing extremes. It will be evident that even though the labelled samples of PBG **1a** and **1b** do not contain 100% of one enantiomer but 83%, the assay by NMR can be done and it showed that **33** and **34** were enantiomerically pure, within the limits of detection by NMR, and that the final amidines were configurationally stable.

The next step was to work out a) the best conditions for using the foregoing imidate esters to form amidines from aminomethylpyrroles under aqueous conditions and b) which of the reagents **26**, **29**, **31** and **34** formed a handleable amidine that in



Scheme 7

addition gave good discrimination by ^{1}H-NMR between the diastereotopic protons of the aminomethyl group. Experiments were carried out on PBG **1**, the dipyrromethane **43** and finally, the aminomethylbilane **7**. Since these reactions were carried out at *ca.* pH 10 and imidate esters have a pK_a of *ca.* 6,[20] the reagents could be used directly either as free bases or as hydrochlorides. First it was shown that the reaction at room temperature of a simple achiral imidate ester **36** with PBG **1**, Scheme 7, was complete in 3 h as shown by TLC on cellulose. After esterification of the disodium salt **37** with dimethyl sulfate, a mono-ester **38** or **39** could be extracted for spectroscopic characterisation. In contrast, there was no detectable reaction, as judged by TLC and NMR, between PBG **1** and the imidate esters **29** and **31** probably due to steric hindrance. A large amount of unchanged **29** and **31** was recovered in each case so the failure was not due to hydrolysis of the reagents. However, the imidate ester **26** did react with PBG **1** to yield the racemic amidine (as **40**), Scheme 7, and the diastereotopic protons at the starred centre were distinguishable by ^{1}H-NMR at 400 MHz (AB multiplet). This encouraged study of the reaction of the (2*R*)-imidate ester **26a** with the (11*S*)-, and (11*R*)-labelled forms of PBG, **1a** and **1b,** respectively. The ^{1}H-NMR signal from the starred centre of the (11*S*)-product **40a** appeared at lower field than that from the (11*R*)-sample **40b**. Finally, the imidate ester **34** was used to convert PBG **1** into the amidine **41** which showed a well resolved AB multiplet from the starred centre in its ^{1}H-NMR spectrum. Analogous preparation of the amidines **41a** and **41b** from (11*S*)- and (11*R*)-labelled PBG **1a** and **1b**, respectively, demonstrated that the starred centre of the (11*S*)-amidine **41a** gave a ^{1}H-NMR signal at lower field than that from the (11*R*)-isomer **41b**.

A step up in complexity led to the known dipyrrolic lactam[21] **42** which was hydrolysed[21] to form the aminomethyldipyrromethane **43**, Scheme 7. This reacted with the racemic imidate ester **26** under the conditions described above to form the amidine **44** which showed an AB multiplet in its ^{1}H-NMR spectrum from the starred centre. Similarly, **43** was converted by **34** into the amidine **45**. Now the starred centre gave a poorly resolved ^{1}H-NMR signal but, interestingly, the signal from the interpyrrolic methylene group was a well resolved AB multiplet despite its being 8 atoms away from the chiral centre.

The foregoing experiments set the stage for work on the aminomethylbilanes **7**, **7a** and **7b**; syntheses of the latter two were described above (see also Scheme 5). There was immediate disappointment in that the imidate ester **26** failed to react with the bilane **7**. However, the other imidate **34** reacted smoothly and converted the (*S-aminomethyl*)bilane **7a** into the (*S*)-amidine **25a** and the (*R-aminomethyl*)bilane **7b** into the (*R*)-isomer **25b**, Scheme 5. Fig. 1(b) shows the ^{1}H-NMR signal from the starred centre of the *R*-sample **25b** with the large signal to higher field and the complementary result in Fig. 1(a) shows the large signal at lower field from the (*S*)-sample **25a**. To ensure that no external factors had affected the chemical shifts, the (*S*)-amidine **25a** was mixed with a larger amount of the (*R*)-amidine **25b** to give the NMR pattern shown in Fig. 2(c). A thoroughly reliable method of assay was thus available for determination of the absolute configuration of labelled bilanes from the enzymic experiments.

Enzymic formation of the labelled bilanes 7c and 7d

(11*S*)-[11-$^2H_1$]PBG **1a** was incubated in the presence of 0.2 M

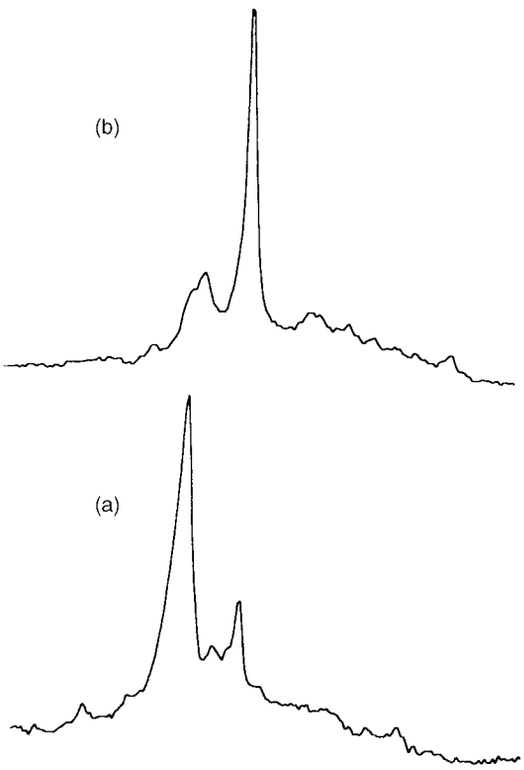


Fig. 1 ^{1}H-NMR signals (400 MHz) from the starred centre of (a) the (*S*)-amidine **25a** and (b) the (*R*)-amidine **25b**. Both spectra were run in CH_3OD.

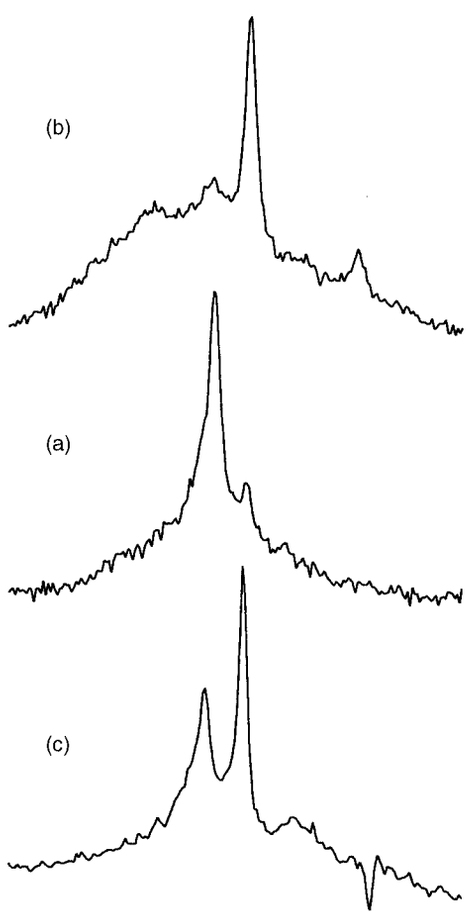
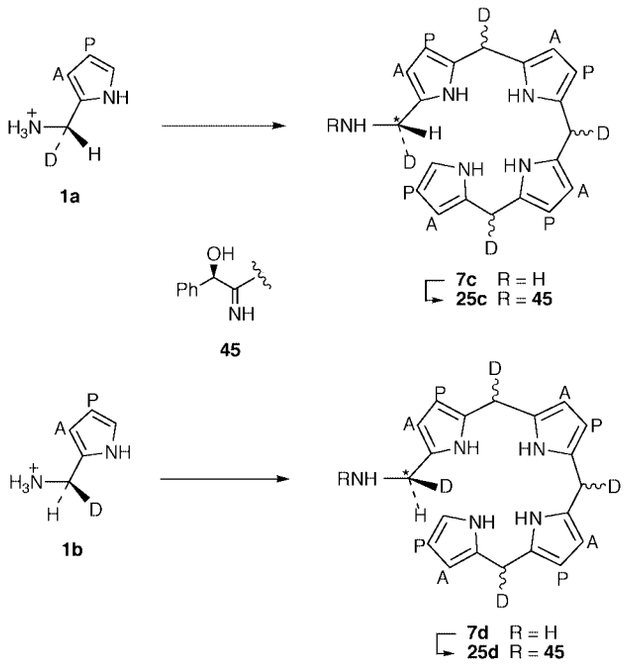


Fig. 2 ^{1}H-NMR signals (400 MHz) from the starred centre of (a) the (*S*)-amidine **25c** from the enzymic experiments, (b) the (*R*)-amidine **25d** from the parallel enzymic run, and (c) a mixture of the synthetic (*S*)-amidine **25a** with a larger quantity of the synthetic (*R*)-amidine **25b**.



Scheme 8

ammonium chloride with amply enough hydroxymethylbilane synthase to convert >95% of **1a** in 0.5 h into the aminomethylbilane,[22] Scheme 8. This was rendered more stable by raising the pH to >13 and excess ammonia was then removed by two rounds of freeze drying. Addition of the imidate ester **34** then converted the bilane into the amidine which displayed the ^{1}H-NMR signal from the starred centre shown in Fig. 2(a); the large signal appeared at low field. This matched Fig. 1(a) proving that this amidine had the (*S*)-configuration **25c** formed from the (*S-aminomethyl*)bilane **7c**.

This entire enzymic preparation was repeated starting from (11*R*)-[11-^{2}H$_1$]PBG **1b** and now the final amidine showed the large ^{1}H-NMR signal at high field, Fig. 2(b), matching the standard spectrum in Fig. 1(b). It followed that this was the (*R*)-amidine **25d** derived from the (*R-aminomethyl*)bilane **7d**. In order to leave no doubt about the signal assignments, a small amount of the (*R*)-amidine **25d** was added to the (*S*)-amidine **25c**. This caused the small high field signal seen in Fig. 2(a) to increase in size and addition of a second portion of the (*R*)-amidine **25d** resulted in a further increase in the size of this signal.

These results proved that (11*S*)-PBG **1a** had been converted into (*S*)-bilane **7c** and the finding that (11*R*)-PBG **1b** had yielded (*R*)-bilane **7d** added complementary strength. It is thus certain that when hydroxymethylbilane synthase acts on PBG **1** and the tetrapyrrole is trapped as the aminomethylbilane **7**, the overall stereochemical outcome at the aminomethyl centre is *retention of configuration*. Further discussion of this finding is best deferred until the conclusion of the following paper[23] when the results for the aminomethylbilane **7** and the hydroxymethylbilane **6** can be considered together.

Experimental

General

For general directions, see ref. 16.

Synthesis of (11*S*)-[11-^{2}H$_1$]porphobilinogen lactam methyl ester 16a and the (11*R*)-[11-^{2}H$_1$]enantiomer 16b

(a) (−)-*N*-Aminoephedrine 11a and (+)-*N*-aminoephedrine 11b. To a cooled (0 °C) stirred solution of (−)-ephedrine hydrochloride (12 g, 0.06 mol) in water (50 cm^3) and 2 M hydrochloric

acid (25 cm^3) was added over a period of 90 min a solution of sodium nitrite (8.4 g, 0.12 mol) in water (50 cm^3). The mixture was stirred at 0 °C for 1 h to complete the precipitation of the product which was collected and dried to give (−)-*N*-nitrosoephedrine (11.4 g, 98%), mp 90 °C (lit.,[8] 92 °C). ν_{max} (Nujol)/cm^{-1} 3350, 2840 and 1445 (NO); δ_H (80 MHz) 1.45 (3 H, d, *J* 6.9, CHCH_3), 2.4 (1 H, br s, OH), 2.93 (3 H, s, NCH_3), 4.67 (1 H, dq, *J* 5 and 6.9, C*H*CH_3), 5.03 (1 H, d, *J* 5, C*H*OH), 7.32 (5 H, s, ArH).

To a stirred mixture of zinc (18 g) in water (90 cm^3) at 0 °C was added dropwise over a period of 30 min a solution of (−)-*N*-nitrosoephedrine (11.64 g, 0.06 mol) in acetic acid (60 cm^3). The mixture was stirred for 1 h at room temperature and then 1 h at 80 °C. The precipitate was filtered off, the filtrate concentrated to about half volume, benzene (90 cm^3) was added and the solution was made alkaline by addition of saturated aqueous potassium carbonate. The equilibrated phases were separated, the aqueous phase extracted several times with benzene and the combined organic phases dried. Evaporation of the solvent gave (−)-*N*-aminoephedrine **11a** (8.3 g, 76%) as a yellow oil which was essentially pure and was distilled *in vacuo*, bp 138–142 °C/8 mmHg (lit.,[8] 138–150 °C/8 mmHg). ν_{max}/cm^{-1} 3300 (br) and 2950; δ_H (80 MHz) 0.82 (3 H, d, *J* 6.7, CHCH_3), 2.57 (3 H, s, NCH_3), 2.74 (1 H, d q, *J* 2.1 and 6.7, C*H*CH_3), 2.3–3.5 (3 H, br s, OH and NH_2), 5.2 (1 H, d, *J* 2.1, CHOH), 7.3 (5 H, m, ArH).

(+)-*N*-Aminoephedrine **11b** was prepared as above from (+)-ephedrine.

(b) The enantiomeric hydrazones 13a and 13b. The aldehyde **12**[24] (3.5 g, 13.8 mmol) and (−)-*N*-aminoephedrine **11a** (5 g, 27.8 mmol) were dissolved in dry benzene (150 cm^3). Molecular sieves (4 Å, 3 g) were added and the solution was heated at reflux for 2 h under argon. The filtered solution was evaporated and the product was chromatographed on silica (0.04–0.063 mm, 3 × 20 cm) using diethyl ether as eluant to give the hydrazone **13a** (5.58 g, 97%) as an oil (Found: M^+, 415.2111. $C_{22}H_{29}N_3O_5$ requires *M*, 415.2107); ν_{max}/cm^{-1} 3375 (br) and 1720; δ_H (100 MHz) 1.06 (3 H, d, *J* 6.7, CHCH_3), 2.68 (4 H, m, $CH_2CH_2CO_2$), 2.86 (3 H, s, NCH_3), 3.39 (1 H, dq, *J* 3 and 6.7, C*H*CH_3), 3.54 (2 H, s, CH_2CO_2), 3.68 (6 H, s, 2 × OCH_3), 4.30 (1 H, br s, OH), 5.20 (1 H, d, *J* 3, C*H*OH), 6.51 (1 H, d, *J* 2.5, pyrrole-H), 7.3 (6 H, m, ArH and HC=N), 8.56 (1 H, br s, NH); *m*/*z* 415 (M^+, 5%), 384 (3), 308 (100, M − C_7H_7O).

The enantiomer **13b** prepared in the same way from **11b** showed identical spectroscopic properties.

(c) The enantiomeric hydrazines 14a and 14c and the unlabelled analogue 14. The acidic protons of the hydrazone **13a** were exchanged by treatment of it (2.7 g, 6.5 mmol) in tetrahydrofuran (THF, 15 cm^3) with D_2O (5 cm^3, 99.8 atom%) for 30 min. The reaction mixture was evaporated to dryness and the procedure was repeated once more. A solution of the final residue in THF (2 dm^3) together with sodium cyanoborodeuteride (1 g, 15.2 mmol, 98 atom% D) and a few milligrams of bromocresol green was stirred under argon in a three-necked flask equipped with a dropping funnel. A solution of *O*-deuteriated toluene-*p*-sulfonic acid mono(deuterium oxide)[11] (2.7 g, 14 mmol, 99.2 atom%) in THF (180 cm^3) was added at room temperature during 90 min to maintain pH 3.5, indicated by the tan colour of the indicator. The sodium salt of toluene-*p*-sulfonic acid precipitated during the reaction. After the mixture had stirred for 30 min more, it was filtered, the solvent was removed *in vacuo* and the residue in CH_2Cl_2 (60 cm^3) was washed with saturated aqueous sodium hydrogen carbonate. The residue from evaporation was chromatographed on silica using ethyl acetate–diethyl ether (8:2) as eluant to give the hydrazine **14a** (2.5 g, 92%) as a pale yellow oil. This product was stable under argon in the deep freezer for several months but rapidly decomposed when exposed to air at room temperature (Found: M^+ − C_7H_7O, 311.1819. $C_{15}H_{23}DN_3O_4$ requires M − C_7H_7O, 311.1829, 95 atom% D); ν_{max}/cm^{-1} 3300 (br) and 1765; δ_H (400 MHz) 0.85 (3 H, d, *J* 6.6, CHCH_3), 2.54 (2 H, t, *J* 6.6, $CH_2CH_2CO_2$), 2.55 (3 H, s, NCH_3), 2.76 (3 H, m, $CH_2CH_2CO_2$ and C*H*CH_3), 3.46 (2 H, ABq, *J* 13.4, CH_2CO_2), 3.63 and 3.68 (2 × 3 H, 2 × s, 2 × OCH_3), 3.9 [0.16 H, s, (11*R*)-NCHD], 4.03 [0.79 H, s, (11*S*)-NCHD], 5.2 (1 H, d, *J* 2, C*H*OH), 6.5 (1 H, d, *J* 2.6, pyrrole-H) 7.3 (5 H, m, ArH) and 8.45 (1 H, br s, NH); *m*/*z* (FD) 419 (M^+ + 1, 100%) and 418 (M^+, 29%).

The enantiomer **14c**, prepared from **13b** as above, gave identical spectroscopic data.

The unlabelled hydrazine **14** was prepared as above from **13a** using sodium cyanoborohydride; δ_H (400 MHz, in part) 3.86 (1 H, d, *J* 13.4, H_S of NCH_2) and 4.02 (1 H, d, *J* 13.4, H_R of NCH_2), all other signals as for **14a**; *m*/*z* (FD) 418 (M^+ + 1, 100%) and 417 (M^+, 40%).

(d) (11*S*)-[11-$^2H_1$]PBG lactam methyl ester 16a and the (11-*R*)-enantiomer 16b. All solvents used in this preparation were deoxygenated and saturated with argon by heating for 2 h under reflux then distillation, both under an argon stream. The hydrazine **14a** (2.4 g, 5.74 mmol) in dry THF (10 cm^3) was added by syringe to pre-reduced Adam's catalyst (0.5 g) in methanol (100 cm^3) and acetic acid (15 cm^3) and the mixture was stirred under hydrogen at room temperature until uptake ceased (*ca.* 1 h). The filtered solution was evaporated and the residue in dichloromethane–methanol (9:1, 50 cm^3) was shaken with saturated aqueous sodium carbonate. The aqueous phase was extracted several times with dichloromethane–methanol (9:1) and the combined organic layers were dried and evaporated. The residue in methanol (25 cm^3) was heated under reflux for 1 h to form PBG lactam methyl ester which started to precipitate. Crystallisation was completed by keeping the mixture at −10 °C for 2 h. The product was collected by centrifugation, washed with methanol and dried to afford the (11-*S*)-[11-$^2H_1$]-lactam **16a** (830 mg, 65%) as off-white prisms, mp 247–249 °C (lit.,[24] 248–249 °C) (Found: M^+, 223.1048. $C_{11}H_{13}DN_2O_3$ requires *M*, 223.1067); δ_H (400 MHz) 2.54 and 2.72 (2 × 2 H, 2 × m, $CH_2CH_2CO_2$), 3.41 (2 H, d, *J* 3.3, CH_2CONH), 3.66 (3 H, s, OCH_3), 4.47 (1 H, br s, NCHD), 5.92 (1 H, br s, *H*NCHD), 6.55 (1 H, d, *J* 1.8, pyrrole-H) and 7.72 (1 H, br s, NH); *m*/*z* 223 (M^+, 100%), 222 (62, M − 1), 192 (11, M − CH_3O) and 150 (65, M − $C_3H_5O_2$).

The enantiomer **16b** was prepared in the same way from **14c** (Found: M^+, 223.1053, $C_{11}H_{13}DN_2O_3$ requires *M*, 223.1067); all other data as for **16a**.

Study of (*S*)-1-amino-2-(methoxymethyl)pyrrolidine (SAMP) 17 as the chiral auxiliary

A solution of the aldehyde[24] **12** (50 mg, 0.2 mmol) and SAMP[13] (52 mg, 0.4 mmol) in benzene (2 cm^3) was heated under reflux for 20 min. The mixture was diluted with dichloromethane, filtered, dried and the solvent was evaporated. The residue was purified by PLC using diethyl ether to give the hydrazone **18** as a yellow oil (40 mg, 54%). ν_{max}/cm^{-1} 3475 and 1735; δ_H (100 MHz) 1.90 (4 H, m, 2 × pyrrolidine-β-CH_2), 2.68 (6 H, m, $CH_2CH_2CO_2$ and NCH_2), 3.38 (3 H, s, OCH_3), 3.5 (2 H, s, CH_2CO_2), 3.66 (6 H, s, 2 × $COOCH_3$), 3.3–3.8 (3 H, m, OCH_2 and CH), 6.5 (1 H, br s, pyrrole-H), 7.23 (1 H, s, NCH), 8.58 (1 H, br s, NH) and a small singlet at 7.36 (~ 10%) rising from the NCH of the *Z*-isomer; *m*/*z* (FD) 365 (M^+ for $C_{18}H_{27}N_3O_5$, 100%).

The hydrazone **18** (40 mg, 0.11 mmol) in THF (1 cm^3) was treated with D_2O (0.1 cm^3) for 20 min before evaporating the solution to dryness; the procedure was repeated once more. A solution of this hydrazone (40 mg, 0.11 mmol), a trace of bromcresol green and sodium cyanoborodeuteriide (30 mg, 0.46 mmol) in dry THF (8 cm^3) was stirred at room temperature under argon. A solution of *O*-deuteriated toluene-*p*-sulfonic

acid mono(deuterium oxide)[11] (80 mg, 0.4 mmol, 99.2 atom%) in THF (4 cm^3) was slowly added at room temperature (45 min) to maintain pH 3.5, indicated by the tan colour of the indicator; the sodium salt of toluene-*p*-sulfonic acid precipitated. The solution was then filtered under argon, the solvent was evaporated and the residue in dichloromethane (5 cm^3) was washed with saturated aqueous sodium hydrogen carbonate. The organic solvent was dried and evaporated and the product was purified by PLC under argon in the dark with methanol–dichloromethane (7:93) as eluant to give the hydrazine **19a** (20 mg, 49%) as an oil. It was unstable and decomposed at 4 °C under argon within a few days; ν_{max}/cm^{-1} 3400 (br) and 1735; δ_H (400 MHz) 1.6, 1.75 and 1.87 (1 H, 2 H, 1 H, 3 × m, 2 × pyrrolidine-β-CH_2), 2.56 (2 H, m, $CH_2CH_2CO_2$), 2.70 (2 H, m, pyrrolidine-α-CH_2), 2.76 (2 H, m, $CH_2CH_2CO_2$), 3.37 (3 H, s, OCH_3), 3.41 (2 H, ABq, CH_2CO_2), 3.35–3.55 (3 H, m, OCH_2 and pyrrolidine-α-CH), 3.64 and 3.66 (2 × 3 H, 2 × s, 2 × CH_3OCO), 3.96 and 3.93 (2 × s, intensity: 3.7/1, HNC*H*D), 6.45 (1 H, d, *J* 2.5, pyrrole-H) and 9.3 (1H, br s, NH). This spectrum showed the diastereotopic excess to be 57% and comparison with the spectrum of unlabelled material below demonstrated at least 95% of the sample was deuteriated.

The unlabelled hydrazine **19** was prepared as above from **18** using sodium cyanoborohydride δ_H (400 MHz, in part) 3.93 (2 H, ABq; *J* 14.4; NCH_2) all other signals as for **20a**; *m*/*z* (FD), 368 ($M^+ + 1$, 100%), 367 (M^+ for $C_{18}H_{29}N_3O_5$, 50%).

Determination of absolute configuration of (11*S*)- and (11*R*)-[11-$^2H_1$]porphobilinogen 1a and 1b

(a) Preparation of camphanamide derivatives. Unlabelled PBG lactam methyl ester **16** (50 mg, 0.22 mmol) in aqueous 2 M potassium hydroxide (0.6 cm^3, 2 mmol) was heated at 70 °C for 5 min under argon and kept at room temperature for 12 h in the dark. (1*S*,4*R*)-Camphanyl chloride **20** (100 mg, 0.46 mmol) in toluene (1 cm^3) was added dropwise to the foregoing ice cooled solution as the mixture was vigorously stirred. Stirring was then continued for 6 h at room temperature while keeping the pH above 7 by dropwise addition of aqueous 2 M potassium hydroxide. The mixture was extracted with dichloromethane (discarded), the aqueous layer was acidified to pH 2 and extracted several times with dichloromethane–methanol (9:1), total volume 70 cm^3. The dried organic solution was evaporated and the amide **21** in methanol (5 cm^3) was treated with an excess of ethereal diazomethane. The product was purified by PLC on silica with dichloromethane–methanol (93:7) as eluant (R_f 0.5) to give the camphanamide of porphobilinogen dimethyl ester **22** (54 mg, 56%) as an oil (Found: M^+, 434.2068. $C_{22}H_{30}N_2O_7$ requires *M*, 434.2053); ν_{max}/cm^{-1} 3430, 1790, 1730 and 1660; δ_H (400 MHz) 0.78 (3 H, s, camphanyl-CH_3), 1.08 and 1.082 (2 × 3 H, each s, 2 × camphanyl-CH_3), 1.66 (1 H, m, camphanyl-CH), 1.88 (2 H, m, camphanyl-CH_2), 2.50 (3 H, m, CH_2CH_2-CO_2 and camphanyl-CH), 2.74 (2 H, m, $CH_2CH_2CO_2$), 3.43 (2 H, s, CH_2CO_2), 3.65 and 3.68 (2 × 3 H, 2 × s, 2 × OCH_3), 4.36 (2 H, 2 × ABq, *J* 6 and 13.4, NHCH_2), 6.46 (1 H, d, *J* 2.6, pyrrole-H), 7.13 (1 H, t, *J* 6, N*H*CH_2) and 8.62 (1 H, br s, NH); irradiation at δ 7.13 converted the 8 signals at 4.36 into one ABq, with the centres 23 Hz apart; the low field doublet corresponds to H_R of $NHCH_2$ and that at high field to H_S; the assignments depend on subsequent work below; *m*/*z* 434 (M^+, 7%), 361 ($M - C_3H_5O_2$, 18), 265 (10), 253 (16), 237 (30) and 223 (100).

The [11*S*-$^2H_1$]isotopomer **22a** was prepared as above *via* **21a** from the [11*S*-$^2H_1$] lactam **16a** (Found: M^+, 435.2118. $C_{22}H_{29}DN_2O_7$ requires *M*, 435.2131); δ_H (400 MHz, in part) 4.33 [0.16 H, d, *J* 6, (11*R*)-NHC*H*D], 4.38 [0.79 H, d, *J* 6, (11*S*)-NHC*H*D] with all the other signals as above; this showed that 95% of the total material was deuteriated and the enantiomeric excess was 66%; *m*/*z* 435 (M^+, 100%), 362 ($M - C_3H_5O_2$, 38), 276 (5), 254 (7), 238 (23) and 222 (100).

The [11*R*-$^2H_1$]isotopomer **22b** was prepared in the same way from **16b** (Found: M^+, 435.2091. $C_{22}H_{29}DN_2O_7$ requires *M*, 435.2131); δ_H (400 MHz, in part) 4.33 [0.79 H, d, *J* 6, (11*R*)-NHC*H*D], 4.38 [0.16H, d, *J* 6, (11*S*)-NHC*H*D], with all other signals as above; these results are complementary to those for the [11*S*] sample.

(b) Degradation by ozone. The plan was to degrade both foregoing labelled amides to yield labelled methyl (1*S*,4*R*)-camphanyl glycinate. Therefore an unlabelled sample **23** was prepared as described by Armarego *et al.*[15] δ_H (100 MHz): 1.09 and 1.11 (2 × 3 H, 2 × s, 2 × camphanyl-CH_3), 0.96 (3 H, s, camphanyl-CH_3), 1.8 (3 H, m, camphanyl CH_2 and CH), 2.5 (1 H, m, camphanyl-CH), 3.75 (3H, s, OCH_3), 4.06 (2 H, 2 × ABq; *J* 6 and 18 NCH_2) and 6.9 (1 H, br s, NH); δ_H (250 MHz , in part) 3.98 (1 H, dd, *J* 5.1 and 18, H_R of NCH_2), 4.19 (1 H, dd, *J* 6 and 18, H_S of NCH_2); all these signals agree closely with those reported;[15] *m*/*z* 269, (M^+). $C_{13}H_{19}NO_5$ requires *M* 269.

To a solution of the [11*S*-$^2H_1$]camphanamide **22a** (27 mg, 0.062 mmol) in dichloromethane (2 cm^3) was added silica (1.5 g, 60–120 mesh) followed by evaporation to dryness. The silica was cooled to −70 °C and a stream of ozone was passed through it for 30 min causing the silica to turn blue by adsorbed ozone. The solid was then warmed to room temperature during 1 h and the degradation products were washed off the silica with ethyl acetate. The residue from evaporation of the extracts was dissolved in methanol (3 cm^3) and treated with an excess of ethereal diazomethane. The (mainly) camphanyl-(2*S*)-[2-$^2H_1$]-glycine methyl ester **23a** (3.3 mg, 20%) was isolated by PLC with ethyl acetate–hexane (1:1) as eluant (R_f 0.4) and identified by comparison with the foregoing unlabelled material (Found: M^+, 270.1339. $C_{13}H_{18}DNO_5$ requires *M*, 270.1341) δ_H (400 MHz, in part) 3.98 [0.79 H, dt, *J* 5.1 and 2.5 (2*S*)-NCHD] and 4.19 [0.16 H, dt, (2*R*)-NCHD]; all other signals were as for the unlabelled material **23**.

The (mainly) camphanoyl-(2*R*)-[2-$^2H_1$]glycine methyl ester **23b** was obtained by degradation of the [11-*R*-$^2H_1$]-isotopomer **22b** as described above for **22a** (Found: M^+, 270.1340. $C_{13}H_{18}DNO_5$ requires *M*, 270.1341); δ_H (400 MHz, in part) 3.98 [0.16 H, dt, (2*S*)-NCHD] and 4.19 [0.79 H, dt, *J* 6 and 2.5, (2*R*)-NCHD]; *m*/*z* 270 (M^+, 9%), 224 (30), 184 (5) and 180 (8).

Preparation of PBG lactam methyl ester 16. The aldehyde **12** (102 mg), hydroxylamine (42 mg) and sodium acetate (50 mg) were stirred in methanol (2 cm^3) at 18 °C for 2 h and the product was isolated as described[24] to give the oxime (100 mg). This was dissolved in 2 M methanolic hydrogen chloride (2 cm^3) and the solvent was evaporated. A solution of the residual hydrochloride in methanol (8 cm^3) was stirred with palladium on carbon (10%, 80 mg) for 0.5 h under hydrogen at room temperature. The filtered solution was concentrated until the PBG dimethyl ester hydrochloride (as **15,** HCl salt) started to crystallise, a process completed by cooling the mixture to 0 °C. The solid was collected and dried (78 mg); it was shown by TLC using methanol–chloroform (1:9) to be free from the corresponding lactam (R_f of lactam, 0.5; R_f of product, 0.1). δ_H (100 MHz) 2.47 (4 H, m, CH_2CH_2CO), 3.46 (2 H, br s, CH_2CO), 3.54 and 3.62 (each 3 H, s, 2 × OCH_3), 4.14 (2 H, br s, CH_2N), 6.5 (1 H, br s, pyrrole-H), 8.0 (3 H, br s, NH_3^+) and 10.0 (1 H, br s, NH). The signals became sharper when the spectrum was determined in $CDCl_3$–CD_3OD (4:1); *m*/*z* (FD) 254, (M − HCl; $C_{12}H_{18}N_2O_4$).

The foregoing crystals in methanol (5 cm^3) were treated with an aliquot (0.2 cm^3) from a solution of sodium (0.5 g) in methanol (10 cm^3). After the mixture had been heated under reflux for 5 min, it was concentrated to low volume when the lactam ester **16** crystallised; it was collected by centrifugation (56 mg, 63% overall from **12**) and identified by comparison with authentic material.[24]

Synthesis of the bilane 7 and the (*S*)- and (*R*)-analogues 7a and 7b $^2H_1$-labelled at the aminomethyl group

The unlabelled bilane **7** had been synthesised earlier[16] and the same approach was used here. However, some changes in procedure are described below and new data from a fuller spectroscopic study are reported.

***tert*-Butyl 5-acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate.** Freshly distilled sulfuryl chloride (4 g, 29.6 mmol) was added to an ice-cooled solution of *tert*-butyl 5-methyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate[25] (10 g, 29.5 mmol) in dichloromethane (125 cm^3) containing finely divided calcium carbonate (10 g). The mixture was stirred at 5 °C for 2 h, with protection from moisture then the solvent was evaporated below room temperature. The residue was redissolved in dichloromethane (150 cm^3) and the filtered solution was evaporated. The residue with sodium acetate (7.5 g, 91 mmol) in glacial acetic acid (200 cm^3) was stirred at 60 °C for 30 min then evaporated. The residue was partitioned between water (50 cm^3) and dichloromethane (50 cm^3, 2 × 25 cm^3). The combined organic extracts were dried and evaporated; the residue by flash chromatography using 3.5:6.5 ethyl acetate–hexane gave the acetoxymethylpyrrole (9.33 g, 80%) as an oil which crystallised on standing. The product gave an ^{1}H-NMR spectrum identical to that from an earlier preparation[25] of it by a different method.

***tert*-Butyl 2,7,12-tris(2-methoxycarbonylethyl)-3,8-bis(methoxycarbonylmethyl)-6′-oxo-1′,2′,5′,6′-tetrahydropyrido[4,3-*a*]tripyrrene-*b*-1-carboxylate hydrochloride and the $^2H_1$-labelled analogues.** A solution of hydrogen chloride in dichloromethane (0.2 M, 4 cm^3) was added to a stirred solution of porphobilinogen lactam methyl ester **16** (91.9 mg, 0.41 mmol) and formyldipyrromethane[25] illustrated in Scheme 5 (237 mg, 0.4 mmol) in dichloromethane (26 cm^3) and methanol (2 cm^3). After 30 min, dry benzene (40 cm^3) was added, the solution was evaporated to dryness at room temperature and this process was repeated. Trituration of the residue with benzene–diethyl ether gave a solid which was collected by centrifugation, washed with diethyl ether (3 × 10 cm^3) and dried *in vacuo* to yield the orange tripyrrene hydrochloride (313 mg, 94%), 123–126 °C (decomp.). δ_H (400 MHz) 1.54 (9 H, s, $C(CH_3)_3$), 2.39 (2 H, t, *J* 7.6), 2.53 (2 H, t, *J* 7.5), 2.61 (2 H, t, *J* 6.8), 2.78 (2 H, t, *J* 7.6), 2.94 (2 H, t, *J* 8.3) and 3.00 (2 H, t, *J* 6.8), (3 × $CH_2CH_2CO_2$), 3.51 (2 H, s, CH_2CO_2), 3.56, 3.62, 3.63, 3.67, and 3.71 (each 3 H, s, 5 × OCH_3), 3.78 (2 H, s, CH_2CO_2), 4.38 (2 H, s, methane CH_2), 4.87 (2 H, s, C*H*$_2$NH), 6.01 (1 H, s, CH_2NH), 7.64 (1 H, s, methene CH), 10.72 (2 H, br, 2 × pyrromethene NH); *m*/*z* (FD) 794 (M^+ − HCl). This tripyrrene had been prepared earlier as its hydrobromide[25] and the present sample was correlated with it spectroscopically.

Using the same procedure, (11-*R*)-[11-$^2H_1$]-porphobilinogen lactam methyl ester **16b** (44.7 mg, 0.2 mmol) and the same formyldipyrromethane (118 mg, 0.2 mmol) gave *tert*-butyl [*R*-*aminomethylene*-$^2H_1$]-2,7,12-tris(2-methoxycarbonylethyl)-3,8-bis(methoxycarbonylmethyl)-6′-oxo-1′,2′,5′,6′-tetrahydropyrido[4,3-*a*]tripyrrene-*b*-1-carboxylate hydrochloride (151 mg, 91%), as an orange solid, mp 123–126 °C (decomp.). The ^{1}H-NMR spectrum was identical to that for unlabelled material except for δ 4.87 (1 H, br, CHD); *m*/*z* (FD) 795 (M^+ − HCl).

Also by the same procedure, (11-*S*)-[11-$^2H_1$]porphobilinogen lactam methyl ester **16a** (45.4 mg, 0.2 mmol) and the formyldipyrromethane (119 mg, 0.2 mmol) gave the [*S*-*aminomethylene*-$^2H_1$]-analogue as its hydrochloride (155 mg, 92%), an orange solid, mp 123–126 °C (decomp.). The ^{1}H-NMR spectrum was identical to that for unlabelled material except for δ 4.87 (1 H, br, CHD); *m*/*z* (FD) 795 (M^+ − HCl).

3,8,13,18-Tetrakis(2-methoxycarbonylethyl)-7,12,17-tris(methoxycarbonylmethyl)-6′-oxo-1′,2′,5′,6′-tetrahydropyrido[3,4-*a*]biladiene-*a*,*c* dihydrobromide 24 and the $^2H_1$-labelled analogues 24a and 24b. This was prepared as earlier[10,16] from the foregoing tripyrrene hydrochloride (294 mg, 0.35 mmol) and 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-formylpyrrole **12** (92.4 mg, 0.37 mmol). The resultant biladiene dihydrobromide **24** (386 mg, 100%) was identified by comparison with the earlier product and it showed δ_H (400 MHz) 2.05 (2 H, m), 2.60 (6 H, m), 2.84 (2 H, m), 2.93 (2 H, m) and 3.01 (4 H, m) (4 × $CH_2CH_2CO_2$), 3.27, 3.37, 3.609, 3.614, 3.67, 3.69 and 3.72 (each 3 H, s, 7 × OCH_3), 3.52, 3.80 and 3.86 (each 2 H, s, CH_2CO_2) 4.98 (2 H, s, C*H*$_2$NH), 5.30 (2 H, s, methane CH_2), 6.21 (1 H, br, CH_2NH), 7.72 and 7.76 (each 1 H, s, 2 × methene CH), 7.78 (1 H, d, *J* 4.4, 19-H), 13.65, 13.66, 13.72, and 13.98 (each 1 H, br, 4 × NH).

Using the same procedure, the foregoing [*R*-*aminomethylene*-$^2H_1$]tripyrrene hydrochloride (146 mg, 0.176 mmol) and the unlabelled formylpyrrole **12** (46.3 mg, 0.183 mmol) gave [*R*-*aminomethylene*-$^2H_1$]-3,8,13,18-tetrakis(2-methoxycarbonylethyl-7,12,17-tris(methoxycarbonylmethyl)-6′-oxo-1′,2′,5′,6′-tetrahydropyrido[3,4-*a*]biladiene-*a*,*c* dihydrobromide **24b** (193 mg, 100%) as a red solid, 131–140 °C (decomp.). The ^{1}H-NMR spectrum was identical to that for unlabelled material except for δ 4.98 (1 H, br, CHD); *m*/*z* (FD) 931 (M^+ − HBr_2).

Also by the same procedure, the foregoing [*S*-*aminomethylene*-$^2H_1$]tripyrrene hydrochloride (148 mg, 0.178 mmol) and unlabelled formylpyrrole **12** (47.9 mg, 0.189 mmol) gave the [*S*-*aminomethylene*-$^2H_1$]-analogue as its dihydrobromide **24a** (201 mg, 100%) mp 131–140 °C (decomp.). The ^{1}H-NMR spectrum was identical to that for unlabelled material except for δ 4.97 (1 H, br, CHD); *m*/*z* (FD) 931 (M^+ − HBr_2).

3,8,13,18-Tetrakis(2-methoxycarbonylethyl)-7,12,17-tris(methoxycarbonylmethyl)-6′-oxo-1′,2′,5′,6′-tetrahydropyrido[3,4-*a*]bilane and the $^2H_1$-labelled analogues. This was prepared as earlier[10,16] by Method B from the biladiene dihydrobromide **24** (374 mg, 0.342 mmol) to afford the bilane (lactam of esterified **7**) (229 mg, 72%) as a pale brown powder, 229–235 °C (decomp.) (lit.,[10,16] mp 225–228 °C (decomp.)). δ_H (400 MHz) 2.33 (2 H, m), 2.49 (6 H, m) and 2.70 (8 H, m) 4 × $CH_2CH_2CO_2$), 3.32, 3.42, and 3.44 (each 2 H, s, 3 × CH_2CO_2), 3.36 (2 H, s, C*H*$_2$CONH), 3.56, 3.58, 3.60, 3.62, 3.64, 3.65, and 3.69 (each 3 H, s, 7 × OCH_3), 3.68, 3.72 and 3.74 (each 2 H, s, 3 × methane CH_2), 4.38 (2 H, br, C*H*$_2$NH), 5.81 (1 H, br, CH_2NH), 6.38 (1 H, br, 19-H), 8.61 (1 H, br, NH), 9.00 (2 H, br, 2 × NH) and 9.17 (1 H, br, NH); *m*/*z* (FD) 957 (M^+ + H + Na) and 934 (M^+ + H). This product was shown to be identical to the earlier[10,16] fully characterised sample.

Using the same procedure, [*R*-*aminomethylene*-$^2H_1$]biladiene dihydrobromide **24b** (162 mg, 0.148 mmol) gave [*R*-*aminomethylene*-$^2H_1$]-3,8,13,18-tetrakis(2-methoxycarbonylethyl)-7,12,17-trismethoxycarbonylmethyl-6′-oxo-1′,2′,5′,6′-tetrahydropyrido[3,4-*a*]bilane (lactam of esterified **7b**) (67.4 mg, 48%), mp 224–229 °C (decomp.). The ^{1}H-NMR spectrum was identical to that for unlabelled material except for δ 3.36 (2 H, d, *J* 3.4, C*H*$_2$CONH), 4.38 (1 H, br, C*H* DNH) and 6.38 (1 H, d, *J* 2.4, 19-H). *m*/*z* (FD) 935 (M^+ + H).

Also by the same procedure, [*S*-*aminomethylene*-$^2H_1$]-biladiene dihydrobromide **24a** (185 mg, 0.169 mmol) gave the [*S*-*aminomethylene*-$^2H_1$]-analogue (lactam of esterified **7a**) (85.2 mg, 54%) mp 226–235 °C (decomp.). The ^{1}H-NMR spectrum was identical to that for *R*-labelled material; *m*/*z* (FD) 935 (M^++H).

Synthesis of chiral imidate esters including single enantiomers

(2*RS*)-2-Phenylbutyramide. Aqueous ammonia (17.5%, 5 cm^3) was added to a stirred solution of (2*RS*)-2-phenylbutyryl chloride (1 g, 5.45 mmol) in diethyl ether (10 cm^3). After 5 min,

the layers were separated and the organic solution was washed with water (5 cm^3), dried, and evaporated. The product was recrystallised from diethyl ether to give amide (694 mg, 78%) as needles, mp 82–83.5 °C (lit.,[26] mp 85–86 °C) (Found: C, 73.6; H, 8.1; N, 8.5. $C_{10}H_{13}NO$ requires C, 73.6; H, 8.0; N, 8.6%); ν_{max} ($CHCl_3$)/cm^{-1} 3480, 3370, 2920, 1660; δ_H (250 MHz), 0.88 (3 H, t, *J* 7.4, $CHCH_2CH_3$), 1.80 (1 H, m, $CHCHHCH_3$), 2.20 (1 H, m, $CHCHHCH_3$), 3.28 (1 H, dd, *J* 8.1 and 7, $CHCH_2CH_3$), 5.44 and 5.50 (each 1 H, br, 2 × NH) and 7.23–7.37 (5 H, m, ArH); *m*/*z* (EI) 163 (M^+, 16%), 135 (M − C_2H_4, 20%), 119 (M − $CONH_2$, 20%) and 91 ($C_7H_7^+$, 100%).

(2*RS*)-2-Phenylbutyronitrile. A solution of the foregoing amide (452 mg, 2.55 mmol) in thionyl chloride (3 cm^3) was heated at reflux for 2 h, then evaporated and flash chromatography of the residue using 1:9 ethyl acetate–hexane followed by Kugelrohr distillation (110 °C/19 mmHg) gave the nitrile (314 mg, 77%) (lit.,[27] bp 107 °C/7 mmHg) (Found: C, 82.9; H, 7.7. $C_{10}H_{11}N$ requires C, 82.7; H, 7.6%); ν_{max} (neat)/cm^{-1} 3100–2850, 2255; δ_H (250 MHz) 1.07 (3 H, t, *J* 7.4, $CHCH_2CH_3$), 1.93 (2 H, m, $CHCH_2CH_3$), 3.73 (1 H, t, *J* 7.2, $CHCH_2CH_3$) and 7.27–7.41 (5 H, m, ArH); δ_C (100 MHz) 11.2 (q, CH_3), 29.0 (t, CH_2), 38.6 (d, CH), 120.8 (s, CN), 127.1, 127.8, and 128.8 (each d, *o*, *m*, and *p*-ArH), 135.9 (s, Ar-C-C); *m*/*z* (EI) 145 (M^+, 32%), 117 (M − C_2H_4, 100%), 116 (M − C_2H_5, 74%), 90 ($C_7H_6^+$, 27%), 89 ($C_7H_5^+$, 30%) and 69 ($CH_3CH_2CHCNH^+$, 26%).

Methyl (2*RS*)-2-phenylbutyrimidate 26 and the corresponding (2*R*)-enantiomer 26a. An ice cooled solution of the foregoing nitrile (187 mg, 1.06 mmol) in diethyl ether (5 cm^3) containing methanol (0.25 cm^3) was saturated with dry hydrogen chloride gas. After 12 h at 0 °C, the solvent was evaporated below room temperature, the residue was washed with diethyl ether and dried at high vacuum to afford the *imidate ester hydrochloride* **26** (243 mg, 100%) as a hydroscopic solid, mp 95–101 °C. δ_H (250 MHz) 0.95 (3 H, t, *J* 7.3, $CHCH_2CH_3$), 2.02 and 2.12 (each 1 H, sextet, *J* 7.5, $CHCH_2CH_3$), 4.30 (4 H, m, overlapping 3 H, s, $CHCH_2CH_3$, OCH_3), 7.32 (3 H, m, *m*, *p*-ArH), 7.47 (2 H, m, *o*-ArH), 11.71 and 12.04 (each 1H, br, NH_2); *m*/*z* (FD) 178 (M − Cl) and 162 (M − HCl − CH_3).

Part of the foregoing imidate hydrochloride was converted into the free base by extraction with dichloromethane from aqueous base (Found: M^+ 177.1150. $C_{11}H_{15}NO$ requires *M* 177.1154); ν_{max} (neat)/cm^{-1} 3310, 2960, 1640; δ_H (250 MHz) 0.86 (3 H, t, *J* 7.3, CH_3), 1.81 and 1.99 (each 1 H, m, CH_2CH_3), 3.35 (1 H, dt, *J* 7.9 and 0.9, $CHCH_2CH_3$), 3.68 (3 H, s, OCH_3), 7.07 (1 H, br, NH) and 7.18–7.28 (5H, m, ArH); *m*/*z* (EI) 177 (M^+, 33%), 162 (M − CH_3, 58%), 91 ($C_7H_7^+$, 100%) and 58 (CH_3OCNH^+, 34%).

Commercially available (2*R*)-2-phenylbutyric acid was carried through exactly the same steps to yield methyl (2*R*)-2-phenylbutyrimidate hydrochloride **26a**, identified by spectroscopic comparison with the foregoing sample.

Methyl (2*RS*)-2-methoxy-2-trifluoromethylphenylacetimidate 29. Sodium methoxide (1.7 g, 31.5 mmol) was added to a stirred solution of the commercially available (2*RS*)-nitrile **27** (1.7 g, 7.9 mmol) in dry methanol (15 cm^3). After 2 h at room temperature, the solution was poured into saturated aqueous sodium hydrogen carbonate (200 cm^3) and extracted with dichloromethane (100 cm^{-3}, 3 × 50 cm^3). The combined organic solution was dried and evaporated. Flash chromatography of the residue using first 1:9 then 3:7 ethyl acetate–hexane gave, after Kugelrohr distillation (95 °C/19 mmHg) recovered nitrile **27** (855 mg, 50%) and later (125 °C/19 mmHg) the *imidate ester* **29** (825 mg, 42%) as an oil (Found: C, 53.2; H, 4.3; N, 6.0. $C_{11}H_{12}F_3NO_2$ requires C, 53.4; H, 4.5; N, 6.0%); ν_{max} ($CHCl_3$)/cm^{-1} 3310, 2940, 1660; δ_H (250 MHz) 3.29 (3 H, d, *J* 1.3, CF_3COCH_3), 3.80 (3 H, s, $CNHOCH_3$), 7.41 (5 H, m, ArH) and 8.30 (1 H, br, NH); δ_C (100 MHz) 54.1 and 54.4 (each s, 2 × OCH_3), 123.9 (q, *J* 290, CF_3), 127.8, 128.5 and 129.4 (each d, *o*, *m*, *p* ArH), 132.3 (s, Ar–C–C), 166.9 (s, C=NH), signal for CH_3OCCF_3 too weak to observe; *m*/*z* (EI) 247 (M^+, 11%), 233 (M − CH_3, 20), 189 ($C_6H_5C(CH_3)OCF_3^+$, 19), 170 ($C_6H_5^+$, 42), 69 (CF_3^+, 23) and 58 ($HNCOCH_3^+$, 100).

Attempted preparation of the foregoing imidate as its hydrochloride by the standard method as follows, led to a different product. An ice cooled solution of nitrile **27** (420 mg, 1.95 mmol) in diethyl ether (10 cm^3) containing methanol (0.5 cm^3) was saturated with dry hydrogen chloride gas. The solution was kept at 0 °C overnight then treated with more hydrogen chloride gas and after a further 24 h at 0 °C, nitrogen was passed through the solution (there was no precipitate) to remove HCl. It was then evaporated below room temperature to give (*2RS*)-*2-methoxy-2-trifluoromethylphenylacetamide* **28** (405 mg, 89%) mp 77–78.5 °C from diethyl ether–hexane (Found: C, 51.7; H, 4.3; N, 6.0. $C_{10}H_{10}F_3NO_2$ requires C, 51.5; H, 4.3; N, 6.0%); ν_{max} ($CHCl_3$)/cm^{-1} 3495 , 3375, 2960, 1700; δ_H (250 MHz), 3.44 (3 H, q, *J* 1.6, OCH_3), 5.99 and 6.68 (each 1 H, br, NH_2), 7.38–7.44 (3 H, m, *m*, *p*-ArH) and 7.44–7.58 (2 H, m, *o*-ArH); *m*/*z* (EI) 233 (M^+, 6%), 190 (M − CONH, 80%), 189 (M − $CONH_2$, 90%), 175 (M − $CONHCH_3$, 45%), 105 (C_6H_5CO, 100%) and 77 (C_6H_5, 50%).

(1*R*,4*S*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxamide. Aqueous ammonia (17.5%, 20 cm^3) was added to a vigorously stirred solution of (+)-(1*R*,4*S*)-camphanyl chloride (1 g, 4.6 mmol) in dichloromethane (20 cm^3) and after 45 min, the separated aqueous phase was extracted with dichloromethane (2 × 10 cm^3). The combined organic solution was washed with aqueous 2 M hydrochloric acid (20 cm^3), dried and evaporated; recrystallisation of the residue from hexane–ethyl acetate gave the *amide* (821 mg, 90%) as needles, mp 209–211 °C (Found: C, 60.6; H, 7.7; N, 7.1. $C_{10}H_{15}NO_3$ requires C, 60.9; H, 7.7; N, 7.1%); ν_{max} ($CHCl_3$)/cm^{-1} 3490, 3380, 2920, 1775, 1680; δ_H (250 MHz), 0.95, 1.10 and 1.11 (each 3 H, s, 3 × CH_3), 1.68, 1.94 and 2.51 (1 H, 2 H, 1 H, each m, CH_2CH_2), 5.75 and 6.39 (each 1 H, br, NH_2); *m*/*z* (EI) 197 (M^+, 10%), 169 (M − C_2H_4, 15%), 151 (M − CO_2H_2, 90%) and 83 ($C_6H_{11}^+$, 100%).

(1*R*,4*S*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonitrile 30. A solution of the foregoing amide (269 mg, 1.36 mmol) in toluene (5 cm^3), containing phosphorus pentoxide (0.25 g) was heated at reflux for 5 h then cooled, poured into saturated aqueous sodium hydrogen carbonate (50 cm^3) and extracted with dichloromethane (3 × 20 cm^3). The combined organic solution was dried and evaporated. The residues by flash chromatography using 3:7 ethyl acetate–hexane and recrystallisation of the product from hexane–diethyl ether, gave *nitrile* **30** (162 mg, 66%) as needles, mp 134–136 °C (sealed capillary) (Found: C, 67.8; H, 7.4; N, 7.8. $C_{10}H_{13}NO_2$ requires C, 67.0; H, 7.3; N, 7.8%); ν_{max} ($CHCl_3$)/cm^{-1} 2930, 1790; δ_H (250 MHz) 1.06, 1.13, 1.14 (each 3 H, s, 3 × CH_3), 1.72 (1 H, ddd, *J* 13.5, 8.6 and 5.0), 1.93 (1 H, ddd, *J* 1.3, 10.3 and 4.8) and 2.16–2.37 (2 H, m, CH_2CH_2); δ_C (100 MHz) 9.6, 16.1 and 16.7 (each q, 3 × CH_3), 28.0 and 31.9 (each t, 2 × CH_2), 52.6 and 54.5 (each s, 2 × quat.-C), 81.6 (s, O*C*CN), 114.3 (s, CN) and 176.4 (s, C=O); *m*/*z* (EI) 179 (M^+).

Methyl (1*R*,4*S*)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]-heptane-1-carboximidate 31. Sodium methoxide (350 mg, 6.42 mmol) was added to a stirred solution of nitrile **30** (569 mg, 3.17 mmol) in dry methanol (10 cm^3) under argon. After 30 min, the solution was poured into saturated aqueous sodium hydrogen carbonate (25 cm^{-3}) and extracted with dichloromethane (3 × 20 cm^3). The combined organic solution was dried and evaporated. Flash chromatography of the residue using first 3:7 then 1:1 ethyl acetate–hexane gave *imidate ester*

31 (355 mg, 53%) mp 69–70.5 °C from diethyl ether–hexane (Found: C, 62.7; H, 8.2; N, 6.7. $C_{11}H_{17}NO_3$ requires C, 62.5; H, 8.1; N, 6.6%); ν_{max} ($CHCl_3$)/cm^{-1} 3310, 2840, 1770, 1645; δ_H (250 MHz) 0.85, 1.01 and 1.09 (each 3 H, s, 3 × CH_3), 1.65–1.73 (1 H, m), 1.82–1.96 (2 H, m), 2.39 (1 H, m, CH_2CH_2), 3.80 (3 H, s, OCH_3) and 7.79 (1 H, br, NH); δ_C (100 MHz) 9.5, 16.3 and 16.7 (each q, 3 × CH_3), 28.9 and 29.8 (each t, 2 × CH_2), 53.0 (q, OCH_3), 53.0 and 54.9 (each s, 2 × quat.-C), 91.7 (s, O-*C*-C=O), 167.1 (s, C=NH) and 178.1 (s, C=O); *m*/*z* (EI) 211 (M^+, 17%), 183 (M − CO, 80%), 167 (M − CO_2, 40%), 155 (M − COC_2H_4, 75%) and 83 ($C_6H_{11}^+$, 75%).

Also obtained from this reaction was *methyl (1S)-1,2,2-trimethyl-3-oxocyclopentane carboxylate* **32** (232 mg, 40%) as an oil which crystallised (mp 24–26 °C) after Kugelrohr distillation (100 °C/19 mmHg) (Found: C, 65.4; H, 8.6. $C_{10}H_{16}O_3$ requires C, 65.2; H, 8.6%); ν_{max} ($CHCl_3$)/cm^{-1} 2980, 1720; δ_H (250 MHz) 0.90, 1.01 and 1.20 (each 3 H, s, 3 × CH_3), 1.80 (1 H, m) and 2.20–2.58 (3 H, m, CH_2CH_2) and 3.66 (3 H, s, OCH_3); δ_C (100 MHz) 19.1, 19.2, and 19.7 (each q, 3 × CH_3), 28.8 and 33.5 (each t, 2 × CH_2), 51.4 (q, OCH_3) 51.5 and 52.3 (each s, 2 × quat.-C), 176.2 and 176.3 (each s, 2 × C=O); *m*/*z* (EI) 184 (M^+) and 156 (M − CO).

Methyl (2*R*)-2-phenyl-2-hydroxyacetimidate hydrochloride 34. (+)-Amygdalin (10 g) in distilled water (30 cm^3) was treated with concentrated sulfuric acid (11 cm^3) and the solution was heated at 85–90 °C (bath temperature) for 50 min during which time a brown oil separated. Water (50 cm^3) was added to the cooled mixture and the mandelonitrile was extracted thrice with benzene. The combined organic layers were washed once with water, dried for 10 min over sodium sulfate and evaporated to give the single enantiomer (*R*)-mandelonitrile **33** (2.11 g). This material is easily racemised so it was immediately converted into the following imidate hydrochloride.

To the (*R*)-mandelonitrile **33** (2.11 g, 15.9 mmol) in dry diethyl ether (7 cm^3) was added dropwise with ice cooling, a solution of hydrogen chloride in dry methanol (3 cm^3, containing 1.5 g (3 equiv.) of CH_3OH and 1.5 g (2.6 equiv.) of hydrogen chloride). After the mixture had been stirred for 2 h at 0 °C, the precipitate was collected by centrifugation, washed twice with dry diethyl ether and dried *in vacuo* to give the imidate hydrochloride **34** (2.1 g, 65%) (Found: M^+ − HCl, 165. $C_9H_{12}NO_2Cl$ requires (M − HCl), 165); δ_H (CD_3OD, 100 MHz) 4.1 (3 H, s, OCH_3), 5.5 (1 H, s, C*H*OH) and 7.4 (5 H, m, ArH); *m*/*z* 165 (M − HCl, 55%), 108 (55) and 107 (M − HCl − C_2H_4ON, 88); $C_9H_{12}NO_2Cl$ requires for M − HCl, 165.

Conversion of PBG into amidine derivatives

The amidine 37. All the experiments that follow involved fragile products that were highly polar and water soluble. Therefore, the chemistry to be applied eventually to bilanes was developed using similar, though simpler, starting materials; the generation of an amidine was studied first on PBG.

A solution of PBG **1** (35 mg, 0.15 mmol) in aqueous 0.2 M sodium hydrogen carbonate buffer (pH10) was treated with a solution of methyl 2-phenylacetimidate hydrochloride **36** (70 mg, 0.4 mmol) in the same buffer (7 cm^3), the pH of the final solution being 9.4. This was stirred at room temperature, the reaction being complete in 3 h as monitored by TLC on a cellulose plate using isopropanol–water–acetic acid (15:5:1); R_f of product, 0.75; R_f of PBG, 0.5. The mixture was extracted twice with chloroform to remove excess reagent, the pH was adjusted to 7.5 with 1 M hydrochloric acid and the water was evaporated at high vacuum. The residue was extracted twice with methanol (2 × 2 cm^3), the extracts were evaporated and the residue was dissolved in [$^2H_4$]methanol (1 cm^3). The solution was evaporated and the residue dried at high vacuum for 3 h. This material, the disodium salt **37**, was homogeneous by NMR and TLC, R_f in isopropanol–water (15:4) 0.5, apart from a small amount of sodium chloride. δ_H (CD_3OD, 100 MHz) 2.2–2.9 (4 H, m, $CH_2CH_2CO_2$), 3.27 (2 H, s, $C_6H_5CH_2$), 3.3 (2 H, s, CH_2CO_2), 4.48 (2 H, s, CH_2N), 6.45 (1 H, d, pyrrole-H), 7.5 and 7.64 (3H and 2 H, 2 × m, ArH). This product gave no M^+ by mass spectrometry, even using FD with application from a solution in acetic acid.

A solution of the foregoing amidine **37** (10mg) in methanol (3 cm^3) was treated with dimethyl sulfate (0.4 cm^3) and stirred for 0.5 h at room temperature, with monitoring by TLC; R_f of product, 0.9; R_f of starting material 0.5 using isopropanol–water (15:4). The reaction mixture was diluted with water (final pH was 2), the product was extracted with dichloromethane and the dried extracts were evaporated to give a monomethyl ester **38** or **39** (7 mg, 70%) which was pure by NMR and TLC, R_f in CH_3OH–CH_2Cl_2 (1:9) 0.15. δ_H ($CDCl_3$–CD_3OD, 100 MHz) 2.55 (4 H, m, $CH_2CH_2CO_2$), 3.46 (2 H, s, CH_2CO_2), 3.58 (2 H, s, CH_2 CN), 3.62 (3 H, s, OCH_3), 4.56 (2 H, s, CH_2N), 6.5 (1 H, d, *J* 3, pyrrole-H) and 7.6 (5 H, m, ArH); the NH groups had exchanged. δ_H ($CDCl_3$, 100 MHz) 2.62 (4 H, m, CH_2-CH_2CO_2), 3.54 (2 H, s, CH_2CO_2), 3.66 (3 H, s, OCH_3), 3.97 (2 H, s, CH_2CN), 4.77 (2 H, d, *J* 5.5, CH_2NH), 6.52 (1 H, br s, pyrrole-H), 7.55 and 7.75 (1 × 3 H, 1 × 2 H, both m, ArH) and 8.1, 8.92 and 9.15 (each 1H, 3 × br s, 3 × NH); *m*/*z* (FD) 358 (M^+, 100%); $C_{19}H_{24}N_3O_4$ requires 358.

The racemic amidine (as 40). A solution of the (*RS*)-imidate hydrochloride **26** (47.5 mg, 0.21 mmol) in 0.2 M carbonate buffer (pH 10, 4 cm^3) containing DMF (0.5 cm^3) was added to a solution of PBG **1** (18.2 mg, 0.08 mmol) in the same buffer (0.5 cm^3). The mixture was stirred for 4 h at room temperature in the dark under argon then adjusted to pH 7–8 with 1 M hydrochloric acid and the excess imidate was extracted into chloroform (3 × 3 cm^3). The aqueous solution was evaporated to dryness at <40 °C under vacuum and the residue was redissolved in D_2O (2 cm^3). The solution was evaporated to dryness and the process was repeated before dissolving the final residue in CD_3OD (1.5 cm^3). This solution was centrifuged and filtered twice until perfectly clear for NMR analysis. The signal from the NCH_2-pyrrole group of the amidine (as **40**) was an ABq with the four signals at δ 4.237, 4.298, 4.309 and 4.370.

The chloroform washings were worked up to give recovered imidate as the free base (26 mg, 71%).

Conversion of (11-*R*)-[11-$^2H_1$]PBG 1b and (11*S*)-[11-$^2H_1$]-PBG 1a into the amidines 40b and 40a. (11-*R*)-[11-$^2H_1$]PBG lactam methyl ester **16b** (19.7 mg, 0.088 mmol) was heated at 70 °C for 5 min, under argon, in argon saturated aqueous 2 M potassium hydroxide (0.6 cm^3) then the resulting solution was kept at room temperature in the dark under argon for 18 h. The solution was adjusted to pH 7–8 with 1 M hydrochloric acid followed by 0.2 M sodium carbonate buffer (pH 10, 0.4 cm^3). To this was added a solution of the (2*R*)-imidate hydrochloride **26a** (58 mg, 0.27 mmol) in the same carbonate buffer (4 cm^3) containing DMF (0.5 cm^3). The remaining steps were as above to give a solution in CD_3OD of the amidine **40b** having the (*R*)-configuration at the starred centre.

Using the same procedure, (11-*S*)-[11-$^2H_1$]PBG lactam ester **16a** (19.8 mg, 0.088 mmol) and the (2*R*)-imidate ester hydrochloride **26a** (54.3 mg, 0.25 mmol) afforded a solution in CD_3OD of the amidine **40a**. ^{1}H-NMR showed that the signal from the starred centre of the sample having the (*S*)-configuration at that site appeared at lower field (δ 4.33) and that from the other diastereoisomer, at higher field (δ 4.26).

The amidine 41. PBG (35 mg, 0.15 mmol) in 0.2 M sodium hydrogen carbonate buffer (1 cm^3, pH 10) was treated with the imidate hydrochloride **34** (80 mg, 0.4 mmol) in the same buffer (8 cm^3) and the solution (final pH 9.4) was stirred at room temperature until complete reaction (2 h), as shown by TLC on cellulose. The product showed R_f 0.7 and starting material

showed R_f 0.5 using isopropanol–water–acetic acid (15:5:1). The solution was extracted twice with chloroform, the pH adjusted to 7.5, the water evaporated and the residue was dried for 3 h at high vacuum. It was then extracted with dry methanol (2 × 2 cm^3), the extracts were clarified by centrifugation and evaporated. The residue in $^2H_4$-methanol (1 cm^3) was centrifuged and the clear solution was evaporated to give **41** as its disodium salt which was pure by NMR and TLC. δ_H (CD_3OD, 400 MHz) 2.38 (2H, m, $CH_2CH_2CO_2$), 2.7 (2H, m, $CH_2CH_2CO_2$), 3.3 (2H, s, CH_2CO_2), 4.37 (2H, ABq, *J* 15.2; the high field part of this quartet corresponds to H_S of NHCH_2), 5.36 (1H, s, CHOH), 6.54 (1H, s, pyrrole-H), 7.3 and 7.5 (5H, 2 × m, ArH). It was not possible by FD-MS to generate M^+ under any conditions tested.

Conversion of (11*S*)-[11-$^2H_1$]PBG 1a and (11-*R*)-[11-$^2H_1$]-PBG 1b into the amidines 41a and 41b, respectively. A suspension of the lactam ester **16a** in aqueous 2 M potassium hydroxide (0.33 cm^3) was heated at 70 °C for 5 min and the solution was kept for 18 h at room temperature in the dark. It was adjusted to pH 8 with 1 M hydrochloric acid and aqueous 0.2 M sodium hydrogen carbonate buffer (0.2 cm^3, pH 10) was added followed by the imidate hydrochloride **34** in the above buffer (2.2 cm^3). The solution (final pH 9.4) was stirred for 2 h at room temperature and the amidine **41a** having mainly the (*S*)-configuration at the starred centre was isolated as for the unlabelled analogue **41**. δ_H (CD_3OD, in part, 400 MHz) 4.32 [0.16 H, s, (11*R*) NHC*H*D], 4.39 [0.79H, s, (11*S*) NHC*H*D]; the other signals were as for the unlabelled analogue **41**.

The lactam **16b** having mainly the (11*R*)-configuration was used in the same way to afford the amidine **41b** having largely the (*R*)-configuration at the starred centre. δ_H (CD_3OD, in part, 400 MHz) 4.32 [0.79 H, s, (11*R*)-NHC*H*D], 4.39 [0.16 H, s, (11*S*)-NHC*H*D]; the rest of the spectrum was identical to that from the unlabelled **41**.

Formation of amidines from dipyrromethanes

Importantly, the solvents used for the next experiment and all those that follow it were deoxygenated and were then saturated with argon (see earlier for methods used).

Reaction of (2*RS*)-2-phenylbutyrimidate hydrochloride 26 with the aminomethyl dipyrromethane 43. The lactam[21] **42** (14.5 mg, 0.035 mmol) was treated overnight in the dark under argon with argon saturated aqueous 0.2 M potassium hydroxide (0.3 cm^3) then the solution was adjusted to pH 7–8 by the addition of hydrochloric acid followed by carbonate buffer (pH 10, 0.5 cm^3). A solution of imidate hydrochloride **26** (57.5 mg, 0.25 mmol) in the same buffer (4 cm^3) containing DMF (0.5 cm^3) was added to the product **43** and the resulting solution was stirred for 2 h at room temperature in the dark under argon. The solution was extracted with chloroform (3 × 2 cm^3) and the aqueous solution was adjusted to pH 7–8 with 1 M hydrochloric acid before evaporation to dryness. The residue was redissolved in D_2O (2 cm^3) and evaporated to dryness; this was repeated. The residue was extracted with CD_3OD (1.5 cm^3), the solution was centrifuged and filtered (twice) to give a clear solution for NMR analysis. The signal from the N-CH_2-pyrrole group of the amidine **44** was an ABq with the four signals at 4.233, 4.271, 4.283 and 4.321.

The chloroform extracts yielded recovered imidate free base (40.2 mg).

The amidine 45. The dipyrromethane **42** was hydrolysed by stirring it (23 mg) with aqueous 2 M KOH (0.4 cm^3) for 3 h at room temperature under argon and then keeping the solution for 18 h in the dark to complete the hydrolysis. The solution was adjusted to pH 7–8 with 1 M hydrochloric acid and aqueous 0.2 M sodium hydrogen carbonate buffer (0.5 cm^3, pH 10) was added followed by the imidate hydrochloride **34** (50 mg) in the same buffer (4 cm^3). The solution, final pH 9.4, was stirred at room temperature for 2 h until the conversion was complete as shown by TLC on cellulose using isopropanol–water (15:5): R_f of product, 0.6; R_f of starting material, 0.5. The solution was extracted twice with chloroform, the pH was adjusted to 8 and then evaporated to dryness and further dried at high vacuum. The residue was extracted with dry methanol (2 × 3 cm^3), the extracts were centrifuged and the clear solution was evaporated. The product was dissolved in CD_3OD (0.5 cm^3), centrifuged clear and the solvent was evaporated to give the amidine **45** which was homogeneous by NMR and TLC. δ_H (CD_3OD, 250 MHz) 2.4 (4 H, m, $CH_2CH_2CO_2$), 2.77 (4 H, m, $CH_2CH_2CO_2$), 3.28 and 3.36 (2 × 2 H, 2 × s, 2 × CH_2CO_2), 3.75 (2 H, ABq, *J* 16.7, 5-H_2), 4.34 (2 H, br s, NCH_2), 5.38 (1 H, s, CHOH), 6.38 (1 H, s, pyrrole-H) and 7.25 and 7.4 (5 H, m, ArH). When the spectrum was measured at 400 MHz, the interpyrrolic CH_2 (5-H_2) appeared as an ABq with $\Delta\nu = 24$ Hz whilst the signal from the NCH_2 group was not clearly resolved.

Synthesis of the standard unlabelled amidine 25 derived from bilane 7 and the analogous [$^2H_1$]-labelled amidines 25a and 25b. An alkaline solution of the aminomethylbilane **7** was prepared by hydrolysis as usual[16] of the corresponding lactam ester (24 mg). The solution was adjusted to pH 8 with 1 M hydrochloric acid and aqueous 0.2 M potassium hydrogen carbonate buffer (0.6 cm^3, pH 10) was added followed by the imidate hydrochloride **34** (20 mg) in the same buffer (2 cm^3). The final solution (pH 9.4–9.7) was stirred until the conversion was complete (2.25 h) as shown by TLC using isopropanol–water–acetic acid (15:4:1); R_f of product, 0.78; R_f of starting material, 0.66. The solution was extracted twice with dichloromethane (each 20 cm^3), then the pH was adjusted to 8 and the water was evaporated. After the powdered residue had been dried for 3 h at high vacuum, it was extracted twice with methanol (each 3 cm^3), the extracts were centrifuged until clear and the solvent was removed. The residue was dissolved in [$^2H_4$]methanol (1 cm^3), the solution was evaporated and the solid was extracted again with [$^2H_4$]methanol (1 cm^3), the solution being centrifuged until clear ready for NMR analysis of the amidine **25**. δ_H (CD_3OD, 400 MHz) 2.3 and 2.7 (each 8 H, $CH_2CH_2CO_2$), 3.3 (8 H, m, CH_2CO_2), 3.6–3.8 (6 H, m, (pyrrole)$_2$CCH_2), 4.38, (2H, ABq, *J* 16.7, $\Delta\nu \approx 20$ Hz NHCH_2; high field part, H_S), 5.5 (1 H, s, CHOH), 6.35 (1 H, s, pyrrole-H) and 7.25 and 7.5 (5H, m, ArH).

The labelled amidines 25a and 25b. Each labelled bilane **7a** and **7b** was prepared by hydrolysis of the corresponding lactam and converted into the corresponding amidine **25a** and **25b** as described for the unlabelled series. The mainly (*S*)-amidine **25a** showed δ_H (CD_3OD, 400 MHz) 4.42 (0.16 H, s, (*R*)-C*H*DNH), 4.43 (0.79 H, s, (*S*)-C*H*DNH). The mainly (*R*)-amidine **25b** showed δ_H (CD_3OD, 400 MHz) 4.40 [0.79 H, s, (*R*)-C*H*DNH], 4.428 [0.16 H, s, (*S*)-C*H*DNH]. The rest of the signals matched in the two samples and also with those from the authentic unlabelled sample **25**. These spectra were recorded one after the other on the same instrument. Though the large signal at *ca.* δ 4.4 from the sample having mainly the (*R*)-CHDN group was clearly at higher field than that from the material having mainly the (*S*)-CHDN residue, slight differences in the preparation of the samples prevented the absolute values of the chemical shifts coinciding exactly. Therefore, the foregoing assignments were confirmed by mixing the (*R*)-amidine **25b** with a smaller quantity of the (*S*)-isomer **25a** to demonstrate coincidence of the small NMR signal from the C*H*DNH group of one with the large signal from the corresponding position of the other, Fig. 2(c).

Enzymic preparation of $^2H_1$-labelled aminomethylbilanes and determination of their configuration by formation of amidines. The (11*S*)-PBG **1a** prepared as above by hydrolysis of the

corresponding lactam **16a** (39 mg) was converted enzymically[22] into the aminomethylbilane **7a** by first adjusting the hydrolysate to pH 9 with 0.1 M phosphoric acid. This solution was made 0.2 M with respect to NH_4Cl and then was at pH 8.05. Hydroxymethylbilane synthase[22] (130 000 units) was added and the mixture (20.8 cm^3) was shaken at 37 °C for 30 min. The solution was cooled on ice, adjusted to pH 12.2 with aqueous 1 M sodium hydroxide and sodium dithionite (50 mg) and 2 M potassium hydroxide (1.6 cm^3) were added. This solution was freeze dried and the residue in aqueous 0.2 M sodium hydrogen carbonate buffer (1 cm^3, pH 10) was again freeze dried to remove all the ammonia. A solution of the final residue in distilled water at pH > 11 was mixed with aqueous 0.2 M sodium hydrogen carbonate buffer (0.8 cm^3, pH 10) and adjusted to pH 9.7 with 1 M hydrochloric acid. The imidate hydrochloride **34** (25 mg) in the above buffer (2 cm^3) was added and the solution was stirred at room temperature in the dark for 2 h, monitoring formation of the amidine **25c** by TLC as described above for the unlabelled analogue **25**. The rest of the work-up of **25c** through to the NMR analysis followed exactly that used above for the synthetic sample **25**.

The (11*R*)-PBG **1b** prepared from the lactam **16b** was enzymically converted into the aminomethylbilane **7d** and then into the amidine **25d** by exactly the same procedure. The ^{1}H-NMR spectra (CD_3OD, 400 MHz) of the amidines **25c** and **25d** were identical, respectively, with the synthetic samples **25a** and **25b**, apart from the expected differences due to ^{2}H at the interpyrrolic methylene groups. The signals from the CHDN residues of each sample are shown in Fig. 2.

It was essential to carry out all the foregoing steps at as low a temperature and as quickly as possible. Otherwise, the background hump evident in Fig. 2(b) became much larger; even then, however, the signals which are quite clear in Fig. 2(a) and 2(b) could still be observed.

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References

1 Part 51. K. Ichinose, M. Kodera, F. J. Leeper and A. R. Battersby, *J. Chem. Soc.*, *Perkin Trans. 1*, 1999, 879.
2 Part of this work has been published in preliminary form: W. L. Neidhart, P. C. Anderson, G. J. Hart and A. R. Battersby, *J. Chem. Soc.*, *Chem. Commun.*, 1985, 924.
3 Reviewed by A. R. Battersby and F. J. Leeper, *Chem. Rev.*, 1990, **90**, 1261.
4 A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, E. McDonald and R. Hollenstein, *J. Chem. Soc.*, *Perkin Trans. 1*, 1983, 3031.
5 R. Radmer and L. Bogorad, *Biochemistry*, 1972, **11**, 904; R. C. Davies and A. Neuberger, *Biochem. J.*, 1973, **133**, 471.
6 C. Jones, P. M. Jordan and M. Akhtar, *J. Chem. Soc.*, *Perkin Trans. 1*, 1984, 2625.
7 A. H. Jackson, W. Lertwanawatana, G. Procter and S. G. Smith, *Experientia*, 1987, **43**, 892.
8 H. Takahashi, H. Noguchi, K. Tomita and H. Otamasu, *Yakugaku Zasshi*, 1978, **98**, 618; D. L. Trepanier, V. Spanemaris and K. E. Wiggs, *J. Org. Chem.*, 1964, **29**, 668.
9 A. D. Abell, B. K. Nabbs and A. R. Battersby, *J. Am. Chem. Soc.*, 1998, **120**, 1741.
10 A. R. Battersby, E. Hunt, E. McDonald, J. B. Paine III and J. Saunders, *J. Chem. Soc.*, *Perkin Trans. 1*, 1976, 1008; A. R. Battersby, C. J. R. Fookes, M. J. Meegan, E. McDonald and H. K. W. Wurziger, *J. Chem. Soc.*, *Perkin Trans. 1*, 1981, 2786.
11 D. E. Horning and J. Muchowski, *Can. J. Chem.*, 1968, **46**, 3665.
12 M. M. Kreevoy and J. E. C. Hutchins, *J. Am. Chem. Soc.*, 1969, **91**, 4329.
13 D. Enders and H. Eichenaur, *Chem. Ber.*, 1979, **112**, 2933.
14 H. Klein and A. Steinmetz, *Tetrahedron Lett.*, 1975, 4249.
15 W. L. F. Armarego, B. A. Milloy and W. Pendergast, *J. Chem. Soc.*, *Perkin Trans. 1*, 1976, 2229.
16 A. R. Battersby, C. J. R. Fookes, K. E. Gustafson-Potter, E. McDonald and G. W. J. Matcham, *J. Chem. Soc.*, *Perkin Trans. 1*, 1982, 2413.
17 M. J. Hunter and M. L. Ludwig, *J. Am. Chem. Soc.*, 1962, **84**, 3491.
18 I. A. Smith, *Chem. Ber.*, 1931, **64**, 427; *cf.* J. W. Walker and V. K. Krieble, *J. Chem. Soc.*, 1909, 1369.
19 D. E. Neilson, D. A. V. Peters and L. H. Roach, *J. Chem. Soc.*, 1962, 2272.
20 G. Hafelinger, *The Chemistry of Amidines and Imidates*, ed. S. Patai, Wiley, New York, 1975, p. 15.
21 A. R. Battersby, D. A. Evans, K. H. Gibson, E. McDonald and L. Nixon, *J. Chem. Soc.*, *Perkin Trans. 1*, 1973, 1546.
22 A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, E. McDonald and R. Hollenstein, *J. Chem. Soc.*, *Perkin Trans. 1*, 1983, 3031.
23 J.-R. Schauder, S. Jendrezejewski, W. L. Neidhart, G. J. Hart and A. R. Battersby, *J. Chem. Soc.*, *Perkin Trans. 1*, 1999, 2691, following paper.
24 A. R. Battersby, C. J. R. Fookes, K. E. Gustafson-Potter, E. McDonald and G. W. J. Matcham, *J. Chem. Soc.*, *Perkin Trans. 1*, 1982, 2427.
25 A. R. Battersby, S. Kishimoto, E. McDonald, F. Satoh and H. K. W. Wurziger, *J. Chem. Soc.*, *Perkin Trans. 1*, 1979, 1927.
26 D. A. Shirley, M. J. Danzig and F. C. Canter, *J. Am. Chem. Soc.*, 1953, **75**, 3278.
27 P. E. Gagnon, J. L. Boivin and C. Haggart, *Can. J. Chem.*, 1956, **34**, 1662.

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