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The chemical synthesis of porphobilinogen an important intermediate of the biosynthesis of the "pigments of life"

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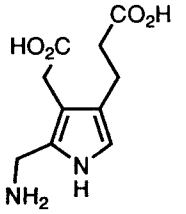
ABSTRACT

Porphobilinogen is the second dedicated intermediate in the biosynthesis of «pigments of life». Only very few alkylpyrroles have been isolated from natural sources so far. The absence of stabilising substituents confers to porphobilinogen a high reactivity. The chemical synthesis of porphobilinogen had to take its sensitivity into account. The published synthesis of this unusual pyrrole are reviewed. The synthetic strategies used are analysed and compared with the biosynthesis.



1

Porphobilinogen

Figure 1

INTRODUCTION

Pyrroles as Natural Products

Relatively few mono-pyrrolic natural products have been reported in the literature [1-4]. Most of these natural mono-pyrroles are stabilised by an electron-withdrawing substituent or by an aromatic ring. Without these substituents the electron rich pyrrole ring is easily polymerised or auto oxidised[5-8]. Porphobilinogen (= PBG **1**) a trialkylsubstitued pyrrole is a remarkable exception to this rule (see figure 1).

The lack of stabilising substituents confers a high reactivity to porphobilinogen[9-12]. The biosynthesis of the tetrapyrrolic «pigments of life» makes use of this high reactivity. About 10^{10} tons of chlorophyll and more than $4*10^5$ tons of heme are synthesised each year [13-22].

Porphobilinogen is the second dedicated intermediate in the biosynthesis of tetrapyrroles [23-29]. The tetrapyrrolic pigments like **2** - **4** are universally distributed and have therefore been named "pigments of life" (see figure 2) [30,31]. They are used as cofactors for many central processes of life like photosynthesis, oxygen transport and catalysis of unusual chemical reactions [32,33].

The tetrapyrrolic skeleton of all «pigments of life» is synthesised in a highly convergent way, starting with 8 molecules of 5-aminolevulinic acid (**5**) (see figure 3). 5-aminolevulinic acid (**5**) is condensed to porphobilinogen (**1**), which tetrameroidise to form uroporphyrinogen III (**6**).

The tetrameroidisation [34] of porphobilinogen (**1**) could be achieved without the help of an enzyme [9-12]. Porphobilinogen (**1**) has a strong tendency to form the uroporphyrinogens. The chemical reactivity of porphobilinogen (**1**) leads to the formation of the next biosynthetic intermediate without the help of an enzyme. This enzymatic transformation might be called an example of a chemomimetic biosynthesis [35-38].

For the dimeroidisation of 5-aminolevulinate to porphobilinogen (**1**) a ΔG = -16.9 kcal/mol and for the tetrameroidisation of porphobilinogen (**1**) to uroporphyrinogen III a ΔG = -34.6 kcal/mol were calculated for the gas phase reactions [37]. The biosynthesis of tetrapyrroles liberates free energy [37,38]. This observations were taken as arguments in favour of a spontaneous formation of tetrapyrroles [39].

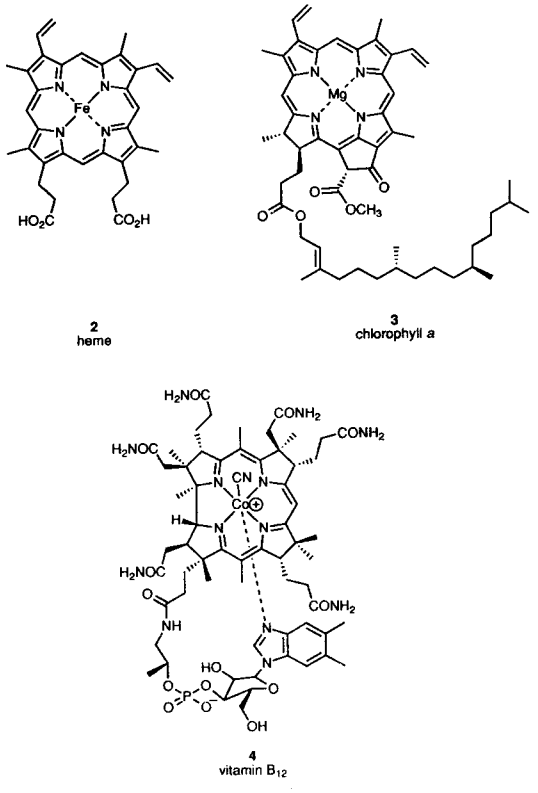


Figure 2

The importance of the «pigments of life» and the elegance of the biosynthetic pathway was a strong motivation to develop chemical synthesis of porphobilinogen (1). In recent years the need for good analytical methods to determine low levels of lead poisoning has renewed the interest in the synthesis of porphobilinogen (1) [40-43]. Another motivation was the need for porphyrin-based pigments for the use in photodynamic therapy. [44,45]

SYNTHESIS OF PORPHOBILINOGEN

Introduction

The first synthetic efforts were undertaken to prove the structure of porphobilinogen (1) [46,47]. Later synthetic methods were developed in order to obtain porphobilinogen (1) labelled at a specific positions for the use in biosynthetic studies [48-58]. A renewed interest to develop synthetic methods for porphobilinogen

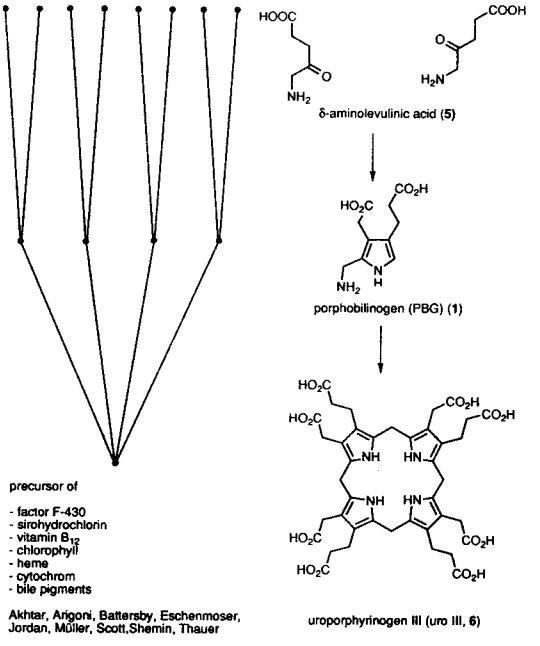


Figure 3

(1) has been stimulated by the possibility to use the known sensitivity of porphobilinogen synthase (=PBGS, also called aminolevulinate dehydratase, E.C. 4.2.1.24) against lead poisoning [42,43,59-61]. Photodynamic therapy especially well suited for the treatment of superficial, localised tumors has renewed the interest in practical and efficient routes towards porphobilinogen and suitable derivatives thereof [44,45]. Finally a series of analogues of porphobilinogen (1) were synthesised to study the chemical behaviour and possible biosynthetic pathways leading to the natural product [44,62-65]. The number of fundamentally different approaches to the synthesis porphobilinogen (1) or its analogues reported in the literature is limited. The synthetic efforts toward porphobilinogen (1) has been reviewed [66,67]. In this review special attention will be given to the most recent results.

Six different synthetic strategies have been used so far for the synthesis of porphobilinogen (1) (see figure 4).

Porphobilinogen (1) was synthesised for the first time using a classic Knorr synthesis [46,47]. The development of this strategy is mainly due to *MacDonald* [68]. He developed with his group the chemistry necessary to modify the substituents obtained using the Knorr reaction. The major problems are the introduction of the acetic acid side chain and the introduction of the methylamino

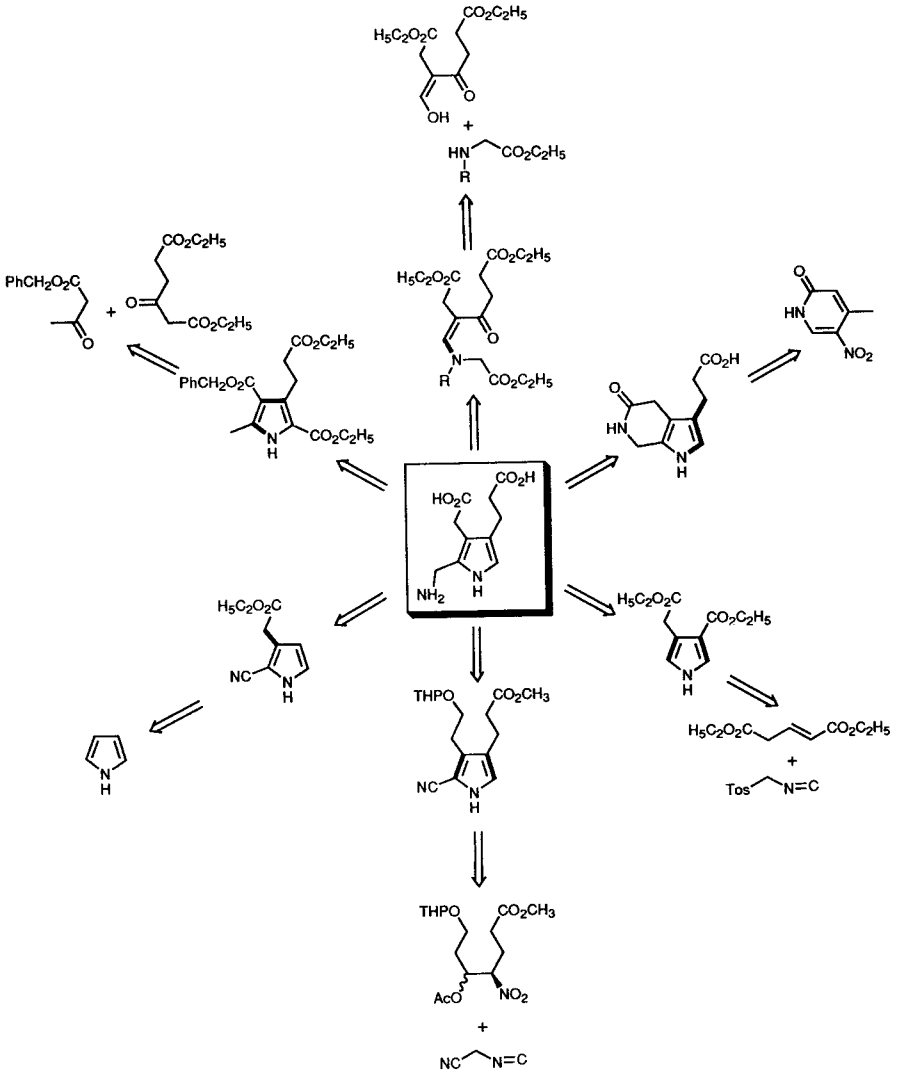


Figure 4

group. The removal of the ester group, used to protect the α-position of the pyrrole, proved to be difficult as well.

Plieninger [69] and later *Evans* [70] reported a second synthesis. The pyrrole ring was formed by condensation of a C_3-unit with a C-N-unit, using the variant of Kleinspehn of the Knorr synthesis. [69] *Evans* [70] achieved the same ring closure in a stepwise fashion. The acetic acid and the propionic acid side chains are in place right from the beginning, which is an advantage of this strategy.

Anderson and collaborators started with the unsubstituted pyrrole [71,72], introducing step by step the acetic acid side chain, the nitrile group as precursor of the methylamino group and finally the propionic acid side chain.

Frydman and *Rapoport* [73] reported a completely different strategy

using a pyridine derivative as starting material. The key synthetic intermediate is an azaindole which is hydrogenated to give the porphobilinogen lactam. The major problems are the introduction of the propionic acid side chain and the reduction step.

Ganem reported a synthesis of porphobilinogen (1) [45] based on the methodology developed by *van Leusen*. [74] The addition of TosMIC to an α,β-unsaturated ester gave a β,β'-disubstitued pyrrole. Vilsmeier-Haack formulation allowed to introduce the missing substituent in the α-position. Finally the propionic acid side chain had to be elaborated starting from the ester function.

The most recent synthesis reported by *Adamczyk* [42,43,59-61] uses a [2 + 3] cycloaddition. The acetic and propionic acid side chains are already present in the starting material and only a few synthetic transformations are necessary to obtain the porphobilinogen (1).

Synthesising the Pyrrole Ring in a Knorr Reaction

MacDonald and his collaborators developed in a systematic study the methods necessary for the synthesis of porphobilinogen (1)[75,76] and its regioisomers [68] (see figure 5).

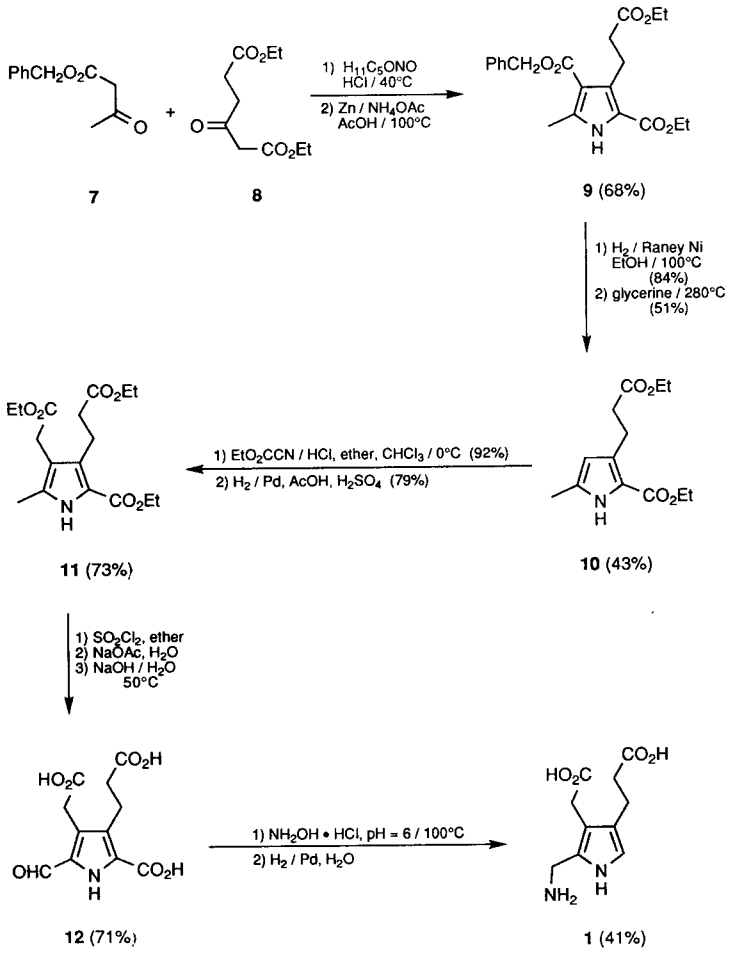


Figure 5

They started with diethyl β-ketoadipate (**8a**) [77] which was submitted to amyl nitrite and then treated with zinc in acetic acid in the presence of benzylacetoacetate (**7**) to give in 68 % yield the tetrasubstituted pyrrole **9** [75]. Nine steps were necessary to adjust the substituents on the pyrrole ring. First the benzyl protecting group was removed by treatment of the pyrrole **9** with Raney nickel and the acid group was decarboxylated in glycerol at 260 to 280 °C. The pyrrole **10** could be acylated in a sort of Houben-Hoesch reaction with ethyl cyanoformate in 92 % yield [76,78]. The glyoxalate was reduced catalytically in 79 % yield to give the triester **11**, containing the complete carbon skeleton of porphobilinogen (**1**) plus the 5-ethylcarboxylate as protecting group for the α-position. Treating this pyrrole **11** with sulfuryl chloride in ether followed by hydrolysis furnished the aldehyde **12** in excellent yield. The tricarboxylic acid **12**, which was treated with hydroxylamine hydrochloride at almost neutral pH give the oxime, which had undergone decarboxylation [46,47]. The oxime was reduced in a diluted solution in the presence of palladium black as catalyst to give a good yield of porphobilinogen (**1**).

Treibs and *Ott* developed a modification of the synthetic pathway described before (see figure 6) [79].

The use of the tert-butyl ester of acetoacetate (**13**) [80] as partner in the Knorr pyrrole synthesis [81] allowed an easy deprotection and decarboxylation. The Mannich type alkylation of **14** lead to the pyrrole **15**. Quaternisation with methyl iodide followed by substitution with potassium cyanide in ethanol produced the acetic acid side chain. The nitrile was then hydrolysed to the ethylcarboxylate, which gave the central precursor **16**.

Another modification of this synthetic pathway is due to *Kenner* (see figure 7) [50,82]. Also in this synthesis the β-oxoadipate **8a** and **8b** were used in the *Knorr* pyrrole synthesis but with acetyl acetone (**17**) as partner. The advantage of this sequence is that acetyl acetone (**17**) is a highly reactive precursor which usually gives good and reproducible yields of the corresponding pyrroles **18a** and **18b**.

The thallium mediated oxidative rearrangement of the acetyl group furnishes the ester of the acetic acid side chain **19a** and **19b**. This gives in a few steps the central intermediate for the porphobilinogen synthesis according to *MacDonald*.

Kenner also developed a modified sequence from this central pyrrole to porphobilinogen (**1**), in order to have a more reproducible procedure in hands (see figure 8) [82].

Using the benzyl ester as protecting group for the α-position of the pyrrole ring **19b** [50] oxidation with lead tetraacetate could be achieved in good yield. Substitution with phthalimide in DMSO yielded the precursor **20**. The deprotection of the benzyl ester was very delicate. Working in anisole as solvent was necessary in order to trap the benzyl group. Under these special conditions a 63 % yield of the fully protected pyrrole could be obtained. Deprotection of the phthalimide with hydroxylamine or N-methylhydrazine gave esterlactam of porphobilinogen **21a**. The hydrolysis of this

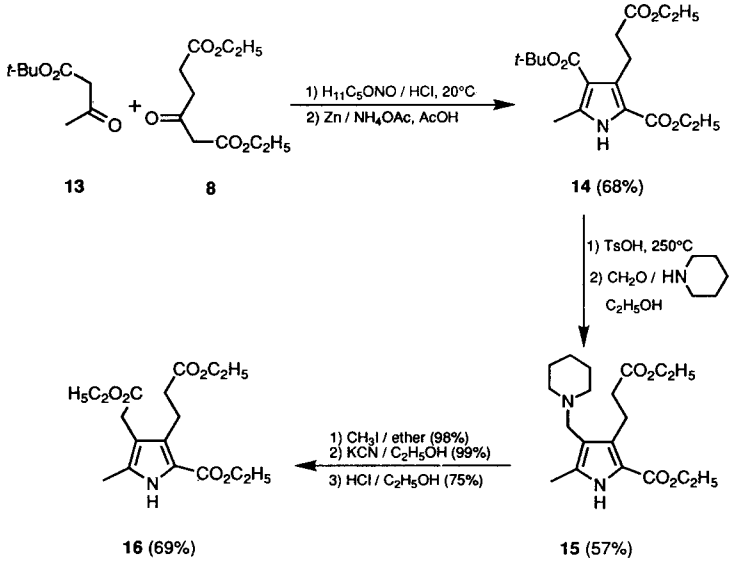
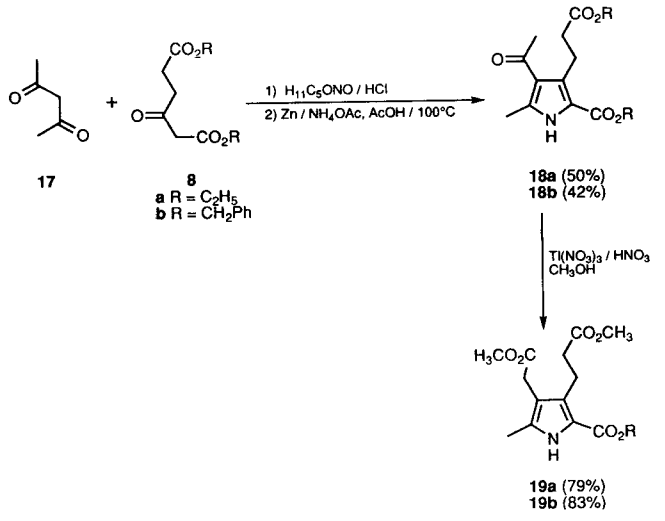


Figure 6



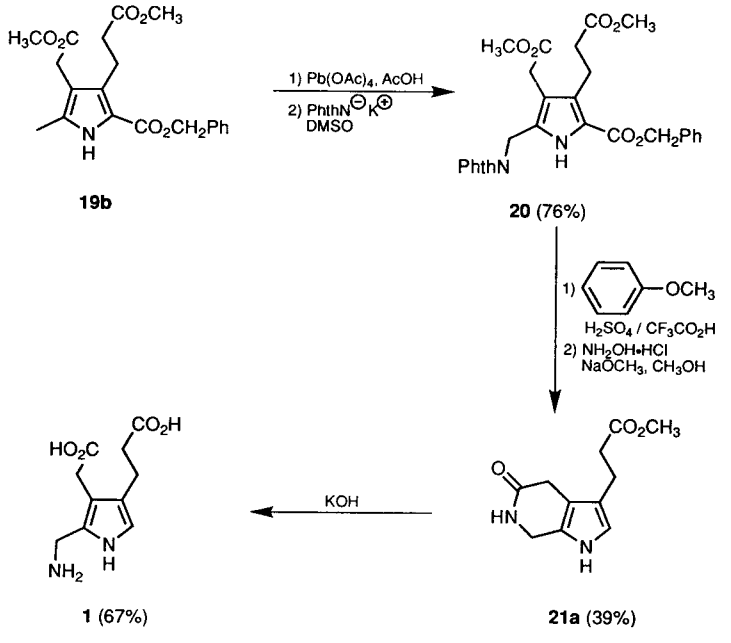
17

8
a R = C_2H_5
b R = CH_2Ph

18a (50%)
18b (42%)

19a (79%)
19b (83%)

Figure 7



19b

20 (76%)

21a (39%)

1 (67%)

Figure 8

precursor lead to good and reproducible yields of porphobilinogen (1).

These general synthetic schemes and slight variants of them were used to obtain porphobilinogen (1) or pyrroles derived from porphobilinogen labelled at different positions [48-50,83,84].

Synthesising the Pyrrole Ring with modified Knorr Reactions

Plieninger [69] and by *Evans* [70] developed an alternative synthesis of porphobilinogen (1). The major advantage of these two synthesis is, that the carbon skeleton is constructed first and only then the pyrrole ring is created.

In *Plieninger*'s synthesis all the carbons necessary for the creation of porphobilinogen (1) are assembled before the creation of the pyrrole ring (see figure 9).

Plieninger [69] synthesised the diester **22** necessary for the *Kleinspehn* version of the *Knorr* synthesis in three steps and in 31.6 % overall yield from commercial starting materials (figure 9) (see also the alternative synthesis of **22** reported in [85]). The pyrrole ring is formed using the diethyl ester of oximinomalonic acid as partner for the *Knorr* synthesis.

Evans synthesis[70] uses the compound obtained by formylation from the commercially available diethyl ester of 4-oxopimelate. This precursor is transformed in two steps into the N-substituted α-free pyrrole ring **24** using the N-dimethoxybenzyl ethyl glycinate as partner (see figure 10).

The yield of this two step pyrrole synthesis is low (20 to 30 %) and the procedure is delicate. Deprotection of the dimethoxybenzyl group is critical and special precautions have to be taken. A mixture of sulphuric acid and trifluoroacetic acid in anisole as a solvent has to be used. The α-free pyrrole is then treated with cyanogen bromide in the presence of zinc chloride to obtain the cyano pyrrole **25** which had already been obtained by another route in *MacDonald*'s group. This precursor **25** can be transformed in 6 steps into porphobilinogen **1** using *MacDonald*'s protocol [76] in a total yield of 6 % for the 6 steps.

Starting from Pyrrole

Anderson and his collaborators [71] decided to start from pyrrole itself and to introduce the side chains afterwards. They developed two synthetic pathways to porphobilinogen (1) starting from pyrrole (see figures 11 and 12). Vilsmeier-Haak acylation of pyrrole immediately followed by Friedel-Crafts type acylation with ethyl oxalyl chloride gave the disubstituted pyrrole **26** in good yield (see figure 11). Decarbonylation catalysed by palladium followed by reduction with Raney nickel allowed to obtain the acetic acid side chain in three steps in 40 % yield compound **27**.

The compound **27** was the key starting material for the two synthetic pathways to porphobilinogen (1) developed in *Anderson*'s group. The pyrrole **27** was treated with chlorosulfonyl isocyanate at -42°C first and then with DMF to give a mixture of the regioisomeric cyano compounds [71]. The mixture had to be separated with medium-pressure liquid chromatography. The pyrrole **28** was iodinated and then protected with the benzoyl

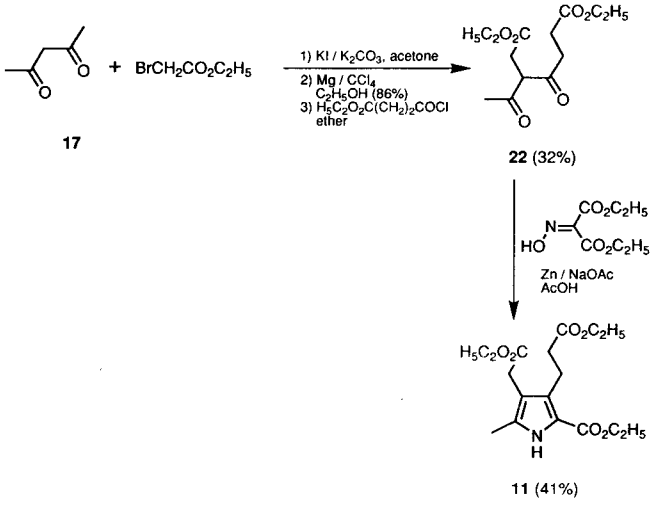


Figure 9

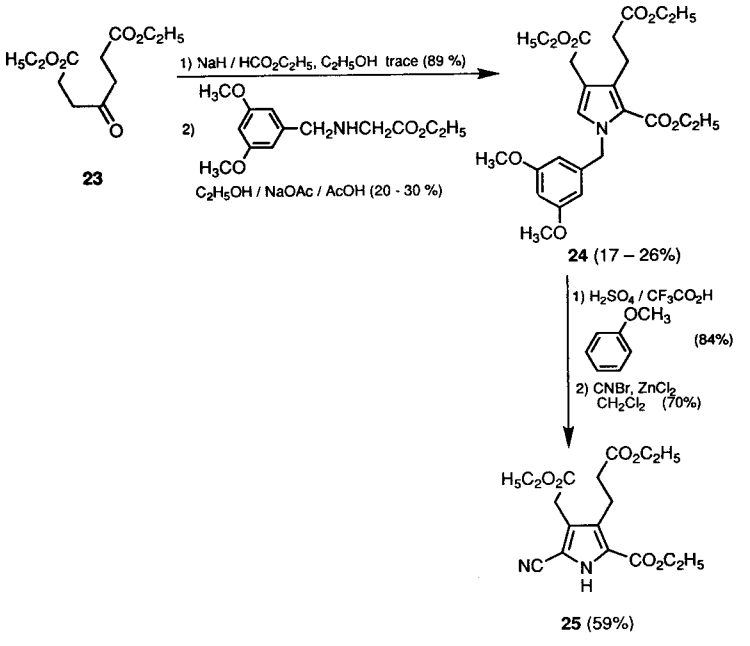


Figure 10

group. **29** reacted with ethyl acrylate in the presence of palladium acetate in a Heck reaction to give the cinnamate **30**. The cinnamate **30** could be transformed into the porphobilinogen lactam-ester and then hydrolysed according to a known procedure developed in *MacDonald*'s group.

Using a Vilsmeier-Haack reaction to introduce the carbon at C2 as an aldehyde was a substantial improvement of *Anderson*'s synthetic procedure (see figure 12) [72]. Iodination gave **31**, which could be separated from its regioisomer.

Repeating the sequence, introduction of the N-benzoyl group and Heck reaction, yielded the corresponding cinnamate partially in the deprotected form. Work-up with aqueous ammonia produced the N-deprotected pyrrole **32** as the only product. The aldehyde was transformed into its oxime. The oxime was reduced in the presence of palladium black in a slightly acidic ethanol solution to give the ammonium salt by simultaneous reduction of the cinnamate and the oxime. The ammonium salt could be transformed into the desired lactam ester **21b** by treatment with sodium ethoxide.

Starting from a Pyridine Ring

Frydman and *Rapoport* [73] reported a different approach for the synthesis of porphobilinogen (**1**). Modified versions of this sequence have been reported later by *Battersby*'s group [49,86]. The key intermediate of this sequence is an azaindoles, which itself is obtained from as pyridone derivative **34** (see figure 13).

The starting material in all three versions of this synthesis is the 4-methyl-5-nitro-2-pyridone which can be obtained in two steps from the 2-amino-4-methylpyridine (**33**). The pyridone is the building block for the lactam ring of porphobilinogen lactam.

In the *Frydman-Rapoport* synthesis [73] the O-methyl ether of the 4-methyl-5-nitro-2-pyridone is acylated with diethyl oxalate to obtain the α-ketoester **34**. Reduction of **34** with palladium on carbon or with zinc in acetic acid gives the key azaindole. Alkylation of the azaindole in a Mannich reaction either with formaldehyde and dimethylamine or with formaldehyde and morpholine yields **35**. Treatment with diethylmalonate followed by hydrolysis yields the diacid 2-pyridone **36**, which contains all the carbon atoms of porphobilinogen (**1**) plus the carboxyl group to protect the α position. Reduction of the pyridone followed by heating the lactam diacid in water gives the porphobilinogen lactam, which is hydrolysed to give porphobilinogen (**1**).

Starting from the pyridone **33** *Battersby*'s group developed a modification of the *Frydman* synthesis (see figure 14) [49,86].

Treating the 4-methyl-5-nitro-2-pyridone under Vilsmeier-Haak conditions (phosphoroxychloride and DMF) yields the compound

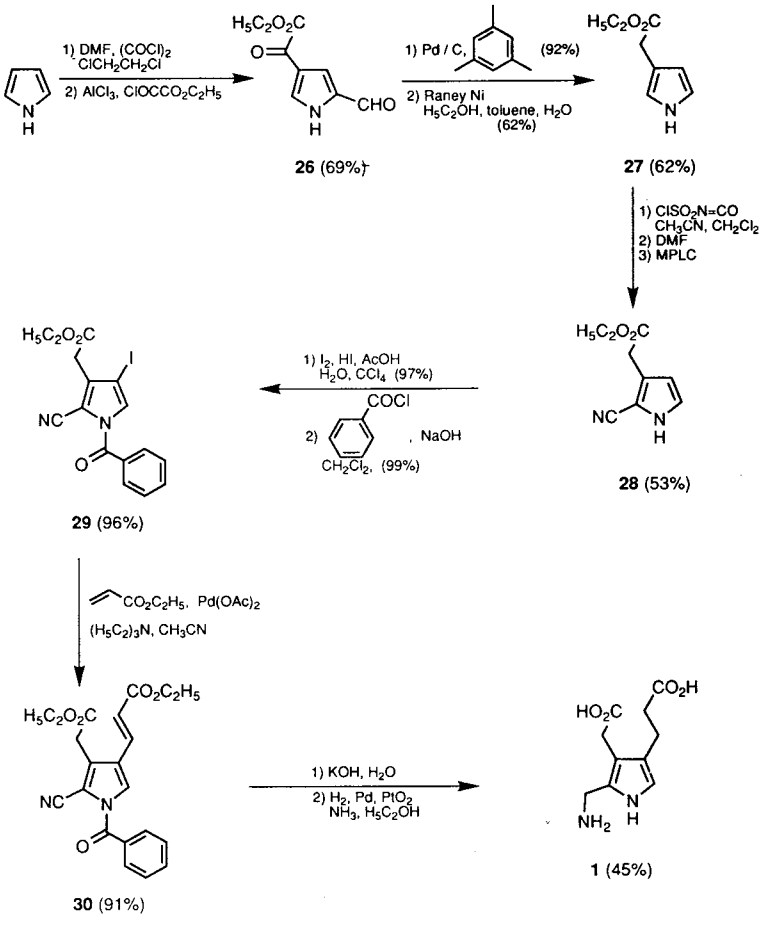


Figure 11

37. Hydrolysis with sodium hydroxide in water acetone first and then treatment with sodium benzyl oxide in benzyl alcohol gave directly the precursor for the azaindole formation. The azaindole **38** was obtained via reduction with zinc in acetic acid. Knoevenagel condensation gave the dibenzyl-protected cinnamate. Palladium catalysed reduction leads directly to the porphobilinogen lactam **21c**.

Synthesising the Pyrrole Ring according to *van Leusen*

Ganem [45] used the procedure developed in the group of *van Leusen* [74] for his synthesis of porphobilinogen (**1**). The motivation for the development of his synthesis was the potential use of porphobilinogen (**1**) or analogues of porphobilinogen in photodynamic therapy. [44] Under optimised conditions the lithium salt of TosMIC was reacted with diethyl glutaconate (**39**) [87,88] at low temperatures to give the 3,4-disubstituted pyrrole **40** in 70 to 80 % yield (see figure 15).

To introduce the missing α-substituent Vilsmeier-Haack conditions were used. The wanted regioisomer was the predominant product (8 : 1). The mixture of the two regioisomers were transformed further without separation. Conversion to the oxime followed by hydrogenation and basic workup gave the

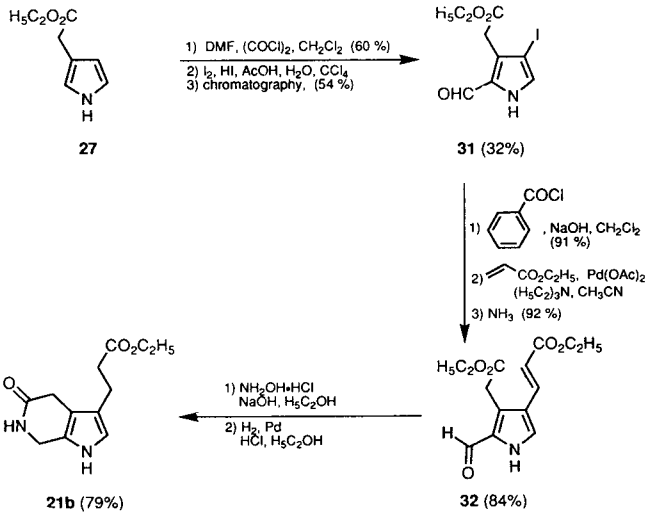


Figure 12

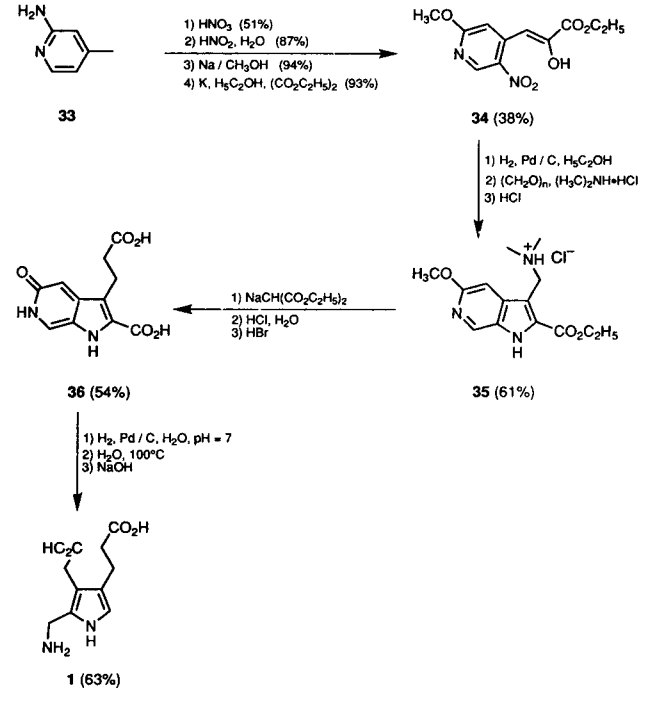


Figure 13

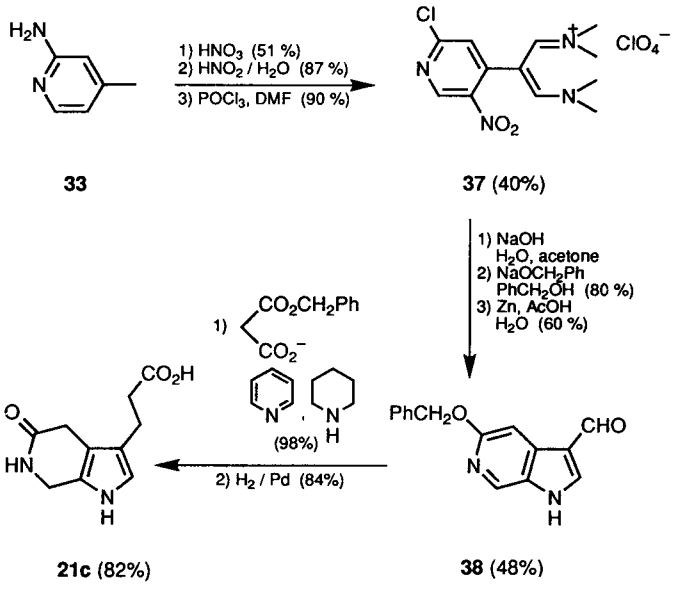


Figure 14

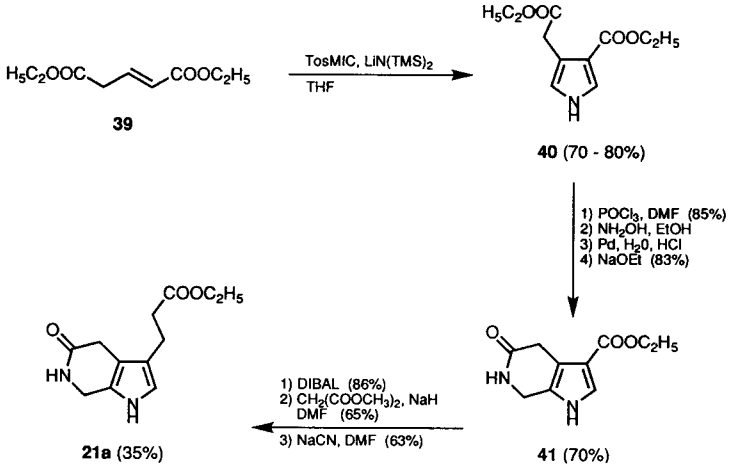


Figure 15

desired lactam **41** in good yield (83 % yield reported presumably starting from the pure regioisomer). To elaborate the propionic acid side chain, the β-ester was reduced with DIBAL in 83 % yield. The hydroxy group could be substituted in an interesting process without any further activation. Heating the alcohol with the sodium salt of dimethyl malonate in DMF the diester could be obtained, which was deesterified and decarboxylated by treatment with NaCN in refluxing DMF. The lactam ester **21a** is a known

intermediate in the synthesis of porphobilinogen (**1**). Starting from the diethyl glutaconate the lactam **39** can be obtained in 17.3 % yield assuming that now loss occurs due to the chromatographic separations of the two regioisomers obtained in the Vilsmeier-Haak formylation. Even assuming that the total yield may be reduced by the chromatography needed this is certainly one of the most efficient synthesis of porphobilinogen (**1**).

Synthesising the Pyrrole Ring in a [3 + 2] Cycloaddition

In view of preparing porphobilinogen like haptens for the preparation of various immunoreagents a new synthesis was developed by *Adamczyk* and his collaborators at the Abbot Laboratories. [42,43,59] *Adamczyk* reported two similar synthetic sequences for porphobilinogen (**1**) itself. [42,60,61] The retrosynthetic strategy uses a formal [3 + 2] cycloaddition of a in situ generated nitro olefin to either a isocyanatoacetate [89] or to isocyanatoacetonitrile. [90,91] This strategy is based on early work in the group of *van Leusen*[74] which was modified by *Barton*'s group. [89,92]

3-Buten-1-ol is transformed in two steps and 44 % into the protected propanal **42**. Condensation of the propanal **42** with the commercially available methyl 4-nitrobutyrate in a Henry reaction gave the hydroxy nitro compound. Acetylation of this nitro compound yielded the desired α-acetoxynitro compound **43** in 50 % yield over the two steps (see figure 16).

The key step of the pyrrole synthesis is the utilisation of either benzyl isocyanoacetate or isocyanoacetonitrile, both of them have to be freshly prepared. [89-91] Using the benzyl isocyanoacetate in excess a 63 % yield of the pyrrole **44a** could be obtained. Cleaving the tetrahydropyranyl ether with pyridinium *p*-toluenesulfonate, followed by Jones oxidation, esterification of the crude acid with diazomethane and finally hydrogenation gave the 2-carboxy-3,4-substituted pyrrole **45** in 26 % yield overall. Activation of the carboxy group with N-hydroxy succinimide followed by reduction of the active ester with sodium borohydride immediately followed by oxidation with manganese (IV) oxide yielded the aldehyde **46** in 18 % yield. From this aldehyde **46** porphobilinogen (**1**) was obtained by treatment with hydoxylamine hydrochloride in methanol followed by hydrogenation using Adam's catalyst and finally basic hydrolysis. The overall yield of this three step sequence was 22 %. The total yield of this fifteen step synthesis from commercial starting material was only 0.14 % (see figure 17). [61]

In the second synthesis a threefold excess of the freshly isocyanoacetonitrile is used in the crucial [3 + 2] cycloaddition (see figure 16). The yield (81 %) of this pyrrole forming step could be considerably improved compared with the first version. [42,60,61] Deprotection of the tetrahydropyranyl ether, oxidation

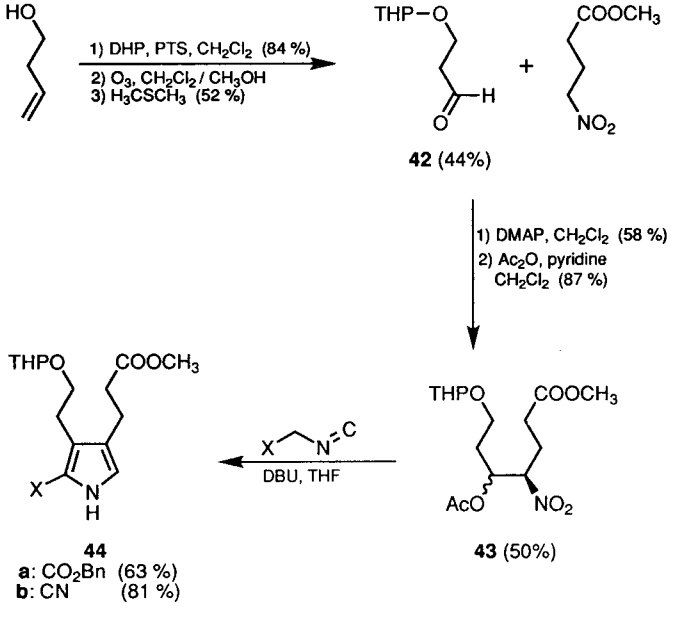


Figure 16

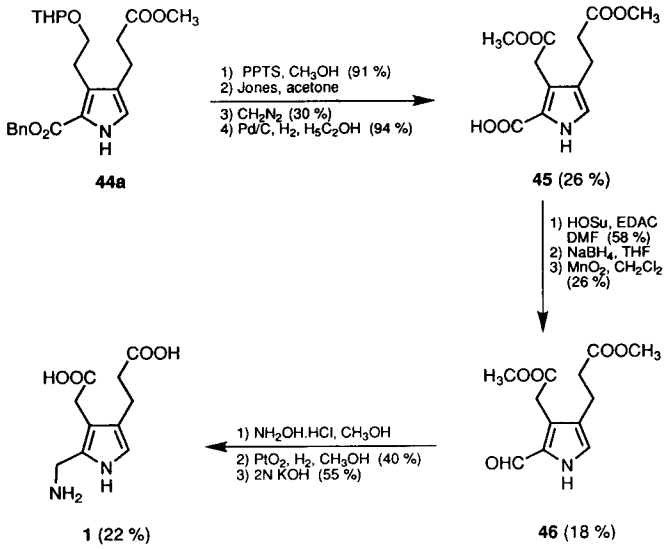


Figure 17

with Jones reagent and esterification with diazomethane gave the 2-cyano pyrrole **47** in 54 % yield over the two steps. Reduction with Pd black-PtO_2 in ammonia saturated ethanol gave the porphobilinogen lactam which could be hydrolysed via a known procedure. This second version is considerably shorter than the first version, only ten steps and the overall yield is 2.7 % starting from 3-buten-1-ol or 6.3 % starting from the nitrobutyrate.

Synthesis of Analogues and Model Compounds

The motivation to synthesise porphobilinogen (1) have changed considerably since the first successful synthesis have been reported in the late fifties and early sixties. Historically the efforts to synthesise porphobilinogen were undertaken in order to prove the structure of this sensitive and reactive molecule.

In the context of studies addressing the questions of the mechanisms of porphobilinogen deaminase and uroporphyrinogen III cosynthetase, analogues of porphobilinogen (1) were synthesised and their incorporation into the tetrapyrroles was studied. In recent years analogues and model compounds of porphobilinogen were synthesised in order to obtain a clearer picture about the chemistry of porphobilinogen and its precursor. [44,62] The other major motivation was the need of porphobilinogen analogues in order to obtain antibodies, which can be used in analytical tests in order to detect lead poisoning. [42,43] Finally derivatives of porphobilinogen have been synthesised recently to model one or the other of the many mechanistic proposals for the PBGS catalysed reaction. [65,93-95]

In order to study the reactivity and the ease of formation of the azafulvenium ion, which may be postulated as intermediate during the formation of the hydroxybilane, model compounds where the amino group has been replaced by a hydroxy group and the hydroxy analogue of porphobilinogen were synthesised. [44,96]

The synthesis recently reported by *Ganem* and collaborators is an example of this approach. [45] The motivation for his synthesis of porphobilinogen (1) is the potential to self-assemble into uroporphinoids. The so-obtained pigments should then be studied in photodynamic therapy.

For the development of an immunoassay for detection of lead poisoning various immunogens for the generation of anti-porphobilinogen antibodies were synthesised. For this project a series of analogues of porphobilinogen and porphobilinogen (1) itself were synthesised. [42,43,59-61] The [3 + 2] cycloaddition methodology was used to obtain a series of derivatives.

In an effort to imitate the mechanism proposed by *Shemin* [97] for the biosynthesis of porphobilinogen a novel methodology for the synthesis of pyrroles has been developed. To imitate the postulated key step, the aldol reaction between the two substrates, the *Mukaiyama* aldol reaction was chosen. [98,99] Starting from levulinic acid the two starting materials **50**, respectively **51** for the aldol reaction, could be easily obtained in two respectively three steps (see figure 19). [63,64,100-102]

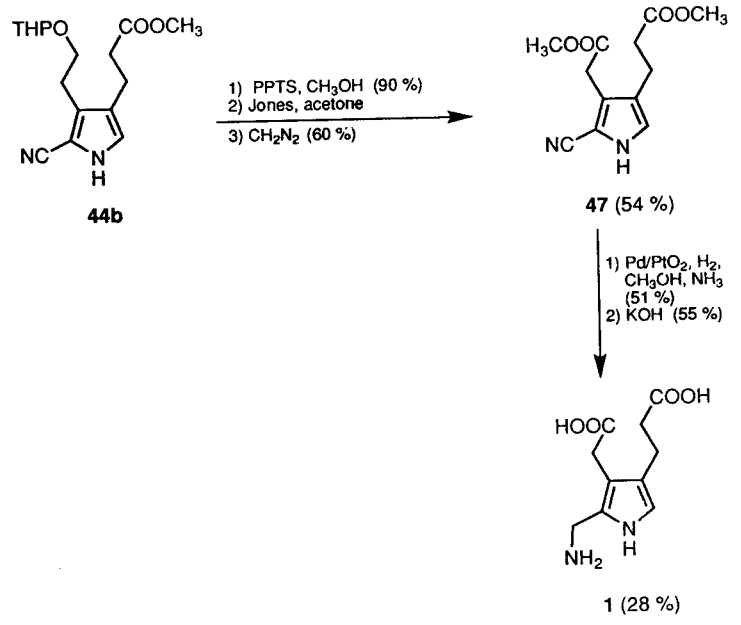


Figure 18

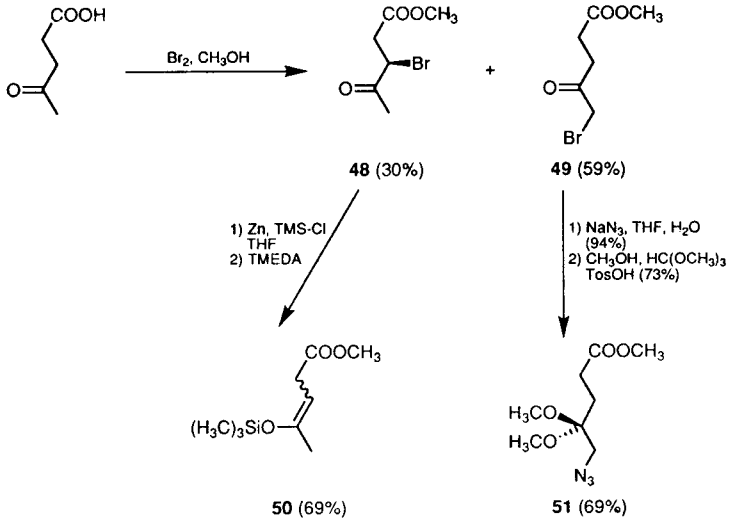


Figure 19

Under the bromination conditions the carboxylic acid was esterified and a combined 89 % yield of the two regioisomeric bromides **48** (30 %) and **49** (59 %) could be obtained. [101,103] The two bromides **48** and **49** can be separated by careful destillation. The 3-bromo compound **48** is reductively silylated to give the silyl enol ether **50** in 69 % yield. The 5-bromo compound **49** is used to obtain the azido acetal **51** in two simple steps and 69 % yield. [64,101] These two starting materials are submitted to the optimised *Mukaiyama* crossed aldol reaction conditions to give the desired compound **52** in 71 % yield as a 2 : 1 mixture of the diastereoisomers (see figure 20). The aldol product could be catalytically reduced with Palladium on charcoal or submitted to the Staudinger reaction. 81 % respectively. 73 % yield of the corresponding pyrrole **53** could be isolated.

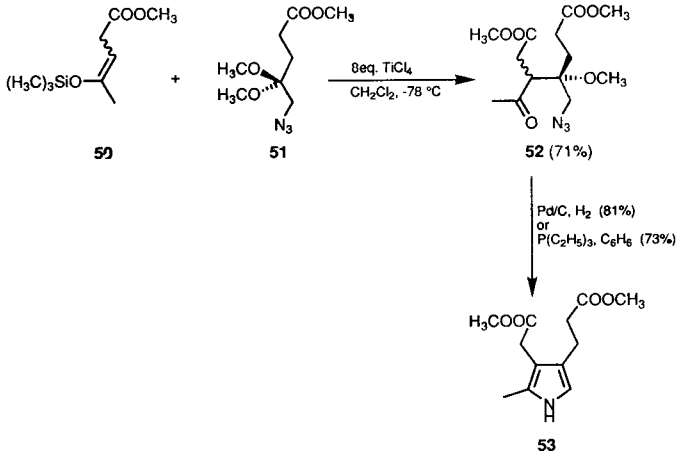


Figure 20

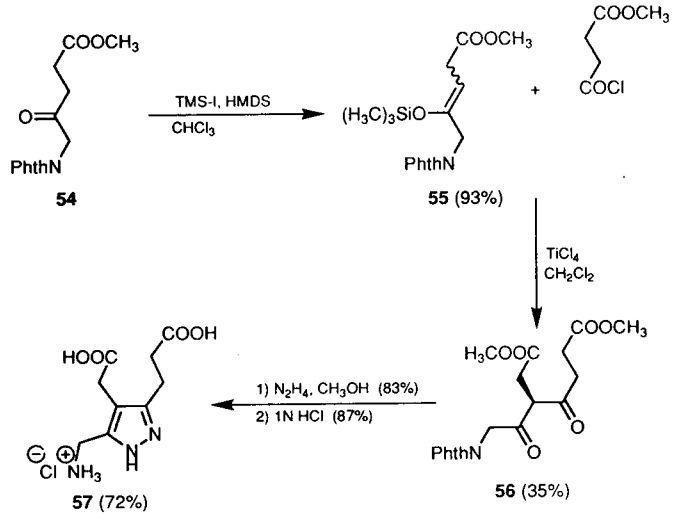


Figure 21

The dicarboxylic acid of **53** had been postulated as product of the enzymatic reaction between levulinic acid and 5-aminolevulinic acid. Comparison of the synthetic and the enzyme formed product proved that the structure postulated by *Shemin* was not correct.[95]

Recently a synthesis of a pyrazole analogue of porphobilinogen has been reported using the same strategy. [65] The silyl enol ether **55** was generated in a regioselective manner from the methyl ester of the phthalimido protected 5-aminolevulinate (**54**) in 93 % (see figure 21).

Coupling this silyl enol ether **55** with the succinic acid monomethyl ester monochloride at -78 °C with 1 equivalent of $TiCl_4$ as catalyst produced the diketone **56** in 35 % yield. Treatment of this diketone **56** with hydrazine at room temperature in methanol gave the fully protected pyrazole in 83 % yield. Hydrolysis with 1N HCl in water at reflux for 24 h allowed to isolate the pyrazole analogue of porphobilinogen **57** in 87 % yield. The pyrazole analogue **57** will be tested as inhibitor of the enzyme porphobilinogendeaminase.

The synthesis of the two model compounds the pyrrole **53** and the pyrazole **57** are designed after the biosynthetic process. In both synthesis the C(3)-C(4) bond is formed first followed by the formation of the N-C(2) bond. All the side chains as well as the functional groups are in place. The only taks which has to be fulfilled after the key pyrrole froming step is the removal of the protecting groups.

COMPARISON OF THE CHEMICAL SYNTHESIS WITH THE BIOSYNTHESIS

Comparing the Strategies of the Chemical Synthesis with the Biosynthesis

The convergent biosynthesis of porphobilinogen (**1**) has attracted the interests of chemists since its discovery. The same starting material is used twice and incorporated in an asymmetric fashion into the final product. Two bonds are formed to connect the two starting materials in a Knorr type synthesis. The pyrrole ring is obtained via a cyclization forming the N-C(2) and the C(3)-C(4) bonds (see figure 22). [97] This elegant biosynthesis is characterised by its high efficiency.

Comparing the chemical synthesis with the biosynthetic process allows to reach interesting conclusions.:

The first synthetic schemes for the synthesis of porphobilinogen (**1**) were developed in the group of *MacDonald* [46,47,68] and later a modified version was reported by *Kenner*'s group (see figures 4, 5, 7 and 8). [50,82] Both approaches use the Knorr synthesis as key step. The retrosynthetic analysis of these early synthesis is in close analogy to the biosynthetic process. The N-C(2) and C(3)-C(4) bonds are formed. Unfortunately a relatively lengthy procedure is necessary to obtain the correct functionalised side chains. In *MacDonald*'s synthesis eight steps have to be executed after the Knorr pyrrole synthesis and therefore the total yield is 6,3 % starting from commercial starting material. In *Kenner*'s version [50,82] all the carbon atom are already present after the Knorr synthesis but still six steps are necessary to obtain porphobilinogen (**1**) in a slightly improved overall yield of 6.9 %. The synthesis reported by *Plieninger* [69] and *Evans* [70] use an alternative [3 + 2] cyclization scheme (see figure 4, 9 and 10). In this process the N-C(2) and the C(4)-C(5) bonds are formed in the key step. The essential parts of the carbon skeleton, with the exception of the N-C(5) piece, are assembled before the pyrrole synthesis. The yield of the Kleinspehn variant of the Knorr synthesis is only moderate, so that the overall yields of these two synthesis are not satisfactory. *Plieninger*'s synthesis allows to obtain porphobilinogen in nine steps in 2.6 % overall yield, whereas the synthetic sequence developed in *Evans*'s group gives only a 0.9 % overall yield.

The strategy developed in *Anderson*'s group is completely different (see figure4, 11 and 12). [71,72] The "naked" pyrrole is the starting material and the side chains have to be introduced one by one. None of the bonds of the pyrrole ring have to be formed, but all of the side chains have to be introduced during the synthesis. In the first synthesis eleven steps are needed to selectively add the side chains in an 8.9 % overall yield. In the second variant [72] the same number of steps are necessary but the overall yield could be improved to 9.2 %.

In the *Frydman Rappoport* synthesis [73] of porphobilinogen (**1**) the pyridine ring is used as a masked equivalent of 5-amino levulinic acid (see figure 4, 13 and 14). The starting pyridine ring contains already C(4) of the final product. Therefore C(5) has to be introduced and then the bonds C(4)-C(5) and N-C(5) are formed sequentially. After the formation of the pyrrole ring the propionic acid side chain has to be added and the oxidation state of the acetic acid side chain and the methylamino group have to be adjusted. Overall 13 steps are necessary. Despite the length of the synthesis the overall yield was 7.9 %. In *Battersby*'s version, [49,104], which uses the same retrosynthetic analysis, the number of steps was considerably reduced. Only nine steps are necessary and the overall yield reported is 12.9 % (see figure 22).

The synthesis reported by *Ganem* [45] forms the pyrrole ring in a highly efficient way using *van Leusen*'s TosMIC methodology (see figure 22). [74] From the retrosynthetic standpoint this is still another [3 + 2] cyclisation scheme. In the key step the bonds C(2)-C(3) and C(5)-C(4) are formed. The pyrrole lacks the α-substituent and the propionic acid side chain is missing also. The α-substituent is introduced via a Vilsmeier-Haak formulation which is unfortunately not completely regioselective. The elaboration of the propionic acid side chain takes profit of an interesting elimination-addition process using dimethyl malonate as nucleophile. Starting from the diethyl glutaconate and assuming

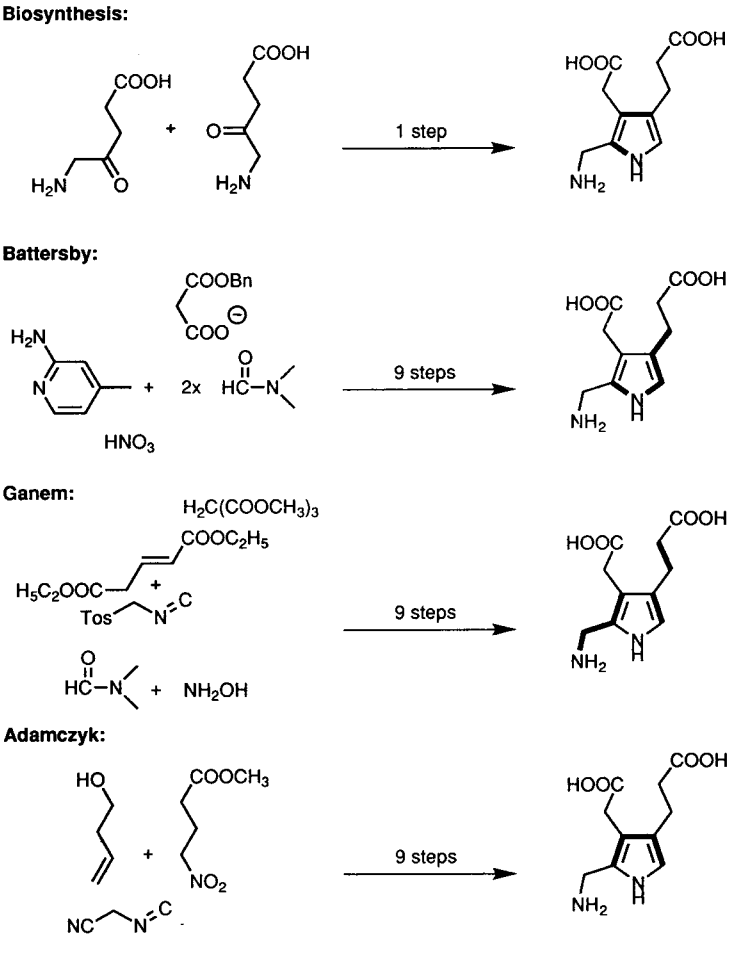


Figure 22

no losses due to the separation of the regioisomers obtained during the Vilsmeier-Haack formylation porphobilinogen (1) is obtained in nine steps and with 11.6 % overall yield.

The *Adamczyk*'s synthesis [42,43,60,61] takes profit of the same [3 + 2] cycloaddition scheme as the synthesis reported by *Ganem*'s group (see figure 22). [45] In *Adamczyk*'s synthesis the two β-side chains are constructed before the cycloaddition process, whereas *Ganem* introduces the propionic acid side chain late in the synthesis. Only the α-substituent had to be added after the formation of the pyrrole ring. Starting with the commercially available methyl 4-nitrobutyrate *Adamczyk* and his collaborators could synthesise porphobilinogen (1) in seven steps and 6.3 % overall yield.

Of the six reported retrosynthetic schemes for the synthesis of porphobilinogen (1) only one uses the biosynthetic Knorr type cyclisation. [46,47,50,68,82] Unfortunately the efficiency of this synthesis suffers from the need to degrade and to build up certain side chains in order to obtain the natural product. The two most recent synthesis use the procedure developed by *van Leusen* to construct the pyrrole ring. [42,43,45,60,61] The overall yield of these synthesis are in a similar range as the yields reported for the *Frydman Rappoport* synthesis. [49,73,104]

Conclusions

The synthesis of porphobilinogen (1) a molecule which is

produced by nature in quantities largely exceeding 10^{10} tons per year has been synthesised several times. The motivations to synthesise porphobilinogen (**1**) has changed. Despite the considerable progress in synthetic methodology the progress in the field of porphobilinogen synthesis has been relatively slow. The three shortest chemical synthesis (*Battersby*, *Ganem* and *Adamczyk*) need nine steps from commercially available starting materials. It is interesting to compare the bonds formed during these chemical synthesis with the bonds formed during biosynthesis (see figure 22). The synthesis with the best reported overall yield was published by *Battersby*'s group and allows to synthesise porphobilinogen in 12.9 % overall yield. The overall yield of *Ganem*'s synthesis is 11.6 % assuming no losses due to the separation of the regioisomers. As has been stated almost twenty years ago in many cases the enzymatic synthesis of porphobilinogen (**1**) is still the best solution. [66] The elegance and the efficiency of the biosynthetic process, which forms porphobilinogen (**1**) from 2 molecules of 5-aminolevulinate in essentially quantitative yield still merits our admiration and stays as a challenge for the synthetic chemist.

Acknowledgement

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