Stereochemistry of Formation of the Hydroxymethyl Group of Hydroxymethylbilane, the Precursor of Uro'gen-III

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A new synthetic route to (11*S*)- and (11*R*)-[11-$^{3}H_1$]porphobilinogen (PBG) is described and the configurations of the products are assigned by degradation to (2*S*)- and (2*R*)-[2-$^{3}H_1$]glycollic acids; these PBG samples are used to establish that hydroxymethylbilane synthase converts PBG into the hydroxymethylbilane with *overall retention of configuration* at the $HOCH_2$– group.

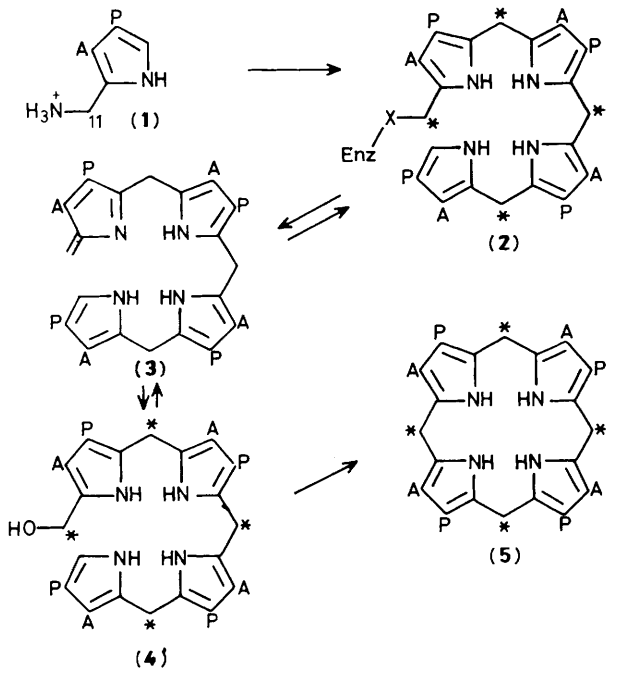
The natural porphyrins, chlorins and corrins, are all biosynthesised from uroporphyrinogen-III (**5**), shortened to uro'gen-III, which is formed from the hydroxymethylbilane[1] (**4**) by a remarkable ring closure and rearrangement process catalysed by the enzyme cosynthetase. The bilane (**4**) is assembled from four molecules of porphobilinogen[2] (**1**), PBG, by the enzyme hydroxymethylbilane synthase† (Scheme 1) and there is strong evidence that release of the bilane (**4**) occurs *via* the azafulvene[3] (**3**).

The long-term aim of our studies is to determine the stereochemistry of formation of all the starred methylene groups of the bilane (**4**) and of uro'gen-III (**5**). We start with the hydroxymethyl group of bilane (**4**).

Pilot studies showed that this problem would require the high sensitivity of tritium rather than deuterium labelling. Further, the earlier synthesis[4] of (*R*)- and (*S*)-[11-$^{2}H_1$]PBG required strongly acidic conditions at high dilution and so was unsuitable for preparing ^{3}H-labelled material. Accordingly, a new synthesis was devised.

† Formerly called PBG deaminase.

The chiral centre was to be introduced by reduction of a 2-formylpyrrole with pinylborane[5] but subsequent S_N2 displacements at the chiral centre could not be expected since hydroxymethylpyrroles and their derivatives react *via* the corresponding azafulvenes (*e.g.* ref. 1). So this reaction mode was blocked by preparing the *N*-trifluoromethanesulphonylaldehyde (**6**) which was reduced with sodium borotritide and the resultant labelled alcohol was reoxidised to yield the labelled aldehyde (**7**). This was reduced with pinylborane, derived from (−)-α-pinene, to give the stable alcohol (**9**). Its illustrated (*R*)-configuration was proved by exact repetition of the asymmetric reduction in the ^{2}H-series (**8**) to yield (**10**) which as its camphanate ester (**11**) was ozonised to yield the *O*-camphanate of [2-$^{2}H_1$]glycollic acid, isolated as its methyl ester (**12**). Having demonstrated that the unlabelled analogue of (**12**) showed a well resolved AB quartet (from $-OCH_2CO-$), the labelled sample (**12**) was correlated by n.m.r. with a standard prepared from authentic (2*S*)-[2-$^{2}H_1$]glycollic acid[6] kindly provided by Professor D. Arigoni. The n.m.r. data also showed that the [^{2}H]alcohol (**12**) contained 88 ± 5% of the illustrated (*R*)-enantiomer, the remainder being the (*S*)-spe-



A = CH_2CO_2H, P = $CH_2CH_2CO_2H$

Scheme 1

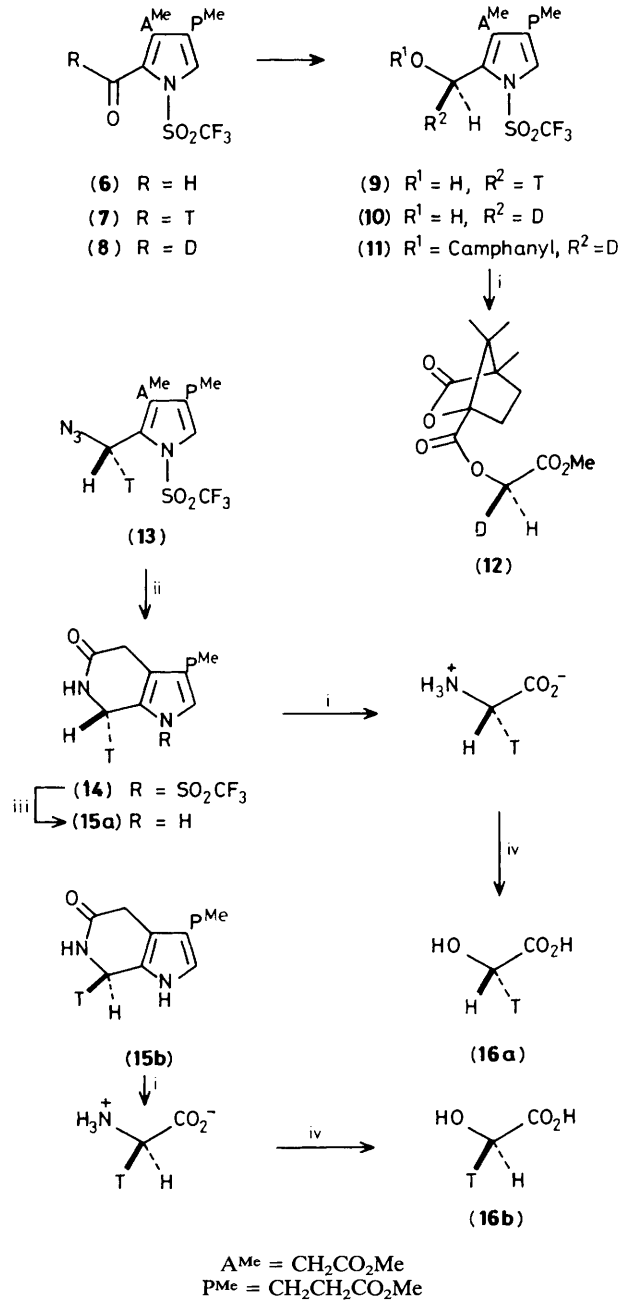
Table 1. Configurational assays on key materials.

	Alcohol (**9**) and enantiomer	PBG lactam esters (**15a**) and (**15b**)	Hydroxymethylbilanes (**18a**) and (**18b**)
Series for (11*S*)-[11-$^3H_1$]PBG	88 ± 5% (*R*)	74 ± 5% (*S*) 74 ± 5% (*S*)	68 ± 5% (*S*)
Series for (11*R*)-[11-$^3H_1$]PBG	96 ± 5% (*S*)	84 ± 5% (*R*) 85 ± 5% (*R*)	74 ± 5% (*R*)

cies, and so by comparison, the same range holds true for the strictly parallel ^3H-series (Table 1).

Conversion of the [$^3H_1$]alcohol (**9**) into the azide (**13**) used Mitsunobu's conditions[7] and the rest of the sequence *via* (**14**) to (11*S*)-[11-$^3H_1$]PBG lactam ester (**15a**) is shown in Scheme 2. The configurational purity of the lactam (**15a**) was determined by ozonolysis to yield glycine and this with nitrous acid gave glycollic acid with retention of configuration.[8] The glycollic acid (**16a**) was assayed enzymically as described later to show that 74 ± 5% of the [^3H]lactam had the (*S*)-configuration (**15a**), the remainder being the enantiomer (**15b**), Table 1. Though some loss of configurational purity had occurred over the sequence (**9**) → (**13**) → (**14**) → (**15a**), the enantiomeric excess was amply sufficient to solve the stereochemical problem. Then the (*R*)-lactam (**15b**) was prepared by repeating the whole sequence in Scheme 2 but now using pinylborane derived from (+)-α-pinene. The configurational purities of the (*R*)-analogue of (**9**) and of lactam (**15b**) were determined as above and the results are collected in the Table 1.

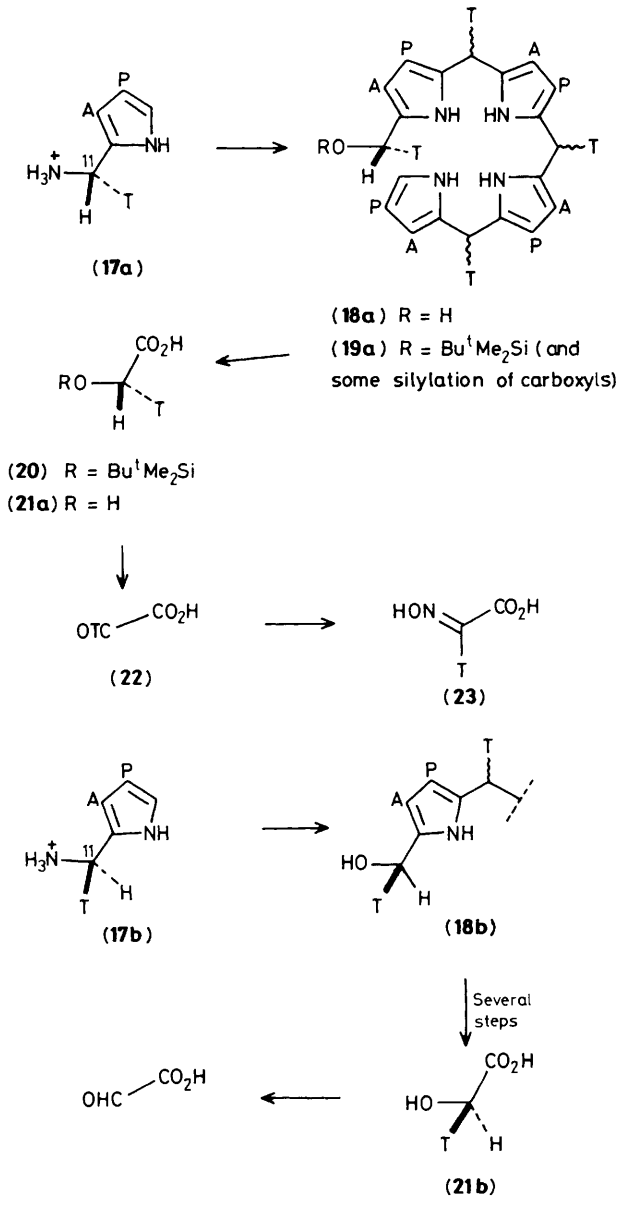
The hydroxymethylbilane (**4**) is generated enzymically at pH 8.0 where it has a half-life of *ca.* 4 min but it is stabilised at high pH.[1] The enzyme was therefore immobilised on Sepharose in a column and the solution of (11*S*)-[11-$^3H_1$]PBG (**17a**)



Scheme 2. *Reagents:* i, O_3; ii, H_2, Pd then Na_2CO_3; iii, MeOH, MeO^-; iv, HNO_2.

prepared by hydrolysis of (**15a**) was passed through it and dripped into alkali at pH >12. The bilane (**18a**) was isolated by persilylation with t-butyldimethylsilyl chloride which reacted with the alcohol group and sufficient of the carboxylate groups to allow solvent extraction of the product (**19a**). This was ozonised, the acid (**20**) was isolated by addition of unlabelled carrier material and was then deprotected to yield [2-$^3H_1$]glycollic acid (**21a**). The entire sequence was then repeated starting with (11*R*)-[11-$^3H_1$]PBG (**17b**) to yield a second sample of glycollic acid (**21b**).

It was planned to assay these two [$^3H_1$]glycollic acids with glycollate oxidase[9] which converts glycollic acid into glyoxylic acid [as (**22**)] with stereospecific removal of the *re*-hydrogen atom.[10] However, for reasons to be given in our full paper, whilst this assay is reliable for $^2H_1$-labelled materials having



essentially 100% of isotope, it has to be modified when ^3H at tracer level is used. The glyoxylic acid must be trapped *as it is formed* by having hydroxylamine in the incubation mixture; the oxime (**23**) is then isolated as its *p*-bromophenacyl ester.

The two glycollic acids (**16a**) and (**16b**) from degradation of the PBG lactams (**15a**) and (**15b**) and also those (**21a**) and (**21b**) from degradation of the bilanes (**18a**) and (**18b**) were all assayed in this way. The results in Table 1 show that (11*S*)-[11-$^3H_1$]PBG (**17a**) is converted by hydroxymethylbilane synthase into hydroxymethylbilane (**18a**) having the (*S*)-configuration at the hydroxymethyl group. The experiments in the (*R*)-series gave the complementary results. There is thus *overall retention of configuration.*

This knowledge is important for future stereochemical research on the ring closure–rearrangement sequence which generates uro'gen-III (**5**). Also, the results show that the bilane (**4**) is formed within the active site of the enzyme as was found when the azafulvene (**3**) was trapped by ammonia;[4] had the azafulvene been released into the medium, bilane [as (**4**)] racemic at the hydroxymethyl group would be the expected result.

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