Tetrahedron Vol. 48, No. 23, pp. 4687-4712, 1992
Printed in Great Britain

0040-4020/92 $5.00+.00

On the Mechanism of Porphobilinogen Deaminase. Design, Synthesis, and Enzymatic Reactions of Novel Porphobilinogen Analogs.†,‡

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(*Received in USA* 22 *August* 1991)

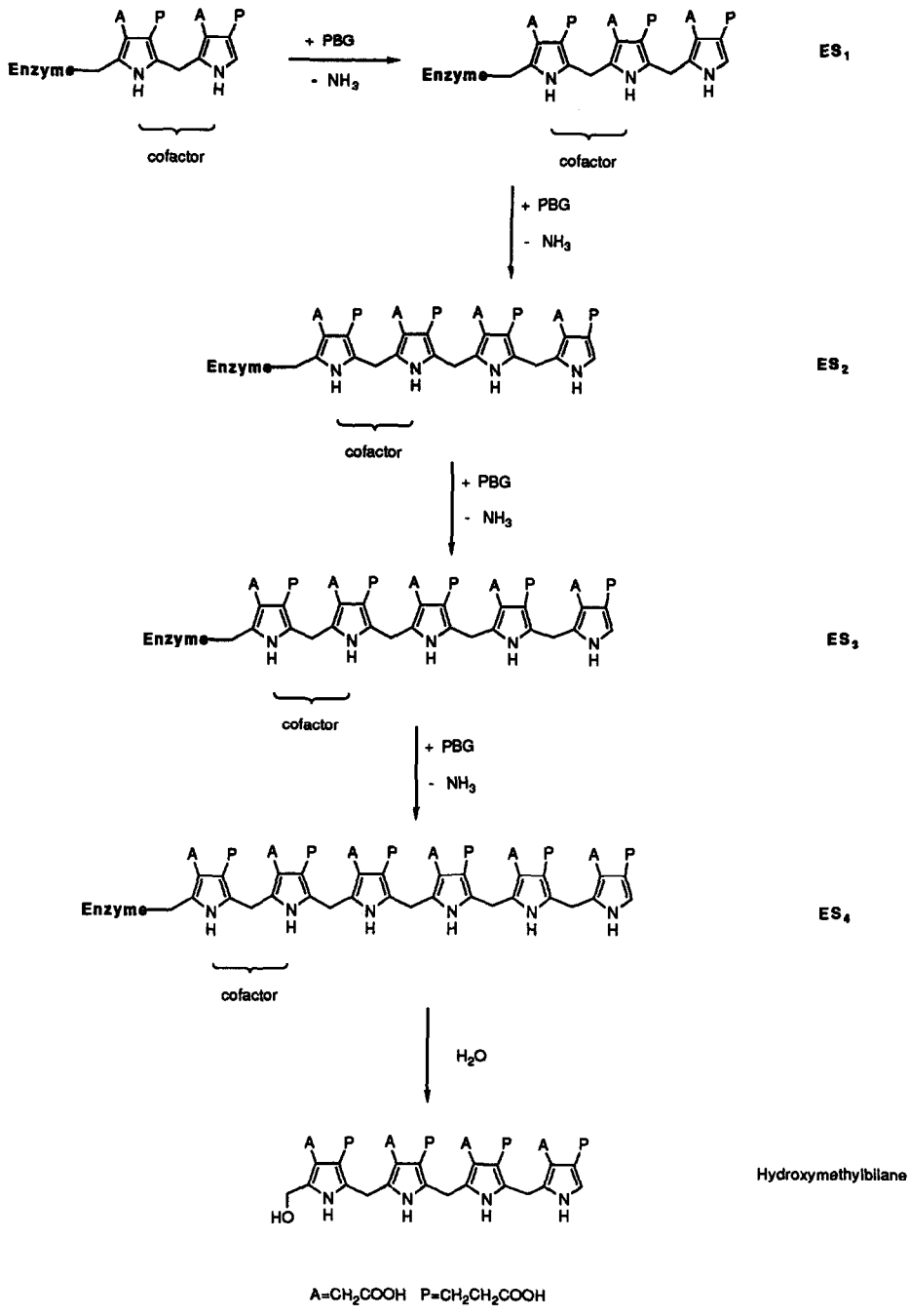
Key Words: New derivatives of porphobilinogen; mechanism study; PBG deaminase.

ABSTRACT: *Three new derivatives of porphobilinogen (PBG;1) were designed and synthesized to study the mechanism of ammonia loss during the tetramerization of PBG, catalyzed by the enzyme PBG deaminase. Two of these compounds are substituted at the C-11 carbon with CH_3 or CF_3, while the third analog is the N-methyl derivative of PBG wherein the pyrrole proton is replaced with methyl. All three compounds reacted, to varying degrees of completion, with PBG deaminase. Our results suggest that activation of substrate by enzyme involves an intermediate which possesses substantial positive charge at the C-11 carbon, though the actual structure of the electrophilic agent is uncertain at the present time. In addition to defining the nature of the initially formed electrophile, our results also reveal something about the nature of the nucleophilic species at the enzyme active site. The results with these three new pyrroles may implicate the first known enzymatic deamination which proceeds through an E1 type pathway.*

INTRODUCTION

Porphobilinogen deaminase (deaminase, also referred to as HMBS, hydroxymethylbilane synthase, EC 4.3.1.8) is the enzyme responsible for condensing four units of PBG, with the loss of four moles of ammonia, to give hydroxymethylbilane (Scheme 1), the linear substrate for uroporphyrinogen III synthase, the cyclizing enzyme of tetrapyrrole biosynthesis.[1] The existence of deaminase has been recognized for well over 30 years,[2] and a number of groups have advanced our understanding of this enzyme. For example, deaminase is an unique enzyme in that it requires a dimer of PBG to serve as cofactor in the polymerization

† A preliminary account of this work was presented at the 199th ACS Meeting, ORGN #428, Boston, MA April 22-27, 1990.
‡ Dedicated to Prof. Gabor Fodor on the occasion of his seventy-fifth birthday.



Scheme 1. Biosynthetic formation of uroporphyrinogen III from PBG

process.[3] It has also been established[4] that the condensation reaction occurs in a head-to-tail fashion, with a monopyrrole unit reacting in four separate and kinetically resolved steps, with the concomitant release of four equivalents of ammonia, to reach an unstable ES_4 complex, *via* the intermediacy of ES_1, ES_2, and ES_3 (Scheme 1).[5] Although hypotheses have been set forth for the mechanism of action of deaminase, there remain several aspects which are not well understood, including the requirement of *two* pyrrole units for the cofactor and the mode of hydrolysis of product off the enzyme.

Irrespective of the mechanism involved in loss of ammonia, clearly the leaving group must be protonated prior to, or concomitant with, its expulsion. While protonation will facilitate loss of ammonia, simply protonating the leaving group is generally not sufficient in *chemical* reactions to promote loss of this poor nucleofuge.[6] Even in biochemical systems, deamination requires the presence of a prosthetic group or cofactor, and still the mechanism invariably proceeds through an E2 or E1cb pathway.[7]

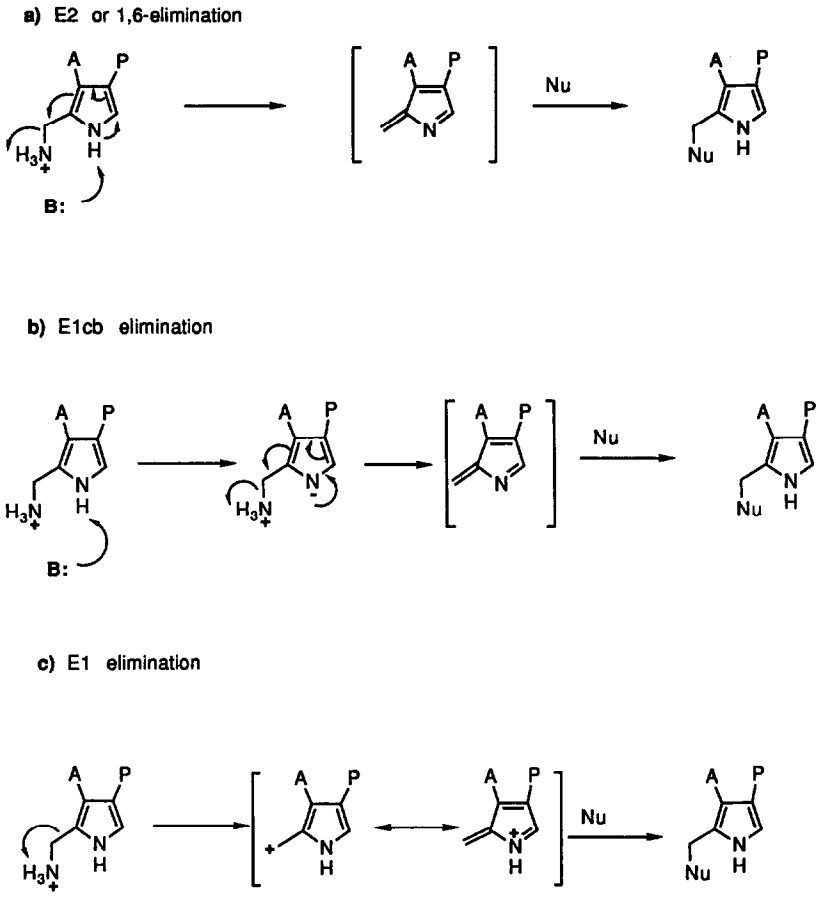
In the case of PBG, there is an inherent structural feature which undoubtedly aids in the elimination process. Thus the leaving amino group is attached to a methylene group α-to the aromatic pyrrole nucleus. This structural feature makes a concerted E2 or 1,6-elimination (Scheme 2a) an attractive mechanistic consideration.[8] The advantages of invoking an E2 pathway are twofold. In the first case, a concerted process would help overcome the poor nucleofugacity of ammonia. Second, as seen in Scheme 2a, the intermediate azafulvene is expected to be relatively stable due to the extended π-conjugation, yet reactive toward nucleophilic attack at the exocyclic methide carbon.

As discussed below, we believe that additional mechanisms must also be considered (Scheme 2). In Scheme 2b, a classical E1cb pathway is depicted wherein prior loss of the pyrrolic proton occurs in the rate limiting step, to form the pyrryl anion. In Scheme 2c, a classical E1 pathway is shown. In this case, loss of ammonia occurs first to give a benzylic-type cation, which presumably is conjugated (at the active-site of the enzyme) and can therefore be drawn in its resonance form of the azafulvenium ion.

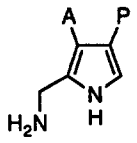
In order to investigate all of these possibilities we designed and synthesized the analogs shown in Figure 1. The N-methyl compound (**12**) would unequivocally define the importance of the pyrrole proton in either an E2 or E1cb mechanism. It was further envisioned that substitution at the benzylic C-11 carbon with CH_3 (**29**) and CF_3 (**36**) would help distinguish between a simple concerted substitution (S_N2), where the presence of an α-substituted amine should significantly retard any S_N2 process, or the unimolecular pathways (E1 or S_N1) where the CH_3 group of compound **29** would be expected to facilitate the unimolecular reaction, while the CF_3 (**36**) group should destabilize any incipient carbocation.

It could be argued that the α-substituted primary amines found in analogs **29** and **36** would predispose any reaction towards the unimolecular pathway. In order to assess the likelihood for the PBG nucleus, containing an unsubstituted primary amine, to undergo a unimolecular reaction, we also examined the chemical solvolysis of the chiral PBG derivative **39** (Figure 2).

Each of the analogs in Figure 1 reacted, to varying extents, with deaminase. We first discuss these results in terms of the mechanistic considerations of Scheme 2, and suggest a possible mechanism for the elimination-addition polymerization of PBG by deaminase.

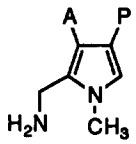


Scheme 2. Possible mechanisms for elimination of ammonia from PBG



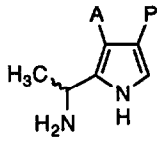
(1)

PBG



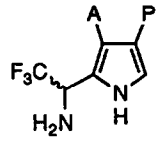
(12)

N-Methyl-PBG



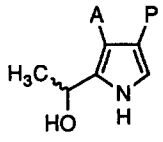
(29)

C-11-Methyl-PBG



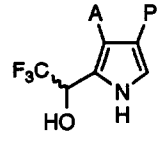
(36)

C-11-Trifluoromethyl-PBG



(23)

C-11-Methyl Hydroxymethyl PBG

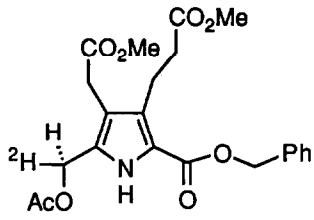


(33)

C-11-Trifluoromethyl Hydroxymethyl PBG

A=CH_2COOH
P=CH_2CH_2COOH

Figure 1. Structures of PBG and the analogs studied in this work



(39)

Figure 2

RESULTS AND DISCUSSION

Synthesis

The syntheses of the PBG derivatives **12, 29** and **36** and their hydroxymethyl analogs **23** and **33** are shown in Schemes 3 to 6.

Enzymology

Table 1 shows the collective results obtained for the five derivatives under typical incubation conditions. It is seen (Figure 3) at pH 8.1, 37°C, and 15 min incubations, compound **12** reacts once to yield ES_1, compounds **23** and **29** (Figure 4) yield observable amounts of ES_1, ES_2, and ES_3,[9] while compounds **33** and **36** show no covalent complexes. Identical results were obtained for **12, 29** and **36** when the reaction was carried out for 15 min at pH 5.2. We also examined the reactions of analogs **33** and **36** for prolonged periods of interaction with enzyme. It was found that at either pH, the CF_3-derivatives will react with enzyme at least once, to form an isolable ES_1 complex. At the normal pH 8.1, compound **36** yielded observable amounts of ES_1 after ~36 hr. At the lower pH, reaction took place in ~ 20 hr, as shown in Figure 5.

Table 1: Formation of Enzyme-Substrate Complexes

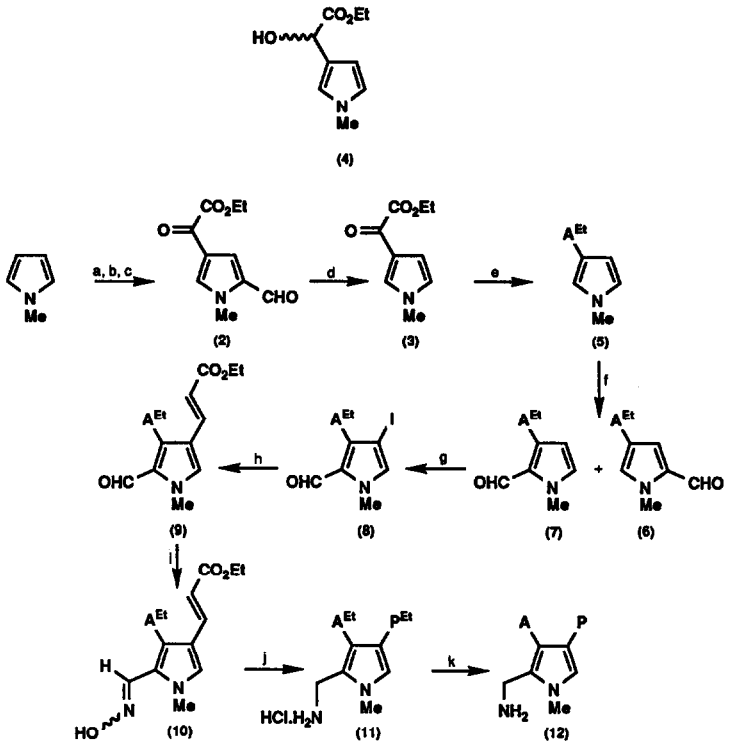
compound	ES_1	ES_2	ES_3
12[a]	+	-	-
23[b]	+	+	+
33[c]	-	-	-
29[b]	+	+	+
36[c,d]	-	-	-
36[e]	+	N.D.[f]	N.D.

a. ES_1 complex inactive.
b. ES_4 complex is not observed with PBG or **23** and **29** for the enzyme, presumably due to rapid decomposition to product.
c. The chemical polymerization of these derivatives is quite apparent and can be observed by conventional spectroscopicmethods.
d. Analyzed as above and by PAGE of reaction at pH 5.2-6.2 in citrate/phosphate buffer.
e. Reaction at pH 5.2 after ~ 20 hrs.
f. Not determined.

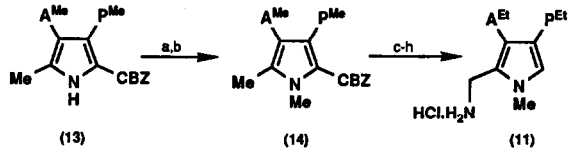
Solvolysis

Figure 6 shows the ^{1}H-NMR of the chiral centers from the solvolysis of compound **39** with (R)-(-)-2-butanol. As seen from the relative peak intensities, 100% racemization of substrate occurred.

In considering the purported mechanism of deaminase (Scheme 2a), we first examined the ability of N-methyl PBG (**12**) to serve as substrate. If the pyrrolic proton is necessary for substrate activation (E2-like or E1cb pathways), then replacement with an alkyl group should inhibit the reaction. Interestingly, deaminase



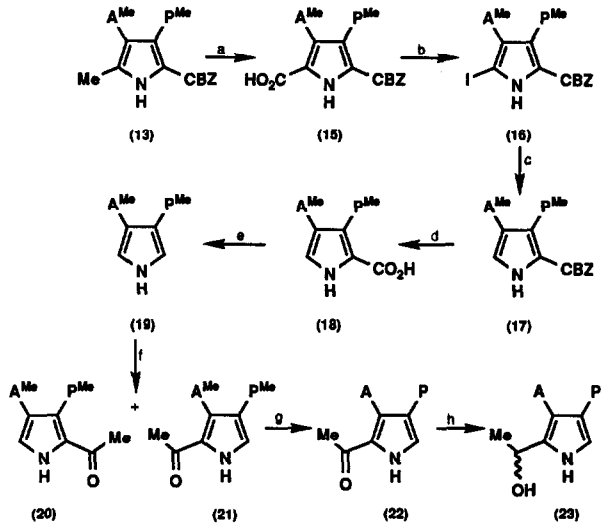
(a) $(COCl)_2$, DMF; (b) $AlCl_3$, $MeNO_2$; (c) EtO_2CCOCl; (d) mesitylene, 10% Pd-C, reflux; (e) Raney Nickel, EtOH-H_2O-toluene; (f) $(COCl)_2$, DMF; (g) I_2/HIO_3, H_2SO_4, in AcOH/CCl_4; (h) ethyl acrylate, $Pd(OAc)_2$, CH_3CN, reflux; (i) $NH_2OH.HCl$, NaOAc; (j) Pd/H_2; (k) NaOH 2N.



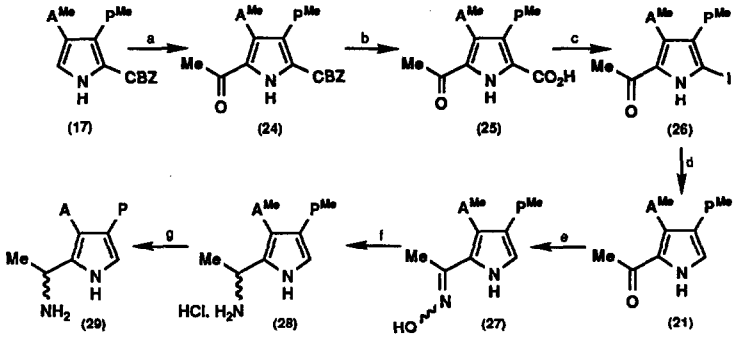
(a) NaH, DMF; (b) MeI; (c) 2 equiv SO_2Cl_2; (d) 10% Pd-C/H_2, Et_3N; (e) $NaHCO_3$, CH_2Cl_2/H_2O, reflux, I_2/KI; (f) 10% Pd-C/H_2, NaOAc; (g) $NH_2OH.HCl$, NaOAc; (h) 10% Pd-C, HCl.

A=CH_2CO_2H, P=$CH_2CH_2CO_2H$, CBZ=CO_2CH_2Ph

Scheme 3. Synthesis of N-Me PBG (**12**).

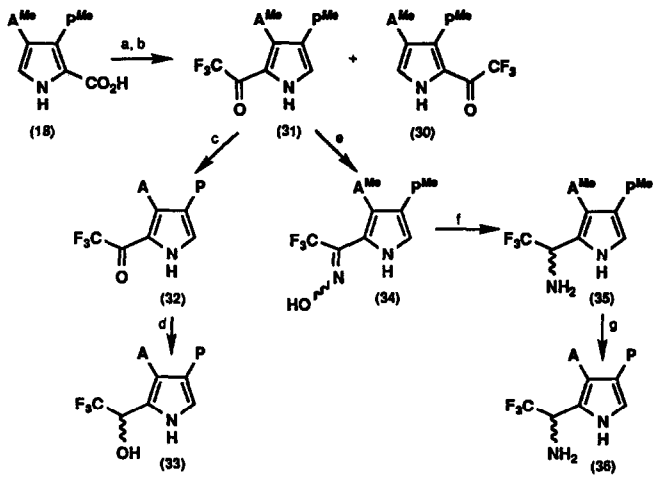


(a) 3 equiv SO_2Cl_2; (b) $NaHCO_3$, CH_2Cl_2/H_2O, reflux, I_2/KI; (c) PtO_2/H_2, Et_3N; (d) 10% Pd-C/H_2; (e) CF_3CO_2H; (f) DMA, $POCl_3$; (g) NaOH 2N; (h) $NaBH_4$.



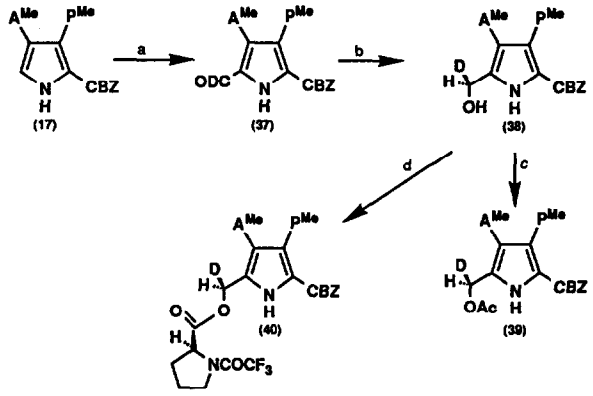
(a) $SnCl_4$, AcCl, 0°C; (b) 10% Pd-C/H_2, EtN_3; (c) $NaHCO_3$, CH_2Cl_2/H_2O, reflux, I_2/KI; (d) $NH_2OH.HCl$, NaOAc, MeOH, reflux; (f) 10% Pd-C/H_2; (g) NaOH 2N.

Scheme 4. Syntheses of the C-11-methyl PBG (**29**) and its hydroxymethyl analog (**23**).



(a) CF_3CO_2H; (b) $(CF_3CO)_2O$; (c) NaOH 2N; (d) $NaBH_4$; (e) $NH_2OH.HCl$, NaOAc, EtOH, reflux; (f) Pd/50 PSI H_2, HCl; (g) NaOH 2N.

Scheme 5. Syntheses of the C-11-trifluoromethyl PBG (**36**) and its hydroxymethyl analog (**33**).



(a) DMF-d_7, $POCl_3$; (b) R-Alpine Borane; (c) Ac_2O, DMAP; (d) RCOCl, Et_3N.

Scheme 6. Synthesis of the chiral compound (**39**).

only incorporates a single unit of **12** to form a stable ES_1 complex (Figure 3), at a rate slightly more slow then that for PBG. However, this ES_1 complex of **12** is no longer reactive toward either N-methyl-PBG, or PBG itself. It is important to point out that deaminase utilizes PBG as *both* nucleophile and electrophile. So, while the pyrrole proton is not necessary for the elimination of ammonia, it appears to be involved in activating the enzyme-substrate complex to act as a nucleophile (see below). In either event, the ability of deaminase to accept the N-methyl derivative indicates that neither an E1cb (Scheme 2c) nor a classical E2-like (Scheme 2a) pathway is in operation.

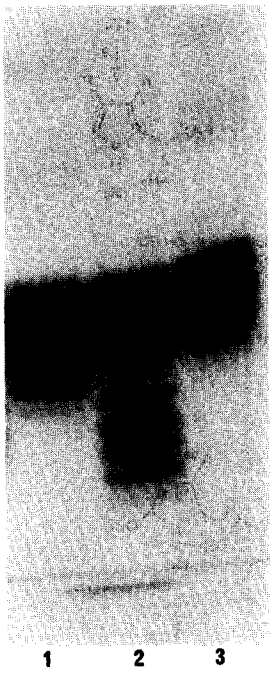


Figure 3

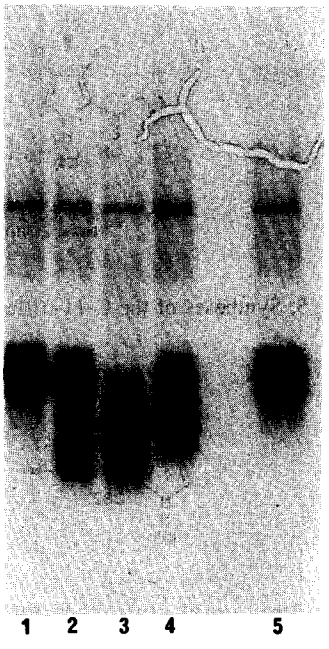
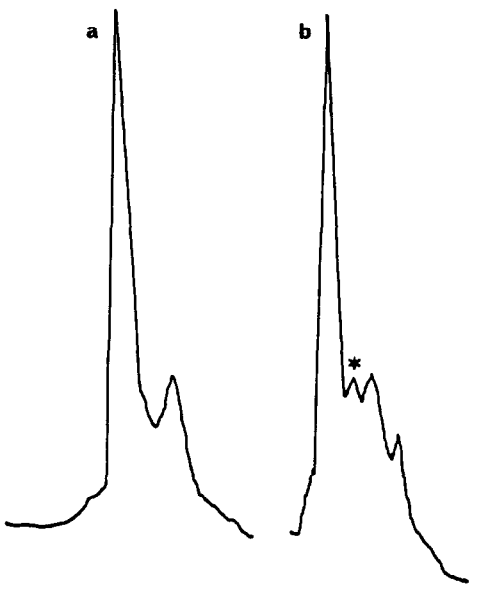


Figure 4

Figure 3. Native PAGE analysis of reaction of N-Me-PBG (**12**) with PBG-deaminase.
lane 1: N-Me-PBG after 15 min at 37°C; the enzyme is a mixture of E and ES_1.
lane 2: PBG, mixture of E, ES_1 and ES_2.
lane 3: Free enzyme; note that four charge isomers are always present. Each of these bands is active and forms a substrate complex (lane 2). Thus the bands of ES_1 largely overlap with those of the free enzyme.

Figure 4. Native PAGE analysis of the C-11-derivatives.
lane 1: C-11-CF_3 alcohol shows no covalent complex.
lane 2: PBG
lane 3: C-11-CH_3 alcohol (**33**).
lane 4: C-11-CH_3 amine (**29**).
lane 5: free enzyme.
Note the similarity of lane 1 with lane 5, as well as lane 2 with lane 3 and 4.



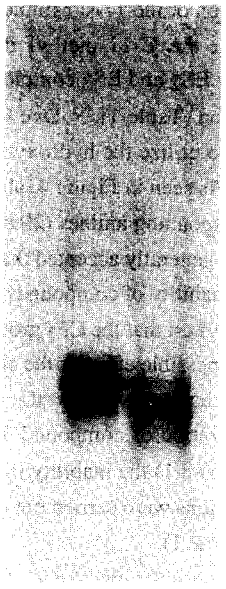


Figure 5a Figure 5b

Figure 5. Complex formation of C-11-CF_3 amine (**36**) after 20 hrs incubation.
a: FPLC analysis of incubation at 22 hrs. A) free enzyme. B) enzyme + **36**. The ES_1 complex is marked with *. The enzyme and ES_1 were isolated by peak shaving of FPLC fractions and analyzed by PAGE.
b: Native PAGE confirming the presence of ES_1.

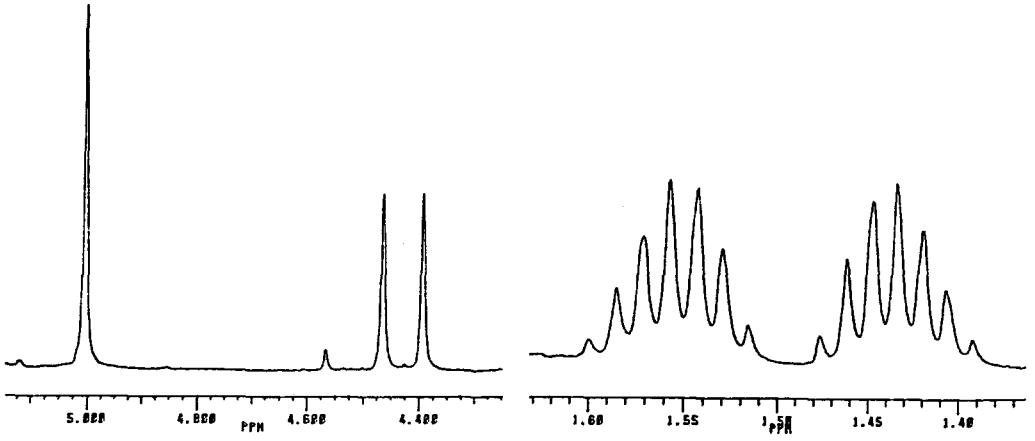


Figure 6. Portion of ^{1}H-NMR spectra of reaction of the chiral derivative (**39**) with (R)-(-)-2-butanol. The peak at ~ 5 ppm is the C-11 H of the starting material, while the peaks at ~4.40 and 4.45 ppm are due to the C-11 H of the ether products. No attempt was made to assign absolute configurations.

The reaction of the 11-substituted analogs (±)**29** and (±)**36** with enzyme, under normal incubation times, revealed that the C-11-methyl derivative (**29**) behaved in a similar fashion as does PBG, yielding preferentially ES_2 and ES_3 complexes. The CF_3-analog (**36**), however, gave no covalent complex after 15 min incubation (Table 1).[10] Due to the accessibility of the alcohols **23** and **33**, and the known[11] ability of the enzyme to utilize the hydroxymethyl-PBG analog, we also examined the action of these alcohols with deaminase. As seen in Figure 4, alcohols **23** and **33** exhibited the same pattern of ES-complex formation as did the corresponding amines (**29** and **36**, respectively).

Since it is generally accepted that the van der Waals radius of trifluoromethyl is nearly identical to that of methyl, the inability of compounds **33** and **36** to react with enzyme cannot be explained on steric grounds,[12] and would suggest that the CF_3 group is influencing the course of the reaction through electronic effects.

One such possible effect is the change in basicity of the amine leaving group. As noted above, regardless of which mechanism is involved, clearly the amine must be protonated prior to its expulsion from substrate. ^{19}F-NMR titration of compound **36** gave a pKa ~ 5.0 for the amino group, so that under typical reaction conditions (pH 8.1) the inability to protonate the leaving group could explain our findings. However, even when incubations were carried out under typical reaction times at pH 5.2, no reaction with compound **36** was observed (Table 1).

Another possible electronic effect could be an increase in the acidity of the C-11 proton. Koch and coworkers have noted[13] that the presence of an α-CF_3 group increases the rate of E2 reactions due to the increased acidity of the proton. In the case of compound **36**, some active-site basic residue could deprotonate the analog to form an unreactive carbanion. However, in deuterated-MeOH/NaOMe at 50 °C, no exchange of the C-11 proton was seen, as evidenced by ^{19}F-NMR (results not shown).

The apparent lack of reaction of the CF_3-derivatives at first seemed quite surprising, and suggested that perhaps the trifluoromethyl group had slowed down the overall rate of the enzymatic reaction such that no complexes were formed in the 15 min incubation. We therefore examined the reaction of analog **36** with deaminase for longer periods of time and found that after ~ 36 hr pH 8.1, or ~ 20 hr pH 5.2, formation of ES_1 complex could be observed (Figure 5). On the other hand, under comparable conditions PBG will react with enzyme in as little as ~ 10 sec, while compound **29** reacts in about 30 sec. While it is difficult to compare these results directly, they suggest that an upper limit on the rate difference for H/CH_3 *versus* CF_3 is ~ 10^3.

The ability of N-methyl PBG (**12**) to react with enzyme rules out an E2 or E1cb pathway. This essentially reduces the problem to differentiating between a S_N2 reaction or an E1 (or S_N1) reaction. The only additional piece of mechanistic information comes from stereochemical studies by Battersby, *et al.*[8c] Using chiral PBG, it was shown that the overall reaction with deaminase provides hydroxymethylbilane with net *retention* of configuration at the CH_2OH center. However, the stereochemical results can be rationalized by either a bimolecular or unimolecular pathway. In the bimolecular case, two consecutive S_N2 processes will produce retention of configuration. For the unimolecular reaction, the same result can be obtained by either of two possible scenarios. Firstly, if the initially formed benzylic cation can conjugate with the ring, the azafulvenium ion is a resonance form, indicating the C-11 carbon has double-bond character. In this case, attack by the nucleophile occurs on only one face, a reasonable proposition for an enzymatic reaction.

In the other possible pathway, if the initially formed cation is formed as an intimate ion pair, then one side of the carbocation is "blocked", and the resulting nucleophilic addition would result in inversion of configuration. In fact, this type of chemistry has been observed in systems very similar to analog **36**, that is CF_3-destablized benzylic carbocations which undergo S_N1 reactions.[14d,15e] Using a chiral 1-aryl-2,2,2-trifluoroethyl sulfonate, Tidwell has shown that for certain solvent conditions, acetolysis results in inversion of configuration for the S_N1 reaction.[14d] Clearly, either of the unimolecular possibilities requires that the hydrolysis step proceeds in an analogous fashion.

Unfortunately, the results with the CF_3 derivative **36** are inconclusive. While there has not been a large amount of work done on the influence by a CF_3 group on bimolecular substitution reactions,[16] it is known for *primary alkyl* halides, rate ratios for α-CH_3:-CF_3 are in the order of 10^4:1,[16e] a number very similar to our estimated 10^3:1. On the other hand, if the reaction is truly S_N2 in nature, one would expect a significant rate difference between the amino-methyl group of PBG and the amino-ethyl group of the C-11 derivative (**29**). The observed difference of $\lesssim$3-fold is not consistent with a S_N2 pathway.

In contrast to the relatively few reports on the effect of a trifluoromethyl group on concerted reactions, there is a wealth of information concerning its effect on *carbocations*.[17] Tidwell and coworkers have extensively studied destabilized carbocations,[14] and in the S_N1 solvolysis of 2,2,2-trifluoroethyl aryl systems, the major influence conferred by the trifluoromethyl group is the slowing of the formation of the cation. In systems where the corresponding CH_3 derivatives are also available, rate ratios for CH_3:CF_3 varied from as little as one order of magnitude, to upwards of 10^9.[14b,d,e;15a,d] Interestingly, in a kinetic study by Richard,[15d,g] it was shown that the rate of nucleophilic or solvent attack on 1-(4-methoxyphenyl)ethyl cation was nearly *identical* for the α-CF_3 derivative and the corresponding CH_3 analog.

A closely related pyrrole system has been the subject of investigation by Tidwell.[18] In 1-(1-methyl-2-pyrrolyl)-2,2,2-trifluoroethyl p-nitrobenzoate, solvolysis was found to react through a carbocationic intermediate, as judged by the Y solvent polarity parameter.[19] The corresponding H analog has also been studied,[20] and the observed rate ratio of H:CF_3 was on the order of ~ 40:1. While the relative rate differences observed between **1** and **36** appear, by our crude methods, to be about 10^3, the electron donation by the N-methyl group is known to have an effect on the electronic nature of the pyrrole ring,[21] as will the two β-substituents (PBG analogs), and may easily account for the 100-fold difference observed between the two systems.

While it has been suggested[8c] that pyrroles favor reaction through the azafulvene, a more careful examination of the literature indicates otherwise.[22] Norris and coworkers have extensively studied pyrrole chemistry and compared it with the corresponding furan and thiophene analogs. They found for simple nucleophilic substitution reactions that unlike the furan or thiophene derivatives, which generally favor a concerted process, the pyrrole nucleus reacts through different pathways.[23] Even in the case of nitro-substituted derivatives, systems which favor $S_{RN}1$ mechanisms in the reaction with simple nucleophiles, the pyrrole system again is anomalous, and reaction products arising from S_N1 processes are also observed.[23a]

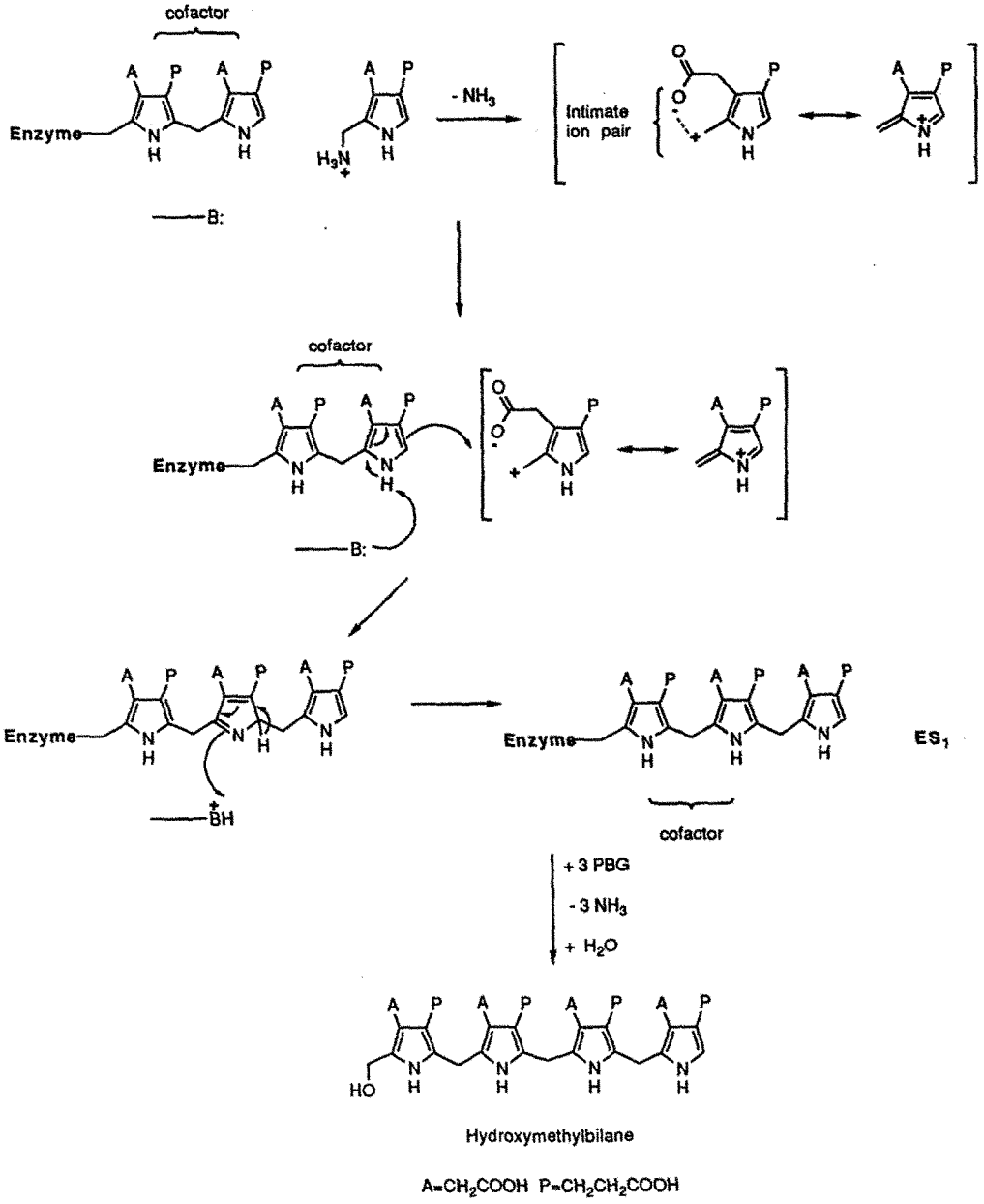
Due to the presence of the aminoethyl group found in analogs **29** and **36**, it is possible that this function shifts the reaction towards the unimolecular pathway. We therefore felt it imperative to investigate whether a porphobilinogen derivative, that is, an *aminomethyl* group, could follow a unimolecular pathway. The

solvolysis of chiral analog **39** in (R)-(-)-2-butanol produced diastereomeric ethers which can be analyzed by NMR. As seen in Figure 6, after ~ 50% reaction, the mixture showed a 1:1 composition of the two ethers. This clearly indicates that even in the case of the aminomethyl system, solvolysis can occur through a S_N1 pathway. It is important to point out that analog **39** is substituted at the α-position with an ester function which *can conjugate with the pyrrole ring*. In other words, even in the presence of the electron withdrawing group, the solvolysis reaction still proceeds through a carbocationic intermediate, and suggests that with a free alpha position, the pyrrole is even more likely to react in an ionic manner. It has also been reported by Noyce[20] that 1-(1-methyl-2-pyrrolyl) p-nitrobenzoate solvolyzes through a carbocationic intermediate.

There is one other point which is relevant to our discussion. It has been shown that the hydroxymethyl analog of PBG reacts ~3x *slower* than PBG itself with deaminase.[11] While we did not quantify the rate differences between **33** and **36**, the alcohol derivative did not behave differently from the amine. That is, going to a better leaving group did not show any increase in reaction rate. If the reaction is indeed, as we propose, an E1 process, the slower rate for the alcohol can easily be rationalized, since it is known[24] that "OH" *per se* cannot participate as a leaving group for an E1 reaction, whereas the protonated "H_2O^+" serves as a good leaving group. Thus the slower nature of the alcohol derivative could be due to the poorer proton affinity of the alcohol relative to the amine.

Based on physical chemical precedence,[18,20,23] the pronounced rate retardation conferred by the CF_3 substituent, and the racemization of **39**, suggest that the *initially* formed cation is benzylic in nature. However, it goes without mention that when one involves an enzyme as catalyst, it is difficult to claim that the reaction pathway seen in the test tube is the same as that seen in the presence of enzyme. Undoubtedly, our results indicate that a more through investigation is necessary. In addition to looking at a detailed kinetic evaluation, using various labeled analogs to measure isotope effects, the question of any stereochemical preference could provide valuable insight. Using NMR techniques we were unable to observe any enantiomeric enrichment with the racemic C-11 compounds in the presence of enzyme. One would assume that the enzymatic reaction would show a stereopreference in the reaction with (±)**29** and (±)**33**. One possibility is that upon formation of the carbocation, there is a "repositioning" of the substituents such that only one "enantiomer" is formed. We hope to be able to evaluate the stereochemistry of the bilanes substituted with CH_3 and CF_3 in ring A, and will report on this at a later date.

It is interesting to note that the reaction of a dipyrromethane with deaminase, in which the second pyrrole is N-methylated, results in the formation of a "dead-end" ES_2 derivative. This finding is further support for the importance in removing the pyrrole proton for activation of the *complex* as nucleophile. Using the results obtained with analogs **12**, **29**, and **36**, we can formulate a putative mechanism for the formation of ES complexes as shown in Scheme 7. Binding of substrate likely initiates, through conformational effects, the positioning of the active-site base[26] which is responsible for activation of the cofactor (or ES-complex) by removing the pyrrole proton. The dissociation of ammonia results in the formation of the benzylic cation, which may either exist in the resonance form of the azafulvenium ion, or equally possible, as an intimate ion pair with the acetate side-chain,[27] thereby "protecting" one side of the benzylic cation, and attack by the nucleophile occurs to the "free" side of the substrate. This sequence is then repeated for the other three PBG



Scheme 7. Mechanistic hypothesis for the polymerisation of PBG to give hydroxymethylbilane involving the carbocation as the electrophile.

molecules to form the unstable ES_4 complex, which then *must be hydrolyzed off the enzyme in an analogous fashion*, to give hydroxymethylbilane.

While we have focused on the mode of action of complex formation, it is clear that there remain additional mysteries associated with deaminase. We are presently using ^{19}F- and ^{13}C-NMR spectroscopy to understand the formation of bilane, as well as the question of stereochemistry.

CONCLUSIONS

The present study has been aimed at understanding the biochemical elimination of ammonia from PBG upon reaction with PBG deaminase. The ability of N-Me-PBG (**12**) to react with enzyme rules out a classical E2 or E1cb pathway. Our current understanding of pyrrole chemistry, coupled with the pronounced effect by CF_3 substitution on the rate of reaction appears to be more consistent with an intermediate carbocation. The fact that the *chemical* solvolysis of chiral PBG analog **39** can proceed through a S_N1 pathway is further support for the feasibility of an E1 (S_N1) elimination of ammonia. Our results clearly suggest that elimination of ammonia initially forms a cation with substantial positive character at the C-11 carbon. To the best of our knowledge, this finding represents the first, albeit indirect, indication of an enzymatic deamination which proceeds through a carbocationic intermediate.

EXPERIMENTAL SECTION

Chemistry

Instrumentation and general procedures. All solvents and reagents were purified and dried when necessary by standard literature methods. Column chromatographies and TLC were carried out with silica gel. Melting points are uncorrected. ^1H- and ^{13}C- NMR (200 or 500 MHz) spectra were recorded in $CDCl_3$, unless stated otherwise.

Ethyl 2-formyl-1-methylpyrrole-4-glyoxalate (2).[28a] A solution of oxalyl chloride (133 mmol, 11.6 mL) in 50 mL of 1,2-dichloroethane (DCE) was added dropwise to a solution of DMF (133 mmol, 10.3 mL) in 100 mL of DCE, cooled to 0 °C under N_2, over a period of 20 min, and allowed to mix for an additional 20 min. A solution of 1-methylpyrrole (125 mmol, 11.1 mL) in 100 mL of DCE was then added slowly. After stirring for 20 min , the reaction was cooled to 0 °C, and $AlCl_3$ (510 mmol, 68 g) was added, followed 10 min later by nitromethane (413 mmol, 22.4 mL) and ethyl oxalyl chloride (187.5 mmol, 21 mL). The reaction was stirred 4 h at room temperature, then poured into 1.5 L of ice/water, and stirred for an additional 3 h. The 2 phases were separated, and the aqueous phase further extracted with CH_2Cl_2. The residue was chromatographed on silica gel (AcOEt-hexanes ,2:3). The solid was recrystallized from CH_2Cl_2-hexanes to give 16.7g (64%) of **2** ; mp 56 °C; ^1H-NMR δ 1.40 (t, 3 H, J=7, $CO_2CH_2CH_3$), 3.99 (s, 3 H, N*Me*), 4.37 (q, 2 H, J=7, $CO_2CH_2CH_3$), 7.55 (s, 1 H, Ar*H*-3), 7.87 (s, 1 H, Ar*H*-5), 9.63 (s, 1 H, *CHO*); ^{13}C-NMR δ 14.20, 35.52, 62.78, 122.57, 123.43, 133.75, 133.85, 162.06, 178.29, 180.83; MS m/e 209 (M^+, 5), 136 (100); Anal. Calcd for $C_{10}H_{11}NO_4$, C 57.42, H 5.26; found, C 57.29, H 5.28.

Ethyl 1-methylpyrrole-3-glyoxalate (3).[28a] A solution of **2** (81.35 mmol, 17 g) in mesitylene (165 mL) was heated at reflux with 10% Pd-C (2.35 g) for 10 h, then cooled and filtered through Celite. The oily residue was purified by chromatography (AcOEt-hexanes, 2:3) to afford 13.1 g (89%) of **3**; ^{1}-NMR δ 1.31 (t, 3 H, J=7, $CO_2CH_2CH_3$), 3.62 (s, 3 H, N*Me*), 4.27 (q, 2 H, J=7, $CO_2CH_2CH_3$), 6.54, 6.66, 7.55 (3 s, 3 H, Ar*H*); ^{13}C-NMR δ 13.87, 36.56, 61.74, 110.55, 121.18, 124.02, 131.18, 162.98, 178.12; MS m/e 181 (M^+, 47), 108 (100); Anal. Calcd for $C_9H_{11}NO_3$, C 59.67, H 6.08; found, C 59.56, H 6.14.

3-[(Ethoxycarbonyl)methyl]-1-methylpyrrole (5).[28] A suspension of commercial Raney Nickel (50 mL) was added to a solution of **3** (63 mmol, 11.4 g) in toluene-EtOH-H_2O (2:1:1, 800 mL). The reaction was stirred at room temperature overnight under N_2. TLC (AcOEt-hexanes, 1:2) indicated that the reaction was incomplete (presence of the alcohol **4**). An additional 50 mL of Raney Nickel were added and the reaction was refluxed for 4 h, after which TLC revealed no remaining starting material and no alcohol. The reaction mixture was cooled, brine was added, and the products were extracted with AcOEt. **5** was obtained as an oil (10 g, 95%) after chromatography (AcOEt-hexanes, 1:4 to 1:2); ^{1}H-NMR δ 1.24 (t, 3 H, J=7, $CO_2CH_2CH_3$), 3.44 (s, 2 H, CH_2CO_2Et), 3.56 (s, 3 H, N*Me*), 4.12 (q, 2 H, J=7, $CO_2CH_2CH_3$), 6.04 (m, 1 H, Ar*H*-5), 6.50 (m, 1 H, Ar*H*-4), 6.51 (s, 1 H, Ar*H*-2); ^{13}C-NMR δ 13.88, 32.76, 35.88, 60.17, 108.60, 115.26, 120.14, 121.42, 172.25; MS m/e 167 (M^+, 21), 94 (100); Anal. Calcd for $C_9H_{13}NO_2$, C 64.67, H 7.78; found, C 65.00, H 7.75. **4** was isolated and characterized from a previous experiment; ^{1}H-NMR δ 1.24 (t, 3 H, J=7, $CO_2CH_2CH_3$), 3.56 (s, 3 H, N*Me*), 4.15, 4.23 (2 m, 2 H, $CO_2CH_2CH_3$), 5.07 (s, 1 H, C*H*OH), 6.08 (m, 1 H, Ar*H*-5), 6.49 (m, 1 H, Ar*H*-4), 6.62 (s, 1 H, Ar*H*-2); MS m/e 183 (M^+, 33), 110 (100).

3-[(Ethoxycarbonyl)methyl]-2-formyl-1-methylpyrrole (7).[28] A solution of oxalyl chloride (94.35 mmol, 8.3 mL) in CH_2Cl_2 (100 mL) was added dropwise to a solution of DMF (94.35 mmol, 7.5 mL) in CH_2Cl_2 (100 mL) at 0 °C for 30 min. The reaction was stirred 10 min more at 0 °C, then a solution of the pyrrole **5** (59.9 mmol, 10 g) in CH_2Cl_2 (100 mL) was added dropwise over 30 min. The reaction was stirred for 1 h at 0 °C, then 500 mL of $NaHCO_3$ solution was added. The 2 phase system was stirred vigorously for 1 h at room temperature, acidified, and the products extracted with CH_2Cl_2. The oily residue was chromatographed (AcOEt-hexanes as eluent, 1:4, 500 mL; 1:3, 3 L). The first fractions gave the 3-[(ethoxycarbonyl)methyl]-5-formyl-1-methylpyrrole **6** (6 g, 51%); ^{1}H-NMR δ 1.27 (t, 3 H, J=7, $CO_2CH_2CH_3$), 3.47 (s, 2 H, CH_2CO_2Et), 3.91 (s, 3 H, N*Me*), 4.15 (q, 2 H, J=7, $CO_2CH_2CH_3$), 6.84, 6.88 (2 s, 2 H, Ar*H*), 9.49 (s, 1 H, C*H*O); ^{13}C-NMR δ 13.76, 31.91, 35.88, 60.43, 116.32, 123.54, 130.96, 131.23, 171.22, 179.04. The second set of fractions contained the 2-formylpyrrole **7** (1.63 g, 14%); ^{1}H-NMR δ 1.24 (t, 3 H, J=7, $CO_2CH_2CH_3$), 3.75 (s, 2 H, CH_2CO_2Et), 3.90 (s, 3 H, N*Me*), 4.13 (q, 2 H, J=7, $CO_2CH_2CH_3$), 6.12 , 6.80 (2 s, 2 H, Ar*H*), 9.73 (s, 1 H, C*H*O); ^{13}C-NMR δ 13.82, 31.82, 36.39, 60.70, 110.41, 127.92, 129.28, 130.53, 170.67, 178.00; MS m/e 195 (M^+, 25), 122 (100); Anal. Calcd for $C_{10}H_{13}NO_3$, C 61.54, H 6.67; found, C 61.62, H 6.73.

3-[(Ethoxycarbonyl)methyl]-2-formyl-4-iodo-1-methylpyrrole (8).[29] A solution of the pyrrole **7** (3.37 mmol, 657 mg) in 0.1M solution of iodine in CCl_4 (2.71 mmol, 27 μL) containing 1M iodic acid in water (0.674 mmol, 680 μL) and 3.5% sulfuric acid in glacial acetic acid (1.7 mL) was stirred at room temperature under N_2. The reaction was shown complete by TLC (AcOEt-hexanes, 1:1) after 2.5 days. The solution was washed with an aqueous solution of sodium thiosulfate, 5% $NaHCO_3$, then brine. After chromatography (AcOEt-hexanes, 1:3), **8** was obtained (973 mg, 90%); mp 58 °C; (CH_2Cl_2 -hexanes); ^{1}H-NMR δ 1.23 (t, 3 H, J=7, $CO_2CH_2CH_3$), 3.69 (s, 2 H, CH_2CO_2Et), 3.88 (s, 3 H, N*Me*), 4.12 (q, 2 H, J=7, $CO_2CH_2CH_3$), 6.89 (s, 1 H, Ar*H*), 9.65 (s, 1 H, C*H*O); ^{13}C-NMR δ 14.07, 32.68, 36.85, 61.16, 67.46, 129.30, 131.94, 134.60, 169.90, 178.14; MS m/e 321 (M^+, 43), 293 (81), 248 (100); Anal. Calcd for $C_{10}H_{12}INO_3$, C 37.38, H 3.74; found, C 37.46, H 3.75.

3-[(Ethoxycarbonyl)methyl]-2-formyl-4-carbethoxyvinyl-1-methylpyrrole (9). A solution of the **8** (3.03 mmol, 973 mg), Et_3N (4.55 mmol, 635 μL), ethyl acrylate (6.06 mmol, 660 μL), and palladium diacetate (42 mg) in CH_3CN (10 mL) was stirred at reflux under N_2 for 18 h. The reaction was cooled, diluted with CH_2Cl_2 and washed with 0.5N HCl and brine. The residue was chromatographed (AcOEt-hexanes, 1:2) to give **9** (751 mg, 85%); mp 94-95 °C (CH_2Cl_2-hexanes); ^{1}H-NMR δ 1.27 (t, 3 H, J=7, $CH_2CO_2CH_2CH_3$), 1.32 (t, 3 H, J=7, CH=CHCO$_2$CH$_2$C*H*$_3$), 3.88 (s ,2 H, CH_2CO_2Et), 3.95 (s, 3 H, N*Me*), 4.17 (q, 2 H, J=7, $CH_2CO_2CH_2CH_3$), 4.24 (q, 2 H, J=7, CH=CHCO$_2$C*H*$_2$CH$_3$), 6.16 (d, 1

H, J=16, C*H*=CHCO$_2$Et), 7.18 (s, 1 H, Ar*H*), 7.57 (d, 1 H, J=16, CH=C*H*CO$_2$Et), 9.80 (s, 1 H, C*H*O); ^{13}C-NMR δ 13.74, 13.97, 29.29, 36.84, 59.89, 60.97, 115.56, 119.13, 127.91, 129.29, 130.20, 134.69, 166.78, 169.85, 178.36; MS m/e 293 (M$^+$, 100), 265 (25); Anal. Calcd for $C_{15}H_{19}NO_5$, C 61.41, H 6.53; found, C 61.34, H 6.56.

Oxime of 4-carbethoxyvinyl-3-[(ethoxycarbonyl)methyl]-1-methylpyrrole (10). A solution of **9** (4.9 mmol, 1.44 g), hydroxylamine hydrochloride (7.36 mmol, 512 mg) and NaOAc (515 mg) in MeOH (40 mL) was stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2 and washed with water. The product **10** was recristallized from CH_2Cl_2-hexanes (1.2 g, 79%); mp 140-141°C; ^{1}H-NMR δ 1.23 (t, 3 H, J=7, CH$_2$CO$_2$CH$_2$C*H*$_3$), 1.28 (t, 3 H, J=7, CH=CHCO$_2$CH$_2$C*H*$_3$), 3.69 (s, 2 H, C*H*$_2$CO$_2$Et), 3.72 (s, 3 H, N*Me*), 4.12 (q, 2 H, J=7, CH$_2$CO$_2$C*H*$_2$CH$_3$), 4.20 (q, 2 H, J=7, CH=CHCO$_2$C*H*$_2$CH$_3$), 6.07 (d, 1 H, J=16, C*H*=CHCO$_2$Et), 6.98 (s, 1 H, Ar*H*), 7.56 (d, 1 H, J=16, CH=C*H*CO$_2$Et), 7.77 (s, 1 H, NO*H*), 8.13 (s, 1 H, C*H*NOH); ^{13}C-NMR δ 14.12, 14.37, 30.52, 37.16, 60.11, 61.06, 114.37, 119.15, 119.62, 124.42 126.79, 138.44, 141.78, 167.68, 170.86; MS m/e 308 (M$^+$, 83), 290 (23), 262 (52), 172 (100); HRMS calcd for $C_{15}H_{20}N_2O_5$, 308.1372; found, 308.1361.

2-Aminomethyl-4-[2-(ethoxycarbonyl)ethyl]-3-[(ethoxycarbonyl)methyl]pyrrole hydrochloride (11). A solution of the unsaturated oxime **10** (2 mmol, 612 mg) in 20 mL EtOH was hydrogenated at room temperature overnight in the presence of palladium black (125 mg). The reaction was filtered, 350 μL 6N HCl were added, and the solvent was evaporated at room temperature under vacuum. The product **11** was purified by recrystallization from CH_2Cl_2-hexanes (364 mg, 55%); mp 168-170 °C; ^{1}H-NMR δ 1.22, 1.27 (2 t, 2x3 H, J=7, 2 CO$_2$CH$_2$C*H*$_3$), 2.45 (t, 2 H, J=7, C*H*$_2$CH$_2$CO$_2$Et), 2.67 (t, 2 H, J=7, CH$_2$C*H*$_2$CO$_2$Et), 3.42 (s, 2 H, C*H*$_2$CO$_2$Et), 3.79 (s, 3 H, N*Me*), 4.08-4.15 (m, 4 H, 2 CO$_2$C*H*$_2$CH$_3$), 6.40 (s, 1 H, Ar*H*), 8.71 (br s, 3 H, N*H*$_3$Cl); ^{13}C-NMR δ 14.02, 14.21, 20.05, 30.69, 33.26, 34.86, 35.27, 60.38, 62.23, 115.65, 120.50, 121.88, 172.97, 174.73; MS m/e 296 (M$^+$- HCl, 27), 209 (100); Anal. Calcd for $C_{15}H_{25}ClN_2O_4$, C 54.14, H 7.52; found, C 53.98, H 7.56.

1-Methyl PBG (12).[30] The pyrrole **11** (0.063 mmol, 21 mg) was hydrolyzed in 2N NaOH (0.5 mL) at room temperature for 3 h. Then the solution was diluted with water, Amberlite IRC-50 was added, and the solution allowed to stand until the pH was neutral, then filtered and lyophilized; ^{1}H-NMR (D$_2$O) δ 2.37 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$H), 2.63 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$H), 3.39 (s, 2 H, C*H*$_2$CO$_2$H), 3.60 (s, 3 H, N*Me*), 4.19 (s, 2 H, C*H*$_2$NH$_2$), 6.59 (s, 1 H, Ar*H*); ^{13}C-NMR (D$_2$O) δ 18.26, 29.45, 30.01, 35.30, 115.49, 118.25, 118.73, 126.08, 178.01, 179.88.

Benzyl 1,2-dimethyl-4-[(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl] pyrrole-5-carboxylate (14).[31] A solution of the 1H-pyrrole **13**[32] (13.4 mmol, 5 g) in DMF (15 mL) was added dropwise at 0 °C under N$_2$ to a suspension of NaH (14.75 mmol, 590 mg) in 25 mL DMF. After the formation of hydrogen ceased, MeI (14.75 mmol, 0.92 mL) was added and the reaction was stirred 2 h at room temperature. Then 10% NaHCO$_3$ was added to quench the reaction, and the product was extracted with ether. The product **14** was purified by chromatography (AcOEt-hexanes,1:4) and obtained as an oil (4.9 g, 95%), but which slowly crystallized upon standing at 0°C for several days; mp 55-56 °C (CH_2Cl_2-hexanes); ^{1}H-NMR δ 2.14 (s, 3 H, *Me*), 2.42 (t, 2 H, J=8, C*H*$_2$CH$_2$CO$_2$Me), 2.94 (t, 2 H, J=8, CH$_2$C*H*$_2$CO$_2$Me), 3.41 (s, 2 H, C*H*$_2$CO$_2$Me), 3.56, 3.62 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.75 (s, 3 H, N*Me*), 5.24 (s, 2 H, C*H*$_2$Ph), 7.30-7.40 (m, 5 H, Ph*H*); ^{13}C-NMR δ 10.48, 21.23, 29.67, 33.01, 34.92, 51.21, 51.78, 65.52, 112.81, 118.07, 127.93, 128.37, 130.72, 134.66, 136.08, 161.11, 172.15, 173.48; MS m/e 387 (M$^+$, 45), 328 (28), 296 (35), 91 (100); Anal. Calcd for $C_{21}H_{25}NO_6$, C 65.12, H 6.46; found, C 65.09, H 6.51.

5-Benzyloxycarbonyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl] pyrrole-2-carboxylic acid (15).[4d] Sulfuryl chloride (16.9 mmol, 1.36 mL) was added to a solution of

13[32] (5.36 mmol, 2 g) in CH_2Cl_2 (20 mL). The reaction was stirred 2 h at room temperature under N_2, then the mixture was poured into boiling acetone-water (2:1, 90 mL), and let reflux in an open flask for 15 min. The reaction was cooled down, any remaining acetone evaporated *in vacuo*, and the product extracted with CH_2Cl_2. The solvent was evaporated and the residue redissolved in ether. The acid was extracted into 10% $NaHCO_3$. The aqueous solution was acidified with 6N HCl, and the acid **15** reextracted into CH_2Cl_2 (1.5 g, 70%); ^{1}H-NMR δ 2.49 (t, 2 H, J=8, C*H*$_2$CH$_2$CO$_2$Me), 2.96 (t, 2 H, J=8, CH$_2$C*H*$_2$CO$_2$Me), 3.58, 3.64 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.86 (s, 2 H, C*H*$_2$CO$_2$Me), 5.29 (s, 2 H, C*H*$_2$Ph), 7.26-7.40 (m, 5 H, Ph*H*), 9.33 (m, 1 H, N*H*), 10.08 (s, 1 H, CO$_2$*H*); ^{13}C-NMR δ 19.90, 29.76, 34.31, 51.47, 51.99, 66.77, 121.99, 122.44, 124.30, 128.43, 128.50, 130.53, 135.06, 159.93, 164.30, 171.69, 173.33.

Benzyl 2-iodo-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole-5-carboxylate (16).[4d,33] A mixture of **15** (3.74 mmol, 1.5 g) and $NaHCO_3$ (975 mg) in H_2O-CH_2Cl_2 (11:13 mL) was heated at reflux. A solution of iodine (1.1 g) and potassium iodide (1.13 g) in H_2O (7 mL) was added and heating continued for an additional 30 min. After addition of sodium bisulfite to destroy the excess of iodine, the product was extracted with CH_2Cl_2 and the compound **16** purified by chromatography (AcOEt-hexanes) (1.45 g, 80 %); ^{1}H-NMR δ 2.50 (t, 2 H, J=8, C*H*$_2$CH$_2$CO$_2$Me), 3.01 (t, 2 H, J=8, CH$_2$C*H*$_2$CO$_2$Me), 3.45 (s, 2 H, C*H*$_2$CO$_2$Me), 3.56, 3.63 (2 s, 2x3 H, 2 CO$_2$*Me*), 5.31 (s, 2 H, C*H*$_2$Ph), 7.25-7.40 (m, 5 H, Ph*H*), 10.35 (m, 1 H, N*H*); ^{13}C-NMR δ 21.01, 32.04, 34.42, 51.37, 51.92, 66.35, 76.49, 123.04, 123.51, 128.13, 128.24, 128.38, 130.32, 135.48, 159.97, 171.23, 173.23.

Benzyl 4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl)]pyrrole-5-carboxylate (17).[4d,33] A solution of **16** (2.99 mmol, 1.45 g) in 25 mL MeOH containing Et_3N (1.25 mL) and Adams' catalyst (platinum oxide, 145 mg) was hydrogenated at room temperature for 3 h. The solution was filtered, diluted with H_2O, and extracted with CH_2Cl_2. Chromatography (AcOEt-hexanes, 1:1) gave the pure product **17** (769 mg, 72%); ^{1}H-NMR δ 2.60 (t, 2 H, J=8, C*H*$_2$CH$_2$CO$_2$Me), 3.08 (t, 2 H, J=8, CH$_2$C*H*$_2$CO$_2$Me), 3.54 (s, 2 H, C*H*$_2$CO$_2$Me), 3.64, 3.70 (2 s, 2x3 H, 2 CO$_2$*Me*), 5.32 (s, 2 H, C*H*$_2$Ph), 6.83 (d, 1 H, J=3, Ar*H*), 7.32-7.44 (m, 5 H, Ph*H*), 9.88 (m, 1 H, N*H*); ^{13}C-NMR δ 20.26, 30.32, 34.61, 51.27, 51.78, 65.81, 116.84, 118.86, 122.10, 128.03, 128.13, 128.39, 129.45, 135.93, 160.72, 172.36, 173.55.

4-[2-(Methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole-5-carboxylic acid (18). A solution of the **17** (1.02 mmol, 367 mg) in THF (3.5 mL) containing 10% Pd-C (37 mg) was hydrogenated at room temperature overnight. The catalyst was filtered off and the solution evaporated. The product **18** was obtained as a solid (263 mg, 96%), which was used in the next step without further purification; ^{1}H-NMR δ 2.54 (t, 2 H, J=8, C*H*$_2$CH$_2$CO$_2$Me), 2.97 (t, 2 H, J=8, CH$_2$C*H*$_2$CO$_2$Me), 3.45 (s, 2 H, C*H*$_2$CO$_2$Me), 3.58, 3.62 (2 s, 2x3 H, 2 CO$_2$*Me*), 6.80 (d, 1 H, J=2.5, Ar*H*), 10.00 (m, 1 H, N*H*); ^{13}C-NMR δ 20.18, 30.43, 34.60, 51.51, 52.00, 116.92, 118.80, 122.79, 130.35, 164.39, 172.85, 174.20.

4-[2-(Methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole (19). A solution of **18** (2.82 mmol, 758 mg) in anhydrous TFA (6 mL) was stirred at room temperature under N_2 for 2 h. The solution was diluted with AcOEt and washed twice successively with H_2O, 10% $NaHCO_3$, and saturated $NaHCO_3$. The product **19** was obtained as an oil (435 mg, 69%), and used without further purification; ^{1}H-NMR δ 2.55 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 2.75 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.46 (s, 2 H, C*H*$_2$CO$_2$Me), 3.63, 3.64 (2 s, 2x3 H, 2 CO$_2$*Me*), 6.46, 6.56 (2 m, 2x1 H, Ar*H*), 8.70 (m, 1 H, N*H*).

2-Acetyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole (21). *A. From the pyrrole* **19**.[34] A solution of dimethylacetamide (0.78 mmol, 73 µL) and phosphorus oxychloride (0.78 mmol, 73 µL) in CH_2Cl_2 (1 mL) was stirred at 0°C for 30 min. The pyrrole **19** (0.39 mmol, 88 mg) in CH_2Cl_2 (1 mL) was then added, and the reaction was mixed at room temperature under N_2

overnight. The reaction was treated as for **7** and the products separated on preparative TLC, eluted 3 times (AcOEt-hexanes, 1:1). The upper band was identified as the 5-acetyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole **20** (39 mg, 38%); ^{1}H-NMR δ 2.41 (s, 3 H, *Me*CO), 2.53 (t, 2 H, J=8, C*H*$_2$CH$_2$CO$_2$Me), 3.01 (t, 2 H, J=8, CH$_2$C*H*$_2$CO$_2$Me), 3.45 (s, 2 H, C*H*$_2$CO$_2$Me), 3.61, 3.64 (2 s, 2x3 H, 2 CO$_2$*Me*), 6.84 (d, 1H, J=8, Ar*H*), 9.70 (m, 1 H, N*H*); ^{13}C-NMR δ 20.61, 27.14, 30.38, 34.86, 51.60, 52.00, 117.43, 122.88, 128.51, 129.09, 172.30, 173.23, 187.43. The lower band contained the desired isomer **21** (38 mg, 36 %); mp 99 °C (CH$_2$Cl$_2$-hexanes); ^{1}H-NMR δ 2.39 (s, 3 H, *Me*CO), 2.54 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 2.74 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.64, 3.67 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.80 (s, 2 H, C*H*$_2$CO$_2$Me), 6.73 (d, 1 H, J=3, Ar*H*), 9.44 (m, 1 H, N*H*); ^{13}C-NMR δ 20.01, 27.33, 30.92, 34.61, 51.64, 52.15, 120.82, 121.19, 124.70, 129.86, 171.74, 173.39, 187.62; MS m/e 267 (M$^+$, 100), 235 (82), 208 (78); Anal. Calcd for $C_{13}H_{17}NO_5$, C 58.40, H 6.41; found, C 58.28, H 6.35.

B. From the 5-iodopyrrole **26**.[33] The 5-iodopyrrole **26** (0.304 mmol, 120 mg) was hydrogenated as for **17**. Compound **21** (74 mg, 92%) was pure by TLC (AcOEt-hexanes, 1:1) and used without further purification.

2-(1-Hydroxyethyl)-4-[(2-ethoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl] pyrrole (23).[30] The pyrrole **21** was hydrolyzed as for **12** to give **22**. Sodium borohydride was added to an aqueous solution of **22** and the solution shaken for a few minutes, then freeze-dried to afford **23**; ^{1}H-NMR (D$_2$O) δ 1.49 (d, 3 H, J=6.5, CHOH*Me*), 2.41 (m, 2 H, C*H*$_2$CH$_2$CO$_2$H), 2.65 (m, 2 H, CH$_2$C*H*$_2$CO$_2$H), 3.36, 3.41 (2 d, 2 H, J=16.5, C*H*$_2$CO$_2$H), 4.98 (q, 1 H, J=6.5, *CH*OHMe), 6.61 (s, 1 H, Ar*H*); ^{13}C-NMR (D$_2$O) δ 21.36, 22.27, 33.13, 38.83, 62.23, 114.92, 122.84, 124.27, 132.06, 182.32, 183.97.

Benzyl 2-acetyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole-5-carboxylate (24). Tin (IV) chloride (0.61mmol, 71 µL) was added at 0 °C to a solution of the pyrrole **17** (0.35 mmol, 127 mg) and acetyl chloride (0.52 mmol, 37 µL) in CH$_2$Cl$_2$ (1 mL). After 45 min at 0 °C, the reaction was quenched with H$_2$O, extracted with CH$_2$Cl$_2$ and the product **24** was recrystallized from ether (132 mg, 95%); mp 99 °C; ^{1}H-NMR δ 2.46 (s, 3 H, *Me*CO), 2.48 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 2.98 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.59, 3.67 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.83 (s, 2 H, C*H*$_2$CO$_2$Me), 5.30 (s, 2 H, C*H*$_2$Ph), 7.30-7.40 (m, 5 H, Ph*H*), 9.80 (br s, 1 H, N*H*); ^{13}C-NMR δ 19.88, 27.76, 30.46, 34.44, 51.54, 52.20, 66.86, 121.89, 121.96, 128.0, 130.94, 135.13, 160.17, 171.34, 173.28, 178.29; MS m/e 401 (M$^+$,7), 91 (100); Anal. Calcd for $C_{21}H_{23}NO_7$, C 62.82, H 5.78; found, C 62.82, H 5.80.

2-Acetyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole-5-carboxylic acid (25). The pyrrole **24** (0.43 mmol, 173 mg) was hydrogenated and treated as for **18**. **25** was obtained as a solid and recrystallized from CH$_2$Cl$_2$ (128 mg, 96%); mp 178-179 °C; ^{1}H-NMR δ 2.47 (s, 3 H, *Me*CO), 2.54 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 2.98 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.61, 3.68 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.83 (s, 2 H, C*H*$_2$CO$_2$Me), 10.40 (s, 1 H, N*H*); ^{13}C-NMR δ 19.76, 27.64, 30.44, 34.46, 51.88, 52.22, 122.12, 122.32, 128.55, 131.23, 163.31, 171.85, 173.77, 189.31; MS m/e 311 (M$^+$, 42), 279 (100), 252 (56); Anal. Calcd for $C_{14}H_{17}NO_7$, C 54.02, H 5.47; found, C 53.76, H 5.45.

2-Acetyl-5-iodo-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole (26). The acid **25** (1.65 mmol, 513 mg) was decarboxylated as for **16**. The 5-iodopyrrole **26** (557 mg, 86%) was purified by chromatography (AcOEt-hexanes, 1:1); mp 133-135 °C (CH$_2$Cl$_2$-hexanes); ^{1}H-NMR δ 2.33 (s, 3 H, *Me*CO), 2.43 (t, 2 H, J=8, C*H*$_2$CH$_2$CO$_2$Me), 2.67 (t, 2 H, J=8, CH$_2$C*H*$_2$CO$_2$Me), 3.63, 3.67 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.82 (s, 2 H, C*H*$_2$CO$_2$Me), 10.06 (s, 1 H, N*H*); ^{13}C-NMR δ 21.60, 27.05,

31.19, 34.19, 51.67, 52.19, 76.37, 121.64, 129.10, 134.07, 171.61, 173.09, 186.66; MS m/e 393 (M^+, 31), 266 (100); Anal. Calcd for $C_{13}H_{16}INO_5$, C 39.69, H 4.07; found, C 39.58, H 4.10.

2-Acetyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole oxime (27). The oxime **27** was obtained in the same way as for **10** and purified by filtration through a short plug of silica gel (AcOEt-hexanes, 3:1) (69 mg, 88%); mp 147 °C (CH_2Cl_2); ^{1}H-NMR δ 2.14 (s, 3 H, *Me*C=NOH), 2.53 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 2.71 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.62 (s, 8 H, 2 CO$_2$*Me* + C*H*$_2$CO$_2$Me), 6.49 (m, 1 H, Ar*H*), 8.95 (m, 1 H, N*H*); ^{13}C-NMR δ 11.90, 20.37, 30.89, 34.74, 51.57, 51.99, 114.72, 116.36, 123.76, 125.78, 150.17, 172.42, 173.67; MS m/e 282 (M^+,100), 265 (74), 223 (48); HRMS calcd for $C_{13}H_{18}N_2O_5$, 282.1216; found, 282.1219.

2-(1-Aminoethyl)-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl] pyrrole hydrochloride (28).[30] The ketoxime **27** (0.25 mmol, 69 mg) was hydrogenated following the procedure for **11**; ^{1}H-NMR δ 2.26 (s br, 3 H, *Me*), 2.42 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 2.59 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.52, 3.56 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.68 (s, 2 H, C*H*$_2$CO$_2$Me), 4.22 (m, 1 H, C*H*NH$_2$Me), 6.65 (m, 1 H, Ar*H*); ^{13}C-NMR δ 19.63, 28.53, 30.56, 33.92, 51.35, 51.49, 51.71, 121.58, 124.21, 129.09, 130.38, 172.11, 173.58; MS m/e 267 (M^+-1, 7), 253 (32), 238 (20), 194 (51), 180 (93), 166 (100); HRMS calcd, 253.1314; found, 253.1307.

C-11-Methyl PBG (29).[30] The pyrrole **28** was hydrolyzed as above for **12** to give an enantiomeric mixture;[35] ^{1}H-NMR (D_2O) δ 1.41, 1.68 (2 d, J=5.5, 7, 3 H, *Me*), 2.41 (m, 2 H, C*H*$_2$CH$_2$CO$_2$H), 2.61 (m, 2 H, CH$_2$C*H*$_2$CO$_2$H), 3.37, 3.70 (2 m, 2 H, C*H*$_2$CO$_2$H), 4.62 (m, 1H, C*H*NH$_2$Me), 6.69, 6.97 (d+s, J=7, 1 H, Ar*H*); ^{13}C-NMR δ 20.76, 20.96, 21.29, 26.35, 28.02, 33.78, 37.93, 47.17, 123.45, 124.81, 125.71, 126.70, 134.49, 182.58.

2-Trifluroacetyl-4-[2(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole (31). A solution of the **18** (1.48 mmol, 400 mg) was stirred in anhydrous TFA (5 mL) at room temperature under N_2 for 2 h. Then TFAA (7.4 mmol, 1.05 mL) was added and the reaction let stir overnight. The solution was diluted with AcOEt and washed several times with H_2O, followed by 10% $NaHCO_3$ and finally saturated $NaHCO_3$. To separate the 2 isomers **30** and **31**, several chromatographies were necessary: first on a silica gel column (MeOH-CH_2Cl_2, 1.5 : 98.5), followed by preparative TLC run twice in MeOH-CH_2Cl_2 (1:99), and finally twice more in MeOH-CH_2Cl_2 (1:49). The upper band gave the compound **30** as a solid (164 mg, 34 %); mp 96 °C (CH_2Cl_2-hexanes); ^{1}H-NMR δ 2.54 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 3.02 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.52 (s, 2 H, C*H*$_2$CO$_2$Me), 3.59, 3.65 (2 s, 2x3 H, 2 CO$_2$*Me*), 7.01 (d, 1 H, J=3.5, Ar*H*), 9.82 (m, 1 H, N*H*); ^{13}C-NMR δ 20.67, 29.83, 33.21, 51.52, 52.08, 116.78 (q, $^1J_{CF}$=291), 119.27, 122.42, 127.44, 137.38, 168.41 (q, $^2J_{CF}$=36), 171.99, 173.60. The second band contained the solid **31** (120 mg, 25 %); mp 99-100 °C (CH_2Cl_2-hexanes); ^{1}H-NMR δ 2.53 (t, 2 H, J=7.5, C*H*$_2$CH$_2$CO$_2$Me), 2.73 (t, 2 H, J=7.5, CH$_2$C*H*$_2$CO$_2$Me), 3.62, 3.66 (2 s, 2x3 H, 2 CO$_2$*Me*), 3.85 (s, 2 H, C*H*$_2$CO$_2$Me), 6.90 (d, 1 H, J=3.5, Ar*H*), 9.69 (br s, 1 H, N*H*); ^{13}C-NMR δ 19.49, 30.60, 34.32, 51.66, 52.07, 116.69 (q, $^1J_{CF}$=290), 123.12, 126.07, 126.42, 129.30, 166.60 (q, $^2J_{CF}$=35), 171.19, 173.17; MS m/e 321 (M^+, 45), 289 (90), 262 (79), 229 (41), 202 (100); Anal. Calcd for $C_{13}H_{14}F_3NO_5$, C 48.59, H 4.39; found, C 48.72, H 4.43.

2-Trifluoroacetyl-4-(2-carboxyethyl)-3-(carboxymethyl)pyrrole (32).[30] The pyrrole **31** (0.049 mmol, 16 mg) was hydrolyzed as for **12** to afford compound **32**; ^{1}H-NMR (D_2O) δ 2.43 (t, 2 H, C*H*$_2$CH$_2$CO$_2$H), 2.71 (t, 2 H, CH$_2$C*H*$_2$CO$_2$H), 3.75 (s, 2 H, C*H*$_2$CO$_2$H), 7.20 (s, 1 H, Ar*H*); ^{13}C-NMR (D_2O) δ 21.38, 34.63, 38.50, 117.68 (q, $^1J_{CF}$=289), 123.50, 129.51, 130.93, 134.60, 170.00 (q, $^2J_{CF}$=35), 179.84, 183.10.

2-(1-Hydroxy-2,2,2-trifluoroethyl)-4-(2-carboxyethyl)-3-(carboxymethyl)pyrrole (33).[30] The ketone **32** was treated with sodium borohydride as for **23**; ^{1}H-NMR (D_2O) δ 2.42 (t, 2 H, J=7.5, $CH_2CH_2CO_2H$), 2.64 (t, 2 H, J=7.5, $CH_2CH_2CO_2H$), 3.39, 3.41 (2 d, 2 H, J=19, CH_2CO_2H), 5.26 (q, 1 H, $^2J_{HF}$=7, $CHCF_3OH$), 6.71 (s, 1 H, Ar*H*); ^{13}C-NMR (D_2O) δ 22.15, 33.82, 38.65, 65.54 (q, $^2J_{CF}$=33), 117.12, 118.06, 121.75, 123.26, 181.56, 183.84.

2-Trifluroacetyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole oxime (34). The ketone **31** (0.143 mmol, 46 mg), hydroxylamine hydrochloride (0.715 mmol, 50 mg) and sodium acetate (0.715 mmol, 60 mg) in absolute EtOH (2 mL) were heated at reflux under N_2 for 60 h. The solution was diluted with water and the product extracted with CH_2Cl_2. The 2 isomers of **34** were purified on preparative TLC eluted 3 times in CH_2Cl_2-MeOH (97:3) (29 mg, 60%); ^{1}H-NMR δ **34a** (minor isomer): 2.59 (t, 2 H, J=7.5, $CH_2CH_2CO_2Me$), 2.76 (t, 2 H, J=7.5, $CH_2CH_2CO_2Me$), 3.59 (s, 2 H, CH_2CO_2Me), 3.64, 3.66 (2 s, 2x3 H, 2 CO_2Me), 6.69 (d, 1 H, J=3, Ar*H*), 8.44 (br s, 1 H, O*H*), 8.92 (m, 1 H, N*H*); **34b** (major isomer): 2.58 (t, 2 H, J=7.5, $CH_2CH_2CO_2Me$), 2.73 (t, 2 H, J=7.5, $CH_2CH_2CO_2Me$), 3.57 (s, 2 H, CH_2CO_2Me), 3.61, 3.63 (2 s, 2x3 H, 2 CO_2Me), 6.74 (d, 1 H, J=8, Ar*H*), 9.20 (br s, 1 H, O*H*), 10.30 (m, 1 H, N*H*); ^{13}C-NMR δ 20.31, 31.02, 34.42, 51.70, 52.06, 115.21, 119.28, 119.44, 123.68, 172.36, 173.63; MS m/e 336 (M^+, 87), 319 (53), 304 (55), 287 (74), 277 (100), 245 (79), 203 (80); HRMS calcd for $C_{13}H_{15}F_3N_2O_5$, 336.0933; found, 336.0931.

2-(1-Amino-2,2,2-trifluoroethyl)-4-[2(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]pyrrole (35). A solution of the oxime **34** (0.093 mmol, 31.3 mg) in MeOH (15 mL) containing 6N HCl (16 µL) was shaken under hydrogen pressure (50 PSI) over palladium black (10 mg) for 2 days. The solution was then filtered, diluted with CH_2Cl_2, and washed with 10% $NaHCO_3$. The residue was purified on preparative TLC (CH_2Cl_2-MeOH, 95:5) to give the amine **35** (25 mg, 85%); ^{1}H-NMR δ 2.56 (t, 2 H, J=7, $CH_2CH_2CO_2Me$), 2.74 (t, 2 H, J=7, $CH_2CH_2CO_2Me$), 3.44 (s, 2 H, CH_2CO_2Me), 3.65 (2 s, 2x3 H, 2 CO_2Me), 4.67 (q, 1 H, J_{HF}=7, $CHCF_3NH_2$), 6.54 (d, 1 H, J=3, Ar*H*), 8.80 (m, 1 H, N*H*); ^{13}C-NMR δ 20.38, 29.58, 34.64, 50.50 (q, $^2J_{CF}$=33), 51.55, 52.02, 113.83, 115.87, 121.74, 122.36, 172.40, 173.79; MS m/e 322 (M^+, 60), 305 (29), 273 (34), 263 (40), 253 (71), 235 (100); Anal. Calcd for $C_{13}H_{17}F_3N_2O_4$, C 48.43, H 5.32; found, C 48.32, H 5.32.

2-(1-Amino-2,2,2-trifluoroethyl)-4-(2-carboxyethyl)-3-(carboxymethyl)pyrrole (36).[30] The diester **35** was hydrolyzed as for **12** to give the enantiomeric mixture **36**;[35] ^{1}H-NMR (D_2O) δ major isomer: 2.42 (m, 2 H, $CH_2CH_2CO_2H$), 2.64 (m, 2 H, $CH_2CH_2CO_2H$), 3.40 (2 d, 2 H, J=17, CH_2CO_2H), 4.88 (q, 1 H, J_{HF}=6, $CHCF_3NH_2$), 6.70 (d, 1 H, Ar*H*); ^{13}C-NMR (D_2O) δ 22.14, 32.88, 38.50, 49.83 (q, $^2J_{CF}$=23), 116.61, 117.37, 118.70, 123.02, 181.46, 183.74; ^{1}H-NMR (D_2O) δ minor isomer: 2.42 (m, 2 H, $CH_2CH_2CO_2H$), 2.64 (m, 2 H, $CH_2CH_2CO_2H$), 3.53 (2 d, 2 H, J=21, CH_2CO_2H), 5.24 (m, 1 H, $CHCF_3NH_2$), 6.78 (d, 1 H, Ar*H*).

Benzyl 2-d-formyl-4-[2-(methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl] pyrrole-5-carboxylate (37). A solution of d_7-DMF (6.42 mmol, 500 µL) and phosphorus oxychloride (6.42 mmol, 520 µL) in CH_2Cl_2 (4 mL) was stirred at room temperature under N_2 for 30 mn. Then the α-free pyrrole **17** (2 mmol, 720 mg) was added, the reaction stirred overnight and treated as for **7**. The product **37** was recristallized from CH_2Cl_2-hexanes (740 mg, 95%); mp 78-79 °C; ^{1}H-NMR δ 2.55 (t, 2 H, J=7.6, $CH_2CH_2CO_2Me$), 3.02 (t, 2 H, J=7.6, $CH_2CH_2CO_2Me$), 3.61, 3.68 (2 s, 6 H, 2 CO_2Me), 3.84 (s, 2 H, CH_2CO_2Me), 5.33 (s, 2 H, CH_2Ph), 7.30-7.45 (m, 5 H, Ph*H*), 9.47 (s br, 1 H, N*H*); ^{13}C-NMR δ 19.52, 28.93, 34.12, 51.33, 52.12, 66.78, 123.86, 124.78, 128.39, 130.26, 130.62, 134.94, 159.94, 171.00, 173.09.

Benzyl 2-hydroxy-d-methyl-4-[(2-methoxycarbonyl)ethyl]-3-[(methoxycarbonyl) methyl]pyrrole-5-carboxylate (38). A solution of the pyrrole **37** (0.95 mmol, 369 mg) in R-alpine Borane (0.5 M in THF, 3.8 mmol, 7.6 mL) was stirred at room temperature under N_2 overnight. The excess of borane was destroyed with acetaldehyde. The solvent was evaporated, the residue redissolved in ether and 2-aminoethanol (4.2 mmol, 260 μL) added to hydrolyze the borane complex. The solution was filtered from the precipitated borane and purified by chromatography (AcOEt-hexanes, 1:1) to give **38** as a white cristalline solid (306 mg, 83%); mp 125 °C; ^{1}H-NMR δ 2.49 (t, 2 H, J=7.75, C*H*$_2$CH$_2$CO$_2$Me), 2.98 (t, 2 H, J=7.75, CH$_2$C*H*$_2$CO$_2$Me), 3.36 (m, 1 H, O*H*), 3.49 (s, 2 H, C*H*$_2$CO$_2$Me), 3.58, 3.66 (2 s, 6 H, 2 CO$_2$*Me*), 4.50 (s, 1 H, C*H*DOH), 5.25 (s, 2 H, C*H*$_2$Ph), 7.29-7.37 (m, 5 H, Ph*H*), 9.75 (s br, 1 H, N*H*); ^{13}C-NMR δ 20.24, 29.32, 34.76, 51.42, 52.35, 66.04, 114.51, 117.93, 128.19, 128.29, 128.51, 130.12, 134.23, 134.85, 160.71, 173.32, 173.52. Althought it has been claimed[8c] this procedure yields up to 90% ee, we were never able to achieve this percentage of selectivity. The chiral purity reported[8c] was actually the result of a complex series of transformations, which as noted by the authors, are neither free from racemization, nor unambiguous in the presence of tracer levels of tritium (the isotope used in the noted reference). We therefore attempted to measure directly the purity of the alcohol by ORD/CD, as well as by NMR in the presence of the chiral europium shift reagent, Tris[3-(heptafluoropropyl)-hydroxymethylene-*d*-camphorato] europium(III), but were unable to observe evidence for any ee. The alcohol was finally derivatized using (S)-(-)-N-(trifluoracetyl)propyl chloride (below).

Benzyl 2-acetoxy-d-methyl-4-[(2-methoxycarbonyl)ethyl]-3-[(methoxycarbonyl) methyl]pyrrole-5-carboxylate (39). A mixture of the pyrrole **38** (0.38 mmol, 150 mg), acetic anhydride (0.77 mmol, 73 μL) and DMAP (0.08 mmol, 10 mg) in CH_2Cl_2 (2 mL) was stirred at room temperature under N_2 for 2 days. The reaction mixture was diluted and washed with $NaHCO_3$. The product **39** was purified by chromatography (AcOEt-hexanes, 1:3) to give a cristalline solid (141 mg, 83%); mp 110 °C (CH_2Cl_2-hexanes); ^{1}H-NMR δ 1.99 (s, 3 H, *Me*CO$_2$), 2.50 (t, 2 H, J=7.9, C*H*$_2$CH$_2$CO$_2$Me), 2.97 (t, 2 H, J=7.9, CH$_2$C*H*$_2$CO$_2$Me), 3.52 (s, 2 H, C*H*$_2$CO$_2$Me), 3.57, 3.63 (2 s, 6 H, 2 CO$_2$*Me*), 5.01 (s, 1 H, C*H*DOAc), 5.26 (s, 2 H, C*H*$_2$Ph), 7.29-7.36 (m, 5 H, Ph*H*), 9.73 (s br, 1 H, N*H*); ^{13}C-NMR δ 20.27, 20.66, 29.18, 34.48, 51.06, 52.80, 56.40 (d, J_{CD}= 89), 66.03, 116.82, 118.87, 126.81, 127.51, 127.77, 129.06, 129.79, 135.66, 160.54, 171.20, 171.81, 173.42.

Benzyl 4-[(2-methoxycarbonyl)ethyl]-3-[(methoxycarbonyl)methyl]-2-[(N-trifluoro acetylprolyloxy)-d-methyl]pyrrole-5-carboxylate (40). A solution of the pyrrole **38** (0.05 mmol, 20 mg), N-trifluoroacetylprolylchloride (0.1M in CH_2Cl_2, 0.12 mmol, 1.2 mL) and Et_3N (0.12 mmol, 18 μL) in CH_2Cl_2 (1 mL) was refluxed under N_2 for 4 h. The reaction was diluted and washed with $NaHCO_3$. The product **40** was purified by preparative TLC (AcOEt-hexanes, 1:2) (21 mg, 71%); ^{1}H-NMR δ 2.00, 2.19 (2 m, 2 H, C*H*$_2$C*H*$_2$NCOCF$_3$), 2.51 (t, 2 H, J=7.8, C*H*$_2$CH$_2$CO$_2$Me), 2.97 (t, 2 H, J=7.8, CH$_2$C*H*$_2$CO$_2$Me), 3.51 (s, 2 H, C*H*$_2$CO$_2$Me), 3.61, 3.64 (2 s, 6 H, 2 CO$_2$*Me*), 3.67, 3.79 (2 m, 1 H, C*H*$_2$CH$_2$CH$_2$NCOCF$_3$), 4.51 (dd, 1 H, J= 4.2, 8.7, COC*H*N), 5.08, 5.12 (2 s, 38:62, 1 H, C*H*D), 5.27 (s, 2 H, C*H*$_2$Ph), 7.30-7.40 (m, 5 H, Ph*H*), 9.36(s br, 1 H, N*H*); ^{13}C-NMR δ 20.32, 24.96, 28.35, 29.32, 34.59, 47.24, 51.45, 52.08, 57.75, 60.11, 66.13, 116.96, 119.36, 127.83, 128.28, 128.38, 128.56, 130.02, 135.86, 160.28, 170.74, 171.77, 173.51, 178.34. In order to get an accurate measurement of the ee using integration, a delay of ~25 sec, corresponding to > 5 x T_1 was used to ensure complete relaxation of the proton signals. Under these conditions, it is estimated that the mixture was 62:38, or ~ 25 ± 3% ee.

Solvolysis

For the solvolysis study, compound **39** was solvolyzed with (R)-(-)-2- butanol. A typical reaction involved diluting ~10 mg of the acetate with 200 μL of the alcohol and heating for 1-5 min at 75°C under an Ar atmosphere. Reactions were conveniently monitored by TLC (AcOEt:Hexanes, 40:60). In some cases reactions were stopped at 50% completion, excess butanol distilled off, and the composition determined by ^{1}H-NMR. The diastereomeric ethers gave distinct peaks in the proton spectrum for both chiral centers (δ: -OC*H*D 4.47 and 4.40 ppm, -OC*H*(CH$_3$)$_2$ 1.56 and 1.44 ppm).

Enzymology

PBG deaminase Purification

Enzyme was purified from overproducing *E. coli* as previously described,[36] with the following modifications. Purified enzyme was desalted on a PD-10 column (G-25M Sephadex), lyophylized from H_2O and stored at -20°C. Enzyme so prepared was dissolved in the appropriate buffer and concentration determined by Bradford's method immediately before use.

Enzymatic Reactions

Reaction of compounds **12, 23, 29, 33,** and **36** with deaminase were carried out in 100 mM buffer, KH_2PO_4, 2 mM EDTA at pH 8.1 or citrate/phosphate at pH 5.2, with an enzyme concentration of ~ 0.5 - 1.0 mg/mL. Substrates were taken up in the appropriate buffer such that the substrate reaction concentration was 1-5 mM. Incubations were carried out at 37°C for 15-20 min. For the prolonged incubations, the enzyme-substrate, as well as enzyme alone, were kept at 37°C for upwards of 40 hrs, with activity being assayed every ~ 4-6 hrs. Under these conditions deaminase slowly decomposed.

It should be noted, the synthesis of compounds **23, 29, 33,** and **36** are completely non-stereospecific so that these analogs are enantiomeric mixtures at the C-11 carbon. No attempts were made to separate the enantiomers. Preliminary NMR (^{19}F and ^{13}C) studies revealed no obvious preference for a single enantiomer in the binding of the substrate with enzyme, nor was there any evidence for the catalytic consumption of only one enantiomer in the enzymatic reaction (results not shown).

Analysis of Reaction

Initial analysis of complexes was by non-denaturing polyacrylamide gel electrophoresis (PAGE). The stacking gel was 3% acrylamide, Tris hydrochloride, pH 6.8, with a separating gel of 7.5% acrylamide, Tris hydrochloride, pH 8.8; electrophoresis was at 180 V and 4°C. Further analysis was carried out by anion exchange FPLC[37] on a Mono-Q column, in Tris hydrochloride pH 7.5 with a 0.0 - 0.4M NaCl gradient.

Deaminase from *E. coli* exists in at least four different forms which are always observed by PAGE. These isozymes exhibit no apparent differences in their reaction with PBG, only differing in their total ionic charges, for example a Gln-to-Glu type change. To circumvent the difficulty in interpretation of the polyacrylamide gels, we have adopted the procedure used by others in the area of deaminase study, namely the combined use of PAGE and FPLC.[38] On an anion exchange column, the four isozymes co-migrate so that there is no ambiguity as to whether a particular band seen in the gel is an ES-complex, or an uncomplexed isozyme. In all cases, both PAGE and FPLC were used in conjunction to unequivocably define the reaction intermediates, though we have only included typical displays from both methods.

ACKNOWLEDGMENTS

The authors wish to thank Prof. Thomas Tidwell for his helpful comments and providing us a pre-print of his work. The insightful suggestions and valuable discussions with Prof. Larry Sayre were greatly appreciated. We also thank Prof. David Giedroc for the use of his ORD/CD instrument and helpful comments. The financial assistance of the NIH and the Robert A.Welch Foundation is gratefully acknowledged.

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