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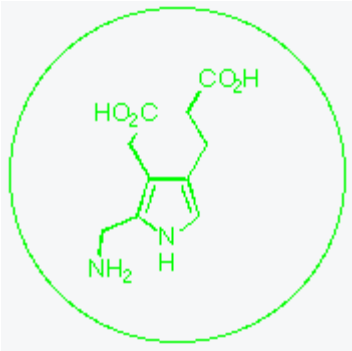
[cl002]

A Chemical Synthesis of Porphobilinogen Imitating the Pathway Proposed by Shemin for the Biosynthesis: Comparing Inhibition Studies with Investigations of Chemical Reactivity



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Abstract

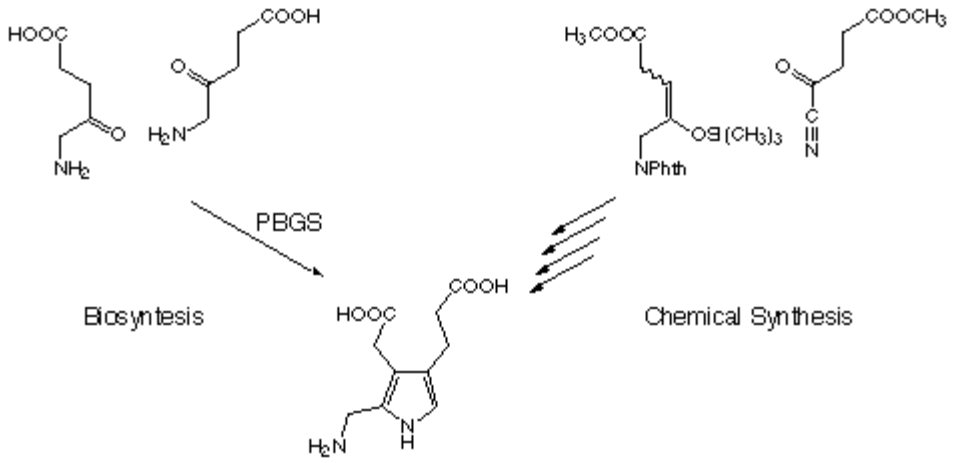

Figure: Biosynthesis compared to the biomimetic synthesis of porphobilinogen

Nature's pathways have been a strong motivation for synthetic chemists. Trying to imitate what has been shown or what is supposed to be a biosynthetic transformation always pursues several objectives: One evident goal is to obtain the natural product via the elegant way used in the natural process. The second not less important goal is to obtain insight into the mechanistic details of the biosynthetic pathway. Finally during the synthetic effort compounds are obtained which are potentially interesting as inhibitors of the natural process. Porphobilinogen, the second dedicated intermediate in the biosynthesis of ´pigments of life[a] was synthesised following the mechanistic rationale proposed 30 years ago by Shemin for the biosynthesis. Substances obtained during this synthetic effort and inhibitors especially synthesised for our studies were tested in order to evaluate the different mechanistic proposals. This biomimetic approach allowed to guide at the same time the synthetic as well as the biosynthetic studies.

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Next chapter: Introduction

Introduction

Importance of the ´pigments of life[a]

The studies of the tetrapyrrolic dyes, which have been called the ´pigments of life[a][1] has attracted the attention of chemists and biologists since their discovery. The importance of the ´pigments of life[a] as crucial cofactors for processes like photosynthesis, oxygen transport, oxidation processes, methane synthesis and for a series of unusual rearrangements illustrates well the central role played by this class of natural products 1 - 4 (see figure 1).[2]

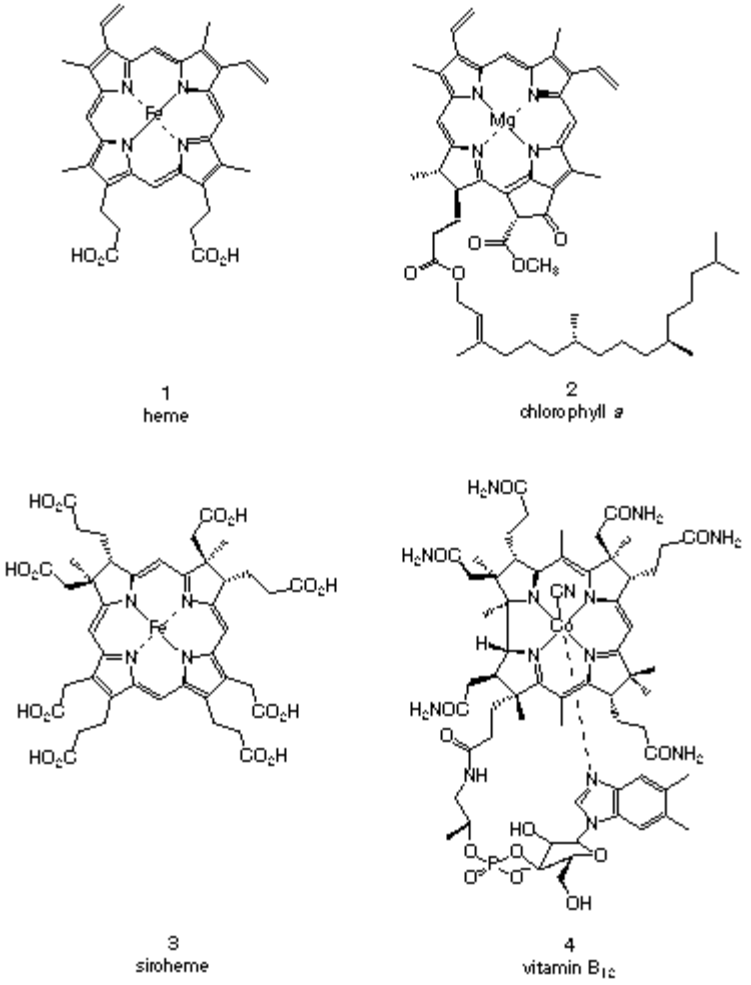
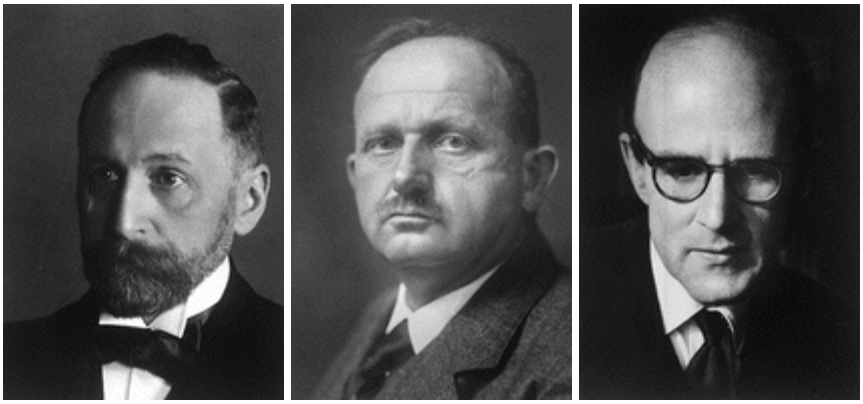


Figure 1: Some important "pigments of life"

Over a period of about 70 years at least six Nobel prices were awarded in Chemistry alone for research obtained working on problems related to the tetrapyrrolic natural products(R. Willstätter,H. Fischer, M.F. Perutz , J.C. Kendrew,

D. Crowfoot-Hodgkin, R.B. Woodward, J. Deisenhofer, R. Huber, H. Michel; see figure 2).

R. Willstätter (1915) H. Fischer (1930) M.F. Perutz (1962)



J.C. Kendrew (1962) D. Crowfoot-Hodgkin (1964) R.B. Woodward (1965)

J. Deisenhofer (1988) R. Huber (1988) H. Michel (1988)

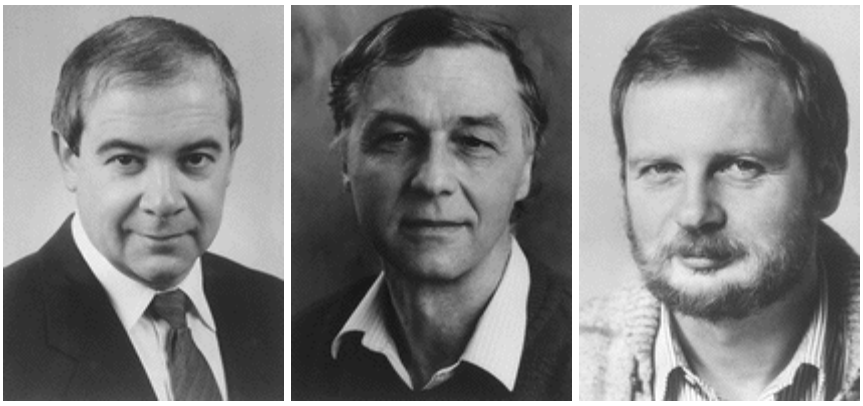


Figure 2: The Nobel price winners R. Willstätter,H. Fischer, M.F. Perutz , J.C. Kendrew, D. Crowfoot-Hodgkin, R.B. Woodward, J. Deisenhofer, R. Huber, H. Michel

Monopyrroles as natural products

Relatively few monopyrrolic natural products have been reported in the literature [3-5]. Most of these natural monopyrroles are stabilised by an electron-withdrawing substituent or by an aromatic ring (see figure 3). Without these substituents the electron rich pyrrole ring is easily polymerised or autooxidised.

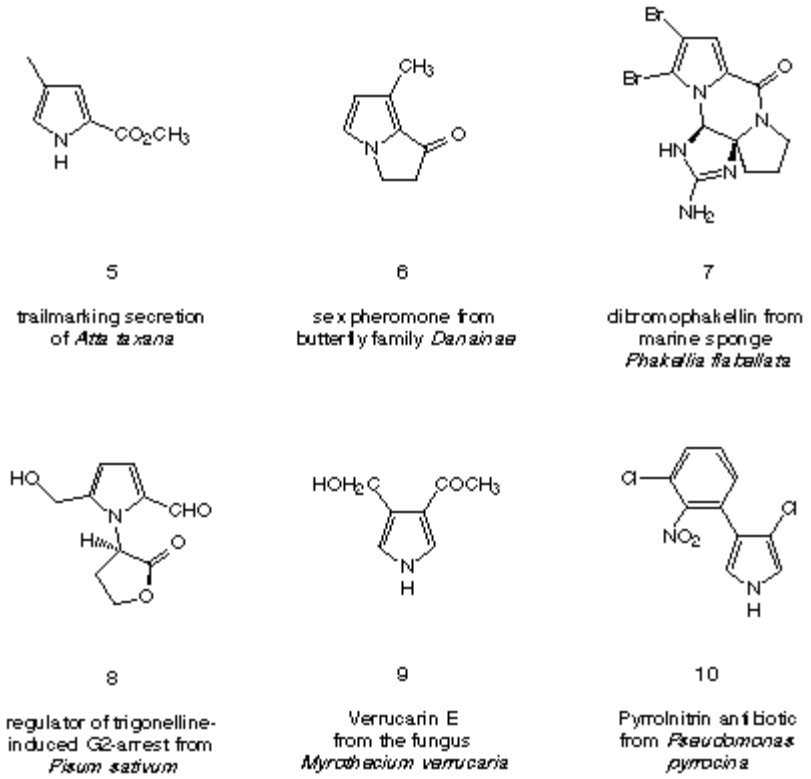
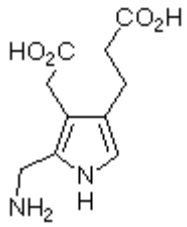


Figure 3: Monopyrrolic natural products.

Their biological functions are as varied as are their structures **5**-**10**.[6-8] Some natural pyrroles are pheromones **6**,[9,10] plant hormones **8** [11] or can be used as antibiotics **10**.[12]

Porphobilinogen (= PBG **11**) a trialkylsubstitued pyrrole is a remarkable exception to this rule (see figure 4). The lack of stabilising substituents confers a high reactivity to porphobilinogen. The biosynthesis of the tetrapyrrolic ´pigments of life[a] 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.[13-16]



11

Porphobilinogen

Figure 4: Porphobilinogen.

Porphobilinogen is the second dedicated intermediate in the biosynthesis of tetrapyrroles.[17-20]

Biosynthesis of porphobilinogen

The tetrapyrrolic skeleton of all ´pigments of life[a] is synthesised in a highly convergent way, starting with 8 molecules of 5-aminolevulinic acid (**12**), 5-aminolevulinic acid (**12**) is then condensed to porphobilinogen (**11**), which itself tetrameroidises to form uroporphyrinogen III (**13**) (see figure 5).

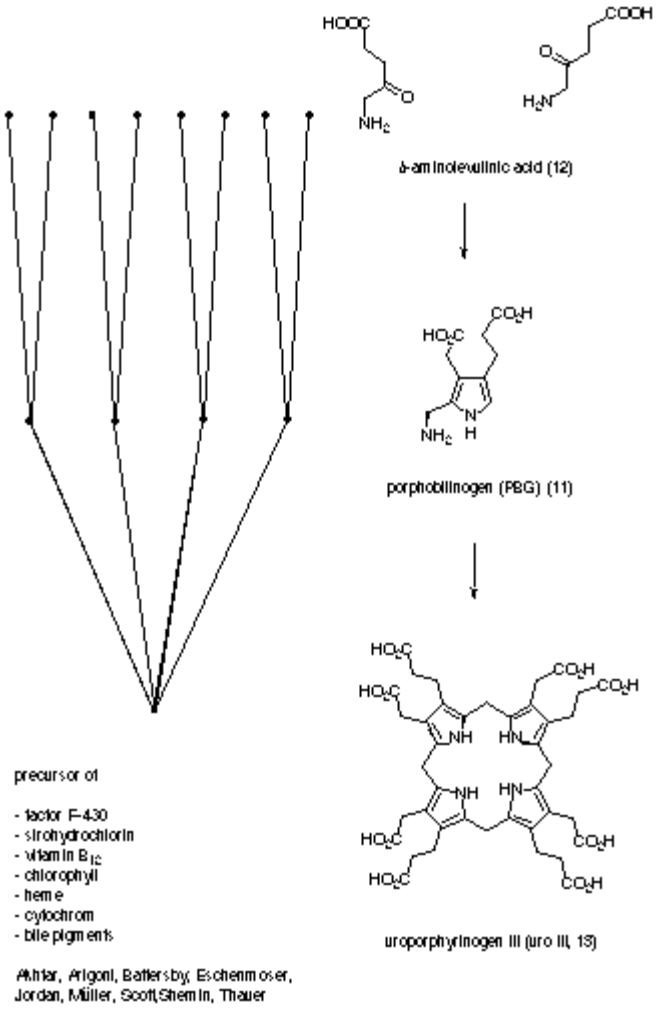


Figure 5: Biosynthesis of Uroporphyrinogen III

The tetrameroidisation [21] of porphobilinogen (**11**) could be achieved without the help of an enzyme (see figure 10).[22,23] Porphobilinogen (**11**) has a strong tendency to form the uroporphyrinogens. The chemical reactivity of porphobilinogen (**11**) 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.[24]

Already early studies of the reactivity showed that tetrameroidzation of PBG **11** could be achieved easily even without the help of an enzyme (see figure 6).[22,23]

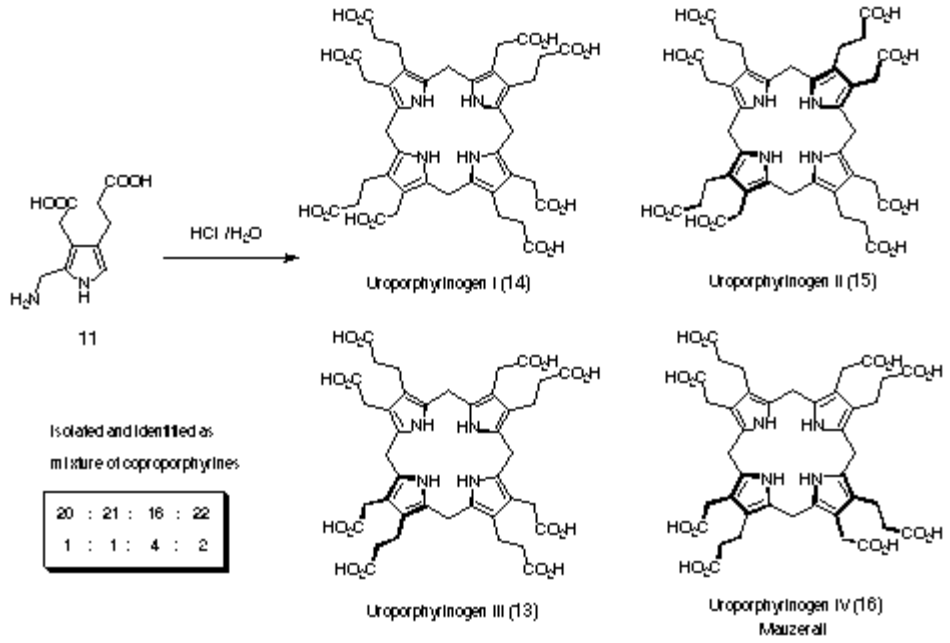


Figure 6: Tetrameroidization of PBG (**11**)

These observations immediately raise the question of the mechanism for the transformation catalysed by porphobilinogen synthase (=PBGS) and of the comparison between the enzyme catalysed mechanism and its chemical analogue the Knorr pyrrole synthesis (see figure 7).

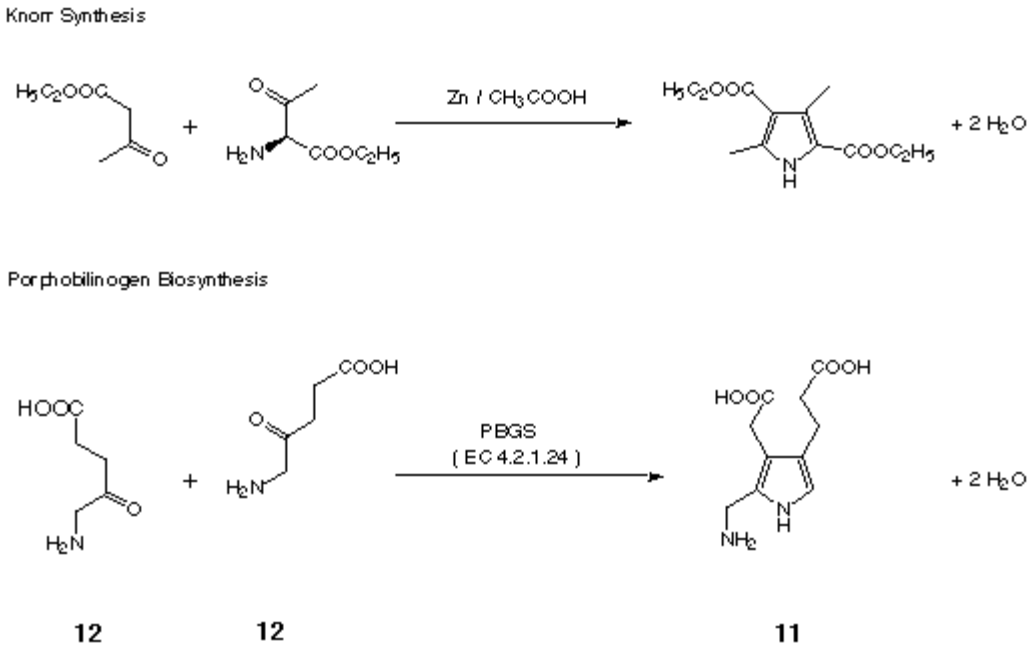


Figure 7: Comparison between Knorr pyrrole synthesis and porphobilinogen biosynthesis.

For the dimeroidisation of 5-aminolevulinate to PBG **11** a DG = -16.9 kcal/mol and for the tetrameroidisation of PBG **11** to uroporphyrinogen a DG = -34.6 kcal/mol were calculated for the gas phase reactions.[25] The biosynthesis of tetrapyrroles liberates free energy.[25,26] This observations were taken as arguments in favour of a spontaneous formation of tetrapyrroles.[27]

The importance of the ´pigments of life[a] and the elegance of the biosynthetic pathway was a strong motivation to develop chemical synthesis of porphobilinogen (**11**). 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 [28-30].

From a mechanistic point of view there exists one big difference between the two processes. The tetrameroidisation can easily be achieved in the absence of an enzyme, whereas it was difficult to realise the dimeroidisation in a reaction vessel without the enzyme.

Previous synthesis of porphobilinogen

The synthesis of PBG **11** has attracted the attention of the chemists for different reasons: In the beginning the synthetic efforts were undertaken to prove the structure;[26,31] afterwards the interest was mainly focused on the synthesis of PBG **11** labelled at a specific positions in order to use it in the studies of the biosynthesis. Labelled and unlabelled PBG **11** or precursors thereof were used in the synthesis of pyrromethanes, tripyrrenes, bilanes and porphyrinogens.[32,33] Despite an exorbitant price which is almost three factors of 10 higher than the price for gold[34] there have been only a limited number of fundamentally different approaches to PBG **11** or to analogues of porphobilinogen been reported in the literature.[35-37]

Six synthetic strategies have been used for the synthesis of PBG **11** (see figure 8).

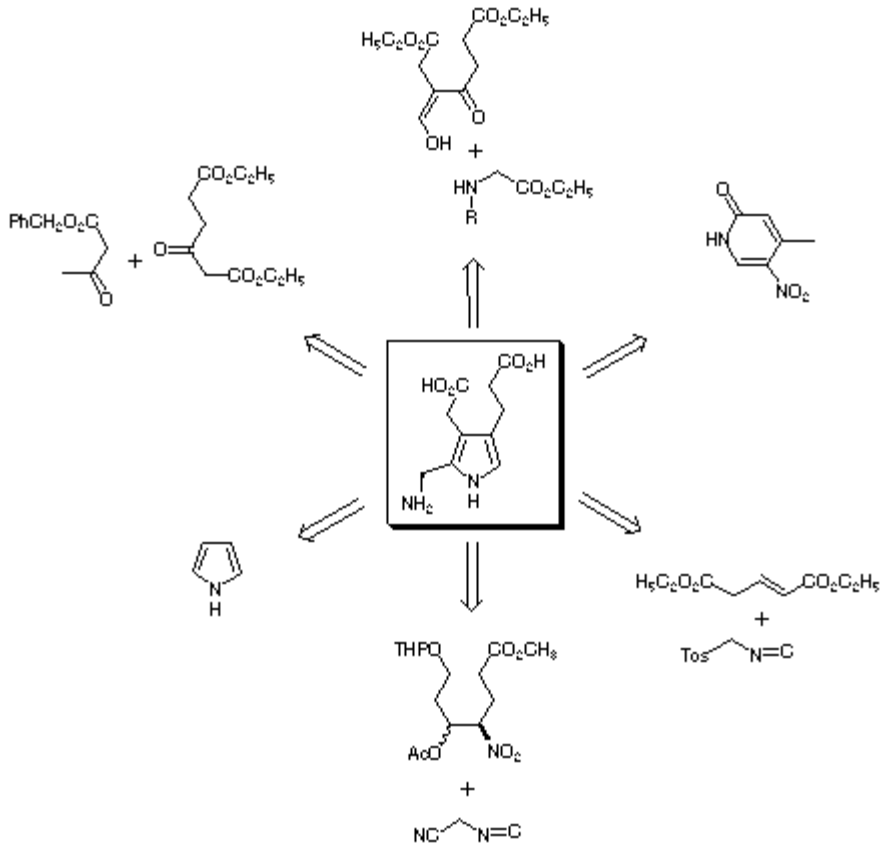


Figure 8: Synthetic strategies for the synthesis of porphobilinogen.

The first and historically the oldest strategy uses a classic Knorr synthesis to obtain a suitable precursor. To obtain the correct substitution pattern the group of MacDonald has invested a considerable amount of work into the modification of the side chains obtained directly from the Knorr synthesis.[38]

In the second strategy developed by the groups of Plieninger[39] and of Evans[40] the pyrrole ring is formed by condensation of a C3-unit with a C-N-unit. In one case the variant of Kleinspehn of the Knorr synthesis is used, whereas in the second case the ring closure is achieved in a stepwise fashion. In this strategy both the acetic acid and the propionic acid side chains are in place right from the beginning.

The third strategy is due to Frydman and Rapoport.[41] They started with a pyridine derivative, which they successfully transformed into an suitably substituted azaindole. Hydrogenation of the azaindole led to the porphobilinogen lactam, which could be hydrolysed to PBG **11**.

The forth strategy stems from Anderson and collaborators, who started with the unsubstituted pyrrole.[42,43] Introducing step by step the acetic acid side chain in the b-position, the nitrile group as precursor of the methylamino group in the a-position and the propionic acid side chain in the b'-position finally gave PBG **11**.

The fifth strategy was developed by the group of Adamckzyk and was published recently.[29,44-46] The condensation of an a-acetoxynitro compound with benzyl isocyanoacetate is used to construct the scaffold of PBG.

Ganem reported a synthesis of PBG **11** based on the methodology developed by van Leusen.[47,48] The addition of TosMIC to an a,b-unsaturated ester gave an advanced pyrrolic precursor of prophobilinogen (**11**). Vilsmeier-Haack formulation introduced the missing a-substituent and finally the propionic acid side chain had to be elaborated.

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Next chapter: Development of a potentially biomimetic methodology

References

Development of a potentially biomimetic methodology

General remarks and comparison of the Shemin mechanism with the Knorr pyrrole synthesis

Concentrating only on the step connecting the two substrate molecules via a covalent bond for the first time, there have been three mechanisms postulated so far for the biosynthesis of porphobilinogen. In two of these mechanisms the central decisive step for the building of the pyrrole ring is the formation of the carbon-carbon bond between C3 of one 5-aminolevulinic acid (12) reacting as a nucleophile and the keto function of the other 5-aminolevulinic acid. It was assumed that the nucleophilic 5-aminolevulinic acid partner is bound to the enzyme as an enamine. One would expect that the formation of the central carbon-carbon bond should be slowest and therefore the rate determining step of the biosynthetic sequence. This sequence is in remarkable contrast to the mechanism of the Knorr pyrrole synthesis (see figure 9).[36,49-51]

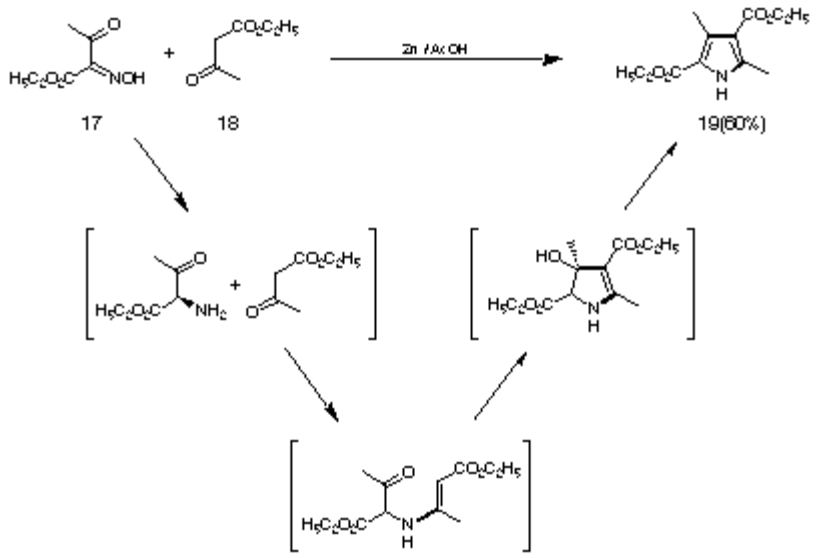

Figure 9: Mechanism for the Knorr pyrrole synthesis.

For the Knorr pyrrole synthesis the steps leading to the pyrrole nucleus are the same, but the sequence is clearly different from the Shemin mechanism for the biosynthesis. In the Knorr synthesis the carbon-nitrogen bond is formed first and the aldol-like carbon-carbon bond forming reaction is therefore an intramolecular process.

First generation of the novel pyrrole synthesis

Following the mechanistic reasoning first proposed by Shemin, one can ask the question if pyrroles can be synthesised using the same sequence of transformations and if it will finally be possible to synthesise even porphobilinogen (**11**) in a biomimetic way using such a methodology?

The Mukaiyama crossed aldol reaction seemed to be ideally suited for our trials to obtain the crucial carbon-carbon bond.[52]

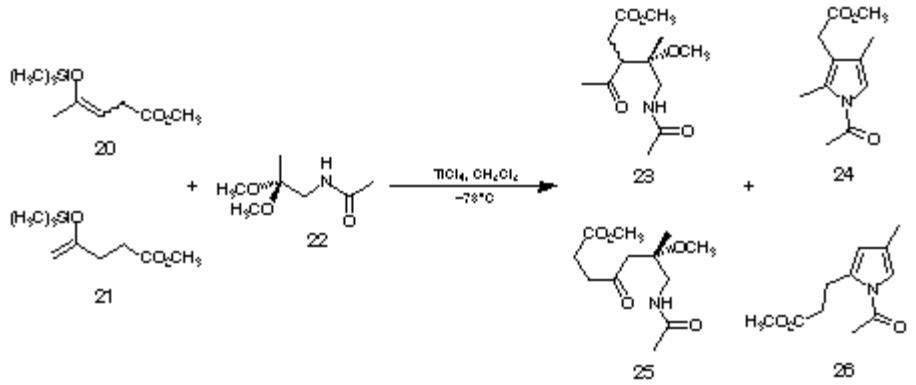

Figure 10: Novel pyrrole synthesis using acetamino acetone (**22**).

Applying the Mukaiyama conditions to the carbon-carbon bond formation between the silyl enol ethers of levulinic acid methyl ester **20** and **21** and the acetal of acetamino acetone (**22**),[53] we were able to isolate small quantities of pyrrole **24** already in our first trials (see figure 10). Optimising the reaction conditions we could isolate up to 48% of the pyrrole **24**. A large excess of titanium tetrachloride was necessary to obtain good yields of the product.

Trials to use this new pyrrole synthesis for the formation of other pyrroles met with mixed success (see figure 11).[53]

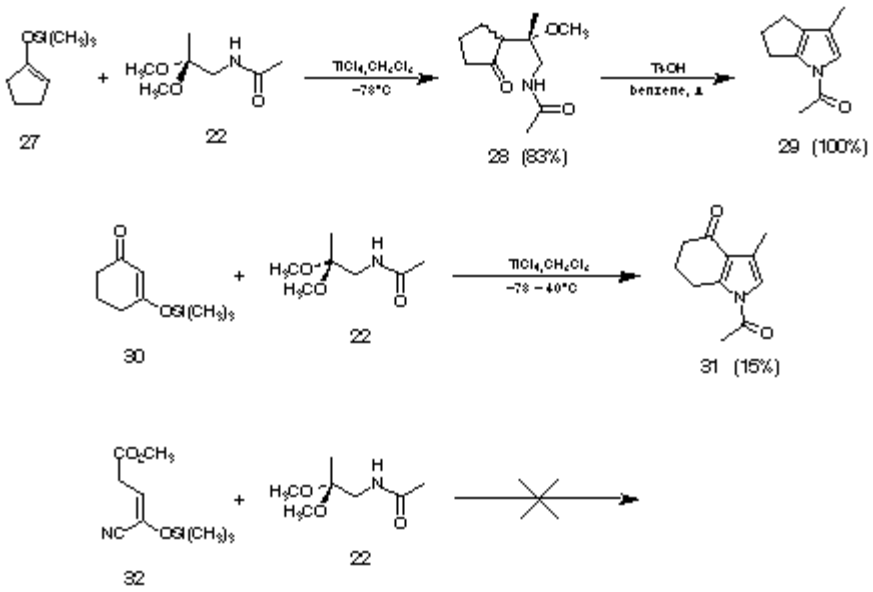

Figure 11: Novel pyrrole synthesis.

Treating the silyl enol ether of cyclopentanone (**27**) with the acetal of acetamino acetone (**22**) gave the mixture of the diastereoisomeric aldol products **28** in 83% yield. These aldol products **28** could be quantitatively transformed into the annelated pyrrole **29** using benzene as solvent and p-toluene sulphonic acid as catalyst. Using deactivated silyl enol ethers like the silyl enol ether from 1,3-cyclohexadione (**30**) the reaction conditions had to be much harsher and still the yield of the pyrrole **31** was disappointingly low. Using the silyl enol ether deactivated by the cyano group **32** we were unable to isolate any pyrrolic product.

Second generation of the novel pyrrole synthesis

We decided to study two ways to improve the crossed aldol reaction:

1) synthesising the pure regioisomers of the silyl enol ethers;

2) replacing the amide function by another protecting group, compatible with the crossed-aldol reaction.

For the synthesis of the isomerically pure silyl enol ether we developed a modification of the procedure of Rubottom[54] for the reductive silylation of the corresponding bromoketone. The reductive silylation had to be carried out in the absence of a base. To avoid problems during work-up the zinc salts had to be precipitated using TMEDA to complex the zinc salts and then adding pentane. As long as most of the zinc salts could be removed by filtration the silyl enol ether **20** could be distilled.

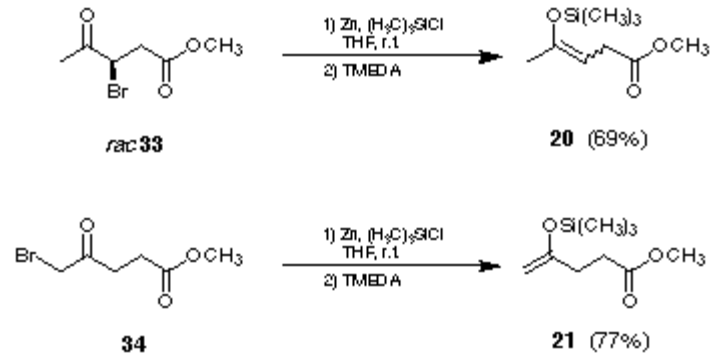

Figure 12: Regioselective synthesis of the silyl enol ethers **20** and **21**.

To synthesise the regioisomeric silyl enol ether **21** the 5-bromo levulinic acid methyl ester (**34**) was treated according to the same procedure and a 77% yield of the silyl enol ether **21** could be isolated (see figure 12).

Instead of the amide protecting group, we decided to use the azido group. Already in our first preliminary trials with the mixture of the silyl enol ethers we were able to isolate the aldol products (see figure 13).[55]

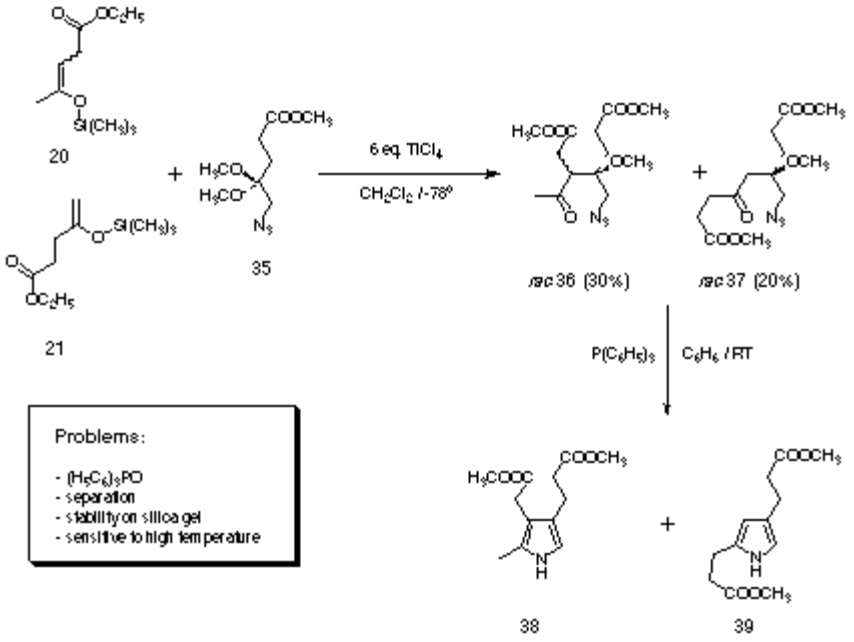

Figure 13: The crossed-aldol reaction followed by the Staudinger reaction applied to the synthesis of the pyrroles **38** and **39**.

The aldol products were treated with triphenylphosphine in benzene to induce a Staudinger reaction.[56] We were able to isolate the pyrroles formed **38** and **39**. The separation of the triphenylphosphineoxide from the alkylpyrroles **38** and **39** was delicate and therefore the yields were not satisfactory. The use of the regioisomerically pure silyl enol ethers **20** and **21** considerably improved the yield of the aldol process (see figure 14).[55]

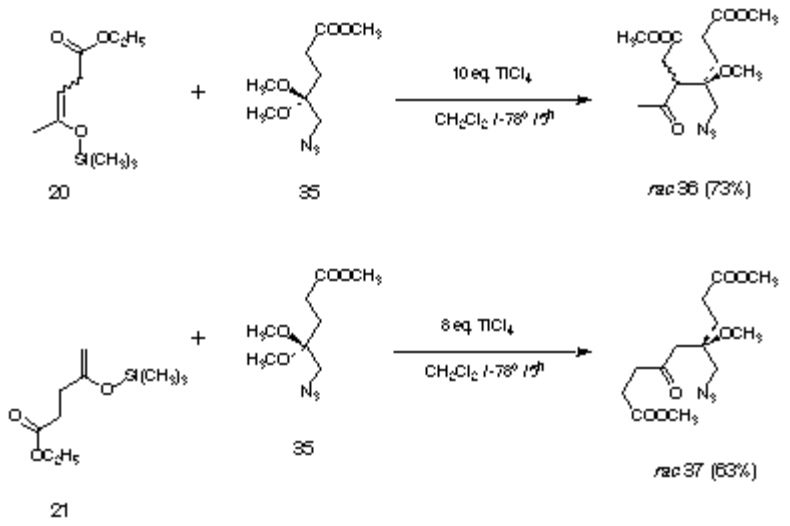

Figure 14: The crossed-aldol reaction using the pure silyl enol ethers **20** and **21**.

Replacing the triphenylphosphine by triethylphosphine, the water soluble triethylphosphine oxide is formed which can be easily removed by extraction (see figure 15).

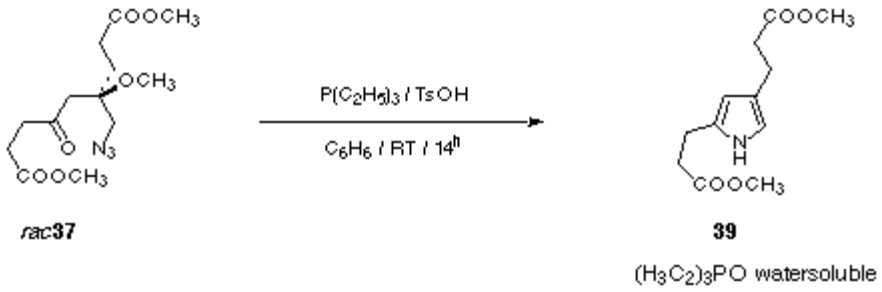

Figure 15: Modified Staudinger reaction.

Catalytic reduction is another mild method to transform the azido group into the corresponding amine. Using palladium on charcoal as catalyst and methanol as solvent the aldol products could be reduced (see figure 16). The amino ketone formed spontaneously the corresponding pyrrole. The work-up using these conditions was very convenient.

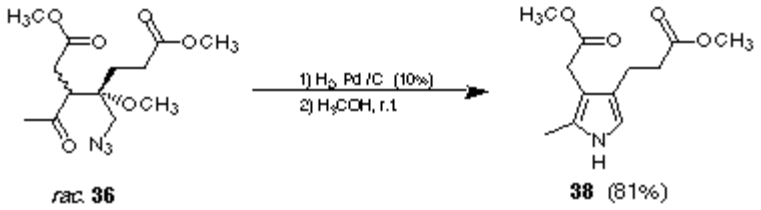

Figure 16: Catalytic reduction

The new two-step pyrrol synthesis allows to synthesise mono-, di-, tri- and tetraalkylpyrroles in good yield (see figure 17). The yield of pyrrole obtained by reduction was extremely low for the annelated products.[55]

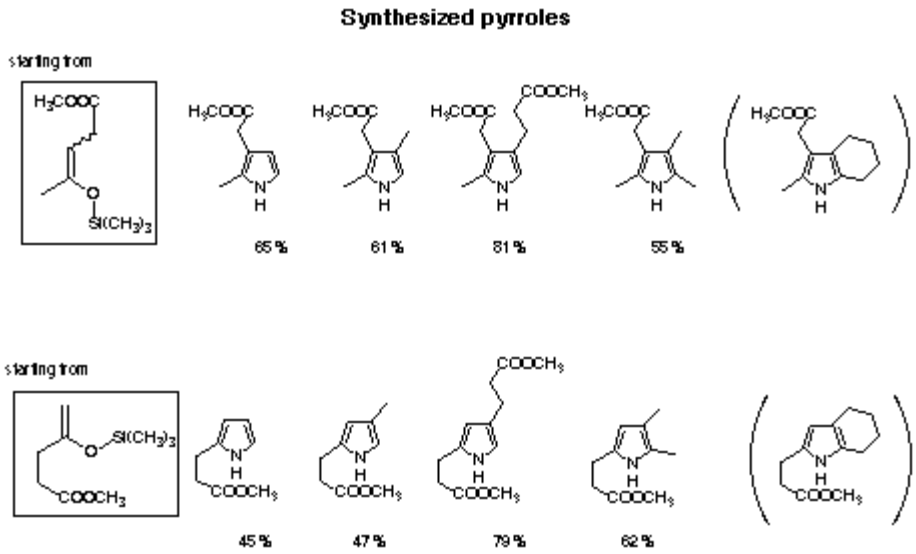

Figure 17: Pyrroles synthesized using the Mukaiyama crossed aldol condensation

The synthesis is complementary to the classical Knorr pyrrole synthesis. It allows to introduce the side chains at the correct positions and with the needed functionalities already in the pyrrole forming step. The reaction conditions for the pyrrole formation are sufficiently mild to allow also the isolation of highly sensitive pyrroles. At this stage of the project we hoped to be able to apply our reaction conditions to a synthesis of porphobilinogen avoiding many of the pitfalls of the former synthesis.

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Next chapter: Chemical Synthesis of Porphobilinogen

References

Chemical Synthesis of Porphobilinogen

Regioselective synthesis of the silyl enol ether component

For the planned synthesis of porphobilinogen and of structural analogues of prophobilinogen we needed the corresponding silyl enol ether of a protected derivative of 5-aminolevulinic acid (see figure 18).

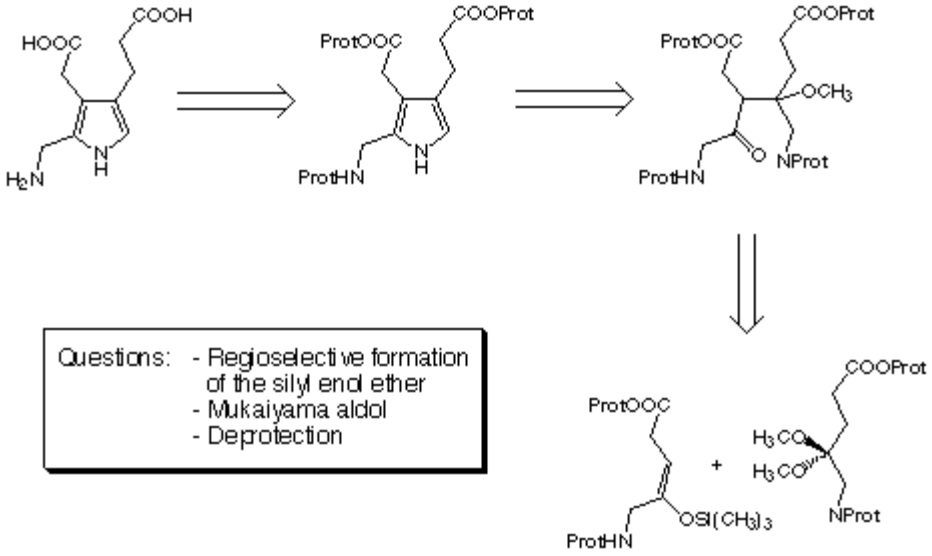

Figure 18: Retrosynthesis for the planned biomimetic approach to porphobilinogen or to structural analogues thereof.

Trials to submit the 5-azido levulinic acid methyl ester **40**, or the 5-tert.-butyldimethylsilyloxy-levulinic acid methyl ester **43** to the conditions worked out by Miller[57] inevitably lead to the unwanted regioisomers of the silyl enol ether die **42** and **45** (see figure 19).

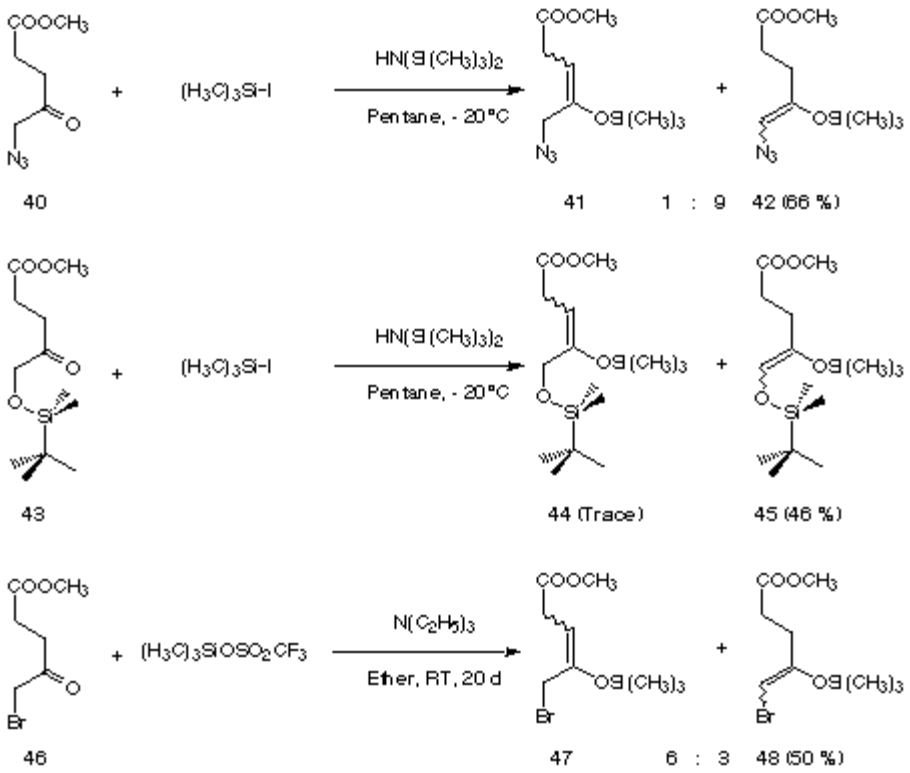

Figure 19: Unsuccessful trials to achieve the transformation of adequately substituted ketones under thermodynamic conditions into the wanted regioisomeric silyl enol ether.

The 5-bromo levulinic acid methyl ester **46** was not stable under Miller's conditions. Even when the conditions developed by House were used no trace of the wanted silyl enol **47** ether could be isolated. However replacing DMF by CH_2Cl_2 as solvent allowed to achieve the transformation at room temperature in the presence of DMAP. Under these conditions a 60 % yield of the silyl enol ethers **47** and **48** could be isolated. But even under these reaction conditions the ratio in favour of the wanted product **47** was only 2 : 1 and 40 % of the product of elimination was isolated as well. Using TMS-triflate in a slight excess in the presence of triethylamine yielded a cleaner mixture of the two regioisomeric silyl enol ethers **47** and **48**. Adding after a short reaction time an additional 20 Mol % excess of 5-bromo methyl levulinate **46** and TMS-triflate and stirring the reaction mixture for another 15 days at room temperature lead to equilibration. Under these conditions 73 % of a mixture could be isolated containing the two wanted diastereomeric silyl enol ethers **47** in 37 %, and the unwanted silyl enol ether **48** in 13 % and the starting material in 23 % yield.

The synthetic problem could be finally solved using the 5-phthalimido-levulinic acid methyl ester **49** submitting it to Miller's conditions but changing the solvent to chloroform (see figure 20).[58] Adding after 17 hours dry hexane allowed to precipitate the salts which had been formed. The wanted silyl enol ether **51** was obtained in 93 % yield as 1 : 1 mixture of the diastereoisomers containing only 4 % of the unwanted silyl enol ether **50** as side product.

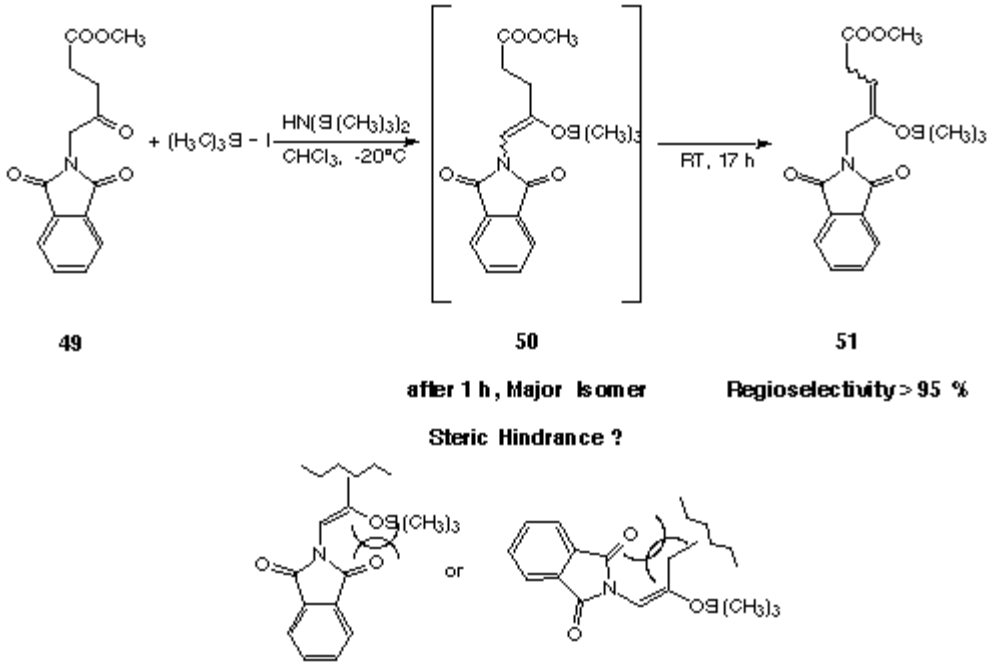

Figure 20: Synthesis of the silyl enol ether **51**.

The silyl enol ether **51** could be stored for months at - 20 °C in the refrigerator. The regioselectivity of the formation of the silyl enol ether is surprising, because one has to assume the methylene group in the a-position to the phthalimido group should be clearly more acidic than the protons at the C3 methylene group. The regioselectivity can probably be attributed to the steric hindrance which can not be avoided if the silyl enol ether towards the position C3 is formed. The X-ray structure of the starting material the 5-phthalimido levulinic acid methyl ester **49** corroborates this proposal (see figure 21).

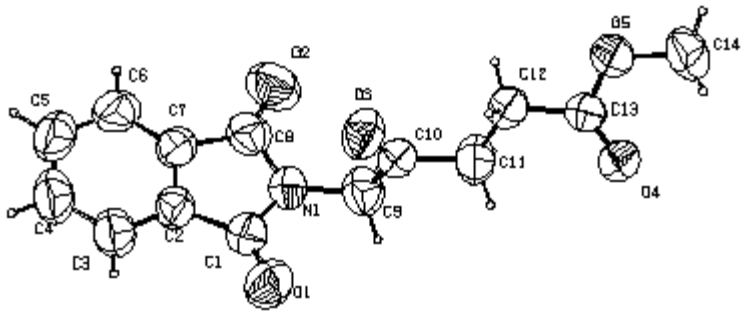

Figure 21: X-ray structure of the 5-phthalimido levulinic acid methyl ester **49**.

The phthalimido group is in an orthogonal position compared to the plane defined by the carbonyl group. In this conformation the oxygen atoms of the phthalimido group avoid the unfavourable steric and electronic interactions with the carbonyl oxygen. At the same time a favourable intramolecular p-p-stacking can be observed.

Trials to couple the silyl enol ether 51

Having the correct regioisomer **51** in our hands experiments were undertaken to couple the silyl enol ether with the acetal of the 5-azido levulinic acid methyl ester **35** under standard conditions.[55] Unfortunately all our trials to couple these two precursors of 5-amino levulinic acid according to Mukaiyama were totally unsuccessful. Despite our intensive efforts to achieve the formation of this crucial C-C-bond we were unable to accomplish this transformation.

In order to check the reactivity in and the potential of the a-phthalimido silyl enol ether for the use in the Mukaiyama crossed aldol reaction we decided to use simple model compounds and to submit them to our reaction conditions. Phthalimido acetone (**52**) can be obtained in one step from chloro acetone and potassium phthalimid.[59] Treating **52** with two equivalents of TMSI and HMDS in $CHCl_3$ during 3 h lead in almost quantitative yield to a mixture containing 90 % of the desired silyl enol ether **53** containing 7 % of the regioisomeric silyl enol ether and 3 % of the starting material (see figure 22).

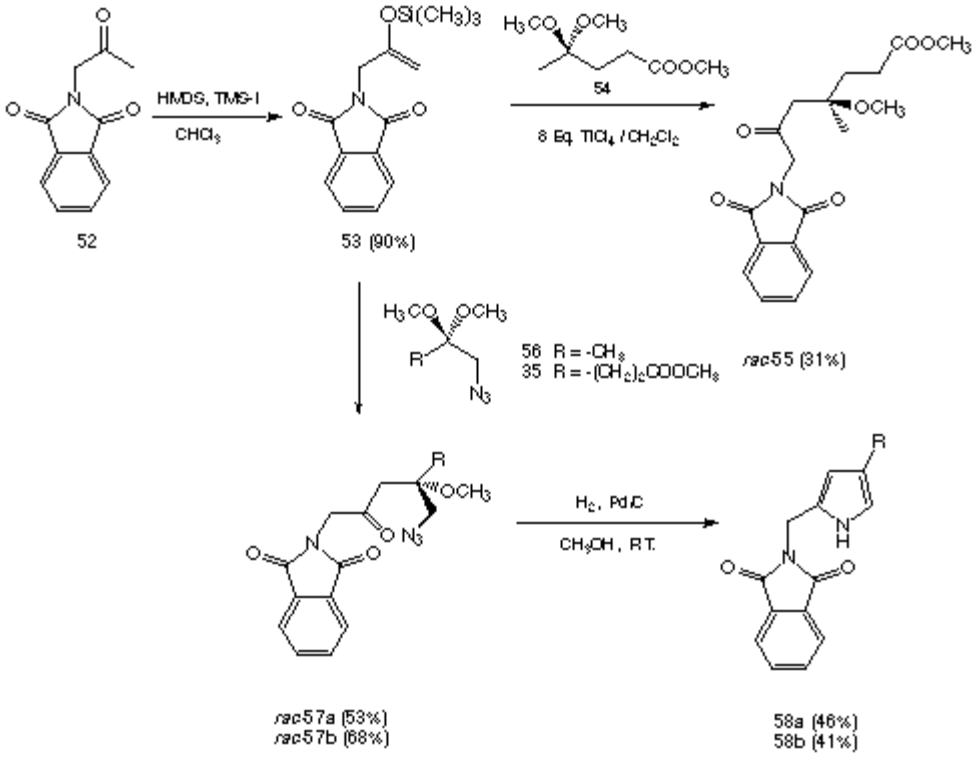

Figure 22: Synthesis and reactivity of the silyl enol ether **53**

Work-up avoiding the contact with water allowed to obtain the product in sufficient purity for our further studies. Also the silyl enol ether **53** could be stored in the deep-freezer for months. Already our first trials to submit the silyl enol ether **53** to the Mukaiyama aldol coupling met with success. Using the dimethylacetal of levulinic acid methyl ester (**54**) as coupling partner allowed to obtain the aldol product *rac* **55** in 31 % isolated yield. Using the same standard conditions the acetals of the azido acetone (**56**) and of 5-azido levulinic acid methyl ester (**35**) could be coupled in 53 respectively 68 % yield. The two aldol products **57a** and **57b** could be reductively transformed in 46 % respectively 41 % to the corresponding substituted pyrrole **58a** and **58b** (see figure 25). Pd on charcoal was used as catalyst and methanol as solvent. Studying a sample which has been taken after 3 h in the ^1H- and ^{13}C-NMR showed the presence of an intermediate, whose spectral datas were in accordance with the Δ^1-pyrrolenine structure. The final product, the pyrrole **58b**, could be crystallised the structure could be determined with an X-ray diffraction study (see figure 23).

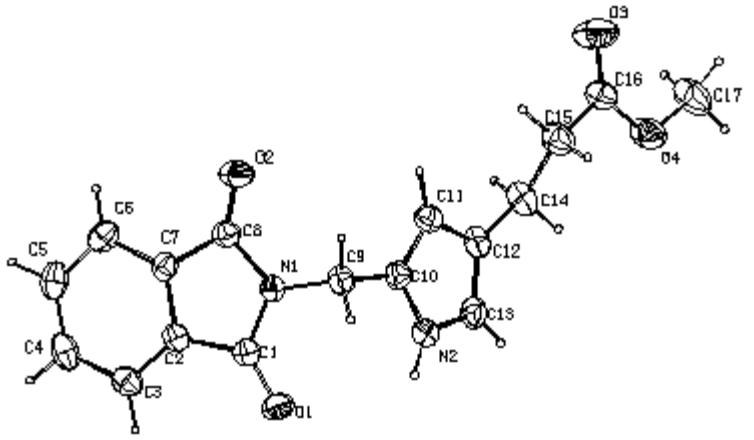

Figure 23: X-ray structure of the pyrrole **58b**

Having shown that the aldol coupling with the model for our silyl enol ether could be successfully achieved, we tried to react the silyl enol ether **51** under our optimized conditions. In order to check the reactivity of **51** in the crossed aldol reaction we first tried the coupling reaction using the dimethyl acetal of benzaldehyde (**59**) (see figure 24).

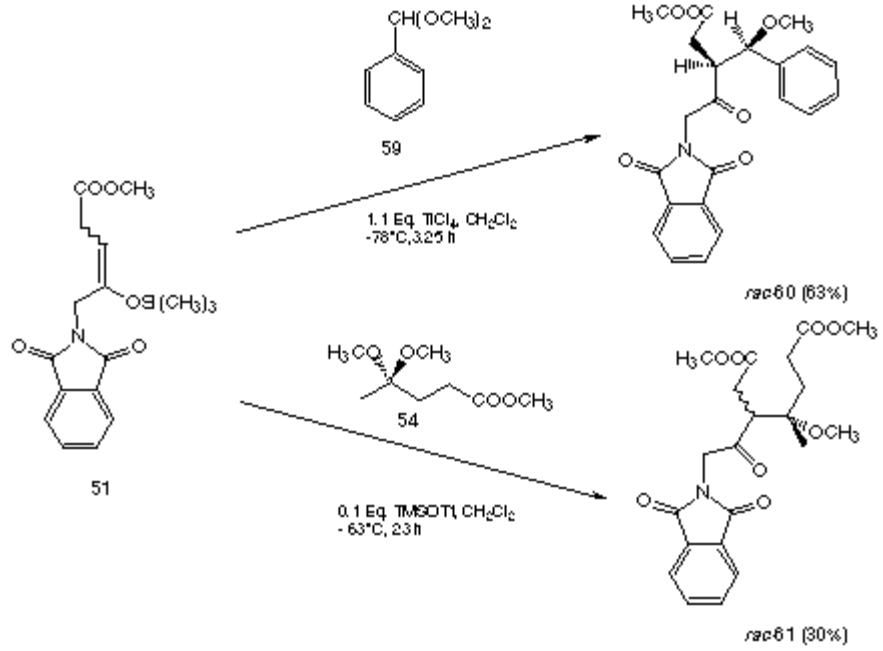

Figure 24: Coupling reactions of the silyl enol ether **51** with model acetals

The reaction of **51** and **59** in the presence of 1.1 equivalents of $TiCl_4$ during 3 1/4 h gave **60** in 63 % after crystallisation. Careful recrystallisation allowed to isolate the main diastereoisomer which could be submitted to X-ray analysis (see figure 25). The relative configuration of the main diastereoisomer is unlike.

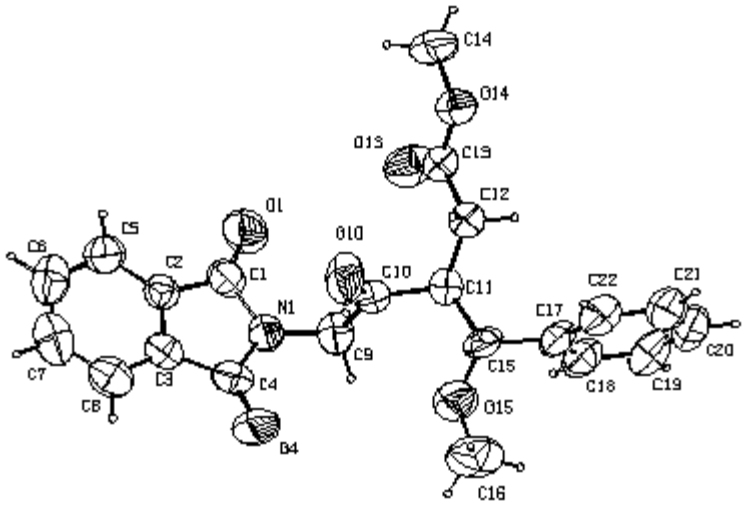

Figure 25: X-ray structure of **60**

Despite our considerable efforts to achieve also the Mukaiyama Aldol coupling using a protected form of 5-amino levulinate, we were unable to isolate products which could be traced back to the central C-C-bond formation. Using $TiCl_4$ as a catalyst for the aldol coupling starting from the silyl enol ether **51** at temperatures below -40 °C no reaction could be observed. Increasing the temperature above -40°C rapid destruction of the reaction partner was observed. Using Lewis acids like TMSOTf[60,61] or the "super-Lewis acid" $B(OTf)_4TMS$ according to Davis[62] the aldol reaction between **51** and the dimethyl acetal of levulinic acid methyl ester (**54**) could be achieved. Using Noyori's conditions [60] whereby 0.11 equivalents of TMSOTf are utilised 30% of one pure diastereoisomer **61** could be isolated. Even when these stronger Lewis acids were used we were unable to achieve the crucial C-C-bond forming process starting from an adequate precursor of 5-amino levulinate. The use of the more reactive, but also more aggressive catalyst TMSI[63] at -80 °C lead to the destruction of both starting materials: the silyl enol ether **51** and the acetal.

Synthesis of the pyrazole analogue of porphobilinogen 64

The only way out seemed to be to increase the inherent reactivity of the carbonyl component. Reacting the silyl enol ether **51** with the succinic acid mono chloride mono methyl ester yielded the b-diketone **62** in 35 % isolated yield (see figure 26).

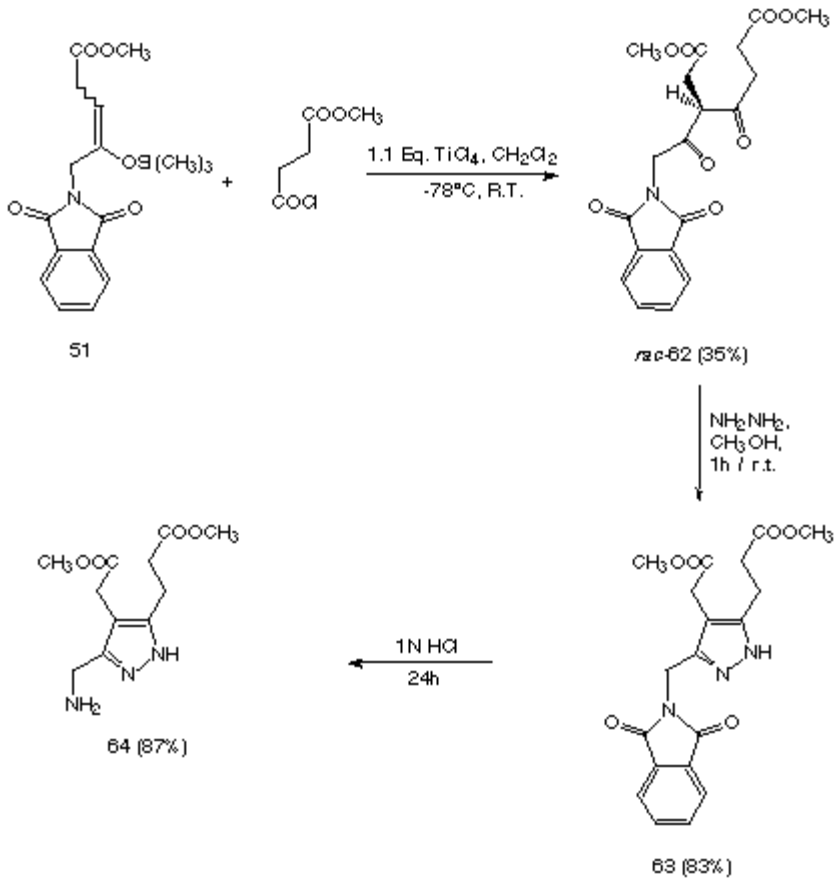

Figure 26: Synthesis of the pyrazole **64**

Treating this diketone with hydrazine hydrate in methanol for one hour yielded the pyrazole **63** in 83 % yield. The protecting groups could be removed by boiling **63** in 1N HCl for 24 h. Using an ion exchange column Amberlite XAD-2 the phthalic acid could be separated from the pyrazole **64** which was obtained in 87 % yield. Crystalisation from acetone/H_2O gave an analytically pure sample.

Structurally the pyrazole **64** has a strong resemblance to PBG. However the reactivity of the two compounds is totally different. Pyrazoles in general a clearly less electron rich than pyrroles. Therefore we could boil the pyrazole for 24 h in 1N aqueous HCl. It is well-known that PBG or even precursors of PBG would not resist to such drastic conditions. It has been reported that PBG forms porphyrines, oligomers of pyrroles and polymers like pyrrole black under such forcing conditions .[64,65]

Pyrazole are present in solution in their two tautomeric forms. The X-ray analysis of the protected pyrazole showed the presence of only one of the two possible tautomeric forms in the solid state (see figure 27).

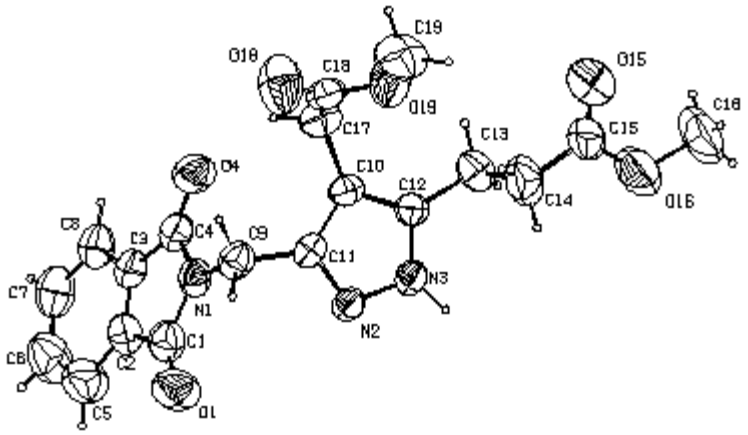

Figure 27: X-ray structure of the pyrazole **63**

In order to get information about the presence of one or two tautomeric forms in solution we measured an $^{15}N,^{1}H$ HMQC-spectrum. The signal ati -170.7 ppm, which can be attributed to the NH of the pyrazole is correlated with the $H_2C(3_1)$-group of the propionic acid side chain. This is a strong indication that in solution the same tautomeric form is present as the one which was observed in the solid state.

Synthesis of porphobilinogen

In order to obtain the porphobilinogen itself we tried to use the mono methyl succinic acid monocyanide (**7**) as activated carbonyl component. In this strategy it should be possible to combine two partners which contain all the carbon, oxygen and nitrogen atoms necessary for the construction of prophobilinogen. Deprotection of the aldol product should then induce the ring closing and aromatisation process. In view of this analysis we reacted the silyl enol ether **51** with the mono methyl succinic acid monocyanide (**65**). The cyano hydrine could be detected in the raw product of the reaction. Extraction against water and purification with column chromatography yielded 35 % of the b-diketone *rac*-**62** as hydrolysis product (see figure 28).

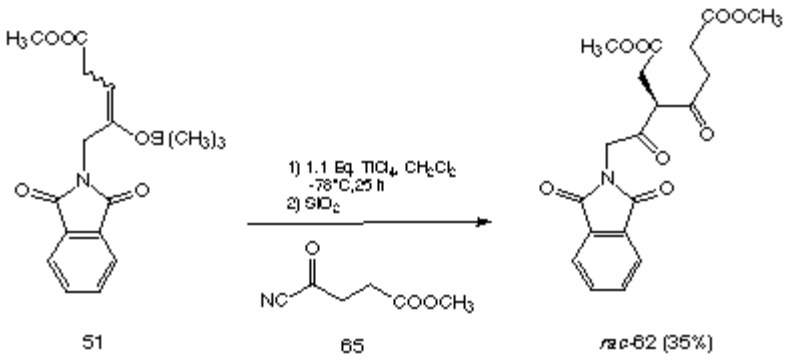

Figure 28: Aldol coupling between mono methyl succinic acid monocyanide (**65**) and the silyl enol ether **51**

Under optimised conditions at 20° C and using $TiCl_4$, which had been freed from HCl by distillation over polyvinyl pyridine, the aldol product *rac*-**66** could be obtained in 60 to 87 % (see figure 29).[58] One diastereoisomer of the aldol product *rac*-**66** could be obtained analytically pure by crystallisation in 47 % yield. Trials to reduce the cyano hydrine directly met with limited success. For the synthesis we protected the unpurified aldol product using acetone enol acetate. The acetylated aldol product *rac*-**67** could be obtained in 56 % yield. Even the reduction of the acetylated cyano hydrine *rac*-**67** proved to be difficult. Finally the cyanohydrine *rac*-**67** could be reduced smoothly at 65° C under 120 atm H_2 in the presence of Raney nickel. After column chromatography we obtained the fully protected

porphobilinogen **68** in 54 % yield analytically pure. Removal of the protecting groups over two steps has already been described in the literature.[33]

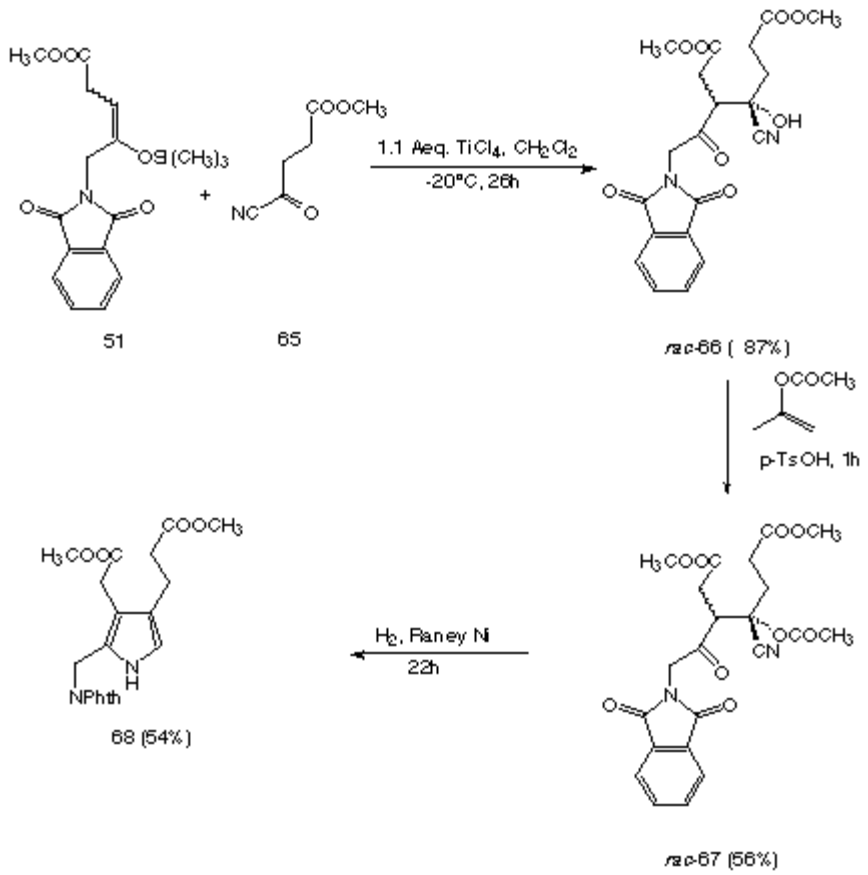

Figure 29: Synthesis of a protected form of porphobilinogen **68**

In conclusion we were able to obtain the protected porphobilinogen **68** in a convergent way starting from two easily obtainable starting materials. The central step of the synthesis is the Mukaiyama aldol reaction between the regioselectively formed silyl enol ether **51** as the nucleophile with succinic acid mono methyl ester mono cyanide (**65**) as electrophile. Reducing the acetylated cyano hydrine *rac*-**67** yields directly the protected porphobilinogen **68**. This synthesis follows the proposal for the biosynthesis made by Shemin exactly 30 years ago. The correctly functionalised side chains are introduced on the level of the two starting materials used for the synthesis of the pyrrole ring. Subsequent functionalisation is therefore not necessary. In this synthetic scheme the same bonds are formed as in the biosynthesis catalysed by porphobilinogen synthase. The overall yield starting from 5-phthalimido methyl levulinate is 25 %. The synthesis can be used to obtain selectively labelled porphobilinogen.

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Next chapter: [Conclusions]

[References]

Conclusions

The motivation for the development of this synthesis has been the proposed pathway for the biosynthesis of PBG (**11**).[36,37,49] In view of the results of our model studies we can interpret the postulated mechanism for the enzymatic formation of PBG (**11**). The foremost task of the enzyme would be to induce the crucial carbon-carbon bond formation (intermediate **69**)(see figure 30).

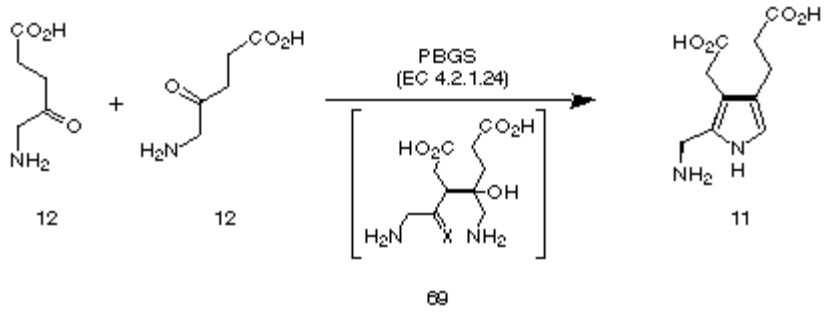

Figure 30: Proposed intermediate of the biosynthesis of porphobilinogen.

Shemin has been the first to propose a mechanism for PBGS drawing a close analogy between PBGS and class I aldolases (see figure 31).[49,66] His postulated mechanism is mainly based on two arguments: The transformation has been formulated in strict analogy of PBGS with aldolases and the sequence of recognition is based on the observed formation of a compound which Shemin called a mixed pyrrole, whose proposed structure however was wrong as could be shown latter.[67]

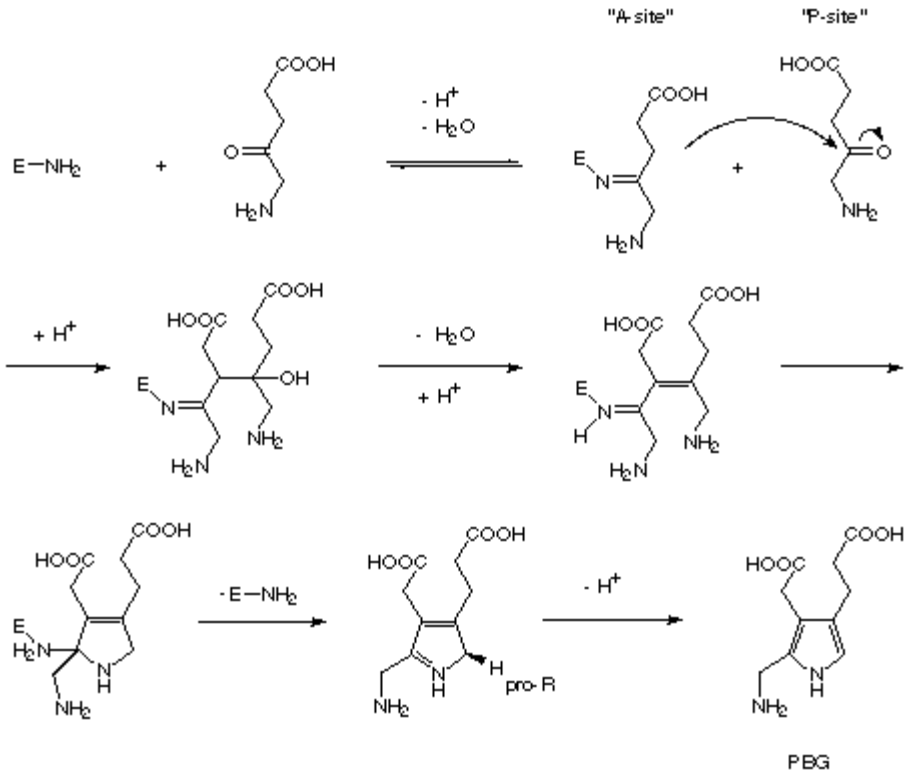

Figure 31: Shemin's mechanism for PBGS.

Despite the knowledge of more than twenty gene derived protein sequences for PBGS from different sources, the sequence of events on the enzyme and the mechanistic details of the transformation are largely unknown still. The following experimental findings are relevant for the mechanism of PBGS (see figure 32).[36]

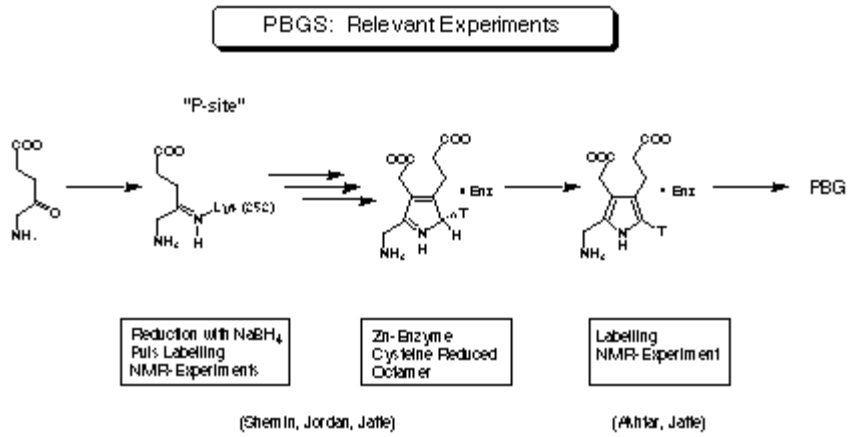

Figure 32: Experiments relevant for the mechanism of PBGS.

The substrate forming the propionic acid side chain is interacting first. At least one of the substrates is forming a covalent bond with the enzyme via a Schiff base. Most of the enzymes isolated so far need Zn^{2+} as an essential cofactor. To bind the Zn^{2+} the cysteines have to be in their reduced form. The enzyme is usually an homooctamer. The deprotonation leading from the pyrrolenine tautomer to the aromatic pyrrole is enantioselective and occurs therefore on the enzyme.[36,68]

To study the order in which the two substrate molecules bind to the enzyme, Jordan performed highly elegant single-turnover experiments.[69-71] Stoechiometric equivalents of labelled substrate and porphobilinogen synthase were rapidly mixed and after about a 100 ms added to a large excess of unlabelled substrate. The position of the radioactive label was determined by degradation. The pulse labelling could also be done using [5-^{13}C] 5-aminolevulinic acid. The ^{13}C-NMR spectrum of the product allowed to identify the position of the label directly.

Starting from his observations Jordan postulated an alternative mechanism for the formation of porphobilinogen (see figure 33).

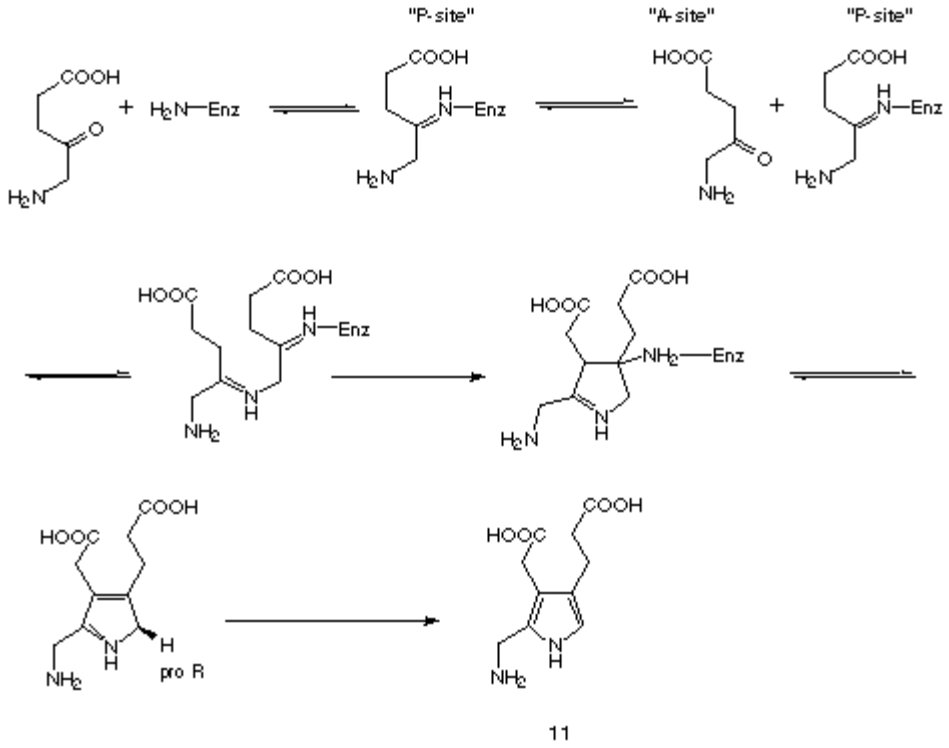

Figure 33: Jordan's mechanism for PBGS (Jordan I)

Jordan postulates that after the formation of the Schiff base between the enzyme and the first substrate molecule, the second substrate molecule forms a new Schiff base to the enzyme bound 5-aminolevulinate. Only after this step follows the aldol reaction and the elimination which leads after deprotonation to the product.

At the same time Jordan postulated a mechanistic alternative (Jordan II),[36,68,72] which combines the events of recognition as indicated by the pulse labelling experiment with the mechanistic thinking of Shemin, which gives preference to the C-C-bond formation in the biosynthetic sequence (see figure 34).

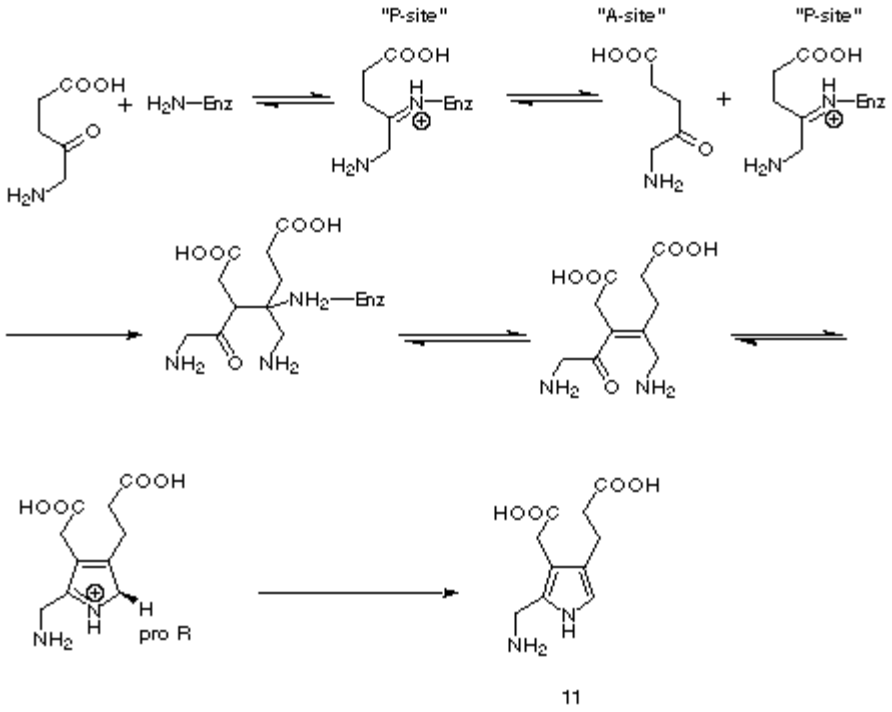

Figure 34: Jordan's second mechanism for PBGS (Jordan II)

Intensive studies using inhibitors which were specifically synthesized in order to test the mechanism of porphobilinogen synthase[73] as well as many other biochemical experiments especially concentrating on the influence of the different metal atoms on the reactivity have not allowed to distinguish unequivocally between the different proposed mechanisms for the biosynthesis of porphobilinogen.[36,74,75] Even the accumulation of over 20 gene derived protein sequences did not allow to decide which of the mechanistic alternatives is used by the enzyme. However due to the cloning and overexpressing of the porphobilinogen synthase large quantities of the enzyme became available. This has allowed to crystallize porphobilinogen synthase from different sources.[76] Very recently the successful crystallisation of porphobilinogen synthase and the structure determination with a resolution of 2 Å has been reported.[77,78]

The structure determination allows for the first time to have clear ideas about the active site and the quaternary structure of porphobilinogen deaminase. The structure clearly shows that the porphobilinogen deaminase is an octamer or better a tetramer of strongly interacting dimers. All active sites are directed towards the outside, so that 8 fully active sites are present. At the active site two lysines could be identified and the positions of the two Zn^{2+} ions has been located near to the active site as well. Another remarkable observation is the fact that the overall structure of porphobilinogen synthase resembles remarkably to the structure of aldolases. These combined observations leads the authors to postulate that the porphobilinogen synthase combines mechanistic and structural aspects of the two classes of aldolases: aldolase I and aldolase II. Therefore they give preference to a mechanism in which the C-C-bond formation is the crucial event of the biosynthetic pathway. It will be very exiting to see how the different approaches, analysis of the gene derived sequences, structural information from the X-ray diffraction data and inhibition results will help to unravel the mechanism of this beautiful enzyme.

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Next chapter: References and notes

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