Global Journal of Science Frontier Research
Chemistry
Volume 12 Issue 2 Version 1.0 February 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Synthesis of Biologically Important Pyrrole Derivatives in Any ^{13}C and ^{15}N Isotope Enriched Form

By Prativa B. S. Dawadi & Johan Lugtenburg
Leiden University, Leiden

Abstract - Recently the synthesis of **[3-^{13}C]-**, **[4-^{13}C]-**, and **[11-^{13}C]-** porphobilinogen, **[^{15}N,^{13}C4]-1H** - pyrrole-**2,3,5 -** tricar - boxylic acid, [1-^{15}N]-3-cyano-4-methyl-1H-pyrrole and **[2-^{13}C]-** and **[3-^{13}C]-**cyano-**4-**methyl-**3-**pyrrolin-**2-**one have been published. Incorporation of **^{13}C** and **^{15}N** in these systems at any position and combination of positions has become accessible. Also mild alkylations of active methylene compounds with **α**-halo carbonyl compounds open up many 3-pyrrolin-2- ones and pyrrole systems based on stable isotope building blocks that have been published. This gives the access to a whole new library of stable isotope enriched pyrroles in any stable isotope enriched form. This is also the case for biliverdin **IXα** which after enzymatic treatment has been converted into **(2R)-**phytochromobilin that reacts with its apoprotein to form intact active phytochrome.

Keywords : [1-^{15}N]-3-Cyano-4-methyl-1H-pyrrole, [3-^{13}C], [4-^{13}C]-, and [11-^{13}C]-porphobilinogen, [^{15}N, ^{13}C4,] -1H-pyrrole-2,3,5-tricarboxylic acid and biliverdin IXα.

GJSFR-B Classification: FOR Code: 030503, 040203,

SYNTHESIS OF BIOLOGICALLY IMPORTANT PYRROLE DERIVATIVES IN ANY 13C AND 15N ISOTOPE ENRICHED FORM

Strictly as per the compliance and regulations of:

Synthesis of Biologically Important Pyrrole Derivatives in Any ^{13}C and ^{15}N Isotope Enriched Form

Prativa B. S. Dawadi[α] & Johan Lugtenburg[σ]

Abstract - Recently the synthesis of **[3-^{13}C]-, [4-^{13}C]-, and [11-^{13}C]-porphobilinogen, [^{15}N,$^{13}C_4$]-1H-pyrrole-2,3,5-tricarboxylic acid, [1-^{15}N]-3-cyano-4-methyl-1H-pyrrole and [2-^{13}C]- and [3-^{13}C]-cyano-4-methyl-3-pyrrolin-2-one** have been published. Incorporation of ^{13}C and ^{15}N in these systems at any position and combination of positions has become accessible.

Also mild alkylations of active methylene compounds with α-halo carbonyl compounds open up many 3-pyrrolin-2-ones and pyrrole systems based on stable isotope building blocks that have been published. This gives the access to a whole new library of stable isotope enriched pyrroles in any stable isotope enriched form. This is also the case for biliverdin **IXα** which after enzymatic treatment has been converted into **(2R)**-phytochromobilin that reacts with its apoprotein to form intact active phytochrome.

Keywords :[1-^{15}N]-3-Cyano-4-methyl-1H-pyrrole, [3-^{13}C], [4-^{13}C]-, and [11-^{13}C]-porphobilinogen, [^{15}N,$^{13}C_4$]-1H-pyrrole-2,3,5-tricarboxylic acid and biliverdin IXα.

I. Introduction

Pyrroles and their derivatives are one of the most important classes of heterocyclic compounds.[1] They exhibit extensive biological and pharmacological properties.[2] Many pyrrole derivatives have shown interesting biological properties such as antibacterial[3], antiinflammatory[4], antioxidant[5], antitumor, antifungal[6] and immune suppressant activities.[7] Highly functionalized pyrroles are subunits of heme, chlorophyll, bile pigments, vitamin B12 and pyrrole alkaloids isolated from marine source.[8] Atrovastatin (Lipitor) is a drug for lowering cholesterol.[9]

Access to stable isotope enriched systems (^{2}H, ^{13}C and ^{15}N) allows the metabolic conversions of these systems to be followed with mass spectroscopic techniques when they have at least three stable isotopes.[10] ^{13}C-NMR Techniques have been used to study the conversion of [5-^{13}C]-aminolevulinic acid into porphobilinogen in vivo in living Rhodobacter sphaerhoides cells.11

Similarly, the conversion of [2-^{13}C]- and [11-^{13}C]-porphobilinogen in the body into uroporphyrinogen III and coproporphyrinogen III has been investigated.[12] Very recently the ^{13}C photo-CIDNAP MAS NMR spectra of membrane fractions of Heliobacillus mobilis that was grown on media containing [4-^{13}C]-aminolevulinic acid have been obtained.[13]

Besides NMR spectroscopy, vibrational techniques such as resonance raman spectroscopy have been applied in heme protein research.[14] In this case some of the vibrations coupled to an electronic transition of the chromophore showed enhanced inelastic scattering up to 10^6 fold.

Access to pyrroles enriched on each position and any combination of positions with stable isotopes such as ^{2}H, ^{13}C and ^{15}N is essential to study the metabolism of important pyrrole derivatives using non-invasive isotope sensitive techniques.The chromophores of heme proteins and photosynthetic reaction centres have been prepared with stable isotope enriched pyrrole building blocks.

Recently, we have published a review paper about the stable isotope enriched systems in heme and (bacterio)chlorophyll protein systems that were known at that time.[15] In the meantime a number of important stable isotope enriched pyrrole systems have been published together with a new method to prepare pyrroles and stable isotope enriched building blocks that allow access to a whole new range of stable isotope enriched pyrroles.

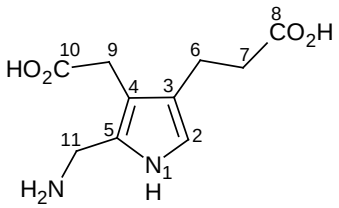
In this paper we focus on those new possibilities that allow access to biliverdin **IXα** which can be converted into (2R)-phytochromobilin, the chromophore of phytochrome via one enzymatic conversion.[16] We mainly focus on ^{13}C and ^{15}N enriched building blocks leading to the labels at all atoms in the molecular skeleton of the pyrroles and tetrapyrrole systems. We have not focused on ^{2}H systems because ^{2}H occupies the peripheral positions on the molecular system and is more prone to isotope loss and scrambling during the synthetic process. However, the schemes for ^{13}C incorporation can easily be adjusted to ^{2}H incorporation as well.

II. Synthesis And Discussion

a) *Synthesis Of [3-^{13}C]-, [4-^{13}C]- And [11-^{13}C]-Porphobilinogen 1.*

Enzymatic incorporation of [11-^{13}C]- and [2,11-$^{13}C_2$]-porphobilinogen 1 (fig. 1) into uroporphyrinogen I and III has been reported.[17,18]

Author α σ : Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA, Leiden. E-mail : p.b.s.dawadi@gmail.com



1

1a: [4-^{13}C]

1b: [3-^{13}C]

1c: [11-^{13}C]

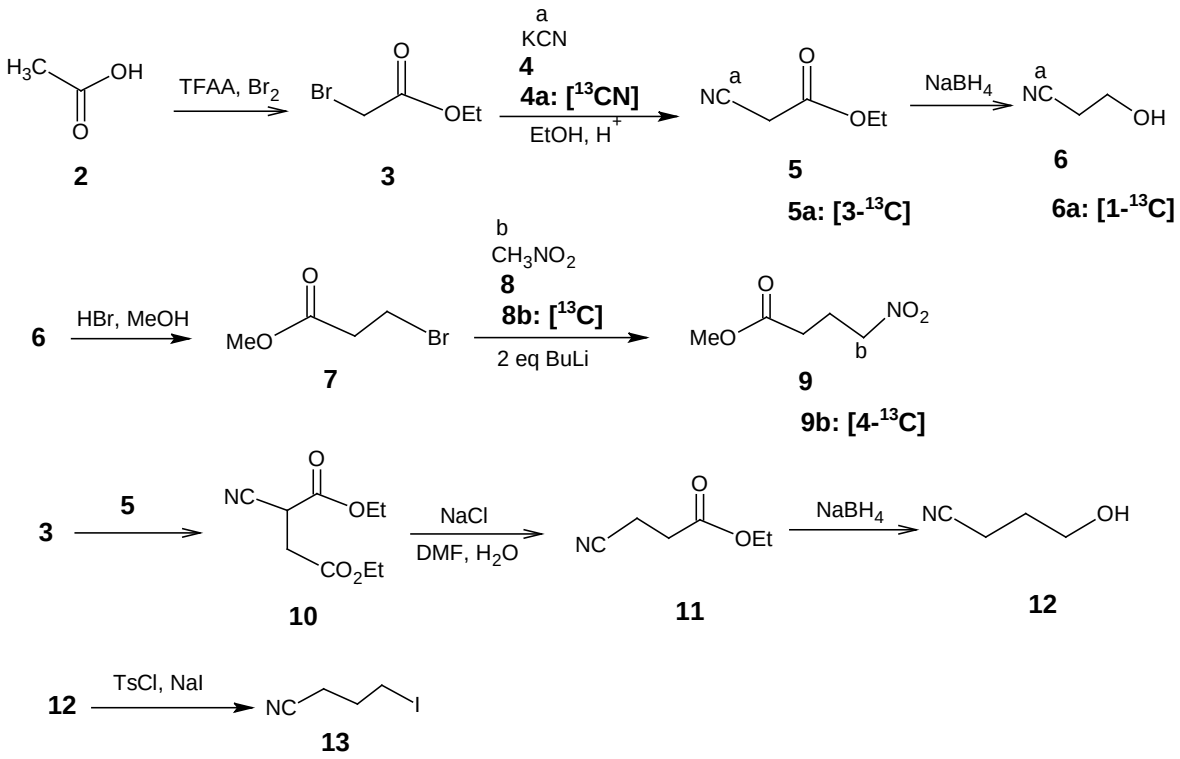
Figure 1 : Structure and numbering of porphobilinogen 1 and its highly enriched isotopomers 1a, 1b and 1c.

Porphobilinogen 1 is a biosynthetic precursor of tetrapyrrole chromophores in heme proteins, photosynthetic antennae proteins, photosynthetic reaction centres and phytochromes. The synthetic access to ^{13}C and ^{15}N enriched porphobilinogen will allow access to enrich stable isotopes in the above mentioned systems at any possible position. With ^{13}C and ^{15}N isotope incorporation in the chromophores of these biologically important proteins can be investigated with noninvasive isotope sensitive techniques.

In figure 1 the structure and numbering of porphobilinogen 1 is depicted. The synthesis of [3-^{13}C]-, [4-^{13}C]- and [11-^{13}C]-porphobilinogen 1a, 1b and 1c via a scheme that allows access to any stable isotopomer and isotopologue has been reported.[19]

Acetic acid 2 is treated with 1 eq of bromine in the presence of trifluoroacetic anhydride to afford a high yield of the 2-bromoacetic acid which is esterified with ethanol into ethyl bromoacetate 3. The bromine is easily substituted for the cyano group with KCN 4 to give ethyl cyanoacetate 5. The ester function is reduced with $NaBH_4$ to give an alcohol function in 3-hydroxypropionitrile 6. Treatment of 6 with aqueous HBr and subsequent esterification afforded methyl 3-bromopropionate 7 in high yield. The S_N2 reaction of reagent 7 with nitromethane 8 in the presence of 2 eq BuLi to obtain methyl 4-nitrobutanoate 9 is somewhat difficult.

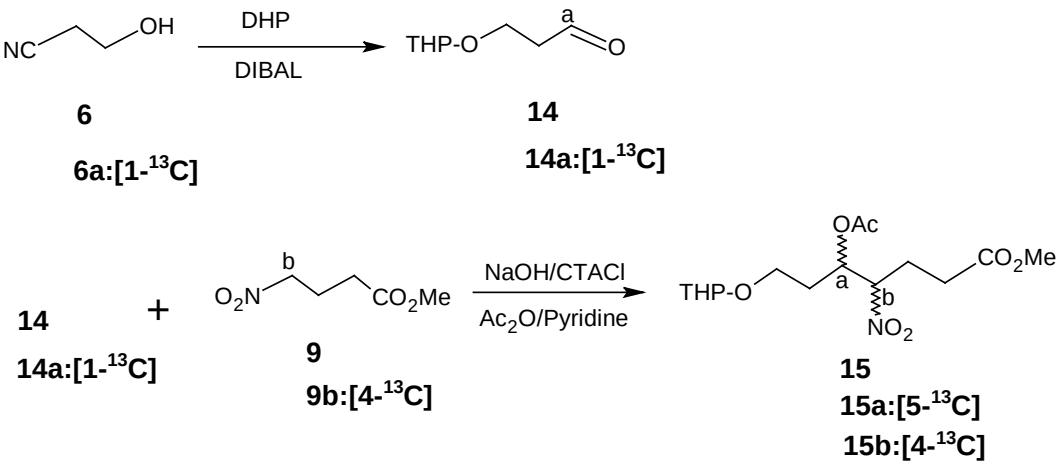
An alternative method to obtain the product 9 is to treat reagents 3 and 5 in the presence of NaOEt to afford diethyl 2-cyanopentanedioate 10.[20] Selective removal of one of the ester functions in NaCl, DMSO, H_2O gave ethyl 3-cyanopropionate 11. Subsequent reduction of the ester function with NaBH4 afforded 4-hydroxybutyronitrile 12 which is further converted into 4-iodobutyronitrile 13. S_N2 substitution of the iodo function with $NaNO_2$ and subsequent conversion of the nitrile function into ethyl carboxylate is expected to give ethyl 4-nitrobutanoate 9 without problem. In porphobilinogen 1 (fig. 1) the carbon atoms 3, 6, 7 and 8 are derived from the compound 9 and carbon atoms 4, 9 and 10 are derived from 3-hydroxypropionitrile 6.



Scheme 1. The synthesis of ethyl [4-^{13}C]-4-nitrobutyrate 9b. The starting compound acetic acid 2 is commercially available in the [1-^{13}C]-, [2-^{13}C]- and [1, 2-$^{13}C_2$] isotopomeric forms.

3-Hydroxypropionitrile 6 is first protected with dihydropyrane via acid catalyzed reaction to afford 3-(tetrahydropyran-2'-yloxy)-propionitrile (scheme 2).[19] DIBAL-H reduction of the nitrile afforded the required 3-hydroxypropanal derivative 14. Henry reaction between the nitro ester 9 and the aldehyde 14 in the presence of a phase transition catalyst and acetylation afforded the nitro derivative 15.

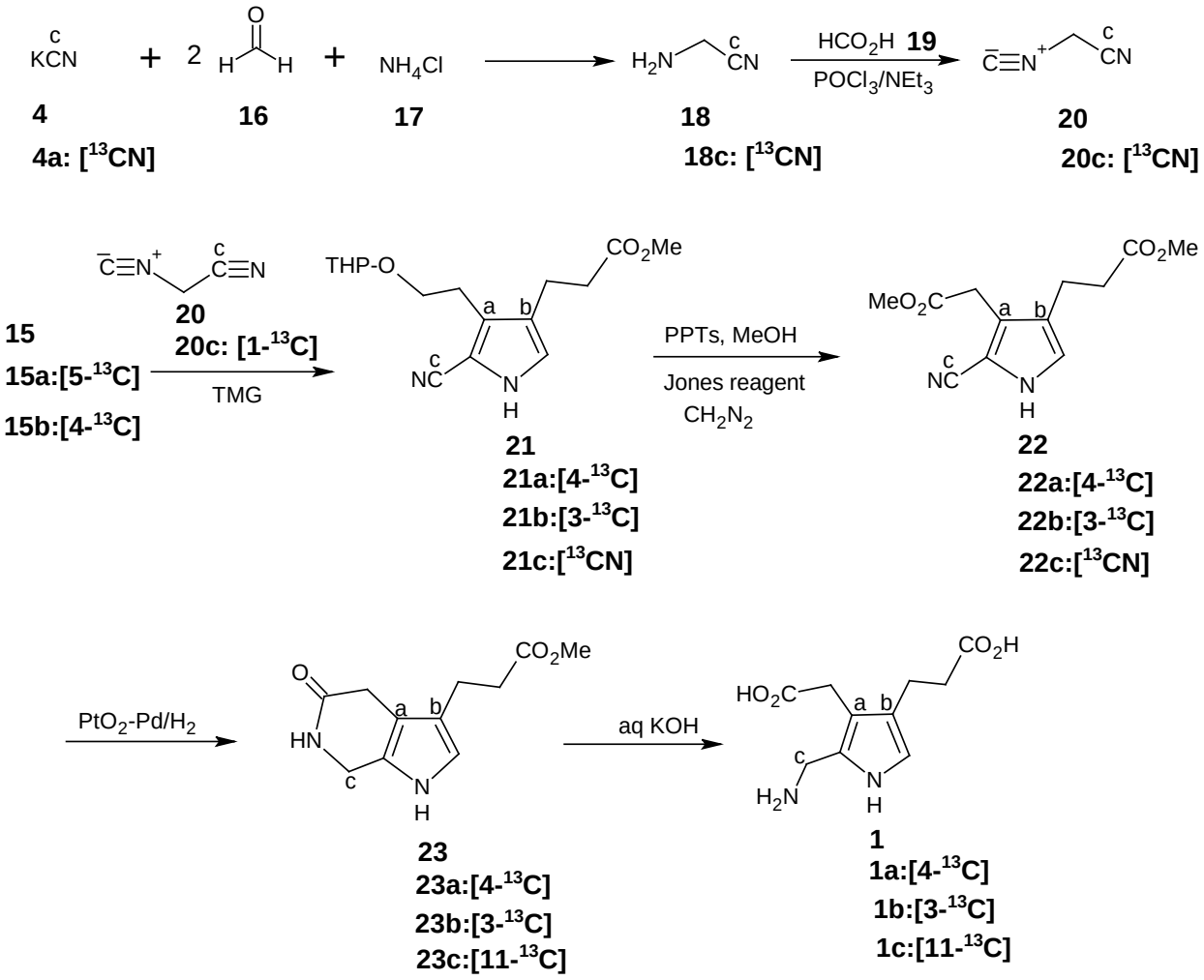
Isocyanoacetonitrile 20 is the building block that provides carbon atoms 2, 5 and 11 and two nitrogen atoms of porphobilinogen 1. It is shown that in scheme 3 a Strecker reaction of KCN 4, formaldehyde 16 and NH_4Cl 17 leads to 2-aminoacetonitrile 18. This molecule reacted with formic acid 19 in acetic anhydride to give the formyl derivative of 2-aminoacetonitrile that upon treatment with $POCl_3$ and triethylamine afforded 2-isocyanoacetonitrile 20. The base (tetramethylguanidine) induced the reaction between compounds 15 and 20 to give 5-cyano-3-(methoxycarbonylethyl)-4 (tetrahydro-pyran-2'-yl-ethyl)-pyrrole 21. The tetrahydropyranyl protective group is removed by acid to obtain the primary alcohol. The alcohol function is converted into the carboxylic acid by Jones oxidation and this acid is subsequently converted into the methyl ester 22. The nitrile and methyl ester functions are catalytically reduced to give an amide function in product 23. Base treatment converted the ester and amide functions into carboxylic acid and a methylene amine function in porphobilinogen 1. The conversion of products 15 and 20 into the pyrrole 21 is a so-called Barton-Zard reacton.[21]



Scheme 2. 3-Hydroxypropionitrile 6 is converted into methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2'-yloxy)-heptanoate 15, 15a and 15b.

The synthetic routes shown in schemes 1-3 have been developed for the synthesis of [3-^{13}C]-, [4-^{13}C]- and [11-^{13}C]-porphobilinogen 1. It is clear that based on commercially available ^{13}C and ^{15}N isotope enriched starting materials any carbon and nitrogen with stable isotope enrichment are accessible in porphobilinogen 1. Schemes 1 and 3 can be modified in such a way that a whole series of vicinal acetoxy and nitro derivatives can be made in the required stable isotope enriched form.

Tertiary butyl isocyanoacetate has been used to prepare pyrroles with methyl carboxylate groups on the 2-position.[22] This building block is accessible in any stable isotope enriched form via reactions analogous to those described in scheme 3. Tosylmethyl isocyanide in all possible stable isotopically labelled forms has been used to prepare pyrroles and other heterocyclic systems via building blocks that can be easily prepared in stable isotope enriched form.[23]



Scheme 3. Preparation of 2-aminoacetonitrile 18 and its conversion into isocyanoacetonitrile 20. Synthesis of [4-^{13}C]-porphobilinogen 1a, [3-^{13}C]-porphobilinogen 1b and [11-^{13}C]-porphobilinogen 1c via base catalyzed condensation of methyl 5-acetoxy-4-nitro-7-(tetrahydropyran-2'-yloxy)-heptanoate 15 and isocyanoacetonitrile 20.

b) Synthesis Of [1-^{15}N]- Formyl-4-Methyl-1H-Pyrrole 24a

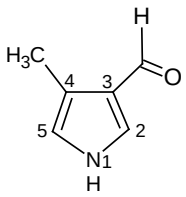
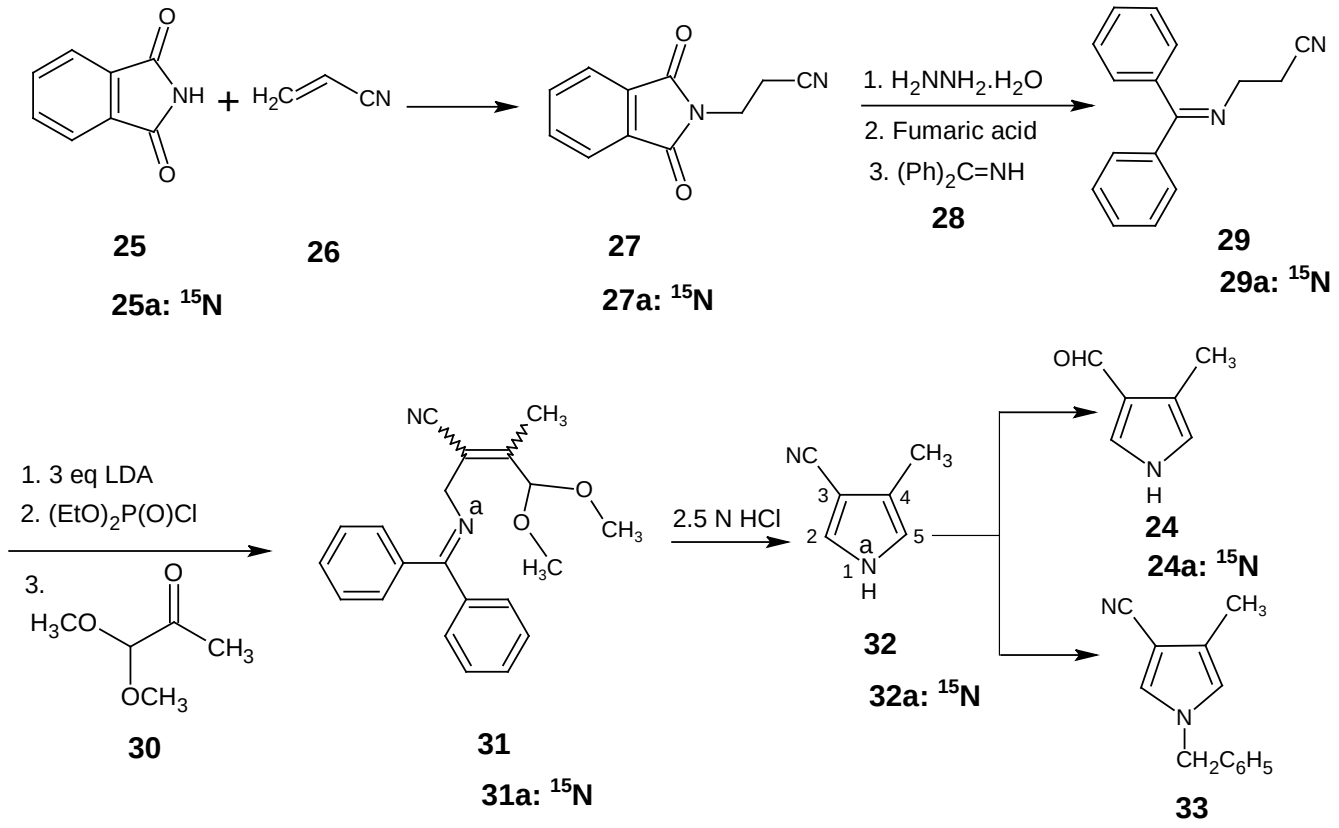


Figure 2. Structure and numbering of [1-^{15}N]-3-formyl-4-methyl-1H-pyrrole 24a.

This pyrrole is a building block for the biologically important tetrapyrrole systems. In Scheme 4 it is indicated that phthalimide 25 treated with acrylonitrile 26 to form β-phthalimidopropionitrile 27. ^{15}N-Phthalimide 25a is commercially available. All possible isotopologues of acrylonitrile are accessible via isotopically enriched 3-hydroxypropionitrile 6 (scheme 1).[24]



Scheme 4. Reactions to prepare [1-^{15}N]-3-formyl-4-methyl-1H-pyrrole 24a starting from[^{15}N]-phthalimide 25a and acrylonitrile 26.

The product 3-[(diphenylmethylene) amino] propionitrile 29 is prepared by first treating β-phthalimidopropionitrile 27 with hydrazine hydrate, then treating the mixture with fumaric acid to obtain the fumaric acid salt of 3-aminopropionitrile followed by reaction with benzophenone imine 28. Product 29 is treated with 3 eq LDA at low temperature and subsequently diethyl chlorophosphate is added followed by 1,1-dimethoxyacetone 30. After the Wittig-Horner reaction compound 31 is isolated as a mixture of E-and Z-forms. In a solution of 2.5 N HCl the acetal and the nitrogen protection groups are removed and the free amino group and aldehyde group reacted to give a high yield of 3-cyano-4-methyl-1H-pyrrole 32. DIBAL-H reduction of the nitrile function in the product 32 afforded 3-formyl-4-methyl-1H-pyrrole 24. Repeating this reaction with commercially available [^{15}N]-phthalimide 25a afforded [1-^{15}N]-3-formyl-4-methyl-1H-pyrrole 24a.

As expected the NH group in the product 32 can be easily alkylated under basic condition. In this case benzyl bromide has been used to obtain N-benzyl-3-cyano-4-methylpyrrole 33 in high yield.

1,1-Dimethoxyacetone 30 or its homologous in any isotope enriched form is easily accessible via a Pummerer reaction of 1-phenyl sulfoxyl acetone.[25] Also many homologous of acrylonitrile are easily accessible. This means synthetic routes shown in scheme 4 will give access to many isotopically labelled pyrroles directly via subsequent functional group transformations.

c) Synthesis Of [1-^{15}N, 2, 2', 3, 3'-^{13}C4]-Pyrrole-2,3,5-Tricarboxylic Acid 34a.

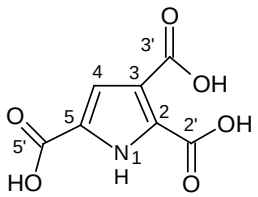
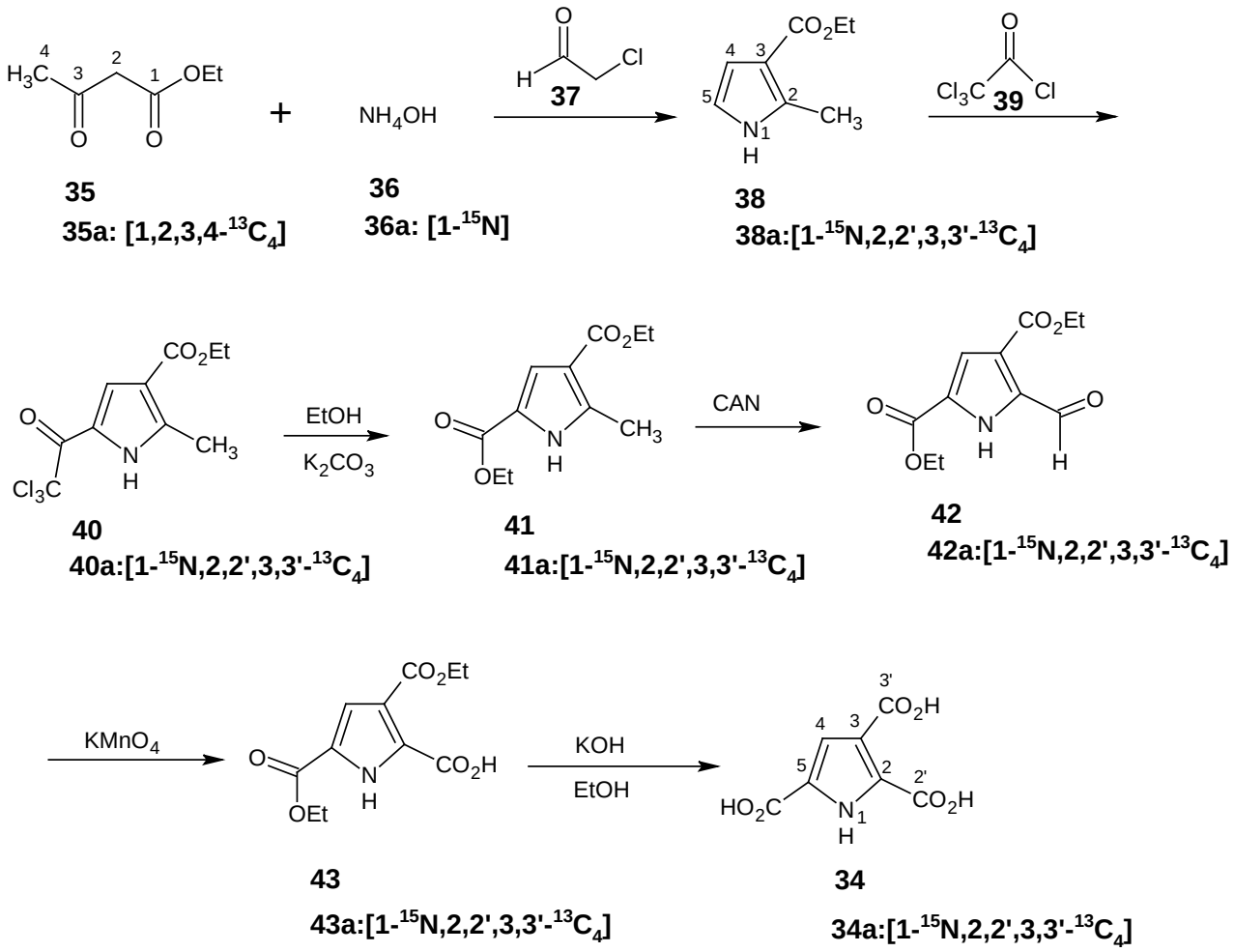


Figure 3. Structure and numbering of [1-^{15}N, 2, 2', 3, 3'-^{13}C4]-pyrrole-2,3,5-tricarboxylic acid 34a.

The synthesis of the product 34a has been reported.[10] Commercially available [^{13}C4]-ethyl acetoacetate 35a is treated with commercially available [^{15}N]-ammonia in water in the presence of 2-chloroacetaldehyde 37. [1-^{15}N, 2,2',3,3'-^{13}C4]-2-Methyl-3-carbethoxypyrrole 38a is obtained via Hantzsch pyrrole synthesis in 35% yield which is in competition with a Feist-Benary reaction leading after HCl treatment to [2,2',3,3'-$^{13}C_4$]-2-methyl-3-carbethoxyfuran. In the Hantzsch pyrrole synthesis the enamine of the 3-keto carboxylate attacks the aldehyde function of the 2-chloro keto molecule. After ring closure and dehydration the pyrrole system is obtained. In the Fiest-Benary reaction the initial reaction is the attack of the anion of the active methylene derivative on the aldehyde function of chloroacetaldehyde and subsequent ring closure.[25]

Product 38a is treated with trichloroacetyl chloride 39 to give a quantitative yield of trichloromethyl keto compound 2-methyl-3-carbethoxy pyrrole 40a. Treatment with ethanol in the presence of potassium carbonate afforded the diester 41a, upon cerium ammonium nitrate oxidation the 2-methyl group is converted into an aldehyde function (compound 42a).

Treatment with potassium permanganate oxidized the aldehyde function into a carboxylic acid function 43a. A final base induced saponification afforded [1-^{15}N, 2,2',3,3'-^{13}C4]-pyrrole-2,3,5-tricarboxylic acid 34a.



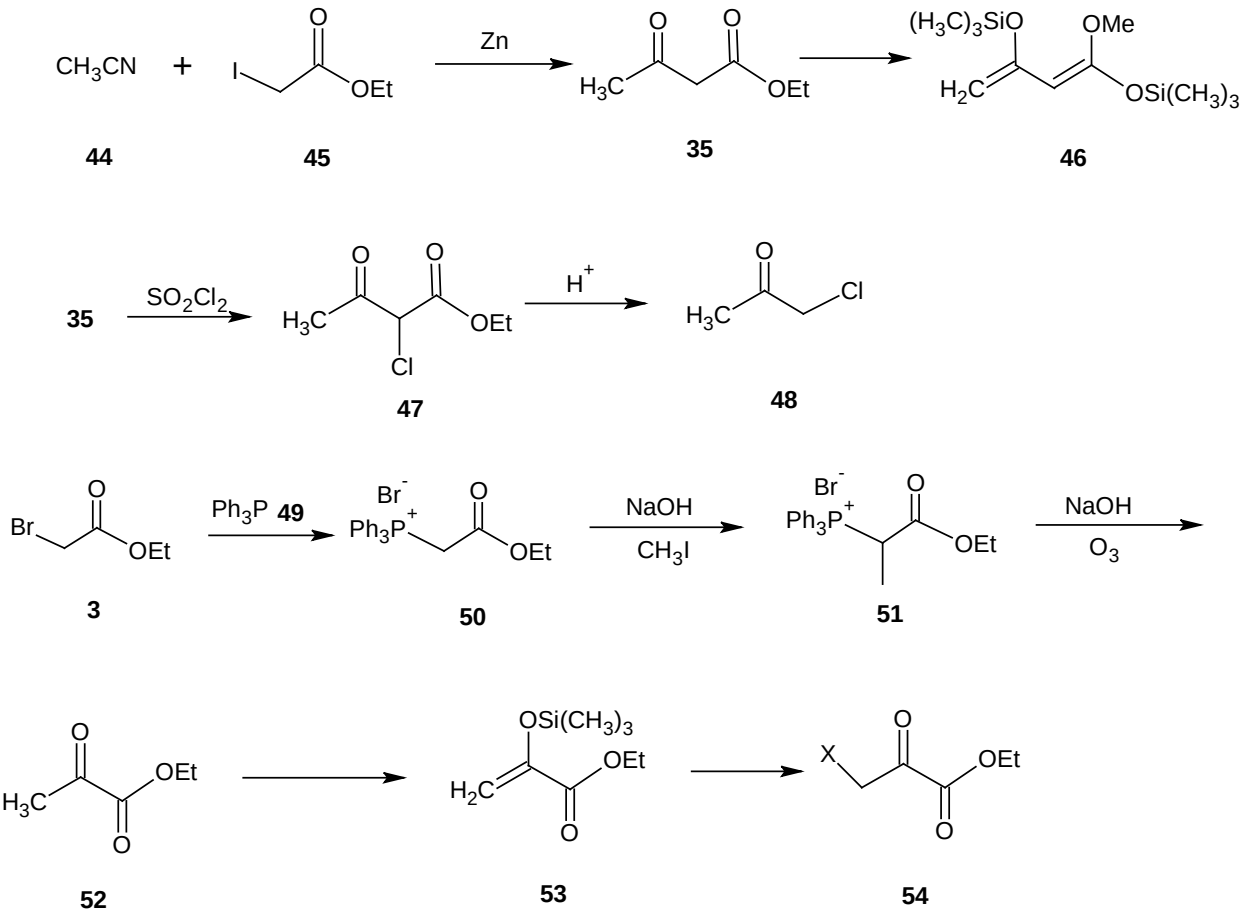
Scheme 5. Synthesis of [1-^{15}N, 2,2',3,3'-^{13}C$_4$]-pyrrole-2,3,5-tricarboxylic acid 34a.

Pyrroles 38a, 40a, 41a, 42a and 43a in scheme 5 have isotope incorporation in ^{15}N on position 1 and ^{13}C$_4$ incorporation on positions 2, 2', 3 and 3'. Via scheme 5 many pyrrole systems can be enriched besides the positions 2, 2', 3 and 3'.

α-Halogenated aldehydes such as 2-chloroacetaldehydes are accessible. Aldehydes that can be easily obtained from stable isotope enriched nitrile esters and alcohol etc.[27]

A general method to prepare the corresponding α-chloroderivatives has been reported.[28] Many α-amino acids are commercially available in stable isotope enriched form. Recently, an efficient method to convert them into 2-chloroaldehydes has been reported.[29] Trichloroacetic acid is commercially available in ^{13}C-enriched form; it can be easily converted into the corresponding chloride.[30]

The Blaise reaction of acetonitrile 44 with zinc enolate of ethyl iodoacetate 45 has been reported.[31] Acetonitrile 44 is commercially available in all possible isotopomers. Zinc derivative of 45 is accessible via ethyl 2-bromoacetate 3 (scheme 1). Many other nitriles and α-bromoesters are accessible in all possible stable isotope enriched forms. Trimethyl silylation of 35 gives the bis(trimethylsilyloxy)butadiene derivatives 46. Treatment with 1 mol of bromine afforded methyl 4-bromo-3-ketobutyrate.[32] This means that besides ethyl acetoacetate 35 many 3-ketoesters are accessible.



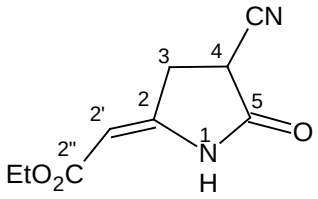
Scheme 6. Preparation of ethyl acetoacetate 35 and ethyl 2-chloro-3-oxobutyrate 47 in any isotopomeric form using acetonitrile 44 and ethyl iodoacetate 45.

Ethyl acetoacetate 35 can be easily monochlorinated by treatment with sulphuryl chloride. Subsequent acid catalyzed hydrolysis and carbon dioxide elimination results in 1-chloroacetone 48. Similarly, many monochloroketones will be accessible in any stable isotope labelled form.

Ethyl bromoacetate 3 treated with triphenyl-phosphine 49 to form triphenyl phosphonium salt 50. After addition of 1eq NaOH and subsequent treatment with CH_3I, the propionate phosphonium salt 51 is formed. Further treatment with base and ozonolysis ethyl pyruvate 52 is formed.[33]

Ethyl pyruvate 52 can be converted in the corresponding trimethyl silyl ether 53.[34] Halogenation of the product 53 afforded the 3-halogenopyruvate 54. Using the reactions discussed in scheme 5 together with the building blocks given in scheme 6 it is clear that a very extended range of pyrroles in all possible stable isotopomeric forms are now accessible.

d) Synthesis Of Ethyl (2Z)-(4-Cyano-5-Oxopyrrolidin-2-Ylidene)Ethanoate 55.



55

Figure 4. Structure and numbering of ethyl (2Z)-(4-cyano-5-oxopyrrolidin-2-ylidene)ethanoate 55.

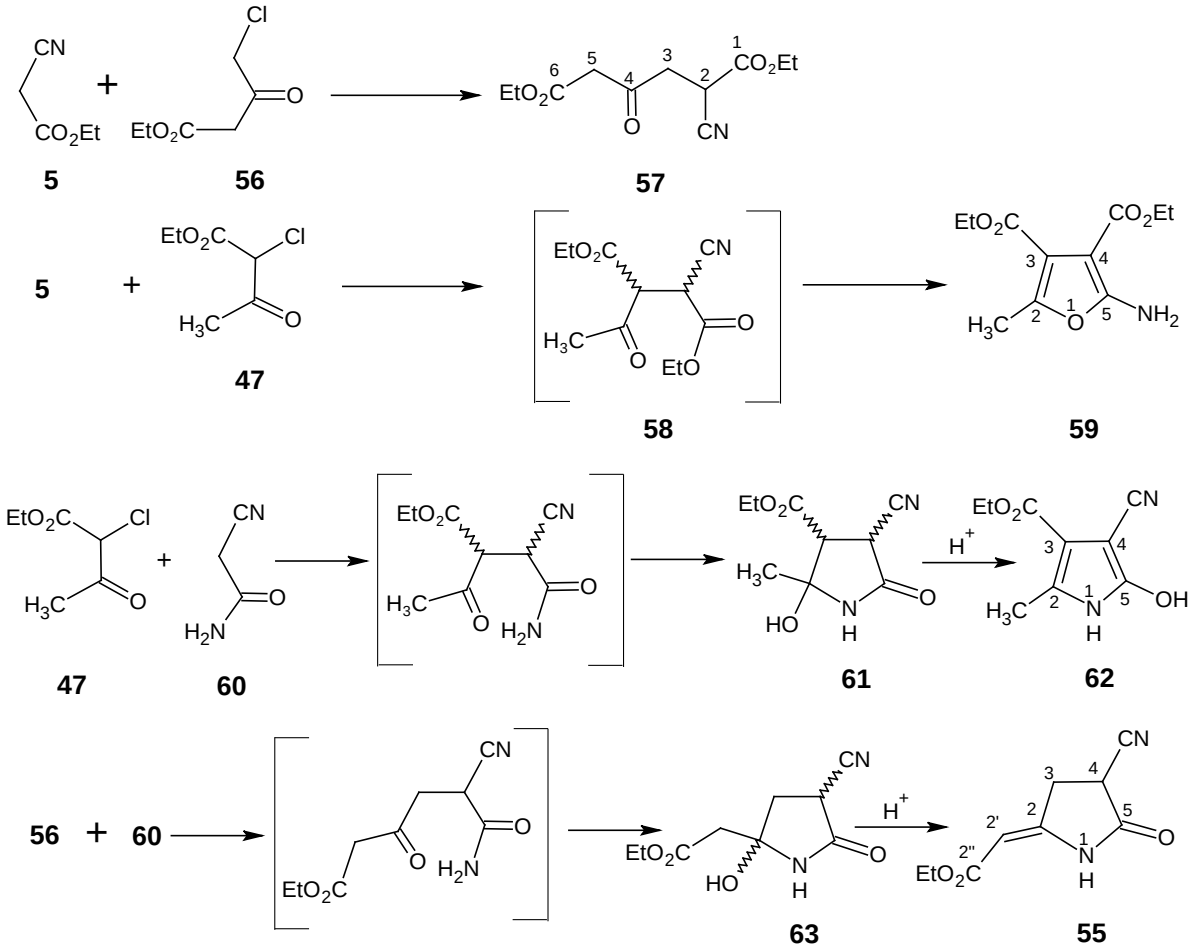
Ethyl cyanoacetate 5 is treated with ethyl 4-chloro-3-oxobutyrate 56 (prepared by chlorination of 46 in scheme 6) in the presence of 1 eq of triethylamine in refluxing toluene (scheme 7).[35] Diethyl 2-cyano-4-oxohexanedioate 57 is the single product in high yield. This product is the result of an S_N2 reaction of the anion of the active methylene compound without competing Feist-Benary reaction in the Hantzsch pyrrole synthesis because the nonnucleophilic base triethylamine cannot give the Hantzsch pyrrole system.

A similar reaction between ethyl cyanoacetate 5 and ethyl 2-chloro-3-oxobutyrate 47 afforded 2-amino-3,4-dicarbethoxy-5-methylfuran 59 in high yield (scheme 7). This molecule has been described in the literature.[36,37] In this case the S_N2 reaction of the anion of ethyl cyanoacetate 5 must have been the first step followed by a base catalyzed cyclization to the furan derivative 59.

It is to be expected that triethylamine induced alkylations of active methylene derivatives with aldehyde or keto functions in both the chloride reagent and the active methylene compound will give 1,4-dicarbonyl systems in Paal-Knorr pyrrole, Paal-Knorr furan and Paal-Knorr thiophene syntheses in high yield.[38]

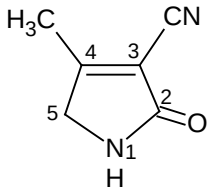
2-Cyanoacetamide 60 is expected to react with ethyl 2-chloro-3-oxobutyrate 47 using triethylamine as a base to give the initial S_N2 reaction on the active methylene carbon without reaction on the amide function due to its higher pKa value.

The carbonyl group and the amide group cyclised to give compound 61. Acid catalyzed dehydration afforded 2-methyl-3-ethoxycarbonyl-4-cyano-5-hydroxypyrrole 62.[35] Similarly, ethyl 4-chloro-3-oxoacetate 56 and 2-cyanoacetamide 60 afforded a high yield of the cyclic derivative 63. Product 55 is obtained via acid catalyzed dehydration of product 63. These 2-oxypyrrole derivatives have important pharmaceutical and biological properties.



Scheme 7. Preparation of ethyl (2Z)-(4-cyano-5-oxopyrrolidin-2-ylidene)ethanoate 55.

e) Synthesis Of [2-^{13}C]-3-Cyano-4-Methyl-3-Pyrrolin-2-One 64a And [3-^{13}C]-3-Cyano-4-Methyl-3-Pyrrolin-2-One 64b.



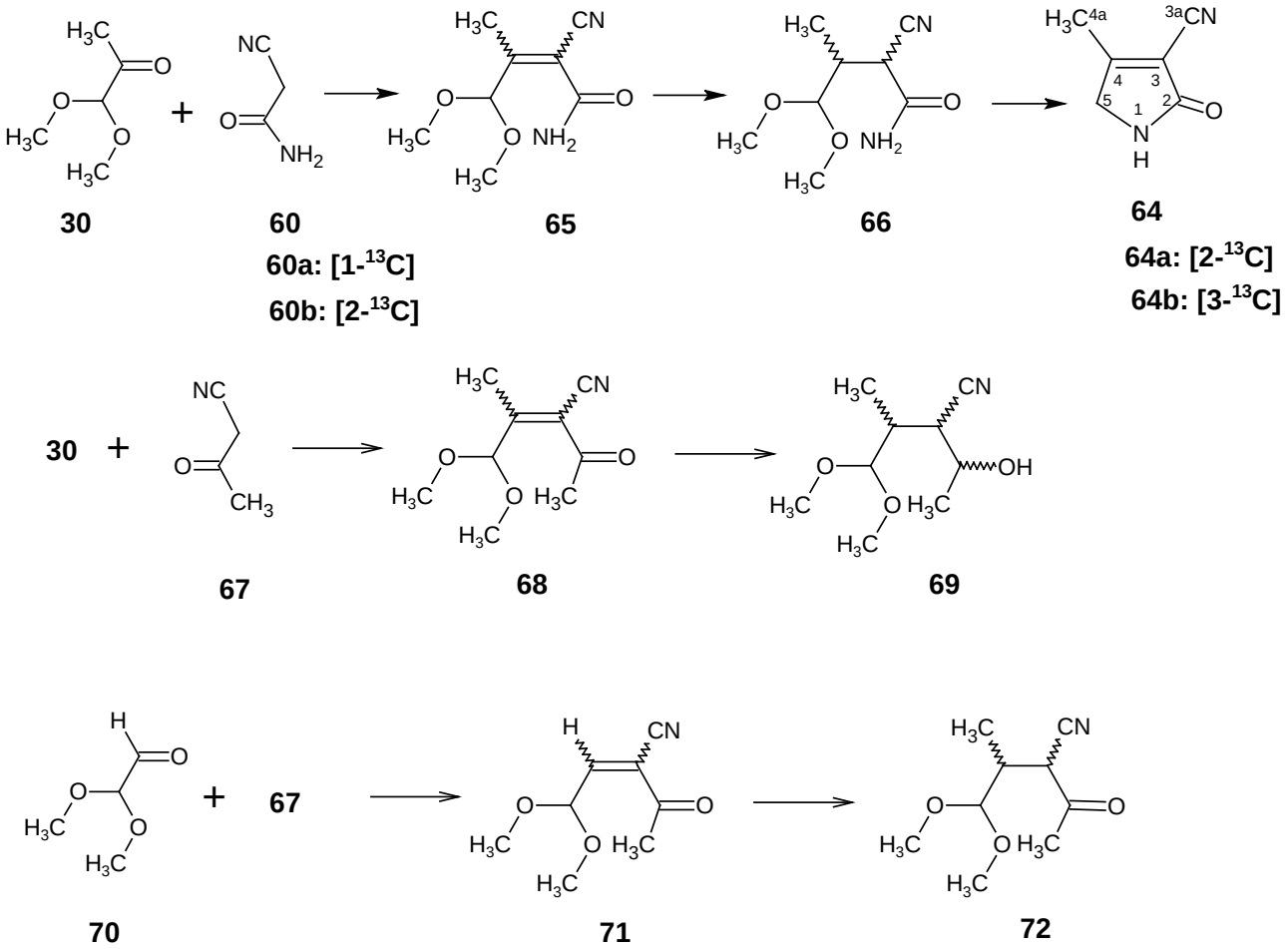
64

64a: [2-^{13}C]

64b: [3-^{13}C]

Figure 5. Structure and numbering of [2-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64a and [3-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64b.

Even earlier than the above discussed alkylation of active methylene reagents, the Knoevenagel reaction between active methylene compounds, ketones and aldehydes to form [2-^{13}C]- and [3-^{13}C]-3-cyano-4-methyl-3-pyrrolin-3-one 64a and 64b, respectively (fig. 5) has been reported.[39] In scheme 8 it is depicted that 1,1-dimethoxyacetome 30 and 2-cyanoacetamide 60 in refluxing toluene in the presence of ammonium acetate and acetic acid afforded a high yield of a mixture of (E)- and (Z)-2-cyano-3-methyl-4,4-dimethoxybut-2-enamide 65. Due to the presence of two electron withdrawing groups in the molecule the acetal function is relatively acid stable. The double bond can easily be reduced by sodium borohydride in ethanol to form the enantiomeric mixtures of 2-cyano-3-methyl-4,4-dimethoxybutanamide 66.



30 **60** **65** **66** **64**

60a: [1-^{13}C]

60b: [2-^{13}C]

64a: [2-^{13}C]

64b: [3-^{13}C]

30 **67** **68** **69**

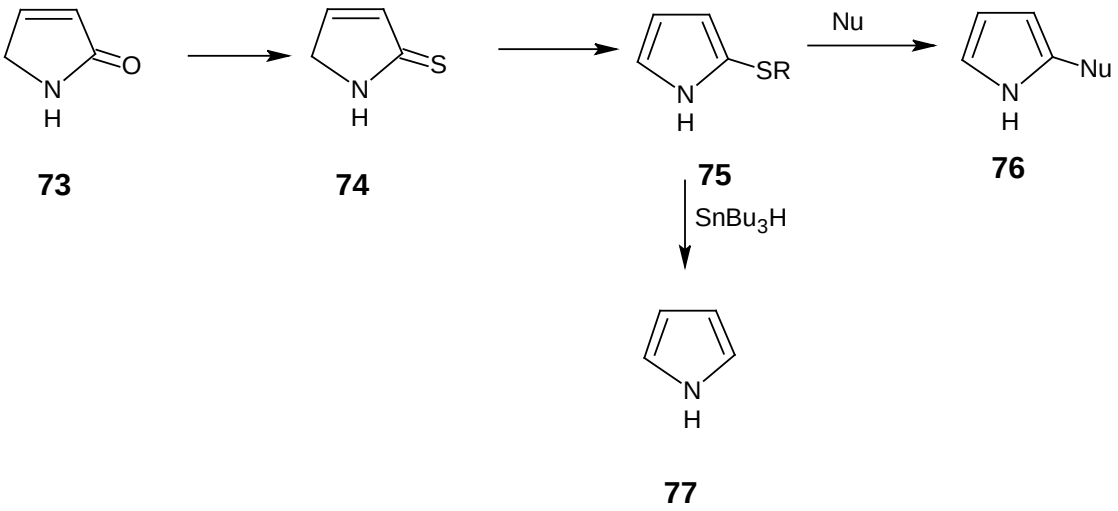
70 **67** **71** **72**

Scheme 8. The preparation of [2-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64a and [3-^{13}C]-3-cyano-4-methyl-3-pyrrolin-2-one 64b from 1,1-dimethoxyacetone 30 and [1-^{13}C]-2-cyanoacetamide 60a and [2-^{13}C]-2-cyanoacetamide 60b, respectively.

Upon mild acid treatment of (E)- and (Z)- 2-cyano-3-methyl-4,4-dimethoxybutanamide 66 afforded 3-cyano-4-methyl-3-pyrrolin-2-one 64 in a high yield. Using [1-^{13}C]-2-cyanoacetamide 60a and [2-^{13}C]-2-cyanoacetamide 60b, [2-^{13}C]-64a and [3-^{13}C]-64b have been prepared, respectively. In order to get to 3-vinyl-4-methyl-3-pyrrolin-2-one 89 for the ring D of biliverdin IXα and phytochromobilin (vide infra), 1,1-dimethoxyacetone 30 is treated with 1-cyanoacetone 67 under Knoevenagel conditions to give an excellent yield of 5,5-dimethoxy-4-methyl-3-cyanopentan-2-one 68 (scheme 8). The reduction of the 3,4 double bond and keto function occurred simultaneously to give an enantiomeric mixture of 5,5-dimethoxy-4-methyl-3-cyanopentan-2-ol 69. Pinner reaction of 69 in aqueous media to convert the nitrile function into the amide function and subsequent ring closure to get 3-(1'-hydroxyethyl)-4-methyl-3-pyrrolin-2-one could not be realized.

As an alternative the Knoevenagel condensation between 1,1-dimethoxyacetaldehyde 70 and 1-cyanoacetone 67 afforded a high yield of 5,5-dimethoxy-3-cyanopent-3-ene-2-one 71. Treatment of 71 with methyl magnesium iodide and cuprous cyanide gave in a high yield of the 1,4-addition product 5,5-dimethoxy-4-methyl-3-cyanopentan-2-one 72. Conversion of product 72 to the required 3-pyrrolin-2-one was not successful. However, 3-pyrrolin-2-ones are now accessible via alkylation reactions of active methylene compounds with amide functions.

Compound 72 (a protected 1,4-dicarbonyl compound) can easily be converted in the corresponding pyrrole, thiophene and furan systems.[25] The scope of the Knoevenagel reaction has been extended.[40] At present various 3-pyrrolin-2-ones are easily available. This is a new approach to pyrrole synthesis. These systems react with Lawesson's reagent to give the corresponding thioamide system 74.[25]



73 **74** **75** **76** **77**

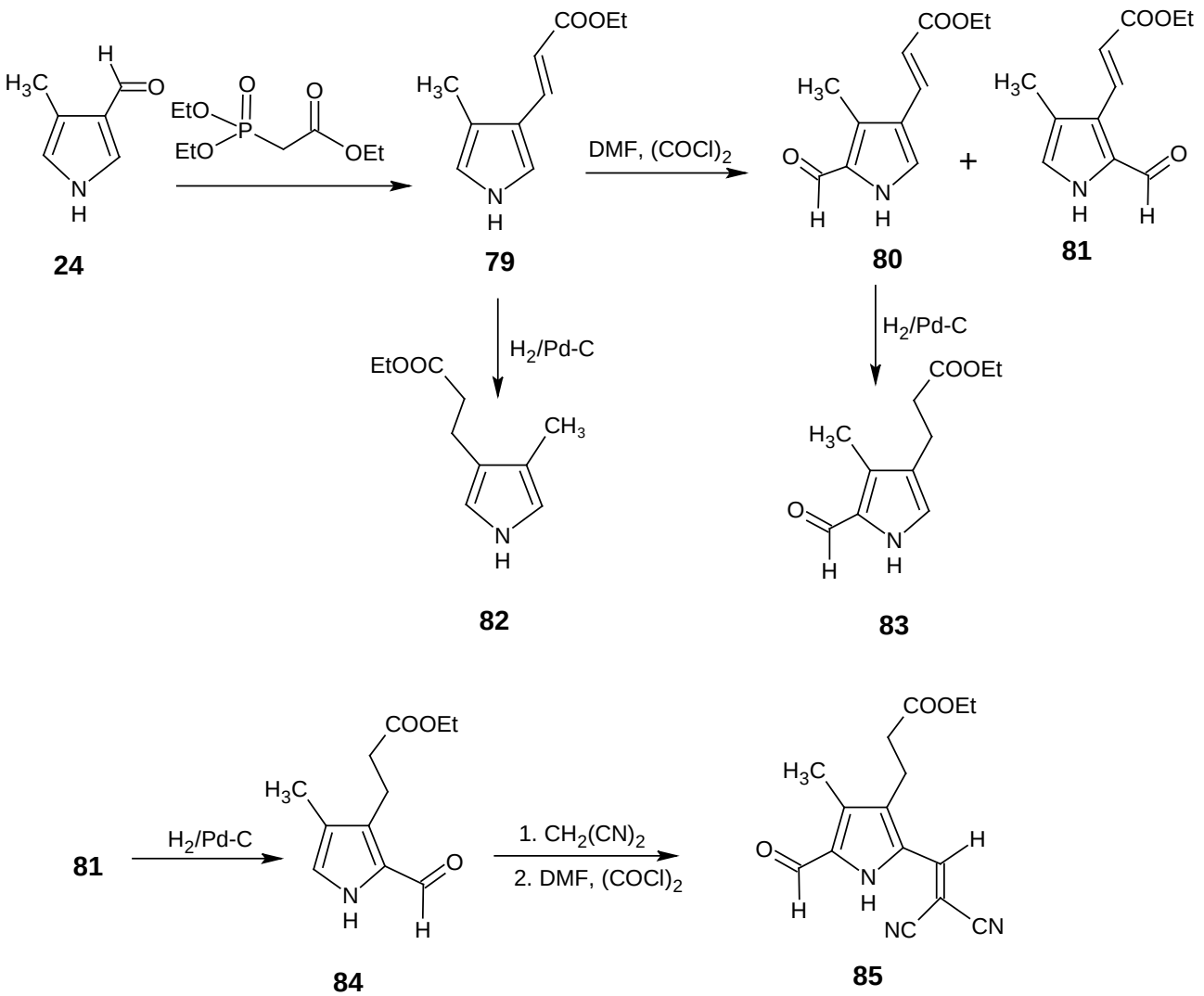
Scheme 9. Conversion of 3-pyrrolin-2-one 73 into 2-substituted pyrrole 76 and pyrrole 77.

The thioamide treated with various alkylhalo - genides to form the 2-thioalkyl substituted pyrroles. The 2-thioalky is easily substituted for many other 2-substituents via various nucleophiles.[41] The thioalkyl group has been substituted for hydrogen via radical reaction with tributyltin hydride to give the pyrrole without a substituent on position 2.[42]

f) Chemoenzymatic Synthesis Of (2R)-Phytochromobilin 80, The Chromophore Of Phytochromes

Biliverdin IXα 78 can be converted into (2R)-phytochromobilin[16] 91 that spontaneously reacts with the apoprotein of phytochromes to form fully active phytochrome (fig. 6).[43,44]

3-Formyl-4-methyl-1H-pyrrole 24 (scheme 4) which can be obtained in any stable isotope enriched form forms the primary building block of both rings B and C of biliverdin IXα 78.

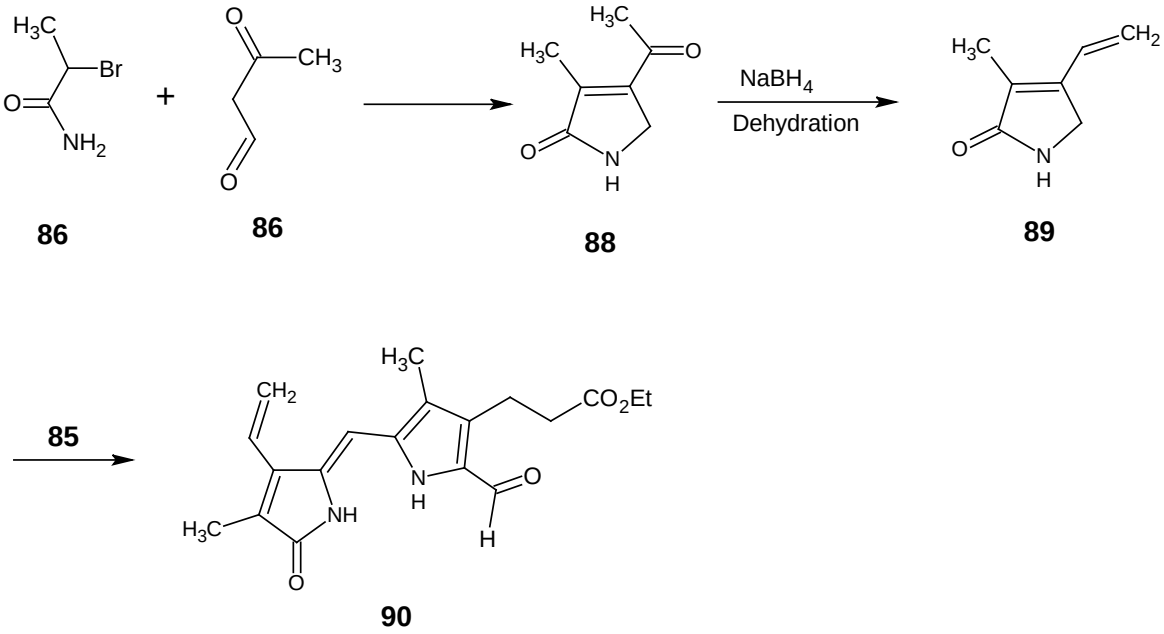


Scheme 10. Conversion of 3-formyl-4-methyl-1H-pyrrole 24 into the necessary building blocks of phytochromobilin 91.

In scheme 10 a synthetic scheme is shown that converts 3-formyl-4-methyl-1H-pyrrole 24 into the necessary building blocks of phytochromobilin 91.

Triethyl phosphonoacetate is obtained from the reaction of ethyl bromoacetate 3 and triethylphosphite. Triethyl phosphonoacetate is treated with 3-formyl-4-methyl-1H-pyrrole 24 (scheme 4) to obtain ethyl 3-(4-methyl-1H-pyrrol-3-yl)acrylate 79.[15] Vilsmeier formylation of product 79 afforded a mixture of the two pyrrole aldehydes which can easily be separated into ethyl 3-(5-formy-4-methyl-1H-pyrrol-3-yl)acrylate 80 and ethyl 3-(2-formyl-4-methyl-1H-pyrrol-3-yl)acrylate 81. Catalytic reductions of the double bond in pyrrole aldehydes 80 and 81 led to 3-[2'-(ethoxycarbonyl)ethyl]-4-methyl-1H-pyrrole-5-aldehyde 83 and 3-[2'-(ethoxycarbonyl)ethyl]-4-methyl-1H-pyrrole-2-aldehyde 84, respectively. Via the Knoevenagel reaction the 2-formyl group in the product 84 is protected as a dicyanovinyl group. A subsequent Vilsmeier formylation afforded in ethyl 3-[2-(2,2-dicyanoethenyl)-5-formyl-4-methyl-1H-pyrrol-3yl] propanoate 85.

In scheme 11 it is indicated that the pyrromethenone building block containing rings A and B is accessible in any stable isotope enriched form.



Scheme 11. Synthesis of pyrromethenone 90 (ring A and ring B) of the phytochrome 91.

2-Bromopropionamide 86 is condensed with 3-oxobutanal 87. Subsequent dehydration of the product gave product 88.[35] Reduction of the carbonyl function and subsequent dehydration results in 3-vinyl-4-methyl-3-pyrrolin-2-one 89. The condensation of product 89 with product 85 and subsequent deprotection of the aldehyde function afforded the product 90.[45]

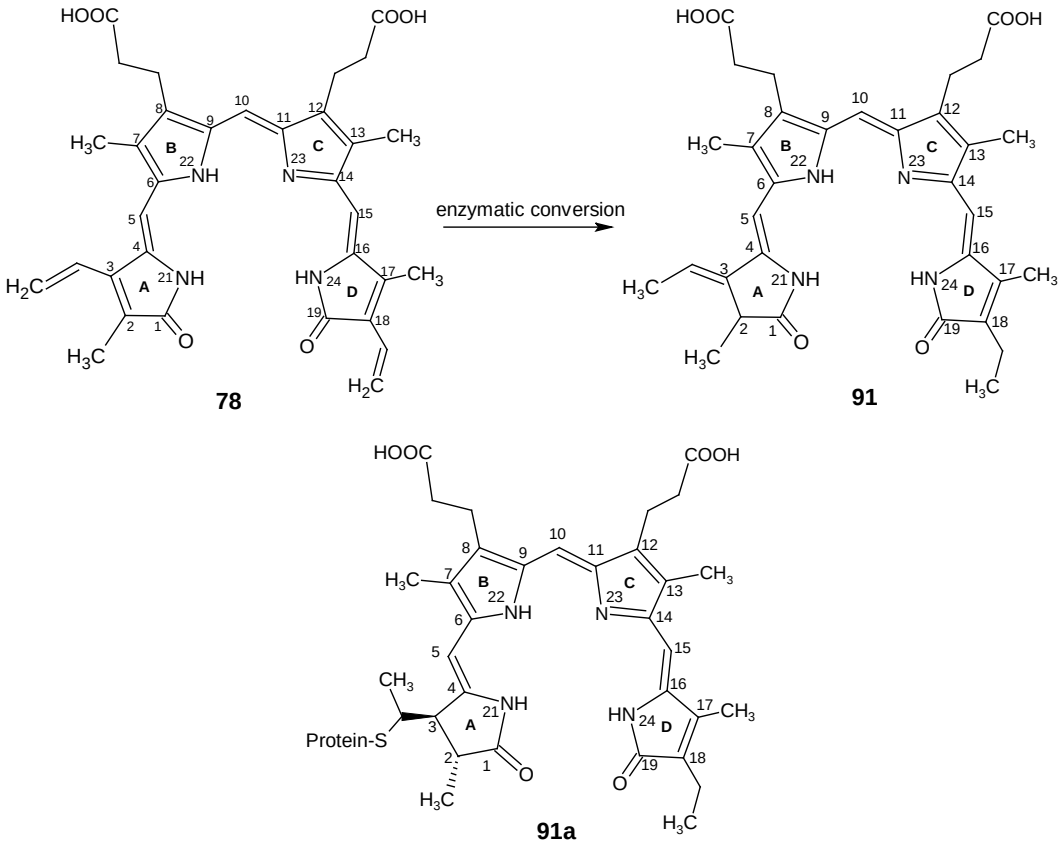
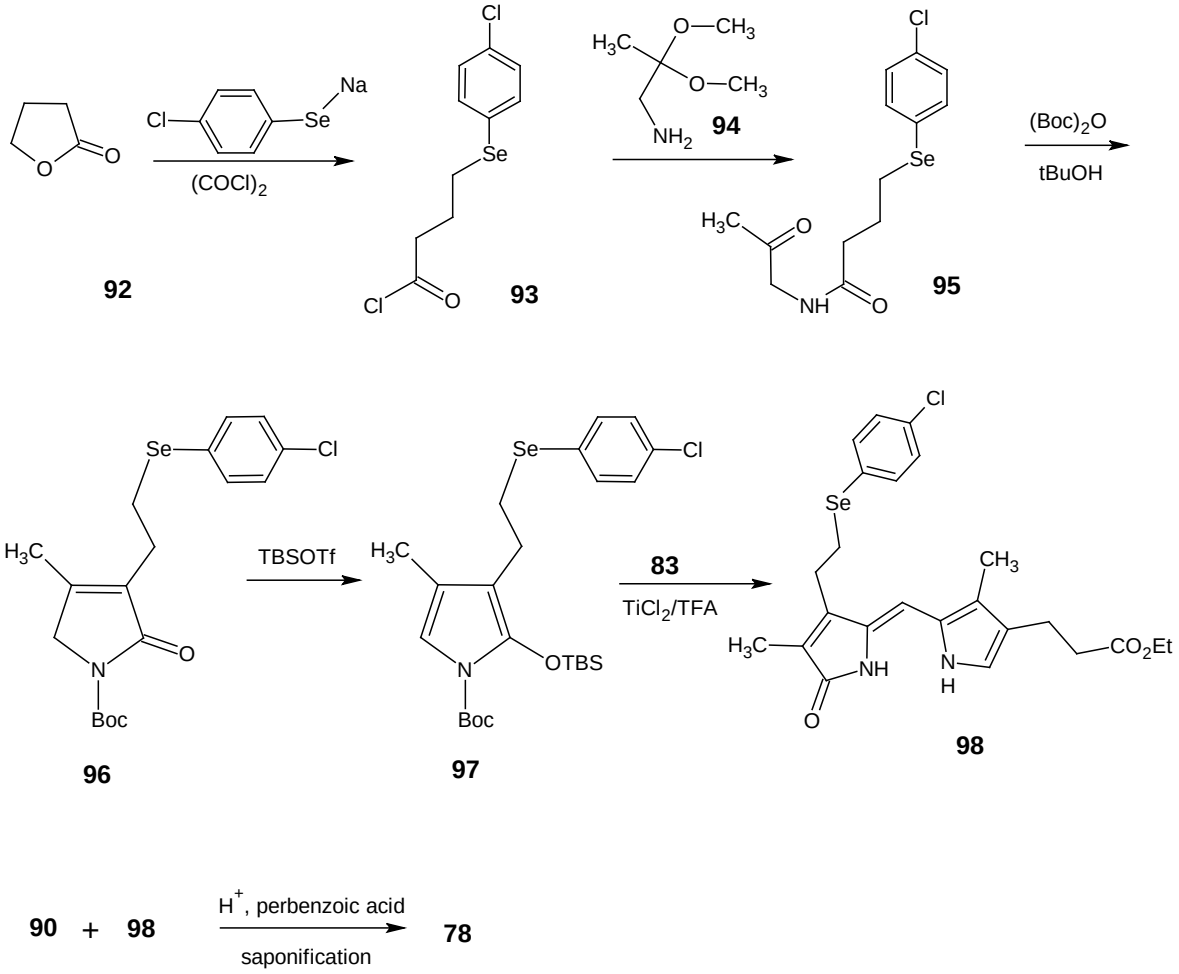


Figure 6. Structure and numbering of biliverdin IXα 78 and (2R)-phytochromobilin 91. Structure 91a represents the linkage of ring A to the protein of the phytochrome.

In scheme 12 it is indicated that the C and D building blocks are accessible in any stable isotope enriched form. Butyrolactone 92 treated with the sodium salt of parachlorobenzene selenol to give the 4-selenophenyl butyrate that treated with oxalyl chloride to form the chloride product 81.[46]

Butyrolactone 92 is accessible in any stable isotope labelled form via a Pinner reaction of 4-hydroxybutyronitrile 12 (scheme 1). Product 93 treated with 2,2-methoxypropylamine 94 to give product 95 after deacetalization. The protected 1-aminoacetone 94 is easily accessible via nitrosation of ethyl acetoacetate 35 (scheme 5).[47]

After introduction of BOC protection of the amide base induced ring closure to the 3-pyrrolin-2-one with tert-butyl alcoholate is effected.[46] Treatment of product 96 with tributyl silyl triflate results in the double protected pyrrole 97. This building block condensed with the pyrrole aldehyde 82 under formation of the pyrromethenone 98.[46] A final acid condensation afforded in the tetrapyrrole system which after oxidative desalination gives the vinyl group in the D ring giving the dimethyl ester of biliverdin IXα. A mild saponification afforded in biliverdin IXα 78.



Scheme 12. Synthesis of biliverdin IXα 78.

III. Conclusion

Nowadays there is a strong synthetic effort in the pyrrole field. Many new synthetic reactions and new pyrroles are worked out and reported. Many of the building blocks that are used in these processes can be made accessible in various stable isotope enriched form. In the near future whole new libraries of stable isotope enriched pyrroles will become available.

IV. Acknowledgment

This paper is written with great indebtedness to investigators who have been involved in pyrrole synthesis and the preparation of ^{13}C and ^{15}N enriched building blocks. We dedicate this paper to future investigators who will use the now accessible isotopomers to unravel the role of medically, pharmaceutically and biologically important pyrrole systems without perturbation at the atomic level.

References Références Referencias

1. Black, D. StC. '1H-Pyrroles', in Science of synthesis, hetarenes and related ring systems, Thieme Verlag, Stuttgart. 2001, 9, 441 – 552.
2. Bellur, E.; Freifeld, I.; Langer, P. Tetrahedron Lett. 2005, 47, 2151-2154.
3. Daidone, G.; Maggio, B.; Schillaci, D. Pharmazie 1990, 45, 441-442.
4. A. Kimura, T.; Kawara, A.; Nakao, A.; Ushiyama, S.; Shimozato, T.; Suzuki, K. PCT Int Appl. CODEN:PIXXD2 WO 2000001688 A1, 200001132000, p 173.

   B. Kaiser, D. G.; Glenn, E. M. J. Pharm. Sci. 1972, 61, 1908-1911.
5. Demir, A. S.; Akhmedov, I. M.; Sesenoglu, O. Tetrahedron 2002, 58, 9793-9799.
6. Meshram, H. M.; Prasad B. R. V.; Kumar, D. A. Tetrahedron Lett. 2010, 51, 3477–3480.
7. Davis, F. A.; Bowen, K.; Xu, H.; Velvadapu, V.; Ballard, C. Tetrahedron 2008, 64, 4174-4182.
8. Reisser, M.; Maas, G. J. Org. Chem. 2004, 69, 4913-4924.
9. Mathew, P.; Asokan, C. V. Tetrahedron 2006, 62, 1708-1716.
10. Skaddan, M. B. J. Labelled Comp. and Radiopharm. 2010, 53, 73–77.
11. Scott, A. I.; Burton, G.; Fagerness, P. E. J. Chem. Soc., Chem Commun. 1979, 199–202.
12. Battersby, A. R.; Hunt, E.; McDonald, E.; Paine III, J. B.; Saunders, J. J. Chem. Soc., Perkin Trans. 1 1976, 1008–1018.
13. Roy, E.; Rohmer, T.; Gast, P.; Jeschke, G.; Alia, A.; Matysik, J. Biochemistry 2008, 47, 4629–4635.
14. Siebert, F.; Hildebrandt, P. Vibrational Spectroscopy in Life Science; Wiley-VCH: Weinheim, 2008.
15. Dawadi, P. B. S.; Lugtenburg, J. Targets in Heterocyclic Systems, 2008, 12, 1-30. Eds. O. A. Attanasi and D. Spinelli.
16. Andel III, F.; Murphy, J. T.; Haas, J. A.; McDowell, M. T.; van der Hoef, I.; Lugtenburg, J.; Lagarias, J. Biochemistry, 2000, 39, 2667–2676.
17. Buldain, G.; Valasinas, A. J. Labelled Comp. and Radiopharm. 1980, 19, 1–5.
18. Burton, G.; Fagerness, P. E.; Hosozawa, S.; Jordan, P. M.; Ian Scott, A. J. Chem. Soc., Chem Comm. 1979, 202-204.
19. Dawadi, P. B. S.; Schulten, E. A. M.; Lugtenburg, J. J. Labelled Compd. Radiopharm. 2009, 52, 341-349.
20. Raap, J.; Wolthuis, W. N. E.; Hehenkamp, J. J. J.; Lugtenburg, J. Amino Acids 1995, 8, 171-186.
21. Barton, D. H. R.; Zard, S. J. Chem. Soc.,Chem. Commun. 1985, 1098–1100.
22. Cappon, J. J.; Witters, K. D.; Baart, J.; Verdegem, P. J. E.; Hoek, A. C.; Luiten, R. J. H.; Raap, J.; Lugtenburg, J. Recl. Trav. Chim. Pays-Bas 1994, 113, 318–328.
23. Van Leusen, A. M. Lect. Heterocyclic Chem. 1980, 5, S111-122.
24. Dawadi, P. B. S.; Lugtenburg, J. Eur. J. Org. Chem. 2008, 2288–2292.
25. Li, J. J. Name Reactions Third Expanded Edition Springer-Verlag Berlin 2000.
26. Bravo, P.; Resnati, G. J. Chem. SOC., Chem. Commun. 1988, 218-219.
27. Mundy, B.P.; Ellerd, M.G.; Favaloro, F. J. Name Reactions and Reagents in Organic Synthesis: 2nd edition, John Wiley & Sons: Hoboken, NJ, 2005.
28. Halland, N.; Braunton, A.; Bachmann, S.; Marigo, M.; Jorgensen, K. A. J. Am. Chem. Soc., 2004, 126, 4790-4791.
29. Dekeukeleire, S.; D'hooghe, M.; T rnroos, K. W.; De Kimpe, N. J. Org. Chem. 2010, 75, 5934-5990.
30. Boullais, C.; Breton, J.; Nabedryk, E.; Mioskowski, C. Tetrahedron 1997, 53, 2505-2512.
31. Creemers, A. F. L.; Lugtenburg, J. J. Am. Chem. Soc. 2002, 124, 6324–6334.
32. Chan, T. H.; Brownbridge, P. J. Chem. Soc., Chem. Commun., 1979, 578-579.
33. Siebum, A. H. G.; Woo, W. S.; Lugtenburg, J. Eur. J. Org. Chem. 2003, 4664-4678.
34. Krebs, A.; Bolm, C. Tetrahedron 2011, 67, 4055 - 4060.
35. Dawadi, P. B. S.; Lugtenburg, J. Tetrahedron Lett. 2011, 52, 2508-2510.
36. Bakavoli, M.; Feizyzadeh, B.; Rahimizadeh, M. Tetrahedron Lett. 2006, 47, 8965-8968.
37. Hu, Y. G.; Li, G. H.; Ding, M. W. Arkivoc. 2008, xiii, 151–158.
38. Kurti, L.; Czako, B. Strategic applications of the named reactions, Elsvier Academic Press, 2005.
39. Dawadi, P. B. S.; Lugtenburg, J. Eur. J. Org. Chem. 2007, 1294–1300.
40. Dawadi, P. B. S.; Lugtenburg, J. Synth. Comm. 2010, 40, 2539-2546.
41. Khalifa, A. F.; Ismail, A. N.; Elghandour, H. H. A.; Zohdi, F. H. Tetrahedron, 1991, 47, 8243-8250.
42. Antonio, Y.; Cruz, M. E. D. L.; Maddox, M. L.; Muchowski, J. M. Can. J. Chem . 1994, 72, 15-22.
43. Rohmer, T.; Lang, C.; Bongards, C.; Gupta, K. B.; Neugebauer, J.; Hughes, J.; Gärtner, W.; Matysik, J. J. Am. Chem. Soc. 2010, 132, 4431-4437.
44. Makhynya, Y.; Hussain, Z.; Bauschlicher, T.; Schwinte, P.; Siebert, F.; Gaertner, W.; Eur. J. Org. Chem. 2007, 1287-1293.
45. Plieninger, H.; Hentschel, K.-H.; Kohle, R.-D. Liebigs Ann. Chem., 1974, 1522-1530.
46. Jacobi, P.; Pippin, D. Org. Lett. 2000, 65, 827–830.
47. Kato, T.; Sato, M.; Yoshida, T. Chem. Pharm. Bull., 1971, 19, 292-296.