Synthesis of analogues of porphobilinogen[1]

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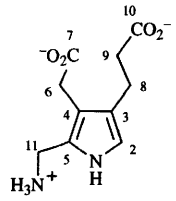
Syntheses are described of several analogues of porphobilinogen intended as substrates and/or inhibitors of porphobilinogen deaminase (hydroxymethylbilane synthase). 2-Methylporphobilinogen 12 has been synthesised from α-methylpyrrole 6, whereas a phosphonate analogue 20 of porphobilinogen, 8,9-didehydroporphobilinogen 26 and 9-fluoroporphobilinogen 38 have all been made from the 1*H*-pyrrolo[2,3-*c*]pyridine 14. The best route to 38 avoids fluoroacrylate 28 because of loss of fluorine during reduction of the double bond.

Porphobilinogen deaminase (PBGD, also known as hydroxymethylbilane synthase) is a remarkable enzyme which catalyses the tetramerisation of porphobilinogen (PBG) **1**† to give hydroxymethylbilane **4**, the precursor of all natural tetrapyrroles including haems, chlorophylls and vitamin B_{12}.[2] All living organisms have to be able to biosynthesise one or more of these tetrapyrroles and therefore inhibitors of the pathway may be valuable antibiotics, herbicides *etc.*, if there is sufficient selectivity between different species. The mechanism of PBGD (Scheme 1) involves successive covalent attachment of each of the four pyrrole rings to the enzyme, to give complexes ES_1 to ES_4, followed by cleavage of the completed bilane (linear tetrapyrrole) **4**. The point of attachment of the first pyrrole is a cofactor **3** which is itself a dipyrromethane made from two molecules of PBG.[3]

A number of analogues of PBG have been made and tested as inhibitors of PBGD[4–11] and Table 1 summarises those that have been published to date. Most effective among these are opsopyrroledicarboxylic acid (entry 1), haemopyrroledicarboxylic acid (entry 3) and isoporphobilinogen (entry 6).‡ In addition to our preliminary account of this work,[1] there have been several reports of further analogues which have been made and tested for covalent attachment to the enzyme[12–17] (Table 2) but their inhibition constants as competitive inhibitors have not been reported. Among these the 11-methyl analogues (entries 3 and 4) and the 3-butyrate analogue (entry 11) can form up to at least the enzyme–tripyrrole complex, whereas



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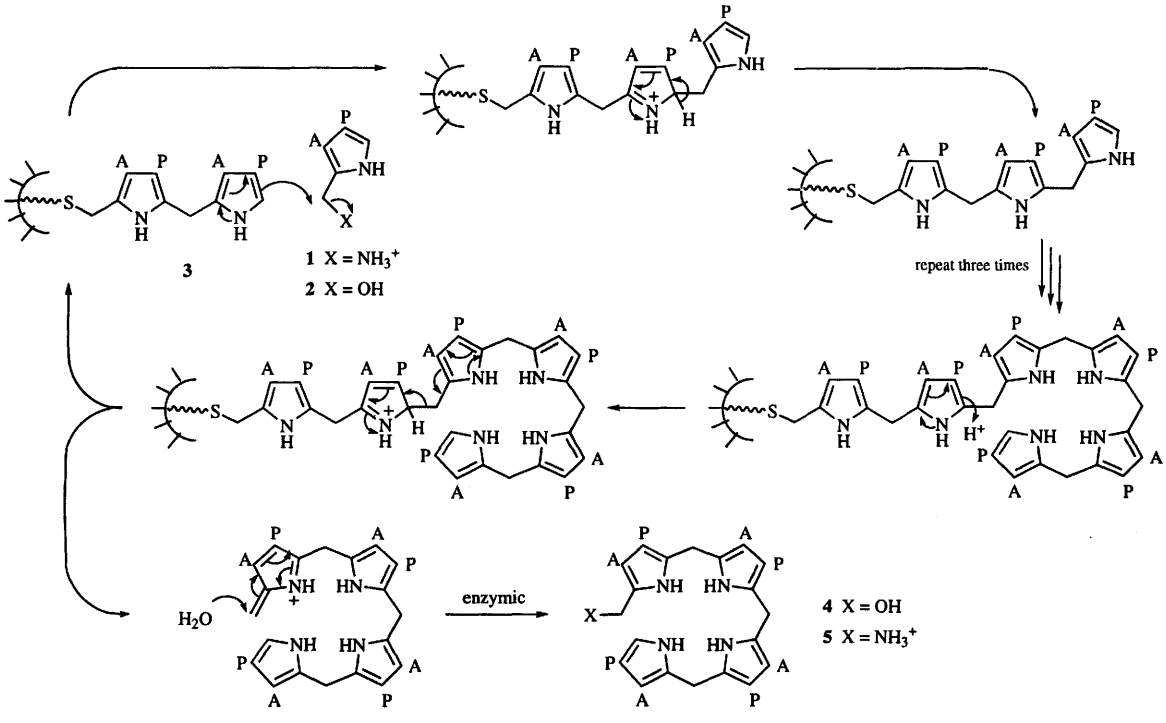
The numbering system for porphobilinogen

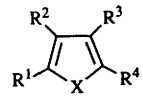
† Systematic name: 5-aminomethyl-4-carboxymethylpyrrole-3-propanoic acid.

‡ Opsopyrroledicarboxylic acid = 4-carboxymethylpyrrole-3-propanoic acid, haemopyrroledicarboxylic acid = 4-carboxymethyl-2-methylpyrrole-3-propanoic acid and isoporphobilinogen = 2-aminomethyl-4-carboxymethylpyrrole-3-propanoic acid.



Scheme 1 The mechanism of PBG deaminase (A = CH_2CO_2H, P = $CH_2CH_2CO_2H$)

Table 1 Analogues of PBG tested as inhibitors of PBGD*



Entry	X	R^1	R^2	R^3	R^4	K_I/μmol dm^3	Inhibition (%)	Notes
1	NH	H	A	P	H	50,[6] 280,[7] 140[9]		*a*
2	NH	CH_2NH_2	A	P	CO_2H		NI[5]	
3	NH	Me	A	P	H	75[10]	NI[6]	*a*
4	NH	H	A	P	Me		NI[6]	
5	NH	$-CH_2NHCOCH_2-$		P	H		NI[6]	
6	NH	CH_2NH_2	P	A	H	510,[7] 75[10]		*a*
7	NH	CH_2NH_2	A	Me	H		40[8]	*b*
8	NH	CH_2NH_2	A	A	H		50[8]	*b*
9	NH	CH_2NH_2	A	H	H	1 150[10]	45[8]	*a,b,c*
10	NH	CH_2NHAc	A	P	H		NI[9]	
11	NH	CH_2NH_2	A	Et	H	370[10]		*a,c*
12	NH	Me	A	P^{Me}	H		NI[10]	
13	NH	Me	H	P	H	60 000[11]		
14	O	H	H	P	H	~100 000[11]		*d*
15	O	H	H	$CH{=}CHCO_2H$	H	~100 000[11]		*d*
16	S	H	H	P	H	~100 000[11]		*d*

* NI = no inhibition; A = CH_2CO_2H; P = $CH_2CH_2CO_2H$; P^{Me} = $CH_2CH_2CO_2Me$. Sources of the PBGD: refs. 5 and 9, *Rhodopseudomonas spheroides*; refs. 6 and 7, spinach; refs. 8 and 11, wheat germ; ref. 10, human erythrocytes. [a] For refs. 6, 9 and 10 approximate K_I values have been calculated from the data quoted in the paper assuming the inhibition is competitive. [b] In ref. 8 the percentage inhibition quoted is after preincubation of the enzyme with the inhibitor (600 μmol dm^{-3}) for 30 min. [c] For entries 9 and 11 greatly increased inhibition (50% and 70%) was observed after preincubation of the enzyme with the inhibitor (600 μmol dm^{-3}) for 30 min (ref. 10). [d] Noncompetitive inhibition.

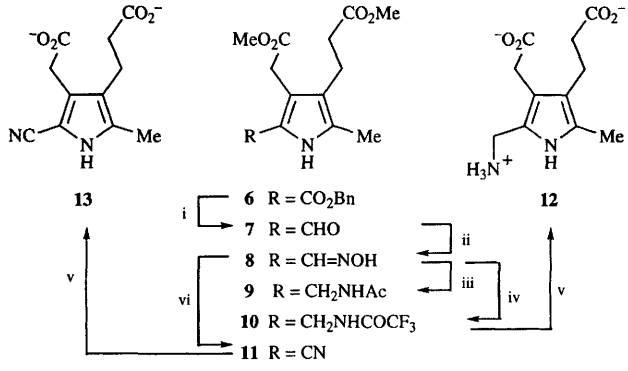
others (*e.g.* entries 1, 2 and 19) become attached to the enzyme but then prevent any further reaction and are thus inactivators. Two analogues, isoporphobilinogen (Table 2, entry 13) and a decarboxylated form of it (entry 17), form covalent adducts with the enzyme which regain their activity on incubation with PBG. It has not been shown whether this is due to displacement of the modified monopyrrole by PBG or by covalent attachment of three PBG molecules and release of a modified bilane product.

Despite the testing of all these analogues, the only compounds that are known to be capable of acting as substrates for the full catalytic cycle of PBGD are the natural substrate, PBG **1**, and the corresponding hydroxymethylpyrrole **2**, both of which give the same hydroxymethylbilane **4** as the product.[18] In the presence of ammonia, PBGD produces aminomethylbilane **5** and this compound is also converted into the hydroxymethylbilane **4** by the enzyme. In other words, no end-product from PBGD has been reported other than the natural one **4**.

In this paper we report the syntheses of some simple analogues of PBG **1** which incorporate only minimal changes in the hope that this would allow the analogues either to be accepted as substrates by PBGD or to be potent inhibitors of the enzyme. The experiments with these compounds, which show that two of them are not only the most potent inhibitors of PBGD yet reported but also the first unnatural substrates for the full enzymic reaction, are described in the following paper.[19]

Results and discussion

The first target compound was 2-methylPBG **12** (Scheme 2).§ It was thought that this might bind to the enzymic cofactor in the same way as PBG but then, with C-2 blocked by the methyl group, the complex would be unable to react with a further molecule of PBG and so the enzyme would be inactivated. The same strategy was employed in studies on 2-bromoPBG (Table 2, entry 1) which were published during the course of the work described here.[12,13]



Scheme 2 Synthesis of α-methyl analogues of PBG. *Reagents:* i, H_2, Pd/C; TFA, TMOF; ii, NH_2OH; iii, Zn, AcOH, Ac_2O; iv, Zn, TFA, TFAA; v, KOH; vi, SiO_2, heat.

The available[20] α-methylpyrrole **6** was chosen as the starting material as it only requires conversion of the benzyloxycarbonyl group into an aminomethyl group. Hydrogenolysis of the benzyl ester gave the acid, which was prone to decarboxylation and so was immediately formylated using trimethyl orthoformate (TMOF) and trifluoroacetic acid (TFA) to give the aldehyde **7** in 80% overall yield. Treatment of the aldehyde with hydroxylamine gave the oxime **8** as a mixture of *E* and *Z* isomers in 89% yield.

The first attempt to convert the oxime **8** into the required aminomethyl group involved reduction with zinc in the presence of acetic anhydride, a method that has been used in a synthesis of PBG from the corresponding oxime.[21] This reduction gave the acetamide **9** in an unoptimised 30% yield. However, difficulties were experienced in the attempted hydrolysis of **9** to give 2-methylPBG **12** due to decomposition under the vigorous conditions required. Therefore the reduction was performed with zinc in the presence of trifluoroacetic anhydride (TFAA) to give the trifluoroacetamide **10**, albeit in only 26% yield. This amide was considerably easier

§ The numbering for the atoms of PBG, given in structure **1**, is that used by, among others, J. Lascelles in *Tetrapyrrole Biosynthesis and its Regulation*, W. A. Benjamin Inc., New York, 1964, p. 42 and by R. B. Frydman, B. Frydman and A. Valasinas in *The Porphyrins*, ed. D. Dolphin, Academic Press, New York, 1979, vol. 6, p. 23.

Table 2 Analogues of PBG tested for covalent attachment to PBGD*

Entry	X	R^1	R^2	R^3	R^4	Complex(es) formed	Notes
1	NH	CH_2NH_2	A	P	Br	ES_1 [12,13]	*a*
2	NMe	CH_2NH_2	A	P	H	ES_1 [14]	*a*
3	NH	$CHMeNH_2$	A	P	H	ES_1, ES_2, ES_3 [14]	
4	NH	CHMeOH	A	P	H	ES_1, ES_2, ES_3 [14]	
5	NH	$CH(CF_3)NH_2$	A	P	H	(ES_1) [14]	*b*
6	NH	$CH(CF_3)OH$	A	P	H	(ES_1) [14]	*b*
7	O	CH_2NH_2	A	P	H	— [15]	
8	O	CH_2NH_2	P	A	H	— [15]	
9	O	CH_2OH	P	A	H	— [15]	
10	NH	CH_2NH_2	A	A	H	ES_1 (ES_2) [16]	*b,c*
11	NH	CH_2NH_2	A	B	H	ES_1, ES_2, ES_3 [16]	
12	NH	CH_2NH_2	P	P	H	ES_1 [16]	*a*
13	NH	CH_2NH_2	P	A	H	ES_1 [16]	*d*
14	NH	CH_2NH_2	A	H	H	ES_1 (ES_2) [16]	*a,b*
15	NH	CH_2NH_2	H	P	H	— [16]	
16	NH	CH_2NH_2	H	A	H	— [16]	
17	NH	CH_2NH_2	P	Me	H	(ES_1) [16]	*b,d*
18	NH	CH_2NH_2	H	H	H	— [16]	
19	NH	CH_2OH	A	P	F	(ES_1) [17]	*a,b*

* Abbreviations as in Table 1 and B = $CH_2CH_2CH_2CO_2H$. HMBS was from *Escherichia coli* in all cases. [a] The complex formed does not react further with PBG. [b] Complexes in brackets are only formed slowly (> 1 h), whereas those not in brackets are formed within 15 min. [c] The complexes formed do react further with PBG but do not release a product. [b] The complexes formed react with PBG, producing a tetrapyrrolic product.

to hydrolyse and treatment with potassium hydroxide in aqueous methanol gave the potassium salt of 2-methylPBG **12** cleanly as judged by ^{1}H NMR spectroscopy. The hydrolysate was used in the inhibition studies without further purification.[19]

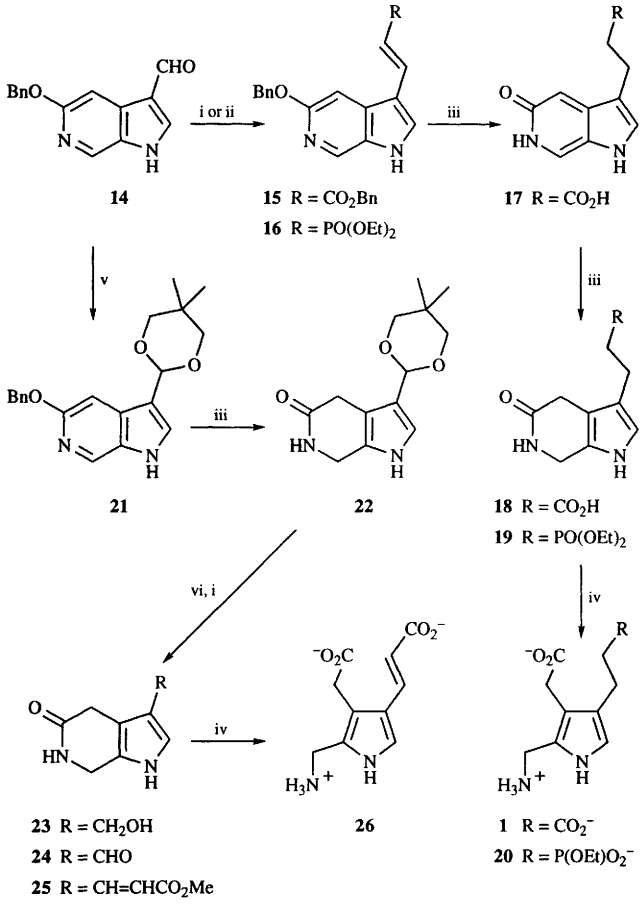
A major by-product that was isolated from the reduction of the oxime **8** in the presence of trifluoroacetic anhydride was the dehydration product, nitrile **11**. This nitrile could be produced in high yields by heating the oxime with silica gel in xylene [22] but all attempts to reduce the nitrile to an aminomethyl group were unsuccessful. The ester groups of nitrile **11** could, however, be hydrolysed to give the 2-methyl-5-cyanopyrrole **13** and this compound was also tested as an inhibitor of PBGD.[19]

Apart from the α-methylpyrroles **12** and **13**, the other analogues of PBG that we hoped to make were all modified on the propionate side-chain. Among the target compounds were the phosphonate analogue (PPBG) **20**, 8,9-didehydroPBG **26** and 9-fluoroPBG (FPBG) **38**. We considered these to be fairly minimal changes which should not affect the mechanism of the reaction and so these compounds could act as substrates for the full enzymic reaction.

A suitable starting material for these syntheses was the 1*H*-pyrrolo[2,3-*c*]pyridine-3-carbaldehyde **14**, which is an intermediate in our standard synthesis of PBG (Scheme 3).[23] This compound has the acetate and aminomethyl side-chains of PBG protected as the benzyloxypyridine ring and the aldehyde at position 3 can be elaborated into a wide variety of different side-chains. The synthesis of PBG involves a Knoevenagel condensation to generate the acrylate **15**, followed by hydrogenation to give PBG lactam **18**, *via* 1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one **17**, and finally hydrolysis to give PBG **1**.

Our synthesis of PPBG **20** followed that of PBG closely. A Horner–Emmons-type reaction of aldehyde **14** with tetraethyl methylenebisphosphonate [generated *in situ* from diethyl methylphosphonate by treatment with lithium diisopropylamide (LDA) and diethyl chlorophosphonate [24]] gave the vinylphosphonate **16** in a disappointing yield of 25%. Catalytic hydrogenation then proceeded smoothly to give the lactam with the phosphonoethyl side-chain **19** in 93% yield. Treatment of **19** with aqueous potassium hydroxide hydrolysed the lactam and the diethyl phosphonate to give the phosphonate monoester **20** as its potassium salt.

We would also have liked to obtain the corresponding phosphonate diacid but reaction of the diethyl phosphonate **19** with trimethylsilyl iodide, although it cleanly removed the ethyl groups (as judged by the ^{1}H and ^{13}C NMR spectra of the product), also resulted in some unidentified reaction at C-2 (loss of the ^{1}H NMR signal for 2-H at δ 6.5) and so we were not able to obtain this fully deprotected material.

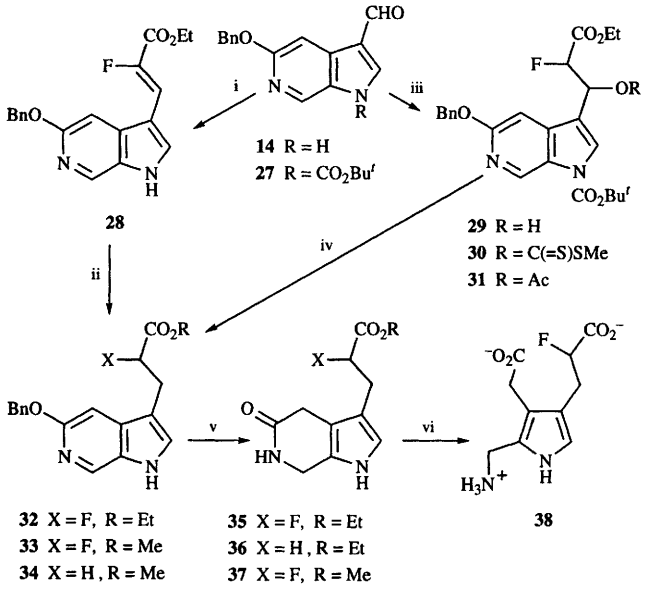


Scheme 3 Synthesis of PBG **1** and its dehydro **26** and phosphonate **20** analogues. *Reagents:* i, $HO_2CCH_2CO_2Bn$, piperidine; ii, $(EtO)_2POCH_2PO(OEt)_2$, LDA; iii, H_2, Pd/C; iv, KOH; v, $Me_2C(CH_2OH)_2$, TsOH; vi, H_3O^+.

For the synthesis of the didehydroPBG **26** a change in the order of the steps was required as hydrogenation of the double bond is more rapid than of the 1*H*-pyrrolo[2,3-*c*]pyridine ring

{as evidenced by the fact that 1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one **17** is an isolable intermediate in the reduction of **15**}. Our first attempt was to obtain the lactam aldehyde **24** by catalytic hydrogenation of aldehyde **14**. However, reduction of the aldehyde group occurred before reduction of the 1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one ring and only the alcohol **23** could be obtained in reasonable yield (70%) after prolonged hydrogenation. To avoid reduction of the aldehyde, it was protected as a cyclic acetal **21**, then hydrogenated and the lactam deprotected to give the aldehyde **24** in 79% yield over the three steps. This aldehyde was unstable and so was used directly in the Knoevenagel condensation with methyl hydrogen malonate to give the acrylate **25** in only 35% yield. The low yield in this reaction may have been due to the insolubility of the aldehyde **24** combined with its low reactivity. In an attempt to improve both these properties, the use of the *N*-tosyl derivative[25] of 1*H*-pyrrolo[2,3-*c*]pyridine **14** was investigated but the hydrogenation of *N*-tosyl-**21** to *N*-tosyl-**22** would not proceed beyond the debenzylated 1*H*-pyrrolo[2,3-*c*]pyridine-5(6*H*)-one. Hydrolysis of the acrylate **25** with potassium hydroxide, as before, gave the potassium salt of didehydroPBG **26**.

For the synthesis of FPBG **38** we initially followed a route similar to the synthesis of PBG in Scheme 3. Thus Knoevenagel condensation of the aldehyde **14** with ethyl hydrogen fluoromalonate gave the fluoroacrylate **28** (58% based on unrecovered **14**) (Scheme 4). The *trans* stereochemistry was



Scheme 4 Synthesis of 9-fluoroPBG **38**. *Reagents:* i, $HO_2CCHFCO_2Et$, piperidine; ii, Mg, MeOH; iii, $(Bu^tOCO)_2O$, DMAP; FCH_2CO_2Et, LiHMDS, DMPU; iv, Ac_2O, pyridine; $NaBH_3CN$, TFA; v, H_2, Pd/C; vi, KOH.

deduced from the J_{HF} value of 38 Hz. Hydrogenation of the fluoroacrylate proved a problem, however, as the standard conditions [10% palladium on carbon in *N*,*N*-dimethylformamide (DMF) or in ethanol] resulted in extensive loss of the fluorine atom to give PBG lactam ethyl ester **36** along with only minor amounts (at best only 25%) of the desired fluorinated lactam **35**. Hudlicky[26] had observed a similar loss of fluorine during the hydrogenation of fluoro-fumaric and -maleic acids but had found that fluorosuccinic acid does not lose its fluorine under the same conditions. He proposed a mechanism by which fluoroalkenes but not fluoroalkanes might lose fluorine during hydrogenation. We therefore looked for alternative methods for reducing double bonds.

Diimide was next tried in an attempt to reduce only the fluoroacrylate double bond to give fluoropropionate **32**. However, diimide failed to react with the fluoroacrylate **28**, under conditions which gave good yields for the reduction of unfluorinated acrylate **15**. It was found that magnesium in methanol did effect reduction of the double bond (as well as ester-exchange) but, unfortunately, the desired product **33** was again contaminated with the corresponding unfluorinated compound **34**. However, the ratio of products (3:1) was much more favourable than with the previous hydrogenation and separation of the fluorinated from the unfluorinated compound was possible by HPLC on silica gel. Due to overlap of the peaks, the fluorinated product **33** could only be obtained pure in 23% yield. The NMR spectrum of the purified **33** showed no detectable **34**.

Hydrogenation of the fluoropropionate **33** proceeded smoothly with no further loss of fluorine (as expected from Hudlicky's results[26]). Hydrolysis of the resulting lactam ester **37** with potassium hydroxide was performed in D_2O and followed by NMR spectroscopy. The reaction mixture was heated at 60 °C for 1 min and then stirred at room temperature for 2 h. As a result of performing the hydrolysis in D_2O, exchange of the protons on the acetate side chain of the FPBG **38** was observed (as has been reported for PBG lactam[27]) but as this should not affect the enzymic experiments, this material was freeze-dried and used in the enzymic experiments without further purification.[19]

Clearly, it would be advantageous to avoid the loss of fluorine during the reduction of the fluoroacrylate **28** and the consequent HPLC separation, which limited the amount of FPBG that could be prepared. Therefore we decided to avoid the elimination of water that generated the double bond of **28** in the first place. Our objective was to react the aldehyde group of 1*H*-pyrrolo[2,3-*c*]pyridine **14** with the enolate from ethyl fluoroacetate to give an α-fluoro-β-hydroxy ester. First, however, it was necessary to protect the pyrrolopyridine N–H group and this was achieved by formation of the *tert*-butoxycarbonyl derivative **27** in good yield using di-*tert*-butyl dicarbonate.[28] Reaction of the aldehyde **27** with the enolate from ethyl fluoroacetate was not straightforward but proceeded in reliably high yields using similar conditions to ones developed by Welch and co-workers[29] in which the enolate is generated using $LiN(SiMe_3)_2$ (LiHMDS) in the presence of 1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one (DMPU) at −85 °C and reacted with **27** at that temperature.

We now wanted to deoxygenate the alcohol **29** and our first attempt was to convert the alcohol to its xanthate **30** in order to use a radical deoxygenation procedure. Unfortunately, reaction with NaH, CS_2 and MeI resulted in elimination to give the *N*-Boc derivative of fluoroacrylate **28** in 63% yield. Next we attempted reductions of the alcohol by an S_N1 mechanism as the cation formed by loss of HO^- from **29** would be stabilised by delocalisation of the lone pair of electrons on the pyrrolic nitrogen. Reaction of alcohol **29** with $NaBH_3CN$ in the presence of ZnI_2 caused loss of the Boc group but not of the OH. Further treatment of this deprotected alcohol with $NaBH_3CN$ in acetic acid did not result in any reaction at all. It appeared, therefore, that the OH is not a sufficiently good leaving group and so it was converted into its acetate **31**. No reaction was observed when acetate **31** was treated with $NaBH_3CN$ in acetic acid but $NaBH_3CN$ in TFA effected both deprotection of the pyrrolic nitrogen atom and reduction of the alcohol to give **32** in 65% yield. This suggests that the TFA causes the deprotection step to occur first and this then facilitates the loss of the acetoxy group in an S_N1 reaction. Clearly reduction of the resulting stabilised cation by $NaBH_3CN$ is faster than loss of the proton α to the ester group which would have led to the fluoroacrylate **28**.

Hydrogenation and hydrolysis of the 1*H*-pyrrolo[2,3-*c*]pyridine **32** proceeded much as for the corresponding methyl ester **33** except that in this case the hydrolysis was performed with KOH in methanol–water and the excess KOH was removed by

treatment with a cation-exchange resin in its ammonium form to give FPBG **38** as its ammonium salt (the resin could not be used in its acid form as PBG is not stable under acidic conditions).

In this paper we have described efficient syntheses of five analogues of PBG **1**, α-methyl derivatives **12** and **13** and propionate-modified analogues **20**, **26** and **38**. The studies of the interactions of these analogues with PBGD is described in the following paper.[19]

Experimental

General directions

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 297 spectrometer using sodium chloride plates for thin film and Nujol mull spectra and 0.5 mm sodium chloride cells for solution spectra. UV–VIS spectra and absorbance readings were taken on a Uvikon 810 spectrophotometer. Proton NMR spectra were recorded on Varian EM390 (90 MHz) or CFT (80 MHz) or on Bruker WH250 (250 MHz) or WH400 (400 MHz) spectrometers. Carbon-13 NMR spectra were run on the WH400 spectrometer at 100 MHz and were broadband proton decoupled. Where indicated assignments were made with the aid of a spectrum acquired using the APT pulse sequence, which gives CH_3 and CH signals positive and CH_2 and quaternary C signals negative. The deuteriated solvent signal was used as standard or, for solutions in D_2O, dioxane or acetone were used as internal standards. Chemical shifts are quoted on the δ scale relative to tetramethylsilane as δ 0.0. Coupling constants (J) are quoted in Hz. Fluorine-19 NMR spectra were run on the WH250 spectrometer at 235 MHz using an external standard of trifluoroacetic acid at δ 0.0 (unless otherwise stated) and were broadband proton decoupled when required. Phosphorus-31 NMR spectra were run on the WH400 spectrometer at 162 MHz using an external standard of phosphoric acid at δ 0.0. Mass spectra were recorded on an A.E.I. MS30 spectrometer and field desorption (FD) spectra on a MS50 spectrometer. Radioactivity was measured using a Packard 2000 CA Tri-Carb liquid scintillation counter on samples dissolved in aqueous or organic scintillation cocktail (10 cm^3).

All solvents were distilled before use. Reagents were purified and solvents for reactions were dried, where required, using standard procedures.[30] Organic solutions were dried using anhydrous magnesium sulfate and evaporated using a Büchi rotary evaporator at water pump pressure at 30–40 °C. Analytical thin layer chromatography (TLC) was perfomed on commercial Merck plates, coated to a thickness of 0.25 mm with Kieselgel 60 (70–230 mesh) silica. Preparative thin layer chromatography (PLC) was performed using plates coated with the same silica to a thickness of 1 mm. Flash chromatography under a moderate pressure of compressed air was carried out using Merck Kieselgel 60 (230–400 mesh) silica gel.

Methyl 5-formyl-4-(methoxycarbonylmethyl)-2-methylpyrrole-3-propanoate 7

A solution of benzyl ester **6**[20] (500 mg, 1.34 mmol) in methanol (30 cm^3) was stirred with sodium carbonate (60 mg) and 10% palladium-on-carbon (100 mg) under an atmosphere of hydrogen at room temperature until uptake of gas ceased (about 2 h). The mixture was filtered through Celite, washing the residue with methanol. The residue on evaporation of the filtrate was dissolved in hydrochloric acid (3 mol dm^{-3}; 25 cm^3) and extracted into dichloromethane (3 × 25 cm^3). The combined extracts were dried and evaporated to give the carboxylic acid as a solid (351 mg, 93%), which was found to decarboxylate readily to give the corresponding α-free pyrrole and hence was used directly in the next step (Found: M^+, 283.1096. $C_{13}H_{17}NO_6$ requires M, 283.1082); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3450 (NH), 3400–2900 (OH), 2950 (CH), 1735 (ester) and 1670 (acid); δ_H(400 MHz, $CDCl_3$) 2.17 (3 H, s, $ArCH_3$), 2.42 (2 H, t, J 7, CH_2CH_2CO), 2.68 (2 H, t, J 7, CH_2CH_2CO), 3.64 and 3.68 (each 3 H, s, OMe), 3.83 (2 H, s, CH_2CO), 9.29 (1 H, br s, NH) and 10.40 (1 H, br s, CO_2H); m/z (EI), 283 (M^+, 13%), 239 ($M^+ - CO_2$, 92), 207 (28), 179 (41) and 166 (100). For the α-free pyrrole: δ_H(400 MHz, $CDCl_3$) 2.14 (3 H, s, $ArCH_3$), 2.44 (2 H, t, J 7, CH_2CH_2CO), 2.70 (2 H, t, J 7, CH_2CH_2CO), 3.44 (2 H, s, CH_2CO), 3.65 and 3.66 (each 3 H, s, OMe), 6.50 (1 H, s, α-H) and 7.74 (1 H, br s, NH).

A solution of the acid (700 mg, 2.47 mmol) in dry dichloromethane (25 cm^3) was stirred with freshly distilled trifluoroacetic acid (5 cm^3) at 0 °C under argon and, after 5 min, freshly distilled trimethyl orthoformate (4 cm^3) was added. After 4 h the reaction mixture was washed with aq. potassium carbonate (12% w/v; 30 cm^3) and the aqueous layer was extracted with dichloromethane (2 × 25 cm^3). The combined organic layers were dried and evaporated and the residue was purified by PLC, eluting with methanol–dichloromethane (5:95), to give the *aldehyde* **7** as needles (571 mg, 86%), mp 77.5–78.5 °C (from dichloromethane–hexane) (Found: C, 55.3; H, 6.4; N, 10.0. $C_{13}H_{17}NO_5$ requires C, 55.7; H, 6.4; N, 10.0%); λ_{max}(MeOH)/nm 310; ν_{max}(Nujol)/cm^{-1} 3250 (NH) and 1750 (2 × C=O); δ_H(400 MHz, CD_2Cl_2) 2.29 (3 H, s, $ArCH_3$), 2.47 (2 H, t, J 7.5, CH_2CH_2CO), 2.74 (2 H, t, J 7.5, CH_2CH_2CO), 3.64 and 3.69 (each 3 H, s, OMe), 3.75 (2 H, s, CH_2CO), 9.47 (1 H, s, CHO) and 10.37 (1 H, br s, NH); δ_C(100 MHz, CD_2Cl_2) 11.6 ($ArCH_3$), 19.3 (CH_2CH_2CO), 29.8 (CH_2CO), 34.7 (CH_2CH_2CO), 51.6 and 52.3 (2 × OCH_3), 121.8, 127.4, 128.4 and 136.3 (4 × pyrrole C) and 171.6 and 173.4 (2 × CO_2Me) and 176.7 (CHO).

Methyl 4-methoxycarbonylmethyl-2-methyl-5-hydroxyiminomethylpyrrole-3-propanoate 8

A solution of the aldehyde **7** (71 mg, 0.26 mmol), sodium acetate (35 mg) and hydroxylamine hydrochloride (30 mg, 0.43 mmol) in methanol (5 cm^3) plus a few drops of water was heated under reflux for 4 h, then poured into water (10 cm^3) and extracted with dichloromethane (3 × 25 cm^3). The combined extracts were dried and evaporated and the residue was purified by PLC, eluting with methanol–dichloromethane (5:95), to give the *oxime* **8** (67 mg, 89%), a mixture of *E* and *Z* isomers, as needles, mp 113–121 °C (from dichloromethane–hexane) (Found: M^+, 282.1234; C, 58.35; H, 6.39; N, 5.06%. $C_{13}H_{18}N_2O_5$ requires M, 282.1215; C, 58.43; H, 6.37; N, 5.24%); λ_{max}(MeOH)/nm 295; ν_{max}(Nujol)/cm^{-1} 3350 (NH), 3300–3050 (OH), 2930 (CH), 1730 (ester) and 1660 (C=N); δ_H(400 MHz, $CDCl_3$) (major isomer) 2.16 (3 H, s, $ArCH_3$), 2.43 (2 H, t, J 7, CH_2CH_2CO), 2.70 (2 H, t, J 7, CH_2CH_2CO), 3.47 (2 H, s, CH_2CO), 3.63 and 3.65 (each 3 H, s, OCH_3), 7.31 (1 H, br s, OH), 8.03 (1 H, s, CH=N) and 9.17 (1 H, br s, NH); (minor isomer) 2.22 (3 H, s, $ArCH_3$), 2.45 (2 H, t, J 7, CH_2CH_2CO), 2.74 (2 H, t, J 7, CH_2CH_2CO), 3.55 (2 H, s, CH_2CO), 3.64 and 3.66 (each 3 H, s, OCH_3), 7.31 (1 H, br s, OH), 8.03 (1 H, s, CH=N) and 9.90 (1 H, br s, NH); δ_C(100 MHz, $CDCl_3$) (major isomer) 11.3 ($ArCH_3$), 19.4 (CH_2CH_2CO), 29.9 (CH_2CO), 35.0 (CH_2CH_2CO), 51.6 and 52.2 (2 × OCH_3), 118.8, 119.1, 120.1 and 128.9 (4 × pyrrole-C), 136.1 (C=N) and 172.0 and 173.7 (C=O); (minor isomer, distinguishable signals) 11.6, 30.2, 118.6, 119.8, 129.0, 140.8, 171.8 and 173.6; m/z (EI) 282 (M^+, 36%), 265 ($M^+ - OH$, 22), 191 (52) and 133 (100).

Methyl 5-acetamidomethyl-4-(methoxycarbonylmethyl)-2-methylpyrrole-3-propanoate 9

The oxime **8** (350 mg, 1.24 mmol) was dissolved in water–acetic acid (2:3; 50 cm^3) and freshly activated zinc dust (490 mg) was added quickly followed by acetic anhydride (10 cm^3). The mixture was stirred at room temperature overnight after which another aliquot of acetic anhydride (5 cm^3) was added. After a further 30 min, the zinc was removed by filtration through

Celite, washing with water, and the filtrate was extracted with dichloromethane (3 × 50 cm^3). The combined extracts were washed with saturated aq. sodium hydrogen carbonate (100 cm^3), dried and evaporated. The residue was purified by PLC, eluting with methanol–dichloromethane (5:95), to give the *acetamide* **9** as an oil (120 mg, 30%) (Found: M^+, 310.1510. $C_{15}H_{22}N_2O_5$ requires *M*, 310.1529); ν_{max}(thin film)/cm^{-1} 3450–3100 (2 × NH), 2940 (CH), 1720 (ester) and 1660 (amide); δ_H(400 MHz, $CDCl_3$) 1.95 (3 H, s, MeCO), 2.11 (3 H, s, ArMe), 2.40 (2 H, m, CH_2CH_2CO), 2.67 (2 H, m, CH_2CH_2CO), 3.40 (2 H, s, CH_2CO), 3.65 and 3.67 (each 3 H, s, OMe), 4.24 (2 H, d, *J* 6, CH_2N), 6.49 (1 H, br t, *J* 6, CH_2NH) and 8.25 (1 H, br s, pyrrole-NH); δ_C(100 MHz, $CDCl_3$) 11.0 ($ArCH_3$), 19.6 (CH_2CH_2CO), 23.2 (CH_3CO), 30.0 (CH_2CO), 34.5 and 34.8 (CH_2CH_2CO and CH_2NH), 51.5 and 52.1 (OCH_3), 111.5, 116.2, 123.7, 125.2 (4 × pyrrole-C) and 171.1, 173.6 and 173.7 (3 × C=O); *m*/*z* (EI) 310 (M^+, 30%), 245 (57), 237 (58), 195 (100) and 122 (75).

Methyl 4-methoxycarbonylmethyl-2-methyl-5-trifluoroacetamidomethylpyrrole-3-propanoate 10

The oxime **8** (60 mg, 0.16 mmol) was dissolved in freshly distilled trifluoroacetic acid (1.35 cm^3) and trifluoroacetic anhydride (6.78 cm^3) at 0 °C and freshly activated zinc dust (95 mg) was added. The mixture was stirred for 90 min at 0 °C, until TLC indicated that the reaction was complete, and the zinc was filtered off through Celite. The filtrate was evaporated to dryness and the residue was dissolved in dichloromethane (15 cm^3), washed with aq. sodium hydrogen carbonate (15 cm^3) and then with water (15 cm^3), dried and evaporated. The residue was purified by PLC, eluting with diethyl ether–hexane (1:1), to give the nitrile **11** as a by-product (10 mg, 18%) and the *trifluoroacetamide* **10** as an oil (20 mg, 26%); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3420 and 3330 (2 × NH), 2940 (CH), 1720 (ester) and 1705 (amide); δ_H(250 MHz, CD_2Cl_2) 2.16 (3 H, s, ArMe), 2.41 (2 H, t, *J* 7.5, CH_2CH_2CO), 2.70 (2 H, t, *J* 7.5, CH_2CH_2CO), 3.48 (2 H, s, CH_2CO), 3.65 and 3.71 (each 3 H, s, OMe), 4.20 (2 H, d, *J* 5.5, CH_2N), 7.76 (1 H, br s, CH_2NH) and 8.36 (1 H, br s, pyrrole-NH); δ_C(100 MHz, $CDCl_3$) 11.0 ($ArCH_3$), 19.5 (CH_2CH_2CO), 30.0 (CH_2CO), 35.1 and 35.4 (CH_2CH_2CO and CH_2NH), 51.5 and 52.4 (2 × OMe), 113.2, 116.8, 122.2 and 124.5 (4 × pyrrole-C), 115.9 (q, J_{CF} 286, CF_3), 157.5 (q, J_{CF} 37, CF_3CO) and 173.6 and 173.9 (2 × CO_2); *m*/*z* (FD) 364 (100%).

2-Methylporphobilinogen 12

The trifluoroacetamide **10** (18 mg, 0.049 mmol) was stirred with a solution of potassium hydroxide (2 mol dm^{-3}) in methanol–water (1:1; 0.6 cm^3) under argon at room temperature for 56 h. The mixture was freeze-dried to give 2-methylporphobilinogen **12** as its potassium salt mixed with potassium trifluoroacetate and potassium hydroxide, and this mixture was used for the enzymic experiments without further purification; δ_H(250 MHz, D_2O) 2.48 (3 H, s, ArMe), 2.57 (2 H, m, CH_2CH_2CO), 2.91 (2 H, m, CH_2CH_2CO), 3.64 (2 H, s, CH_2CO) and 3.94 (2 H, s, CH_2N); δ_C(100 MHz, D_2O) 10.9 ($ArCH_3$), 22.1 (CH_2CH_2CO), 33.6 (CH_2CO), 36.4 (CH_2CH_2CO), 40.2 (CH_2NH_2), 114.6, 118.1, 124.3 and 128.5 (4 × pyrrole-C) and 182.9 and 184.2 (2 × CO_2^-).

Methyl 5-cyano-4-(methoxycarbonylmethyl)-2-methylpyrrole-3-propanoate 11

The oxime **8** (180 mg, 0.58 mmol) was heated under reflux in dry xylene (30 cm^3) with silica (230–400 mesh; 70 mg) under argon for 40 h. The silica was filtered off and the solvent was evaporated under reduced pressure to give the *nitrile* **11** as an oil (137 mg, 81%) (Found: M^+, 264.1132. $C_{13}H_{16}N_2O_4$ requires *M*, 264.1110); λ_{max}(MeOH)/nm 264; ν_{max}(thin film)/cm^{-1} 3400–3200 (NH), 2940 (CH), 2200 (CN) and 1720 (ester); δ_H(400 MHz, $CDCl_3$) 2.13 (3 H, s, ArMe), 2.40 (2 H, t, *J* 8, CH_2CH_2CO), 2.64 (2 H, t, *J* 8, CH_2CH_2CO), 3.55 (2 H, s, CH_2CO), 3.60 and 3.66 (each 3 H, s, OMe) and 9.60 (1 H, br s, NH); δ_C(100 MHz, $CDCl_3$) 11.2 ($ArCH_3$), 19.2 (CH_2CH_2CO), 30.4 (CH_2CO), 34.4 (CH_2CH_2CO), 51.6 and 52.1 (2 × OMe), 97.8 (CN), 114.2, 116.7, 126.3 and 131.8 (4 × pyrrole-C) and 171.4 and 173.5 (2 × C=O); *m*/*z* (EI) 264 (M^+, 100%), 232 (54), 205 (62), 191 (92), 173 (40), 145 (65) and 133 (82).

5-Cyano-4-(carboxymethyl)-2-methylpyrrole-3-propanoic acid 13

The nitrile **11** (30 mg, 0.11 mmol) was stirred in aq. potassium hydroxide (1 mol dm^{-3}; 0.25 cm^3) at room temperature under argon for 3 h. The solution was neutralised with aq. acetic acid (50%) and evaporated to give the potassium salt of the acid **13** as a mixture with potassium acetate (40 mg); δ_H(250 MHz, D_2O) 2.17 (3 H, s, ArMe), 2.36 (2 H, m, CH_2CH_2CO), 2.63 (2 H, m, CH_2CH_2CO) and 3.48 (2 H, s, CH_2CO); δ_C(100 MHz, D_2O) 11.0 ($ArCH_3$), 20.4 (CH_2CH_2CO), 33.4 (CH_2CO), 37.1 (CH_2CH_2CO), 96.5 (CN), 116.5, 119.5, 130.3 and 133.5 (4 × pyrrole-C) and 179.0 and 180.0 (2 × C=O); for mass spectrometry the product was treated with diazomethane to give the diester, *m*/*z* (FD) 264 (100%).

(*E*)-5-Benzyloxy-3-(2-diethoxyphosphorylethenyl)-1*H*-pyrrolo[2,3-*c*]pyridine 16

To a stirred solution of dry diisopropylamine (1.66 cm^3, 12.0 mmol) in dry THF (30.3 cm^3) at 0 °C under argon was added a solution of butyllithium in hexane (1.5 mol dm^{-3}; 8 cm^3, 12.0 mmol). An aliquot of this solution of lithium diisopropylamide (3.33 cm^3, 0.80 mmol) was cooled to −78 °C and a solution of diethyl methylphosphonate (69 mm^3, 0.40 mmol) in dry THF (5 cm^3) was added dropwise. The mixture was stirred for 10 min and then a solution of diethyl chlorophosphonate (69 mm^3, 0.40 mmol) in THF (3 cm^3) was added *via* a cannula under argon and the mixture was allowed to warm to −20 °C. A solution of 1*H*-pyrrolo[2,3-*c*]pyridine-3-carbaldehyde **14**[23] (100 mg, 0.40 mmol) in THF (15 cm^3) was added dropwise, the mixture was allowed to warm to room temperature and then another portion of the solution of lithium diisopropylamide (1.66 cm^3, 0.40 mmol) was added. The mixture was stirred overnight, treated with water (25 cm^3) and extracted with dichloromethane (3 × 25 cm^3). The combined extracts were dried and evaporated and the residue was purified by PLC, eluting with toluene–acetone (3:1), to give recovered aldehyde **14** (33 mg) and the phosphonate **16** (25 mg, 25% based on unrecovered starting material) as an oil (Found: M^+, 386.1376. $C_{20}H_{23}N_2O_4P$ requires *M*, 386.1367); λ_{max}(MeOH)/nm 299, 271 and 218; $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3440 (NH), 3140 (CH=CH), 2970 (CH) and 1610 (Ar); δ_H(250 MHz, CD_3COCD_3) 1.30 (6 H, t, *J* 7, CH_3), 4.08 (4 H, m, CH_2CH_3), 5.43 (2 H, s, $PhCH_2$), 6.20 (1 H, t, *J* 18, CH=CH–P=O), 7.28–7.53 (6 H, m, Ph and 4-H), 7.67 (1 H, dd, *J* 23 and 18, CH=CH–P=O), 7.98 (1 H, d, *J* 3, 2-H), 8.46 (1 H, s, 7-H) and 11.28 (1 H, br s, NH); δ_C(100 MHz, CD_3COCD_3) 16.7 (d, J_{CP} 6, CH_3), 61.8 (d, J_{CP} 5, CH_2CH_3), 68.2 ($PhCH_2$), 99.1 (C-4), 109.2 (d, J_{CP} 192, CH=CH–P=O), 112.8 (d, J_{CP} 25, CH=CH–P=O), 128.1, 128.5, 129.0, 131.7, 132.8, 135.0, 136.0, 139.5 and 142.2 (Ph and C-2, 3, 3a, 7 and 7a) and 159.3 (C-5); δ_P(162 MHz, CD_3COCD_3) 21.2; *m*/*z* (EI) 386 (M^+, 40%), 309 (10) and 91 (100).

3-(2-Diethoxyphosphorylethyl)-4,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one 19

A solution of α,β-unsaturated phosphonate **16** (25 mg, 65 μmol) in *N*,*N*-dimethylformamide (5 cm^3) was stirred with 10% palladium-on-carbon (15 mg) under an atmosphere of hydrogen at room temperature overnight. The suspension was evaporated to dryness, resuspended in methanol (10 cm^3) and the catalyst removed by filtration through Celite. The filtrate was evaporated and the residue was purified by flash chromatography, eluting with methanol–dichloromethane (8:92), to give the *lactam* **19** as an oil (18 mg, 93%) (Found: M^+,

300.1231. $C_{13}H_{21}N_2O_4P$ requires *M*, 300.1238); λ_{max}(MeOH)/nm 217; ν_{max}(thin film)/cm^{-1} 3600–3100 (2 × NH), 2920 (CH), 1645 (C=O), 1230 (P=O) and 1040 (POR); δ_H(400 MHz, CD_3SOCD_3) 1.32 (6 H, t, *J* 6.5, CH_3), 2.00 (2 H, m, CH_2CH_2P=O), 2.50 (2 H, dt, *J* 10 and 6.5, CH_2CH_2P=O, obscured by DMSO signal but visible in CD_3CN), 3.25 (2 H, t, *J* 3, CH_2CO), 4.07 (4 H, quintet, *J* 6.5, CH_2CH_3), 4.35 (2 H, br s, CH_2N), 6.63 (1 H, s, 2-H), 7.81 (1 H, br s, lactam NH) and 10.44 (1 H, br s, pyrrole-NH); δ_C(100 MHz, CD_3SOCD_3) 16.4 (d, J_{CP} 6, CH_3), 18.0 (d, J_{CP} 4, CH_2CH_2P=O), 25.8 (d, J_{CP} 135, CH_2CH_2P=O), 29.0 (CH_2CO), 40.1 (CH_2N), 60.9 (d, J_{CP} 6, CH_2CH_3), 115.3 (C-2), 118.4 (d, J_{CP} 19, C-3), 110.5 and 120.0 (C=C) and 169.5 (C=O); δ_P(162 MHz, CD_3SOCD_3) 33.2; *m*/*z* (FD) 300 (100%).

2-Aminomethyl-4-[2-(ethoxyhydroxyphosphoryl)ethyl]pyrrole-3-acetic acid (PPBG) 20

The lactam phosphonate **19** (7 mg, 25 μmol) was stirred with aq. potassium hydroxide (2 mol dm^{-3}; 0.25 cm^3) under argon at room temperature for 48 h. The solution was then freeze-dried to give the phosphonate monoester **20** as its potassium salt mixed with excess potassium hydroxide, and this mixture was used for the enzymic experiments without further purification; δ_H(400 MHz, D_2O) 1.61 (3 H, t, *J* 7, CH_3), 2.17 (2 H, m, CH_2CH_2P=O), 2.91 (2 H, m, CH_2CH_2P=O), 3.69 (2 H, s, CH_2CO), 4.00 (2 H, s, CH_2N), 4.26 (2 H, quintet, *J* 7, CH_2CH_3) and 6.97 (1 H, s, α-H); δ_C(100 MHz, D_2O) 17.0 (d, J_{CP} 6, CH_3), 19.9 (CH_2CH_2P=O), 28.2 (d, *J* 132, CH_2CH_2P=O), 33.3 (CH_2CO), 36.5 (CH_2N), 61.6 (d, J_{CP} 5, CH_2CH_3), 114.9 (C-5), 123.9 (d, J_{CP} 19, C-4), 114.3 and 131.3 (C=C) and 182.5 (C=O).

3-Hydroxymethyl-4,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one 23

The aldehyde **14**[23] (150 mg, 0.60 mmol) was stirred in *N*,*N*-dimethylformamide (10 cm^3) with 10% palladium-on-carbon (65 mg) under an atmosphere of hydrogen at room temperature for 48 h. The solvent was then evaporated and the residue resuspended in methanol (5 cm^3) and filtered through Celite, washing with methanol. The filtrate was evaporated to give the lactam **23** as an oil (71 mg, 70%); ν_{max}(thin film)/cm^{-1} 3500–3100 (NH and OH), 2950 (CH), 1640 (lactam); δ_H(250 MHz, CD_3SOCD_3) 3.21 (2 H, t, *J* 3, CH_2CO), 4.23 (2 H, s, CH_2O), 4.27 (2 H, m, CH_2N), 4.53 (1 H, br s, OH), 6.59 (1 H, d, *J* 2, 2-H), 7.73 (1 H, br s, CH_2NH) and 10.41 (1 H, br s, pyrrole-NH); *m*/*z* (FD) 166 (100%).

5-Benzyloxy-3-(5,5-dimethyl-1,3-dioxan-2-yl)-1*H*-pyrrolo[2,3-*c*]pyridine 21

A solution of toluene-*p*-sulfonic acid monohydrate (1 g) in toluene (70 cm^3) was heated under reflux with a Dean–Stark trap for 2 h. An aliquot of this solution (3 cm^3) was added to a mixture of 2,2-dimethylpropane-1,3-diol (3.0 g, 28.8 mmol) and aldehyde **14**[23] (2.47 g, 9.8 mmol) in dry toluene (10 cm^3) and the mixture was heated under reflux with a Dean–Stark trap for 4 h. The solution was allowed to cool, washed with aq. sodium hydrogen carbonate (5% w/v; 30 cm^3) and then water (30 cm^3), dried and evaporated. The residue was recrystallised to give the *acetal* **21** (3.16 g, 96%), mp 156.5–158.5 °C (from acetone–diethyl ether) (Found: M$^+$, 338.1617. $C_{20}H_{22}N_2O_3$ requires *M*, 338.1604); λ_{max}(MeOH)/nm 308, 256 and 218; ν_{max}(Nujol)/cm^{-1} 3130br (NH), 2950 (CH) and 1620 (Ar); δ_H(400 MHz, CD_3COCD_3) 0.80 and 1.29 (each 3 H, s, Me), 3.69 (4 H, ABq, *J* 11, $CH_2CMe_2CH_2$), 5.40 (2 H, s, $PhCH_2$), 5.68 (1 H, s, OCHO), 7.12 (1 H, d, *J* 1, 2-H), 7.26–7.55 (6 H, m, Ph and 4-H), 8.35 (1 H, s, 7-H) and 10.41 (1 H, br s, NH).

3-(5,5-Dimethyl-1,3-dioxan-2-yl)-4,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one 22

A solution of acetal **21** (170 mg, 0.50 mmol) in *N*,*N*-dimethylformamide (15 cm^3) was stirred with 10% palladium-on-carbon (65 mg) under an atmosphere of hydrogen at room temperature until TLC indicated that the reaction was finished (about 24 h). The solvent was evaporated and the residue resuspended in methanol (15 cm^3) and filtered through Celite. The filtrate was evaporated and the residue was recrystallised to give the lactam **22** as needles (110 mg, 87%), mp 89–92 °C (from dichloromethane–hexane) (Found: M$^+$, 250.1314. $C_{13}H_{18}N_2O_3$ requires *M*, 250.1317); ν_{max}(Nujol)/cm^{-1} 3300 and 3170br (2 × NH), 2900 (CH) and 1640 (C=O); δ_H(400 MHz, CD_3SOCD_3) 0.71 and 1.17 (each 3 H, s, Me), 3.27 (2 H, t, *J* 3, CH_2CO), 3.53 (4 H, ABq, *J* 11, $CH_2CMe_2CH_2$), 4.25 (2 H, m, CH_2N), 5.28 (1 H, s, OCHO), 6.69 (1 H, d, *J* 2, 2-H), 7.70 (1 H, br s, CH_2NH) and 10.54 (1 H, br s, pyrrole-NH).

3-Formyl-4,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one 24

The acetal **22** (78 mg, 0.33 mmol) was stirred in acetone (5 cm^3) and hydrochloric acid (1 mol dm^{-3}; 1 drop) at room temperature for 10 min. The precipitate was filtered off, washed well with acetone, and dried *in vacuo* to give the *aldehyde* **24** (54 mg, 95%), which was found to be unstable and was therefore used directly in the next step (Found: M$^+$, 164.0586. $C_8H_8N_2O_2$ requires *M*, 164.0586); λ_{max}(MeOH)/nm 238 and 216; δ_H(400 MHz, CD_3SOCD_3) 3.38 (2 H, s, CH_2CO), 4.30 (2 H, s, CH_2N), 7.60 (1 H, d, *J* 2.5, 2-H), 7.82 (1 H, br s, CH_2NH), 9.66 (1 H, s, CHO) and 11.58 (1 H, br s, pyrrole-NH); δ_C(100 MHz, CD_3SOCD_3) 38.9–40.2 (CH_2N and CH_2CO obscured by solvent peaks), 111.6, 122.9, 123.7 and 130.4 (4 × pyrrole-C), 168.81 (CONH) and 185.36 (CHO); *m*/*z* (EI) 164 (M$^+$, 100%), 135 (9), 121 (17), 93 (35) and 80 (11).

3-(2-Methoxycarbonylethenyl)-4,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one 25

A solution of methyl hydrogen malonate in dry pyridine (1 cm^3) was added in portions to a solution of aldehyde **24** (45 mg, 0.27 mmol) in dry pyridine (2 cm^3) and dry piperidine (0.1 cm^3) heated under reflux. After 5 h at reflux the mixture was evaporated and purified by PLC, eluting with methanol–pyridine–dichloromethane (10 : 1 : 89) and extracting the desired silica band with pyridine–dichloromethane (1 : 9), to give the starting aldehyde (15 mg) and the *acrylate* **25** (14 mg, 35% based on unrecovered starting material) as a semi-solid (Found: M$^+$, 220.0839. $C_{11}H_{12}N_2O_3$ requires *M*, 220.0848); δ_H(250 MHz, C_5D_5N) 3.78 (3 H, s, OMe), 3.92 (2 H, t, *J* 3, CH_2CO), 4.53 (2 H, m, CH_2N), 6.43 (1 H, d, *J* 16, CH=C*H*CO), 7.34 (1 H, br s, 2-H), 8.09 (1 H, d, *J* 16, C*H*=CHCO) and 12.40 (1 H, br s, pyrrole-NH); δ_C(100 MHz, CD_3SOCD_3) 38.5–40.4 (CH_2CO and CH_2NH obscured by solvent peaks), 50.9 (OMe), 110.0, 110.6, 116.1 and 122.8 (4 × pyrrole-C),124.8 (CH=*C*HCO), 139.20 (*C*H=CHCO) and 167.4 and 168.3 (2 × C=O); *m*/*z* (EI) 220 (M$^+$, 44%), 201 (10), 131 (10), 91 (20) and 84 (100).

8,9-Didehydroporphobilinogen 26

The lactam ester **25** (9 mg, 41 μmol) was stirred in a mixture of aq. potassium hydroxide (1.5 mol dm^{-3}; 0.3 cm^3) and methanol (0.1 cm^3) under argon at room temperature for 48 h. The solution was then freeze-dried to give dehydroPBG **26** as its potassium salt mixed with excess potassium hydroxide, and this mixture was used for the enzymic experiments without further purification; δ_H(250 MHz, D_2O) 3.72 (2 H, s, CH_2CO), 4.07 (2 H, s, CH_2N), 6.47 (1 H, d, *J* 16, CH=C*H*CO), 7.53 (1 H, s, α-H) and 7.74 (1 H, d, *J* 16, C*H*=CHCO).

Ethyl hydrogen fluoromalonate

A solution of diethyl fluoromalonate (200 mg, 1.11 mmol) in dry EtOH (3 cm^3) was stirred with a solution of potassium hydroxide (63 mg, 1.12 mmol) in dry ethanol (1 cm^3) for 3 h at room temperature and then at 40 °C for 90 min and then evaporated to dryness. A solution of the residue in aq. sodium hydrogen carbonate (20 cm^3) was extracted with diethyl ether (4 × 20 cm^3) and the combined organic layers were dried and

evaporated to give some recovered diester (50 mg). The aqueous layer was acidified with hydrochloric acid (3 mol dm^{-3}) and extracted with ethyl acetate (3 × 20 cm^3). The combined extracts were dried and evaporated to give the monoester as an oil (106 mg, 84% based on unrecovered starting material) (Found: MH^+, 151.0418. $C_5H_7FO_4$ requires *M*, 151.0407); ν_{max}(thin film)/cm^{-1} 3500–3200 (OH) and 1740 (2 × C=O); δ_H(250 MHz, $CDCl_3$) 1.34 (3 H, t, *J* 7, CH_3), 4.34 (2 H, q, *J* 7, CH_2), 5.34 (1 H, d, *J* 48, CHF) and 10.30 (1 H, br s, OH); *m/z* (EI) 151 (MH^+, 2%), 133 (16), 105 (43) and 78 (100).

(*Z*)-5-Benzyloxy-3-(2-fluoro-2-ethoxycarbonylethenyl)-1*H*-pyrrolo[2,3-*c*]pyridine 28

Aldehyde **14**[23] (100 mg, 0.40 mmol) was dissolved in dry pyridine (1 cm^3) and dry piperidine (0.1 cm^3) and heated to 100 °C for 10 min under argon. An aliquot (0.25 cm^3) of a solution of ethyl hydrogen fluoromalonate (60 mg, 0.40 mmol) in dry pyridine (1 cm^3) was added and heating continued under reflux. Further aliquots were added every hour and after 6 h in total the mixture was evaporated *in vacuo*. The residue was purified by PLC, eluting with diethyl ether, to give the starting aldehyde **14** (55 mg) and the *fluoroacrylate* **28** as a solid (35 mg, 58% based on unrecovered starting material), mp 188.5–190 °C (Found: M^+, 340.1231; C, 67.1; H, 5.0; N, 8.3%. $C_{19}H_{17}FN_2O_3$ requires *M*, 340.1223; C, 67.05; H, 5.0; N, 8.25%); λ_{max}(MeOH)/nm 313, 278 and 225; ν_{max}($CHCl_3$)/cm^{-1} 3620 (NH), 2940 and 2830 (CH), 1720 (C=O) and 1620 (Ar); δ_H(400 MHz, C_5D_5N) 1.25 (3 H, t, *J* 7, Me), 4.33 (2 H, q, *J* 7, $MeCH_2$), 5.71 (2 H, s, $PhCH_2$), 7.32 (1 H, t, *J* 7.5, *p*-Ph), 7.42 (2 H, t, *J* 7.5, *m*-Ph), 7.56 (1 H, s, 2-H), 7.67 (2 H, d, *J* 7.5, *o*-Ph), 7.67 (1 H, d, *J* 38, CH=CF), 8.28 (1 H, s, 4-H), 8.64 (1 H, s, 7-H) and 10.72 (1 H, br s, pyrrole-NH); δ_C(100 MHz, C_5D_5N) 14.4 (CH_3), 61.6 (CH_3CH_2), 68.3 ($PhCH_2$), 98.1 and 106.7 (C-2 and 4), 111.1 (d, *J* 9, *C*=CF), 127.9, 128.2, 128.8, 131.5, 131.9, 135.5, 136.5 and 139.3 (Ph, C-3, 3a, 7 and 7a), 145.4 (d, *J* 253, CF), 158.8 (C-5) and 161.7 (d, *J* 33, C=O); δ_F(235 MHz, reference CF_3CO_2H, C_5D_5N) −52.7 (d, *J* 38); *m/z* (EI) 340 (M^+, 64%), 263 (20), 234 (16) and 91 (100).

5-Benzyloxy-3-(2-fluoro-2-methoxycarbonylethyl)-1*H*-pyrrolo[2,3-*c*]pyridine 33

The fluoroacrylate **28** (49 mg, 0.14 mmol) was dissolved in dry methanol (10 cm^3) and magnesium turnings (50 mg) were added. The mixture was heated under reflux until evolution of hydrogen began, then stirred at room temperature for 3 h and evaporated to dryness. Aqueous acetic acid (50%; 5 cm^3) was added and the solution was extracted with ethyl acetate (4 × 15 cm^3). The combined extracts were washed with aq. sodium hydrogen carbonate (30 cm^3), dried and evaporated. The residue was purified by PLC, eluting with diethyl ether–hexane (9:1), to yield a mixture of **33** and **34** (ratio ~3:1) as a colourless oil (20 mg, 41%). Purification by HPLC (Spherisorb S5W semi-preparative silica column), eluting with methanol–THF–dichloromethane (0.5:0.5:99; 2.7 cm^3 min^{-1}; 20 min), gave the pure *fluoro compound* **33** as a solid (10 mg, 23%), mp 153–155 °C (Found: M^+, 328.1228. $C_{18}H_{17}FN_2O_3$ requires *M*, 328.1223); λ_{max}(MeOH)/nm 316, 274 and 234; δ_H(400 MHz, $CDCl_3$) 3.32 (2 H, m, CH_2CHF), 3.75 (3 H, s, OMe), 5.18 (1 H, ddd, *J* 49, 6 and 4, CHF), 5.41 (2 H, s, $PhCH_2$), 6.98 (1 H, s, 2-H), 7.29–7.52 (6 H, m, Ph and 4-H), 8.37 (1 H, s, 7-H) and 8.79 (1 H, br s, NH); δ_C(100 MHz, $CDCl_3$) 28.1 (d, *J* 22, CH_2CHF), 52.4 (OMe), 68.5 ($PhCH_2$), 88.6 (d, *J* 185, CHF), 96.6 (C-4), 107.8 (C-2), 127.6, 127.7, 128.4, 129.8, 129.8, 130.6, 136.5 and 137.9 (Ph, C-3, C-3a, C-7 and C-7a), 157.4 (C-5) and 169.8 (d, *J* 24, C=O); δ_F(235 MHz, reference C_6F_6, $CDCl_3$) −26.9 (dt, *J* 49 and 27); *m/z* (EI) 328 (M^+, 12%), 251 (9), 91 (100).

The non-fluorinated compound **34** was also obtained (6 mg, 10%), mp 107–110 °C; δ_H(400 MHz, $CDCl_3$) 2.66 (2 H, t, *J* 8, CH_2CH_2CO), 2.99 (2 H, t, *J* 7.6, CH_2CH_2CO), 3.65 (3 H, s, OMe), 5.29 (2 H, s, $PhCH_2$), 6.95 (1 H, s, 2-H), 7.19–7.49 (6 H, m, Ph and 4-H), 8.40 (1 H, s, 7-H) and 8.45 (1 H, br s, NH).

5-Benzyloxy-1-*tert*-butoxycarbonyl-3-formyl-1*H*-pyrrolo[2,3-*c*]pyridine 27

A solution of aldehyde **14**[23] (1.16 g, 4.59 mmol) in dry acetonitrile (50 cm^3) was stirred with 4-dimethylaminopyridine (56 mg, 0.46 mmol) and di-*tert*-butyl dicarbonate (1.1 g, 4.59 mmol) at room temperature for 3 h, until TLC indicated that all the starting material had been consumed. Excess di-*tert*-butyl dicarbonate was then destroyed by the addition of 1,2-diaminoethane (8.3 mg, 1.39 mmol). Water (100 cm^3) was added and the mixture was extracted with diethyl ether (2 × 100 cm^3). The organic extracts were washed with brine (3 × 100 cm^3), dried ($MgSO_4$) and evaporated. The residue was purified by flash column chromatography, eluting with dichloromethane, to give the *protected* 1H-*pyrrolo*[2,3-c]*pyridine* **27** (1.36 g, 84%) as an oil (Found: MH^+, 353.1501. $C_{20}H_{20}O_4N_2$ requires *M* + H, 353.1502); R_F 0.67 (EtOAc–light petroleum, 2:1); ν_{max}(thin film)/cm^{-1} 2979 and 2916 (CH), 1748 (NCO_2), 1679 (CH=O) and 1616 and 1574 (C=C and C=N); δ_H(250 MHz, $CDCl_3$) 1.71 (9 H, s, Bu^t), 5.43 (2 H, s, $PhCH_2$), 7.26–7.63 (5 H, m, Ph), 7.65 (1 H, d, *J* 0.5, 4-H), 8.32 (1 H, s, 2-H), 8.94 (1 H, br s, 7-H) and 10.05 (1 H, s, CHO); δ_C(100 MHz, APT, $CDCl_3$) 28.0 (Me_3C), 68.2 (CH_2), 86.5 (Me_3C), 101.6 (C-4), 120.3 (C-3), 127.6, 127.7 and 128.4 (phenyl-CH), 129.2 and 135.9 (C-3a and 7a), 133.7 and 139.6 (C-2 and 7), 137.5 (phenyl-C), 148.1 (C-5), 160.4 (NCO_2) and 184.9 (CHO); *m/z* (CI) 353 (MH^+, 10%), 297 ($MH^+ - C_4H_8$, 3), 281 ($MH^+ - C_4H_8O$, 5) and 253 ($MH^+ - C_4H_8 - CO_2$, 100).

5-Benzyloxy-1-*tert*-butoxycarbonyl-3-(2-ethoxycarbonyl-2-fluoro-1-hydroxyethyl)-1*H*-pyrrolo[2,3-*c*]pyridine 29

A solution of 1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one (DMPU) (927 mm^3, 7.67 mmol) in THF (6 cm^3) was stirred at −85 °C while a solution of lithium bis(trimethylsilyl)amide in THF (1 mol dm^{-3}; 4.6 cm^3, 4.6 mmol) was added. Ethyl fluoroacetate (177 mm^3, 2.3 mmol) was added dropwise as rapidly as possible while not allowing the temperature to rise above −85 °C. After 10 min, a solution of the aldehyde **27** (201 mg, 0.767 mmol) in THF (4 cm^3) was added quickly. After a further 10 min at −85 °C, saturated aq. ammonium chloride–THF (1:1; 2 cm^3) was added. The mixture was warmed to room temperature and extracted with dichloromethane (10 cm^3). The organic layer was washed with water (4 × 10 cm^3) and then aq. sodium metabisulfite (4 × 10 cm^3), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography, eluting with light petroleum (bp 40–60 °C)–ethyl acetate (8:1), to give the *fluoro ester* **29** as an oil (323 mg, 92%), which was shown to be a mixture of diastereoisomers (ratio 3:1) by NMR spectroscopy (Found: MH^+, 459.1931. $C_{24}H_{27}FO_6N_2$ requires *M* + H, 459.1931); R_F 0.59 (EtOAc–light petroleum, 2:1); ν_{max}(thin film)/cm^{-1} 3630–3160 (OH), 2985 and 2933 (CH), 1738 (C=O) and 1614 and 1589 (C=C and C=N); δ_H(250 MHz, $CDCl_3$) (major diastereoisomer) 1.17 (3 H, q, *J* 7, CH_2CH_3), 1.67 (9 H, s, Bu^t), 2.55 (1 H, br s, OH), 4.20 (2 H, q, *J* 7, CH_2CH_3), 5.19 (1 H, dd, *J* 48 and 4.5, CHF), 5.25–5.35 (1 H, m, CHOH), 5.37 (2 H, s, $PhCH_2$), 7.02 (1 H, br s, 2-H), 7.28–7.49 (5 H, m, Ph), 7.78 (1 H, br s, 4-H) and 8.91 (1 H, s, 7-H); (minor diastereoisomer, distinguishable signals) 1.23 (3 H, q, *J* 7, CH_2CH_3), 4.31 (2 H, q, *J* 7, CH_2CH_3), 5.12 (1 H, dd, *J* 48 and 3, CHF); δ_C(100 MHz, APT, $CDCl_3$) (major diastereoisomer) 14.0 (CH_2CH_3), 28.1 (Me_3C), 62.0 (CH_2CH_3), 67.5 (d, J_{CF} 21, CHOH), 68.2 (CH_2Ph), 84.9 (Me_3C), 90.5 (d, J_{CF} 191, CHF), 99.2 (C-4), 116.4 (C-3), 127.7, 127.8 and 128.4 (phenyl-CH), 129.0 and 138.4 (C-3a and 7a), 129.3 and 133.6 (C-2 and 7), 137.6 (phenyl-C), 148.8 (C-5), 159.0 (NCO_2) and 167.5 (d, J_{CF} 23, CO_2Et); *m/z* (CI) 459 (MH^+, 65%), 429 ($M^+ - Et$, 18), 353 ($M^+ - CHFCO_2Et$, 98), 297 ($MH^+ - CHFCO_2Et - Bu^t$, 12), 253 ($MH^+ - CHFCO_2Et - CO_2 Bu^t$, 71), 184 (100).

3-(1-Acetoxy-2-ethoxycarbonyl-2-fluoroethyl)-5-benzyloxy-1-*tert*-butoxycarbonyl-1*H*-pyrrolo[2,3-*c*]pyridine 31

The alcohol **29** (345 mg, 0.746 mmol) was dissolved in pyridine–acetic anhydride (2:1 v/v; 39 cm^3). After 30 min the solution was added to water (50 cm^3) and the mixture was extracted with dichloromethane (3 × 50 cm^3). The combined organic layers were washed with aq. copper sulfate (4 × 50 cm^3), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography, eluting with light petroleum (bp 40–60 °C)–ethyl acetate (9:1), to give the *acetate ester* **31** (314 mg, 84%) as an oil, which was shown to be a mixture of diastereoisomers (ratio 3:1) by NMR spectroscopy (Found: MH^+, 501.2040. $C_{26}H_{29}FO_7N_2$ requires M + H, 501.2038); R_F 0.51 (EtOAc–light petroleum, 1:4); ν_{max}(thin film)/cm^{-1} 2980 and 2933 (CH), 1740 (3 × C=O), 1618 and 1589 (C=C and C=N); δ_H(400 MHz, $CDCl_3$) 1.12 (3 H, t, J 7, CH_2CH_3), 1.55 (9 H, s, Bu^t), 1.89 (3 H, s, CH_3CO), 3.97 (2 H, q, J 7, CH_2CH_3), 5.27 (1 H, dd, J 49 and 3, CHF), 5.31 (2 H, s, $PhCH_2$), 6.33 (1 H, dd, J 25 and 3, CHOAc), 6.97 (1 H, s, 2-H), 7.13–7.36 (5 H, m, Ph), 7.74 (1 H, br s, 4-H) and 8.82 (1 H, br s, 7-H); (minor diastereoisomer, distinguishable signals) 1.05 (3 H, t, J 7, CH_2CH_3), 1.58 (9 H, s, Bu^t), 1.99 (3 H, s, CH_3CO), 4.04 (2 H, q, J 7, CH_2CH_3), 5.11 (1 H, dd, J 49 and 3, CHF), 6.37 (1 H, dd, J 25 and 3, CHOAc), 7.01 (1 H, s, 2-H); δ_C(100 MHz, APT, $CDCl_3$) (major diastereoisomer) 14.0 (CH_2CH_3), 20.2 (CH_3CO), 28.1 (Me_3C), 62.2 (CH_2CH_3), 67.9 (d, J_{CF} 21, CHOAc), 68.1 ($PhCH_2$), 85.0 (Me_3C), 88.9 (d, J_{CF} 194, CHF), 99.4 (C-4), 112.4 (C-3), 125.0 (C-2), 127.7, 127.85 and 128.4 (phenyl-CH), 130.7 and 135.2 (C-3a and 7a), 133.6 (C-7), 137.7 (phenyl-C), 148.2 (C-5), 159.2 (NCO_2), 166.1 (d, J_{CF} 23, CO_2Et) and 169.6 (CH_3CO); δ_F(235 MHz, reference CCl_3F, $CDCl_3$) −201.6 and −201.4 (each dd, J 25 and 49); m/z (CI) 501 (MH^+, 100%), 441 ($M^+ - CH_3CO_2$, 14), 341 ($MH^+ - CH_3CO_2 - Bu^tCO_2$, 98) and 323 ($MH^+ - Bu^tCO_2 - PhCH_2$, 30).

5-Benzyloxy-3-(2-ethoxycarbonyl-2-fluoroethyl)-1*H*-pyrrolo[2,3-*c*]pyridine 32

A solution of acetate ester **31** (405 mg, 0.84 mmol) in dichloromethane (6 cm^3) was added to a solution of sodium cyanoborohydride (394 mg, 6.28 mmol) in trifluoroacetic acid (18 cm^3) at 0 °C. The solution was allowed to warm to room temperature and was stirred for 12 h and then evaporated. The residue was redissolved in chloroform–methanol (95:5; 100 cm^3), washed with aq. sodium hydrogen carbonate (5% w/v; 2 × 50 cm^3), dried ($MgSO_4$) and evaporated. The residual oil was dissolved in ethanol (15 cm^3) and stirred with potassium fluoride (1.46 g, 25.1 mmol) at room temperature for 24 h. Ethyl acetate (100 cm^3) was added and the mixture was washed with aq. sodium hydrogen carbonate (5% w/v; 2 × 50 cm^3), dried ($MgSO_4$) and evaporated. The residue was triturated with diethyl ether to give the *fluoro ester* **32** (186 mg, 65%) as a solid (Found: M^+, 342.1375. $C_{19}H_{19}FO_3N_2$ requires M, 342.1380); R_F 0.58 (EtOAc); ν_{max}(CH_2Cl_2)/cm^{-1} 3392 (NH), 2975 and 2925 (CH), 1765 (C=O) and 1618 and 1562 (C=C and C=N); δ_H(400 MHz, $CDCl_3$) 1.13 (3 H, t, J 7, CH_2CH_3), 3.35 (1 H, ddd, J 28, 15.5 and 6, CH_AH_BCHF), 3.45 (1 H, ddd, J 23.5, 15.5 and 5, CH_AH_BCHF) 4.07 (2 H, q, J 7, CH_2CH_3), 5.27 (1 H, ddd, J 48, 6 and 5, CHF), 5.43 (2 H, s, $PhCH_2$), 7.19–7.52 (6 H, m, Ph and 2-H), 8.13 (1 H, s, 4-H), 8.56 (1 H, s, 7-H) and 10.47 (1 H, br s, NH); δ_C(100 MHz, CD_3CN) 14.3 (CH_2CH_3), 28.3 (d, J_{CF} 22, CH_2CHF), 62.3 (CH_2CH_3), 71.4 ($PhCH_2$), 89.8 (d, J_{CF} 183, CHF), 97.4 (C-4), 110.1 (C-3) and 127.7, 128.9, 129.5, 136.8 and 137.2 (Ph, C-2 and 7) (other signals obscured by noise); δ_F(235 MHz, reference CCl_3F, $CDCl_3$) −188.4 (dt, J 48 and 27); m/z (EI) 342 (M^+, 73%), 324 (3), 296 (4), 265 (28), 91 ($PhCH_2^+$, 100) and 57 (100).

3-(2-Fluoro-2-methoxycarbonylethyl)-4,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one 37

A solution of the fluorinated 1*H*-pyrrolo[2,3-*c*]pyridine **33** (11 mg, 0.40 mmol) in *N*,*N*-dimethylformamide (1.5 cm^3) was stirred with 10% palladium-on-carbon (4 mg) under an atmosphere of hydrogen at room temperature overnight and then evaporated to dryness. The residue was dissolved in methanol (10 cm^3) and filtered through a plug of Celite. The filtrate was evaporated and the residue was purified by flash chromatography, eluting with methanol–dichloromethane (5:95), to give the *lactam* **37** (7 mg, 87%) as small prisms, mp 250–253 °C (decomp.) (from methanol–dichloromethane) (Found: M^+, 240.0896. $C_{11}H_{13}FN_2O_3$ requires M, 240.0882); δ_H(400 MHz, CD_3OD) 2.89–3.04 (2 H, m, CH_2CHF), 3.31 (2 H, obscured by solvent signal, CH_2CO), 4.39 (2 H, t, J 3.5, CH_2N), 5.09 (1 H, ddd, J 48, 6 and 4.5, CHF), 6.59 (1 H, d, J 1.5, 2-H); δ_C(100 MHz, CD_3OD) 26.9 (CH_2CO), 29.4 (d, J 22, CH_2CHF), 41.5 (CH_2NH), 54.1 (OCH_3), 91.0 (d, J 182, CHF), 112.0, 114.1, 118.8 and 120.6 (4 × pyrrole-C), 171.7 (d, J 24, CHFCO) and 174.5 (CH_2CO); δ_F(235 MHz, CD_3OD) −112.58 (dt, J 48 and 25); m/z (EI) 240 (M^+, 12%), 142 (23), 96 (94) and 94 (100).

3-(2-Ethoxycarbonyl-2-fluoroethyl)-4,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-5(6*H*)-one 35

Hydrogenation of the fluorinated benzyloxy-1*H*-pyrrolo[2,3-*c*]pyridine **32**, as for **33** above, gave the *fluorinated lactam ethyl ester* **35** as small prisms, mp 208–212 °C (with evolution of gas and resolidification) and then 215–216.5 °C (from methanol–dichloromethane) (Found: M^+, 254.1067. $C_{12}H_{15}FO_3N_2$ requires M, 254.1067); R_F 0.35 (CH_2Cl_2–MeOH, 9:1); ν_{max}(thin film)/cm^{-1} 3214 (NH), 2919 (CH), 1735 (CO_2) and 1643 and 1613 (CONH and C=C); δ_H(200 MHz, CD_3OD) 1.21 (3 H, t, J 7, CH_2CH_3), 2.89–3.04 (2 H, m, CH_2CHF), 3.34 (2 H, obscured by solvent signal, CH_2CO), 4.16 (2 H, q, J 7, CH_2CH_3), 4.39 (2 H, t, J 3, CH_2N), 5.09 (1 H, ddd, J 48, 6 and 4.5, CHF) and 6.59 (1 H, d, J 2, 2-H); m/z (EI) 254 (M^+, 68%), 209 (M^+ − OEt, 7), 181 ($M^+ - CO_2Et$, 23), 146 ($M^+ - CHFCO_2Et$, 70) and 106 (FCH_2CO_2Et, 100).

9-Fluoroporphobilinogen 38

Method A. The lactam **37** (2 mg, 0.083 mmol) was heated briefly to about 60 °C with a solution of potassium hydroxide in D_2O (2 mol dm^{-3}; 0.5 cm^3) to effect almost complete dissolution. The solution was stirred at room temperature for a further 2 h and then freeze-dried to give the potassium salt of 9-fluoroporphobilinogen **38** as a mixture with excess potassium hydroxide, and this mixture was used for the enzymic experiments without further purification; δ_H(400 MHz, D_2O) 3.06–3.30 (2 H, m, CH_2CHF), 3.59 (s, CH_2CO largely deuteriated), 3.90 (2 H, s, CH_2N), 5.13 (0.5 H, one half of ddd, J 4 and 6.5, the other half is obscured by the solvent signal, CHF) and 6.9 (1 H, s, 2-H); δ_C(100 MHz, D_2O) 28.5 (d, J_{CF} 22, CH_2CHF), 31.9 (m, CD_2CO), 35.6 (CH_2N), 92.0 (d, J_{CF} 180, CHF), 114.0, 115.5, 116.9 and 130.0 (4 × pyrrole-C), 177.5 (d, J_{CF} 21, CHFCO_2^-) and 181.58 ($CH_2CO_2^-$); δ_F(235 MHz, reference CF_3CO_2H, D_2O) −104.5 (m). Analytical HPLC [Nucleosil-N(CH_3)$_2$ 5 μ analytical ion-exchange column], eluting with acetonitrile–aq. ammonium hydrogen carbonate (0.02 mol dm^{-3}) (1:1), gave a single peak (detection at 230 nm; flow rate 0.8 cm^3 min^{-1}; retention time 4.5 min).

Method B. The lactam **35** (24 mg, 0.101 mmol) was stirred with aq. potassium hydroxide (2 mol dm^{-3}; 2 cm^3) and methanol (2 cm^3) at room temperature for 6 h. The methanol was then evaporated *in vacuo* and the remaining aqueous solution was treated with Dowex 50X8-400 (NH_4^+) ion exchange resin until the pH dropped to 8–9. The ion exchange resin was filtered off and the filtrate was evaporated *in vacuo* to give the ammonium salt of 9-*fluoroporphobilinogen* **38** (23 mg, 92%) as a white solid; R_F 0.25 (BuOH–H_2O–CH_3CO_2H, 12:5:3); δ_H(200 MHz, D_2O) 2.89 (1 H, ddd, J 28, 15.5 and 7, CH_AH_BCHF), 3.09 (1 H, ddd, J 26, 15.5 and 4, CH_AH_BCHF), 3.43 (2 H, s, $CH_2CO_2^-$), 4.16 (2 H, s, CH_2N), 4.80–5.09 (0.5 H,

one half of ddd, J 4 and 6.5, the other half is obscured by the solvent signal, $CHFCO_2^-$) and 6.6 (1 H, s, 2-H).

Acknowledgements

We would like to thank Professor Sir Alan Battersby for helpful discussions and the SERC (now EPSRC) for studentships (for M. R. and D. A.).

References

1 Preliminary account of part of this work: F. J. Leeper and M. Rock, *J. Chem. Soc., Chem. Commun.*, 1992, 242.
2 (*a*) A. R. Battersby and F. J. Leeper, *Chem. Rev.*, 1990, **90**, 1261; (*b*) F. J. Leeper, *Nat. Prod. Rep.*, 1989, **6**, 171.
3 (*a*) G. J. Hart, A. D. Miller, F. J. Leeper and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1987, 1762; (*b*) P. M. Jordan and M. J. Warren, *FEBS Lett.*, 1987, **225**, 87; (*c*) G. J. Hart, A. D. Miller, U. Beifuss, F. J. Leeper and A. R. Battersby, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1979.
4 A. T. Carpenter and J. J. Scott, *Biochem. J.*, 1959, **71**, 325.
5 D. S. Hoare and H. Heath, *Biochem. J.*, 1959, **73**, 679.
6 L. Bogorad in *Comparative Biochemistry of Photoreactive Systems*, ed. M. B. Allen, Academic Press, 1960, p. 227.
7 A. T. Carpenter and J. J. Scott, *Biochim. Biophys. Acta*, 1961, **52**, 195.
8 R. B. Frydman and B. Frydman, *Arch. Biochem. Biophys.*, 1970, **136**, 193.
9 R. C. Davies and A. Neuberger, *Biochem. J.*, 1973, **133**, 471.
10 R. B. Frydman and G. Feinstein, *Biochim. Biophys. Acta*, 1974, **350**, 358.
11 S. H. Wilen, D. Shen, J. M. Licata, E. Baldwin and C. S. Russell, *Heterocycles*, 1984, **22**, 1747.
12 A. I. Scott, C. A. Roessner, N. J. Stolowich, P. Karuso, H. J. Williams, S. K. Grant, M. D. Gonzalez and T. Hoshino, *Biochemistry*, 1988, **27**, 7984.
13 M. J. Warren and P. M. Jordan, *Biochemistry*, 1988, **27**, 9020.
14 C. Pichon, K. R. Clemens, A. R. Jacobson and A. I. Scott, *Tetrahedron*, 1992, **48**, 4687.
15 R. E. Danso-Danquah, A. I. Scott and D. Becker, *Tetrahedron*, 1993, **49**, 8195.
16 K. R. Clemens, C. Pichon, A. R. Jacobson, P. Yon-Hin, M. D. Gonzalez and A. I. Scott, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 521.
17 J. Wang and A. I. Scott, *Tetrahedron*, 1994, **50**, 6181.
18 A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, E. McDonald and K. E. Gustafson-Potter, *J. Chem. Soc., Chem. Commun.*, 1979, 316.
19 F. J. Leeper and M. Rock, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2643 (following paper).
20 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.
21 A. D. Miller, PhD Thesis, Cambridge, 1988.
22 J. J. De Voss, PhD Thesis, Cambridge, 1988.
23 B. Frydman, S. Reil, M. E. Despuy and H. Rapoport, *J. Am. Chem. Soc.*, 1969, **91**, 2338; A. R. Battersby, E. McDonald, H. K. Wurzinger and K. J. James, *J. Chem. Soc., Chem. Commun.*, 1975, 493.
24 G. M. Blackburn and M. J. Parrett, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1417 and 1425.
25 H. K. Wurziger, PhD Thesis, Cambridge, 1976.
26 M. Hudlicky, *J. Fluorine Chem.*, 1979, **14**, 189.
27 Y. C. Kim, *Can. J. Chem.*, 1969, **47**, 3259.
28 L. Grehn and U. Ragnarsson, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 296.
29 J. T. Welch, K. Seper, S. Eswarakrishnan and J. Samartino, *J. Org. Chem.*, 1984, **49**, 4720; J. T. Welch and S. Eswarakrishnan, *J. Chem. Soc., Chem. Commun.*, 1985, 186.
30 D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, Pergamon, Oxford, 3rd edn. 1988.

Paper 6/01207H
Received 19*th February* 1996
Accepted 24*th July* 1996