Award Accounts

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Studies on the Structure and Function of Phytochromes as Photoreceptors Based on Synthetic Organic Chemistry#

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We developed an efficient and flexible general method for the preparation of linear tetrapyrrole (bilin) chromophores of phytochromes as photoreceptors, including sterically locked derivatives as photoreceptors. Assembly experiments of the synthesized chromophores with apophytochromes in vitro and in vivo provided us insights into the structure and function of phytochromes.

1. Introduction

Light is vital not only for photosynthesis, but also for directing plant growth and development. The sensing of light in environmental conditions is essential for plants, as vision is for animals. To fine-tune their development according to light intensity, direction, wavelength, and periodicity, they possess three major chromoproteins–phytochromes,[1–5] cryptochromes,[4,6,7] and phototropin.[4,8]

Phytochromes, one of the best-characterized photoreceptors in plants, are a widespread family of red/far-red light responsive photoreceptors first discovered in plants[9,10] and have been recently also discovered in bacteria,[11–13] fungi,[14] and slime molds.[15] They play critical roles in various light-regulated processes, ranging from phototaxis and pigmentation in bacteria to seed germination, chloroplast development, shade avoidance, and flowering in higher plants. All phytochromes have a covalently attached linear tetrapyrrole (bilin) chromophore that absorbs light in red and far-red region.[5,16–22] Three different bilins are used as chromophores: land plant uses phytochromobilin (PΦB),[1] and cyanobacteria use phycocyanobilin (PCB),[23,24] which is also a chromophore of the light-harvesting pigment, phycocyanin, and differs from PΦB only by substitution of the vinyl group at the C18 position with an ethyl group,[1,24,25] whereas other bacteria use biliverdin (BV) (Fig. 1).[14,26,27]

In plant and cyanobacterial phytochromes, the natural chromophores PΦB and PCB are coupled by a thioether bond between the C3^1 position of the A-ring ethylidene side chain and a conserved cysteine residue within the so-called GAF domain of the proteins. Many bacterial phytochromes carry BV as natural chromophore, which is coupled in a different manner to the protein.

Phytochromes mediate various developmental processes in plants, through the photoconversion between the red light-absorbing (Pr) and the far-red light-absorbing (Pfr) forms.[1,10]

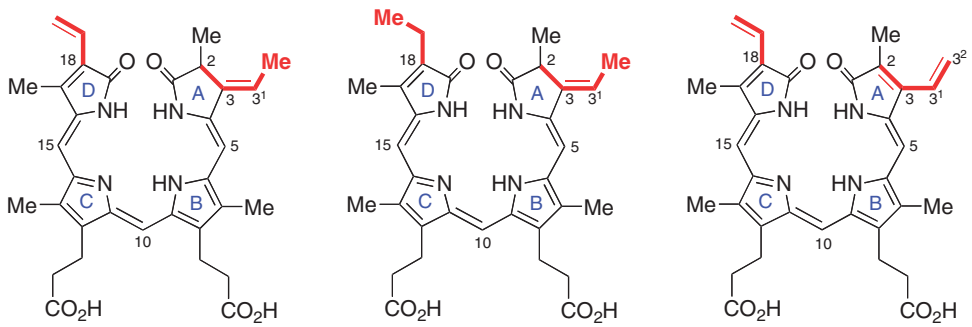

Fig. 1. Structure of three different types of bilin chromophores, PΦB used in plant phytochromes, PCB in cyanobacteria, and BV in other bacteria.

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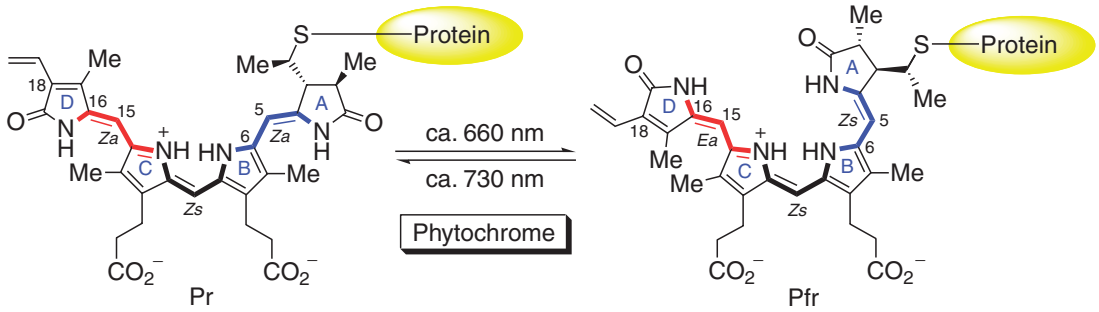


Fig. 2. One of the chromophore structures of the red light-absorbing (Pr) and the far-red light-absorbing (Pfr) forms in the photoreversible conversion of plant phytochromes, which has been recently proposed based on density functional theory (DFT) calculations of resonance Raman spectra.[32]

It is commonly accepted that the first step in the photoconversion from Pr to Pfr is a *Z* to *E* isomerization around the C15=C16 double bond between the C- and D-rings of the bilin chromophores.[28] This photoisomerization generally converts the physiologically inactive red light-absorbing Pr form into the active far-red light-absorbing Pfr form and vice versa. The interchange between the Pr and Pfr forms is essential for light-absorbing biological processes in the phytochrome chromophore function.

During photoconversion, the chromophore also moves around the exocyclic single bonds. In principle, each single bond can adopt either a *syn* or *anti* conformation.[16] Vibrational spectroscopy have provided indirect insight into the conformation of the phytochrome chromophore in the Pr, Pfr, and intermediate states, but the data were not unambiguous and have been interpreted in different ways.[29–32] For example, it has been proposed that the formation of Pfr is accompanied by a *syn*/*anti* rotation around the C14–C15 single bond.[31] More recently, interpretation of resonance Raman spectra of plant phytochromes by density functional theory (DFT) calculations indicated that the C14–C15 single bond is in an *anti* conformation throughout the entire photocycle and that the C5–C6 single bond rotates from *anti* to *syn* upon conversion from Pr to Pfr as shown in Fig. 2.[32]

By photoconverting between Pr and Pfr, phytochromes act as unique light-regulated switches in various signal transduction cascades. Despite intensive physicochemical analysis of various phytochromes, we do not yet understand how contacts between polypeptide and the bilin enable photoconversion between Pr and Pfr, how this transformation reversibly alters the activity of the photoreceptor, or how the holoprotein dimerizes.[22,33]

The development of yeast and bacterial system for the expression of recombinant phytochrome apoprotein has allowed investigation of the biochemical and spectroscopic properties of the reconstituted phytochromes.[34] Moreover, the photophysical and photochemical properties of wild-type phytochrome are quite similar to those of the reconstituted chromoproteins.[35,36] Other strategies, including systematic N- and C-terminal truncations and site-directed mutagenesis of the apoprotein, have been used to study the structural requirements of the chromophore–apoprotein interaction in terms of photochromism.[37–39]

Even though such bilin chromophores could be isolated from natural sources, knowledge of the relationship between the structure of synthetic bile pigments and the biochemical properties of the reconstituted biliproteins prepared by combining them with an apoprotein is quite interesting and important to determine the precise function of the bilin chromophores. However, in contrast to the vertebrate photoreceptor rhodopsin,[40] in the phytochrome field, little had been done to examine the relationships among chromophore structure, its assembly to apoprotein, and photochromism of the holoprotein, because of the difficulty of synthesizing the natural bilin chromophores and their structural analogs.

Gossauer and his co-workers have reported total syntheses of the dimethyl ester derivatives of PΦB and PCB around 1980, but they were unable to assemble these analogs with phytochrome apoprotein.[41–43] Surprisingly, there had been no report regarding the synthesis of the free acid form of PΦB or PCB applicable to assemble with the phytochrome apoproteins when we started our investigation on phytochrome chromophores in 1990.

Therefore, we have been studying on the total syntheses of natural and unnatural bilin chromophores,[44–54] and have succeeded in synthesizing PΦB,[49] PCB,[47,48,53] the modified PCBs,[50,51] BV and its analogs, including sterically locked derivatives,[54] in free acid forms by developing efficient methods for the preparation of each pyrrole ring and a method for coupling them and palladium-catalyzed deprotection of the allyl propanoate side chains of B- and C-rings under mild conditions.

Assembly experiments of the synthesized chromophores with phytochrome apoproteins in vitro and in vivo have allowed us to determine the following: (1) the different role of each substituent on four pyrrole rings of the chromophore in plant phytochrome, (2) structural requirement of bilin chromophore for the photosensory specificity of phytochromes A and B, (3) the binding site of BV chromophore in *Agrobacterium* phytochromes Agp1, and (4) the stereochemistry of the chromophore in Pr and Pfr forms of Agp1 and Agp2. In addition, (5) photoinsensitive single crystals of Pr with a locked chromophore were obtained for X-ray crystallographic analysis of the N-terminal photosensory module of phytochrome Agp1. From these results, it is obvious that an approach based on the synthetic organic chemistry toward the studies on the structure and function of phytochromes is very effective and necessary.

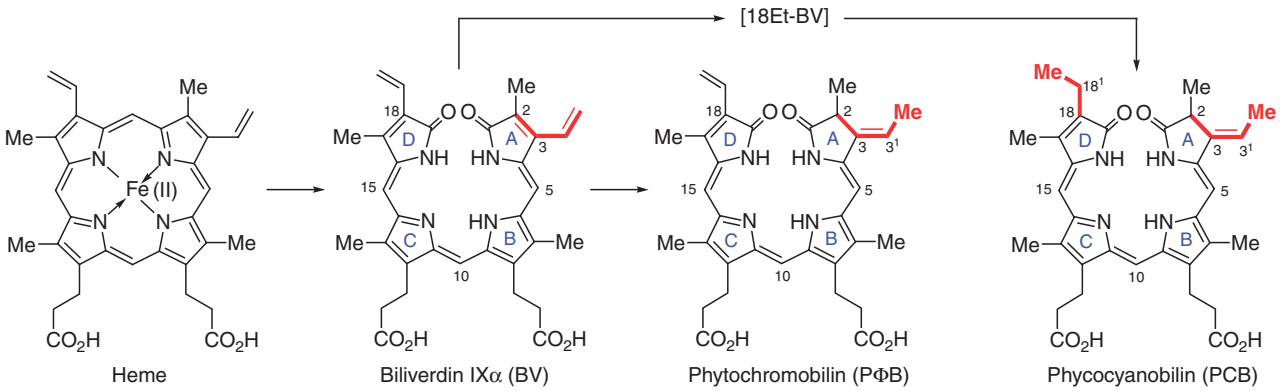


Fig. 3. Biosynthetic pathway of phytochrome chromophores starting from heme.

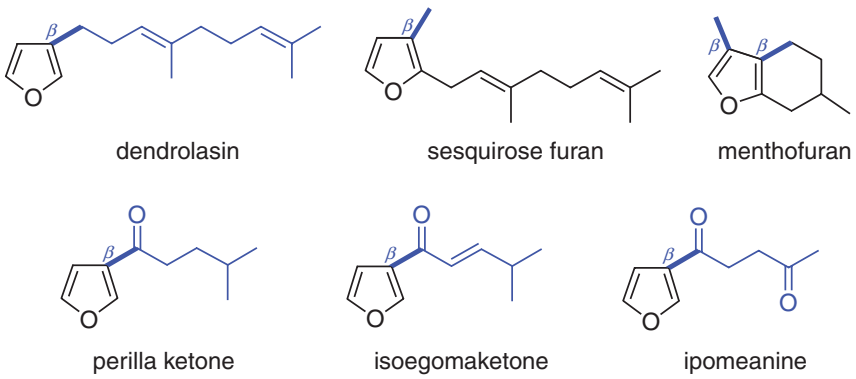


Fig. 4. Typical naturally occurring furans bearing substituent(s) at β-position.

2. Synthesis of Bilin Chromophores of Phytochromes

2.1 Biosynthesis of Bilin Chromophores. Biosynthesis of bilin chromophores of phytochromes begins with the cleavage of the porphyrin ring of heme catalyzed by heme oxygenase.[55,56] Biliverdin IXα (BV), a product by heme oxygenase, is further reduced by ferredoxin-dependent bilin reductases (FDBRs).[57] For phycocyanobilin (PCB) biosynthesis, PCB:ferredoxin oxidoreductase (PcyA), a member of the FDBR family, serially reduces the vinyl group of the D-ring and A-ring of BV to produce 3*Z*/3*E*-PCB via 18^1,18^2-dihydrobiliverdin IXα (shown as 18Et-BV in Fig. 3) as an intermediate.[23,58–60]

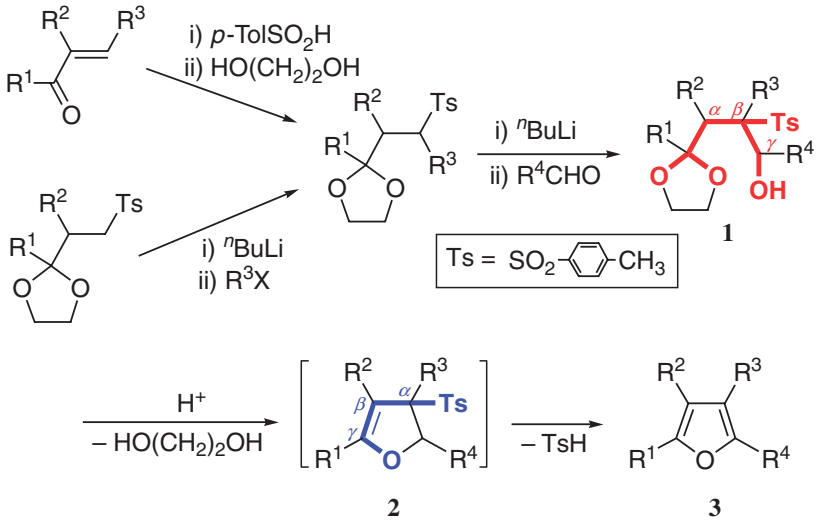
2.2 Background of Our Study on Synthesis of Bilin Chromophores. Before we began to study pyrrole and tetrapyrrole chemistry, we had investigated the synthesis of naturally occurring furans, which have in general substituent(s) at β-position of the furan ring as shown in Fig. 4. It is relatively easier to introduce a substituent to the α-position of furan ring by electrophilic substitution reaction than it is to the β-position, due to the difference in the electron density at each position; however, there was no efficient method to introduce a substituent to the β-position of furan ring.

Therefore, we established a general synthetic method of substituted furans according to Scheme 1. This method was efficient and flexible and allowed us not only to synthesize β-substituted furans but also to introduce additional substituent(s) into the arbitrary position(s) of furan ring via a key intermediate **1**, which has a γ-hydroxy-β-(*p*-tolylsulfonyl)-butanal ethylene acetal framework (Ts in Scheme 1 means a *p*-tolylsulfonyl (=tosyl) group) and was readily converted to the corresponding furan **3** by treating in the presence of an acid catalyst. The conversion of **1** to furan **3** probably proceeds through intermediate **2**, which has a labile γ-alkoxyallylic sulfone framework leading to furan. According to Scheme 1, we could synthesize all of the typical naturally occurring furans shown in Fig. 4.[61–68]

On the other hand, a number of methods for the preparation of pyrroles, which are five-membered heterocyclic compounds similar to furans, have been exploited,[69,70] because they are fundamental constituents of important substances, such as heme, chlorophyll, vitamin B_{12}, and some of them have pharmacological activities themselves.

We tried to apply our synthetic method of furan derivatives mentioned above for the preparation of pyrrole derivatives (Fig. 5). However, we found that pyrroles have characteristic properties different from furans. For example, pyrroles are more unstable than furans under acidic conditions especially when they do not have an electron-withdrawing group to decrease the electron density of the pyrrole ring.

Fortunately, I had a chance to join the group of A. Eschenmoser at ETH for two years from 1981, and studied the synthesis of uroporphyrinogen-octanitrile that was regarded as an origin of tetrapyrrole compounds ubiquitously found in nature.[71,72] After fruitful and valuable experiences at ETH, we studied the regioselective synthesis of substituted pyrroles, porphyrinogen, and porphyrins by developing several new synthetic reactions.[68,73–76] For example, symmetrically substituted porphyrins **8** was synthesized according to a similar



Scheme 1. An efficient and flexible method for the preparation of substituted furans.

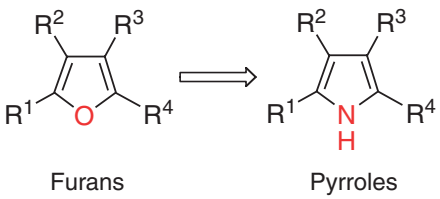


Fig. 5.

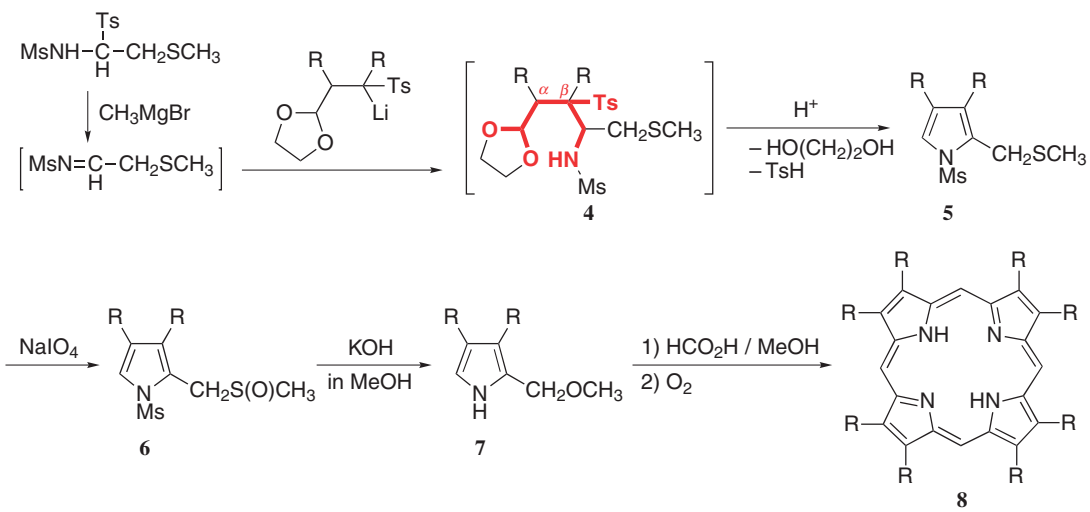
method to that for the preparation of furan derivatives (Scheme 1) through intermediates **4**, as shown in Scheme 2.[75]

In 1990, a biologist of Kanazawa University, K. Wada, introduced me to M. Furuya who was a pioneer in the study of phytochromes in Japan. He asked me about the possibility of introducing a photoreactive labeling group to the natural phytochrome chromophores, phytochromobilin (PΦB) or phycocyanobilin (PCB). Since we had never dealt such linear tetrapyrrole compounds,[16] we first investigated the general properties of such kinds of bilin chromophores by several preliminary experiments.

2.3 Synthetic Strategy of Bilin Chromophores. To establish an efficient and flexible method for the preparation of bilin chromophores of phytochromes, we initially employed the retrosynthetic analysis shown in Fig. 6, which is a similar strategy to that employed by Gossauer and his co-workers to prepare dimethyl esters of PΦB and PCB.[41–43] To prepare free acid forms of the bilin chromophores as final products, we replaced the methyl esters of propanoic acid side chains at the C8 and C12 positions and the benzyl ester at the C5 meso position, which were employed by Gossauer's group, to allyl esters, respectively, based on our preliminary experiments. Furthermore, we developed a new coupling reaction between the C- and D-rings instead of the classical method using a strong base to avoid the hydrolysis of the ester groups.

Preparation of the four different types of pyrrole components, the A- to D-rings, is first described, and then the coupling reaction between the A- and B-rings and the C- and D-rings and the final construction of linear tetrapyrrole (bilin) framework are described.

2.4 Preparation of the A-Ring. We first synthesized the



Scheme 2. Synthesis of symmetrically substituted porphyrins **8** according to the similar method for the preparation of furan derivatives. Ts and Ms mean *p*-tolylsulfonyl and methylsulfonyl groups, respectively.

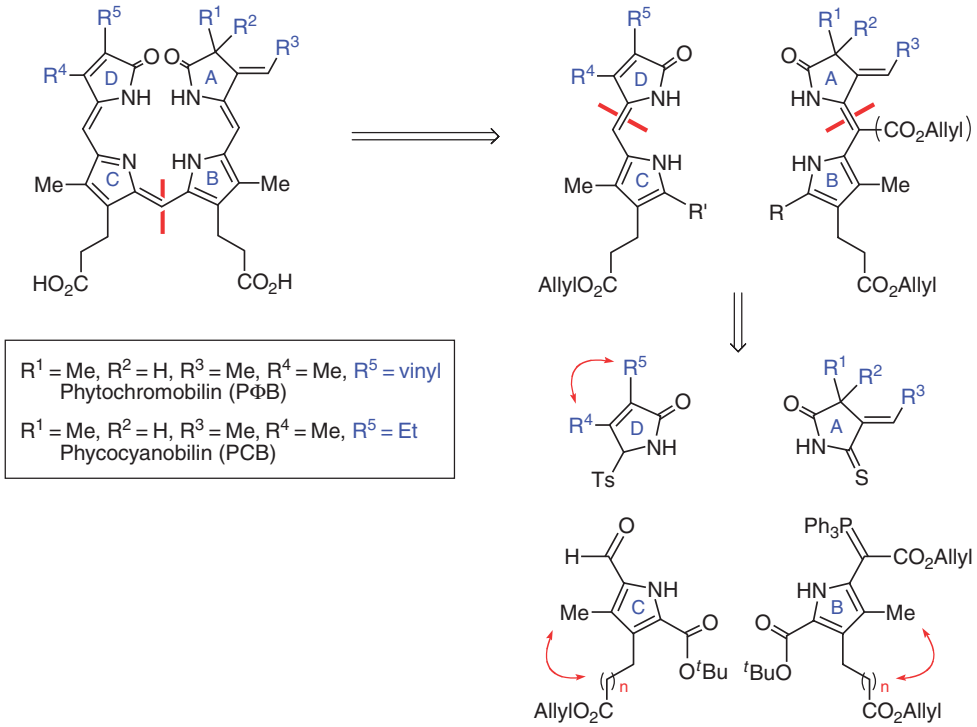
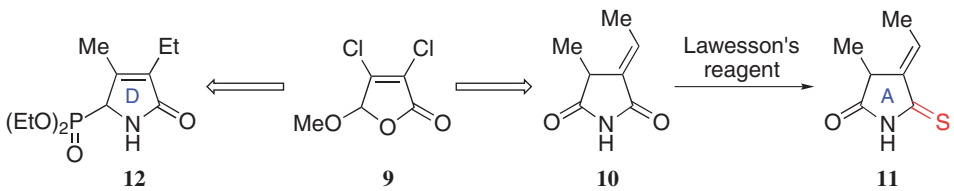


Fig. 6. An initial retrosynthetic strategy toward phytochromobilin (PΦB), phycocyanobilin (PCB), and their analogs.



Scheme 3. Preparation of the A- and D-ring precursors (**11** and **12**, respectively) of phycocyanobilin (PCB) starting from a mucochloric acid derivative **9**.

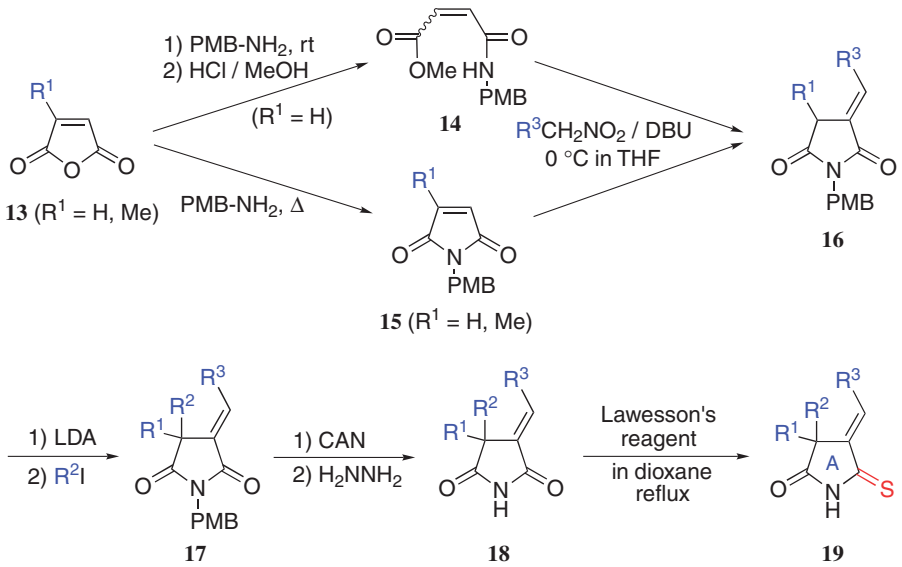
A-ring precursor, 2-ethylidene-3-methyl-1-thiosuccinimide (**11**), which is common to both PΦB and PCB starting from mucochloric acid derivative **9** in good yields (Scheme 3). 2-Ethylidene-3-methylsuccinimide **10** was found to be regioselectively monothiocarbonylated with Lawesson's reagent to give **11**, but the synthesis of **10** still required many steps.[77]

The synthesis of **11** has also been reported by Gossauer and Hinze[42] and Rapoport et al.;[78] however, we could not get reproducible results according to their methods. Though mucochloric acid derivative **9** also allowed us to prepare the D-ring precursor of PCB, diethyl 4-ethyl-1,5-dihydro-3-methyl-5-oxo-2*H*-pyrrol-2-ylphosphonate (**12**), we had to develop another efficient method for the synthesis of **11** which could be conducted on large scale (Scheme 4).[47,51]

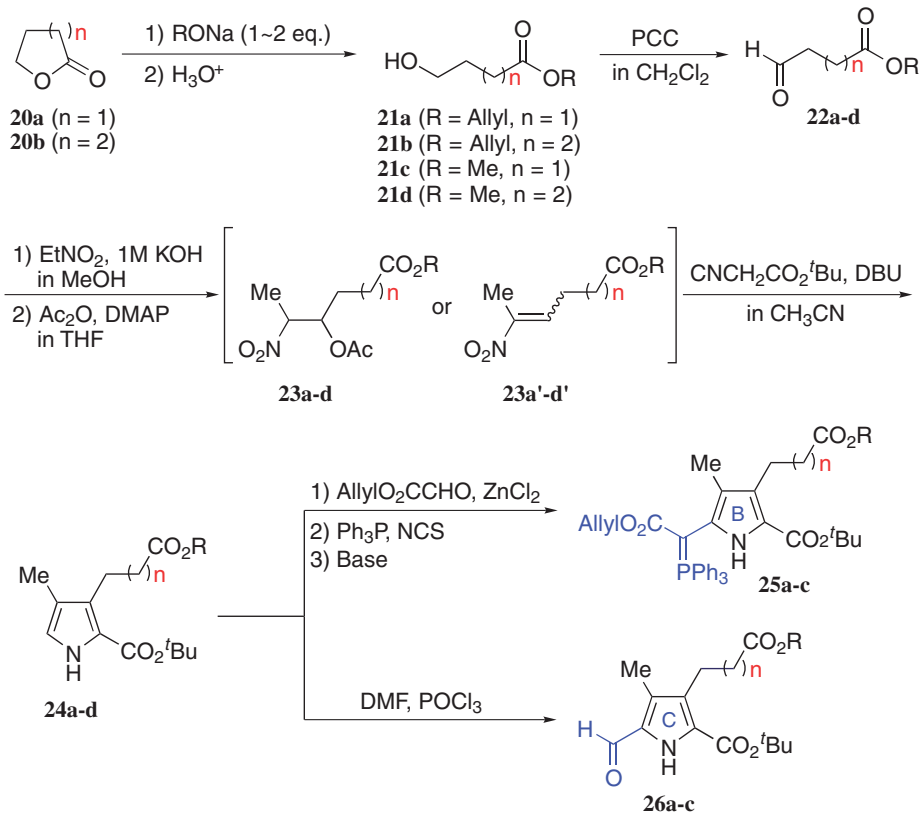
N-Protected 2-alkylidenesuccinimide derivative **16** was efficiently prepared starting from maleic or citraconic anhydride (**13**) through addition/elimination reaction of nitroalkane ($R^3CH_2NO_2$) to amide-ester **14** or succinimide derivative **15** in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). After alkylation of **16** using lithium diisopropylamide (LDA) and an alkylating agent (R^2I), if necessary, and deprotection of *p*-methoxybenzyl (PMB) group by ceric ammonium nitrate (CAN), N-deprotected 2-alkylidenesuccinimide derivative **18** was obtained. Compound **18** was treated with Lawesson's reagent in refluxing 1,4-dioxane to afford the A-ring precursor **19** (R^1, R^3 = Me, R^2 = H) and its analogs in good to moderate yields. According to this method, it was possible not only to change the substituents R^1 and R^3, but also to introduce an additional substituent R^2, which makes the method more efficient and flexible for the preparation of the monothiosuccinimide **19** as the A-ring and its analogs of bilin chromophores, such as PΦB and PCB.

Actually, various kinds of PCB derivatives bearing a modified A-ring, such as 2-norPCB, 2-methylPCB, and 2- or 3-homoPCB, were prepared by applying this method for the structure/function analysis of phytochromes. Further, this method was applicable to synthesize 3,3^1-dihydrogenated PCB derivatives to investigate their non-covalent interaction with phytochrome apoproteins toward the development of affinity chromatography to purify apoprotein.[51]

2.5 Preparation of the B- and C-Rings. We developed an efficient method for the preparation of the B- and C-ring components of bilin chromophores according to Scheme 5,[50] which allowed us not only to synthesize natural chromophores, PΦB and PCB, but also their derivatives having butanoic acid side chain(s) instead of propanoic acid side chain(s), regio-



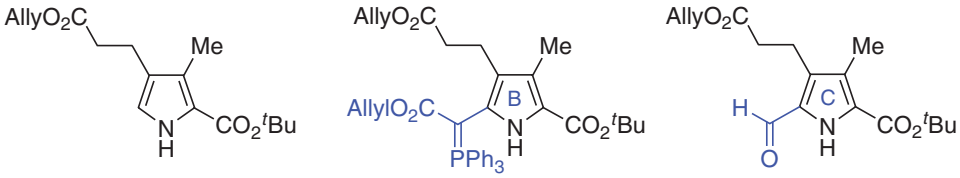
Scheme 4. Synthesis of the monothiosuccinimide **19** including the A-ring precursor (R^1, R^3 = Me, R^2 = H) of PΦB and PCB.



Scheme 5. Synthesis of the B- and C-ring components (**25** and **26**, respectively) of bilin chromophores.

selectively monoesterified derivatives at the C8 or C12 position, and regioisomers with respect to methyl and propanoic acid substituents of the B- and C-rings by exchanging aldehyde and nitroalkane components in Scheme 5 each other.

Previously, we reported a convenient method for the preparation of a pyrrole precursor **24c** common to the B- and C-rings starting from methyl 4-oxobutanoate (**22c**), which was prepared by using rhodium(I)-catalyzed hydroformylation of methyl acrylate according to a modified procedure of the reported method,[79] though it was not so easy to get good reproducible results.[46] Compound **22c** was also available from methyl 4-nitirobutanoate by Nef reaction.[80] Aldehyde **22c** thus obtained was then reacted with nitroethane to give the nitroalcohol in quantitative yield, and this was followed by dehydration[81] with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and CuCl to afford the nitroalkene **23c′** in 69% yield. The reaction of **23c′** with *t*-butyl isocyanoacetate[82] in the presence of DBU gave the desired pyrrole derivative **24c**[83] in reasonable yield.



24a' **25a'** **26a'**

Fig. 7. Regioisomers (**25a′** and **26a′**) of **25a** and **26a** were readily prepared from allyl 4-nitrobutanoate and acetaldehyde through pyrrole **24a′**.

This synthetic method to obtain **24c** was simpler than the reported procedure.[84–86]

We initially planned to prepare acid free bilin chromophores by hydrolysis of the dimethyl ester groups at the C8 and C12 positions of the chromophores. However, it was found that an exocyclic olefin at the C3 position tends to migrate to endocyclic position (C2) under basic conditions at several synthetic stages toward PCB and its derivatives. Thus, methyl ester group of **24c** (R = Me, $n = 1$)[80,85] was changed to other ester groups (R = CH_2Ph, CH_2CCl_3, and Allyl) which are removable under mild conditions prior to the preparation of the B- and C-rings components. Ultimately, allyl ester was chosen as a protecting group of the carboxylic acid side chain, since it turned out to be applicable to the synthesis of PCB derivatives bearing a photoreactive group avoiding migration of the exocyclic olefin of the A-ring in the final stage of the total synthesis of bilin chromophores.[47,87]

Since it was not easy to directly prepare allyl ester derivative of pyrrole compound **24a** (R = Allyl, $n = 1$) according to the above methods, transesterification was required to change the propanoic methyl ester side chain of the intermediary pyrrole derivatives **24c** (R = Me, $n = 1$) to allyl esters for the total synthesis of PCB and its derivatives.[47]

Thus, we developed a new and convenient method, which can provide not only the desired pyrrole **24a** (R = Allyl, $n = 1$) without transesterification, but also other derivatives, such as **24b**–**24d**, which are useful for production of a variety of bilin chromophores depending upon the used alcohols (ROH) and the starting lactones (**20a** and **20b**) as shown in Scheme 5. After treating the lactones (**20a** and **20b**) with sodium allyloxide or methoxide in the corresponding alcohols at room temperature, the resulting crude ω-hydroxy esters (**21a**–**21d**) were oxidized with PCC in CH_2Cl_2 at room temperature to afford the corresponding 4-oxobutanoates ($n = 1$) or 5-oxopentanoates ($n = 2$). In the case of 5-membered lactones **20a** ($n = 1$), it was necessary to use excess amounts of alkoxide to obtain **21a** and **21c** ($n = 1$) in good reproducible yields, probably due to the existence of an equilibrium going back to the starting lactone **20a**.

Aldehydes **22a**–**22d**, thus prepared, were reacted with nitroethane in the presence of a base according to Henry reaction to afford the nitroalcohols, which were then converted to the corresponding pyrroles **24a**–**24d** applying Barton's method[83] as shown in Scheme 5.

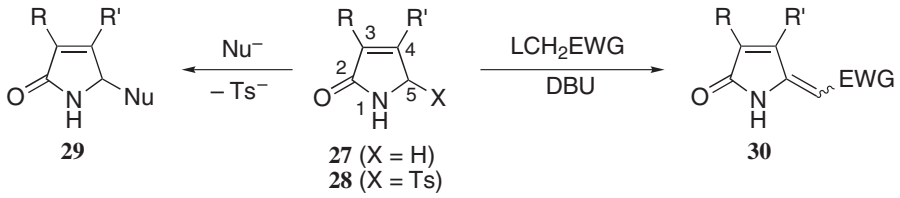
The pyrroles **24a**–**24c** were then converted to ylides **25a**–**25c** as the B-ring precursor, or formylated to **26a**–**26c** as the C-ring precursor by using Vilsmeier–Haack reaction in high yields, by modifying the procedures reported in the literature.[88,89]

In a similar manner, regioisomers (**25a′** and **26a′**) of **25a** and **26a** were prepared starting from allyl 4-nitrobutanoate and acetaldehyde through pyrrole **24a′** (Fig. 7).[50]

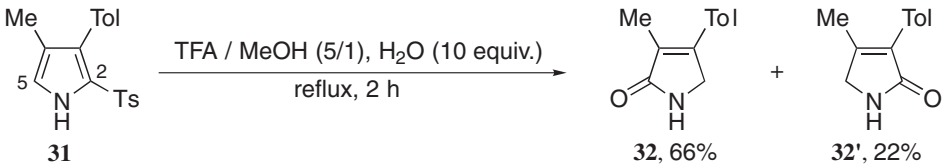
2.6 Preparation of the D-Ring. Modification of the D-ring of bilin chromophores is crucial for analysis of photochromism of phytochrome, because the D-ring connected to the C-ring via the C15 methine bridge has been regarded as the essential site where photoisomerization occurs during photoconversion of phytochromes. Therefore, it was required to establish an efficient and flexible synthetic method for the D-ring precursors to synthesize PΦB, PCB, and their derivatives modified at the C17 and C18 positions toward structure and function analysis of phytochromes.

3,4-Disubstituted 1,5-dihydro-2*H*-pyrrol-2-ones (pyrrolinones) **27** are useful building blocks for the synthesis of biologically important bile pigments such as bilirubin, chlorins,[90] and the prosthetic groups of biliproteins, such as phytochromes.[16,42] A variety of the methods for the synthesis of such 3,4-disubstituted pyrrolinone derivatives have been so far reported:[44,91–93] the modification of the Paarl–Knorr synthesis,[94] intramolecular Horner–Emmons cyclization,[95] condensation of acetoaminoketone with cyanoacetate,[96] and reductive cyclization of the cyanohydrin derivatives of β-ketoester.[97] In addition, direct structural transformation of substituted pyrroles to the corresponding pyrrolinones has been studied. For example, 2-formyl-3-ethyl-4-methylpyrrole has been oxidized by hydrogen peroxide in pyridine to give pyrrolinones concomitantly by the loss of the formyl group.[98] Acid hydrolysis of *t*-butyl 5-bromo-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate[90] and 4-(2-carboxyethyl)-3-carboxymethyl-5-chloropyrrole-2-carboxylic acid[99] has also been investigated. However, neither chemical yield nor regioselectivity of them have been satisfactory.

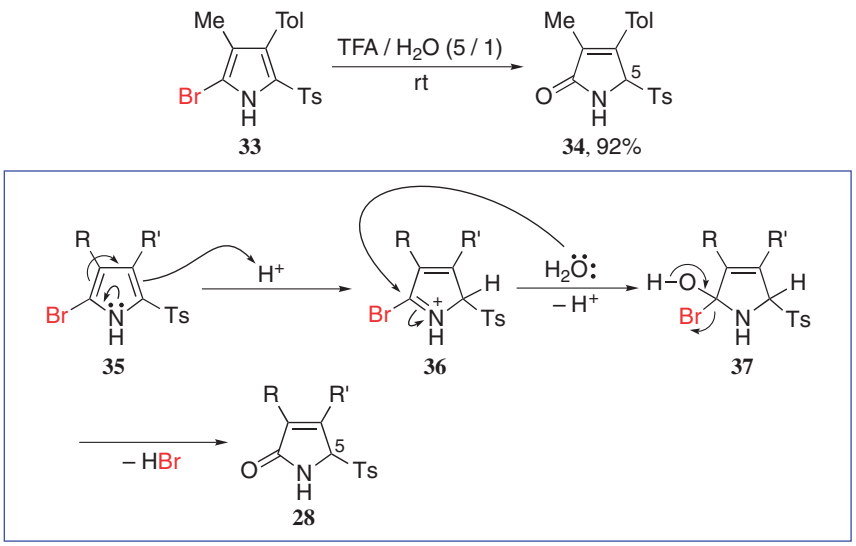
The reported base-catalyzed Knoevenagel-type condensation of an α-unsubstituted pyrrolinones **27** (X = H) with a formylpyrrole as the C-ring requires a subsequent reesterification of the propanoic acid side chain of the resulting CD-ring components with diazomethane. On the other hand, we have reported that readily available *N*-(tosylmethyl)-*p*-toluenesulfonamide ($TsNHCH_2Ts$)[100] and *N*-(2-methylthio-1-tosylethyl)-methanesulfonamide ($MsNHCH(Ts)CH_2SMe$) react as methanimine equivalents with a variety of nucleophiles in the presence of a base to afford the corresponding substitution products through elimination of *p*-toluenesulfinic acid and the subsequent addition of nucleophile toward the intermediary *N*-methylenesulfonamide derivatives in good yields.[73,75] Furthermore, β-[*N*-methane(or toluene)sulfonamidomethyl]ated propanal ethylene acetals, like **4**, which were available by applying the above reaction, could be converted into the



Scheme 6. Reactions of 5-tosylpyrrolinones **28** with nucleophiles (Nu^-) and active methylene compounds (LCH_2EWG) bearing a leaving group (L).



Scheme 7. Hydrolysis of 2-tosylpyrrole **31** under acidic conditions.



Scheme 8. A probable mechanism of regioselective hydrolysis of 2-bromo-5-tosylpyrrole **35** to the corresponding 5-tosylpyrrolinones **28** under acidic conditions.

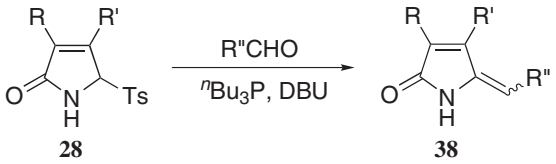
corresponding N-substituted pyrroles, such as **5**, with the aid of acid catalyst in excellent yields (Scheme 2).[73]

Such background prompted us to develop a versatile method for the regioselective synthesis of 3,4-disubstituted 5-(*p*-tolylsulfonyl)pyrrolinones **28**, instead of **27**, as described in the following. Compounds **28** were found to react with various nucleophiles (Nu^-) including hydride (H^-) and active methylene compounds (LCH_2EWG, EWG = electron-withdrawing group) with an appropriate leaving group (L), affording the corresponding **29** and exomethylene compounds **30**, respectively, as shown in Scheme 6.[44] It is most likely that the reaction proceeded through elimination/addition processes as in the cases of $TsNHCH_2Ts$ and $MsNHCH(Ts)CH_2SMe$ mentioned above.[101–103]

Before the preparation of 5-tosylpyrrolinones **28**, we first tried the hydrolysis of a 2-tosylpyrrole, such as **31**, which were readily available by the reaction of *p*-tolylsulfonylmethyl isocyanide and substituted nitroolefins or *β*-acetoxy nitroalkanes in the presence of a base according to Barton's method,[83] under acidic conditions expecting selective protonation at the C2 position due to the strong inductive effect of a tosyl group. Actually, when 2-tosylpyrrole **31** was refluxed in a trifluoroacetic acid (TFA)–MeOH solution containing a small amount of water, product **32** was predominantly obtained in good yield along with its regioisomer **32′**, as shown in Scheme 7.

Formation of **32′** seemed to be due to the competitive initial protonation at both the C2 and C5 positions of **31**. In order to improve the regioselectivity, 2-tosylpyrrole **31** was brominated with trimethylphenylammonium tribromide in CH_2Cl_2 at 0 °C to afford 2-bromo-5-tosylpyrroles **33** in quantitative yield. Then, to a solution of **33** in TFA was added water, and the reaction mixture was allowed to stand overnight at room temperature to give a single product, 5-tosylpyrrolinone **34**, in 92% yield. Compound **34** was converted to **32** in quantitative yield by reducing with $NaBH_4$ ($Nu^- = H^-$) in ethanol according to Scheme 6.[44]

A probable mechanism for regioselective hydrolysis of 2-bromo-5-tosylpyrroles **33** to 5-tosylpyrrolinones **34** is shown in Scheme 8. Initial protonation takes place selectively at the C5 position of **35** (=**33**, when R = Me, R′ = Tol) due to the



Scheme 9. A new Wittig-type coupling reaction of 5-tosylpyrrolinones **28** with various aldehydes to afford the corresponding pyrromethenone derivatives **38** in the presence of PBu_3 and DBU.

strong inductive effect of a tosyl group to afford the intermediates **36**. Then, water attacks the C2 position of **36**, followed by elimination of hydrogen bromide from the resulting addition products **37**, to afford 5-tosylpyrrolinones **28** (=**34**, when R = Me, R′ = Tol).[44]

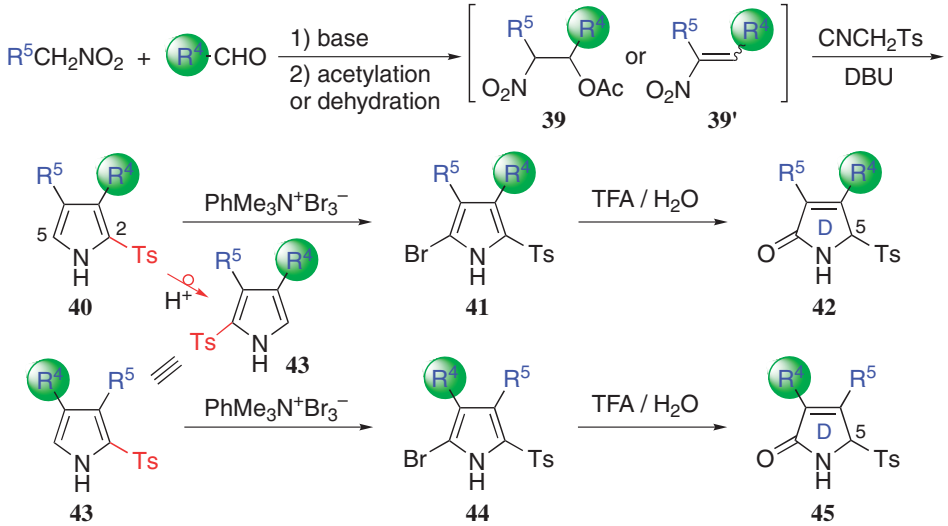
Such 5-tosylpyrrolinones **28** were found to be useful not only for the substitution reaction with nucleophiles and active methylene compounds, shown in Scheme 6, but also for a new Wittig-type coupling reaction with various aldehydes (R″CHO) as shown in Scheme 9. Namely, when 5-tosylpyrrolinones **28** were reacted with aldehydes in the presence of tributylphosphine and DBU, 5-exomethylene compounds **38**, including pyrromethenone derivatives (R″ = 2-pyrrolyl), which are as the CD-ring component of bilin chromophores, were obtained in high yields.[45]

Now, we can depict the general procedure for the preparation of the D-ring in Scheme 10.

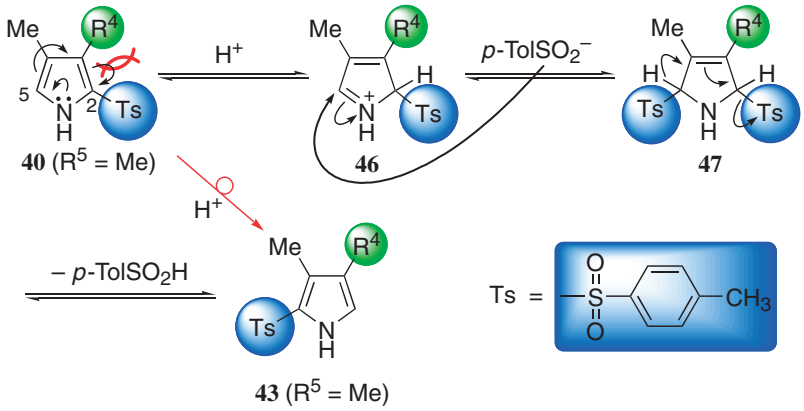
3,4-Disubstituted 2-tosylpyrrole **40** was prepared starting from nitroalkane and aldehyde via nitro-acetate **39** and/or nitroolefin **39′**, which were reacted with tosylmethyl isocyanide[104] by applying Barton's method,[83] followed by bromination with trimethylphenylammonium tribromide to afford 2-bromo-5-tosylpyrrole **41**. Acid hydrolysis of **41** in TFA containing water gave the corresponding 5-tosylpyrrolinone **42** as shown in Scheme 10.

In the course of the investigation of the synthesis of 2-tosylpyrroles **40**, a peculiar phenomenon was observed when NMR spectrum of an isolated 2-tosylpyrrole **40** was taken in $CDCl_3$. That is, the spectrum of **40** showed contamination, even though it was carefully purified on TLC. Structure of the impurity was later confirmed to be regioisomer **43** of compound **40** by comparison with an authentic sample prepared separately. Furthermore, such phenomenon was not observed by using $CDCl_3$ treated with basic alumina. This fact strongly suggested that tosyl group of 3,4-disubstituted 2-tosylpyrroles **40** rearranged from the C2 to C5 position under the mild acidic conditions. Actually, the tosyl group of **40** readily rearranged in chloroform containing trifluoroacetic acid (TFA) (TFA/$CDCl_3$ = 1/9). The ratio of the regioisomers at equilibrium was definitely influenced by the bulkiness of the substituent R^4 (marked with a green circle in Scheme 10) at the C3 position of the starting 4-methyl-2-tosylpyrroles **40** (R^5 = Me). When the substituent R^4 was *t*-butyl group, **40** was completely transformed to **43**.[105]

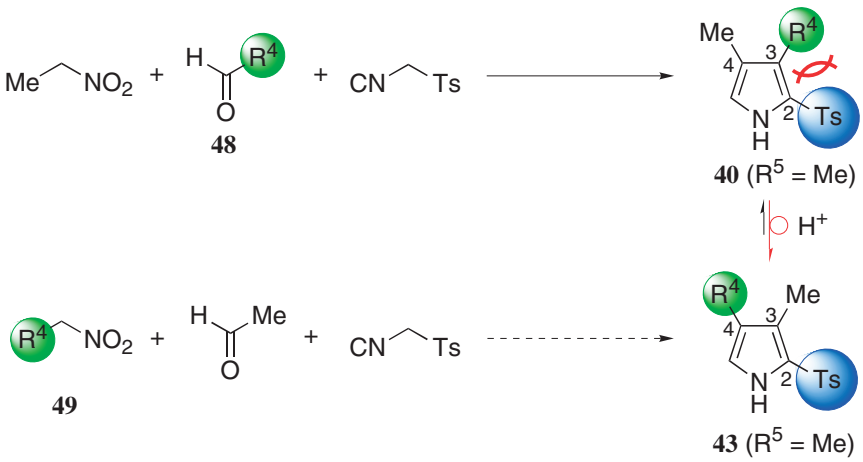
We have proposed that the rearrangement proceeds through the elimination of tosyl cation from the initial cationic intermediate formed by protonation at the C2 position, followed by electrophilic attack of the tosyl cation from the less hindered α-position of the intermediary α-unsubstituted pyrroles.[105] In order to confirm this mechanism, the tosylation of α-unsubstituted pyrroles was examined by utilizing TsX (X = $AlCl_4^-$, $CF_3CO_2^-$, etc.) under several conditions. However, a complicated mixture was obtained in all cases. On the other hand, we found that addition of a catalytic amount of *p*-toluenesulfinic acid remarkably accelerated the rearrangement.[106] From these results, the rearrangement of tosyl group from the C2 to C5 position seemed to proceed through the mechanism shown in Scheme 11. Namely, initial protonation takes place selectively at the C2 position with a tosyl group to afford the cationic intermediate **46** in a similar manner in the case of reversible sulfonation of aromatic compounds, followed by addition of *p*-toluenesulfinate at C5 position to afford the intermediate **47**, which has a tosyl group at both α-positions. From compound **47**, *p*-toluenesulfinic acid must be eliminated to avoid the steric congestion between bulky substituent



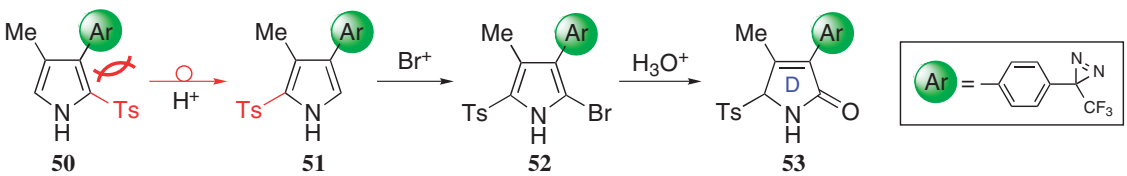
Scheme 10. Synthesis of 2-tosylpyrrole **40** and rearrangement to its regioisomer **43** under acidic conditions. Both pyrroles, **40** and **43**, were converted to the corresponding 5-tosylpyrrolinones, **42** and **45**, respectively, by bromination and subsequent acid hydrolysis. R^4 marked with a green circle shows a bigger substituent than R^5.



Scheme 11. The most plausible mechanism of the arrangement of 2-tosyl group of **40** to the C5 position to afford the regioisomer **43** in the presence of a catalytic amount of *p*-toluenesulfinic acid under acidic conditions.



Scheme 12. Preparation of a sterically unfavorable pyrrole **40** is easier than the direct preparation of the regioisomer **43**. Pyrrole **43** is readily available by acid-catalyzed rearrangement of tosyl group of **40**.



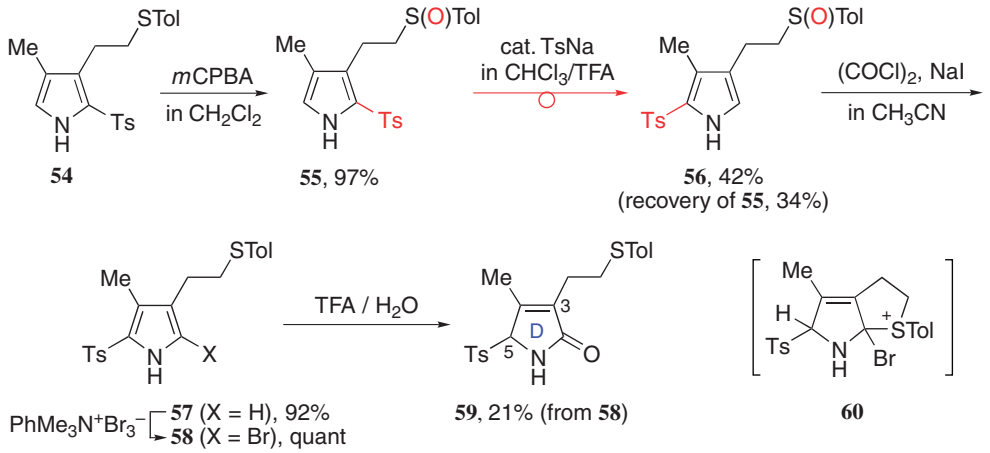
Scheme 13. Preparation of the D-ring **53** with a photoreactive group instead of a vinyl group of phytochromobilin (PΦB).

R^4 and tosyl group found in the starting 2-tosylpyrrole **40**. The ratios of **40**/**43** at equilibrium were proven to be dependent only on the steric requirement between tosyl group and the substituents at the C3 or C4 positions.

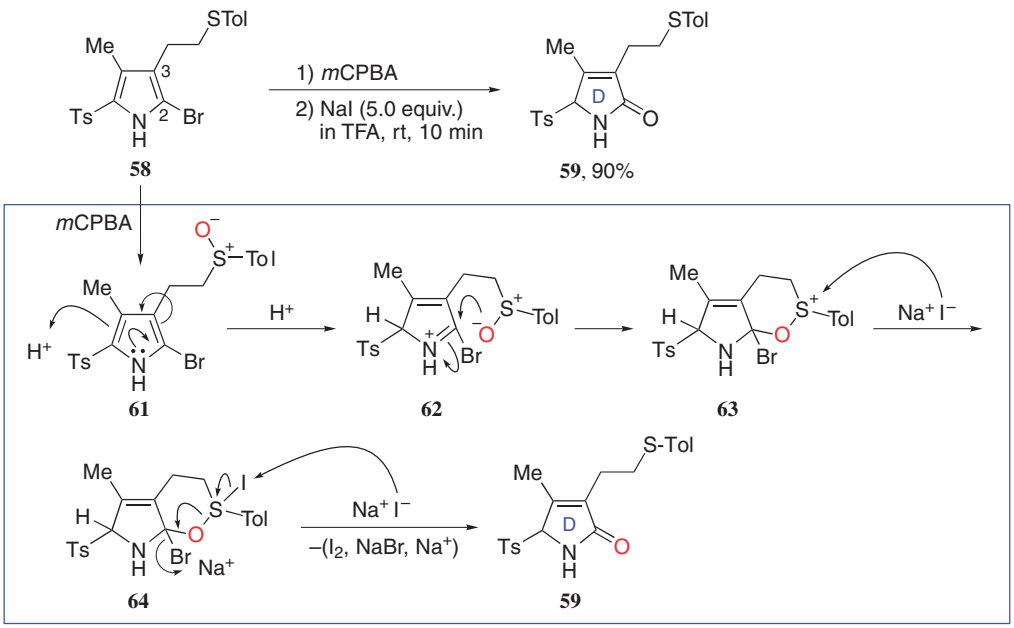
This rearrangement was synthetically very useful. Barton and his co-workers have reported a convenient method for the preparation of substituted pyrrole derivatives through the reaction of isonitrile with a nitroolefin or its equivalent available from nitroalkane and aldehyde.[83] However, we were sometimes confronted with the difficulty of introducing arbitrary substituents at the C3 and C4 positions of pyrroles according to Barton's method. For example, synthesis of the sterically unfavorable 4-methyl-3-substituted 2-tosylpyrrole **40** (R^5 = Me) is much easier than that of its regioisomer, 3-methyl-4-substituted 2-tosylpyrrole **43** (R^5 = Me), because substituent R^4 (marked with a green circle in Scheme 12) at the C3 position of the pyrrole **40** comes from aldehydes **48**, many of which are commercially available. However, substituent R^4 at the C4 position of the pyrrole **43** is from nitro compounds **49**, which are not as easy to obtain as aldehydes **48**.

The rearrangement of tosyl group of 2-tosylpyrroles **40** was successfully applied to the preparation of the D-ring precursor **53** with a photoreactive group instead of vinyl group at the C18 position of phytochromobilin (PΦB) as shown in Scheme 13.[87]

The acid hydrolysis of 2-bromo-5-tosylpyrroles **44** generally proceeded well when the substituent R^4 is a simple alkyl or aryl group.[87] However, when we tried to hydrolyze 2-bromo-5-tosylpyrrole **58** bearing a 2-(tolylthio)ethyl group at the C3 position, which was prepared according to Scheme 14 (**54** → **58**) via rearrangement of tosyl group of the sulfoxide **55**[107] obtained by oxidation of the readily available 2-tosylpyrrole **54**



Scheme 14. Preparation of the D-ring precursor **59** from 2-tosylpyrrole **54** via rearrangement of the tosyl group under mild acidic conditions.



Scheme 15. Preparation of 5-tosylpyrrolinone **59** as a precursor of the D-ring of PΦB from 2-bromo-5-tosylpyrrole **58** via sulfoxide **61** under mild acidic conditions.

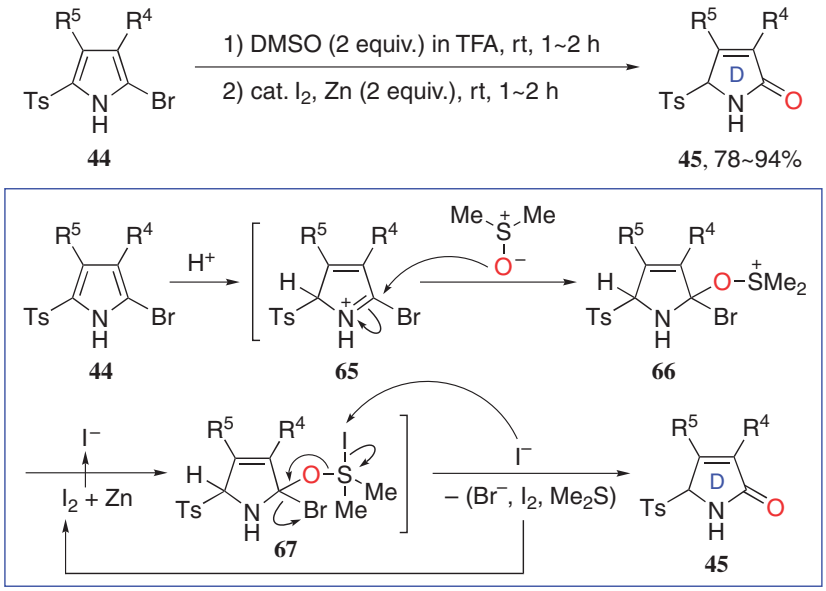
with *m*-chloroperbenzoic acid (*m*CPBA) and subsequent reduction (**56** → **57**) with $(COCl)_2$/NaI in CH_3CN[108] and bromination (**57** → **58**), to the corresponding 5-tosylpyrrolinone **59** as a precursor of the D-ring of PΦB under acidic conditions,[44] the yield of the expected 5-tosylpyrrolinone **59** was disappointingly poor[49] (Scheme 14). This result seemed to be due to the neighboring effect of 2-(tolylthio)ethyl group to form the cyclic intermediate, such as **60**, shown in Scheme 14, to avoid hydrolysis. Many attempts to improve the yield of **59** by acid hydrolysis were unsuccessful.

Ultimately, it was found that 5-tosylpyrrolinone **59** as a precursor of the D-ring of PΦB was available in high yield by treating sulfoxide **61**, which was obtained by oxidizing **58** with *m*CPBA, with NaI in TFA under anhydrous conditions for a short time. A probable reaction mechanism is shown in Scheme 15.[49]

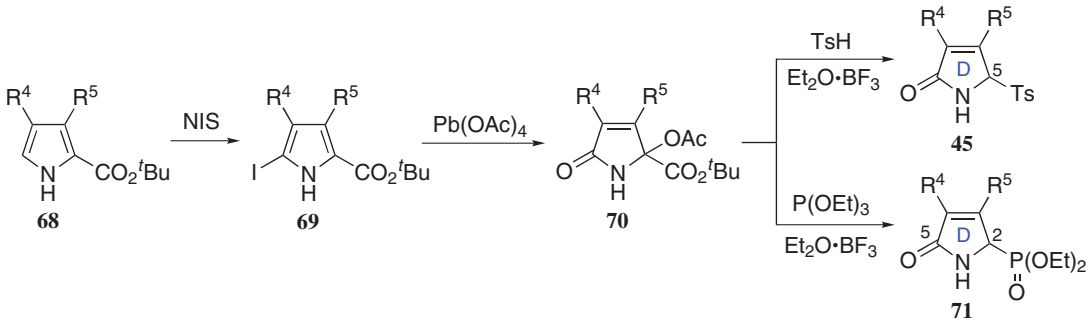
This successful redox method for the conversion of **58** to **59** via sulfoxide **61** prompted us to examine the use of dimethyl sulfoxide (DMSO) as an external nucleophile instead of the sulfoxide moiety in **61** to expand the method to prepare 2-bromo-5-tosylpyrroles **44**, in which a thioether substituent does not exist at the C3 position. Though the expected reaction proceeded well to give the corresponding 5-tosylpyrrolinones **45** by employing DMSO and NaI in TFA, the use of a large excess (5–7 molar amount) of NaI was required to obtain high yield, and a lot of iodine was liberated in progress of the reaction. After many attempts, it became possible to use only a catalytic amount of iodine together with zinc powder, which reduces iodine in situ to reproduce iodide anion as shown in Scheme 16.[52]

More recently, it was found that the iodide salt is unnecessary when zinc powder is used as a reductant in TFA.

Though 3,4-disubstituted 5-tosylpyrrolinones **45** have been so far synthesized from the corresponding 2-bromo-5-tosylpyr-



Scheme 16. An efficient redox method for the conversion of 2-bromo-5-tosylpyrroles **44** to the corresponding 5-tosylpyrrolinones **45**.



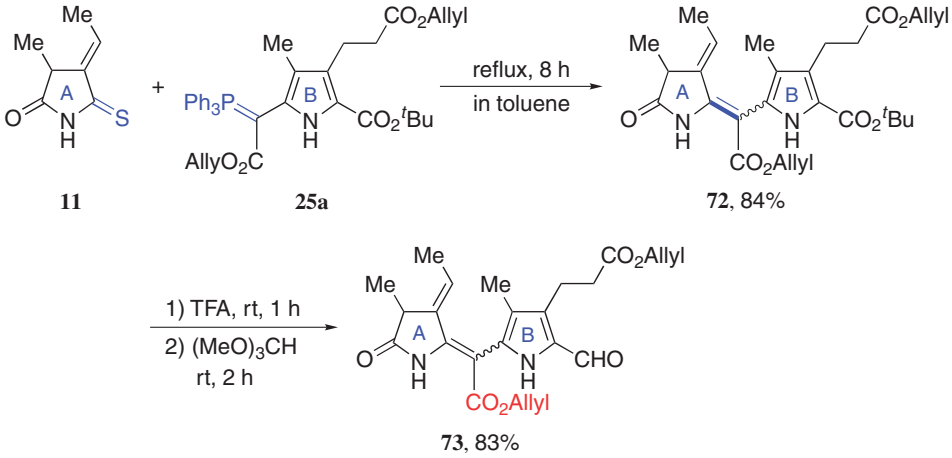
Scheme 17. An alternative method for the preparation of the D-ring precursors **45** and **71** from the 2-pyrrolecarboxylic acid ester derivatives **68**.

roles **44** according to the method described above,[44] we could also establish an alternative method starting from 2-pyrrolecarboxylic acid ester derivatives **68**, which are easily prepared by the reaction of β-acetoxy nitroalkanes and *t*-butoxycarbonylmethyl isocyanide in the presence of a base by applying Barton's method,[83] as shown in Scheme 17. Iodination at the 5-position of **68** with *N*-iodosuccinimide (NIS) afforded the corresponding iodinated products **69**[109] in quantitative yields. Iodinated pyrroles **69** were then oxidized with $Pb(OAc)_4$ to give the pyrrolinone derivatives **70** in excellent yields. When compounds **70** were treated with *p*-toluenesulfinic acid in the presence of diethyl ether–boron trifluoride (1/1), the desired 5-tosylpyrrolinones **45** were obtained in high yields.

Similarly, compounds **70** underwent a substitution reaction with triethyl phosphite to give the corresponding diethyl 3,4-disubstituted 1,5-dihydro-5-oxo-2*H*-pyrrol-2-ylphosphonates **71**[77,110] as an alternative D-ring precursor, probably through an elimination/addition mechanism accompanied by a decarboxylation reaction. However, it required a strong base, such as *t*-BuOK, for the subsequent Horner–Emmons-type coupling reaction with a formylpyrrole as the C-ring to afford the corresponding pyrromethenone derivative as the CD-ring component.[77,110]

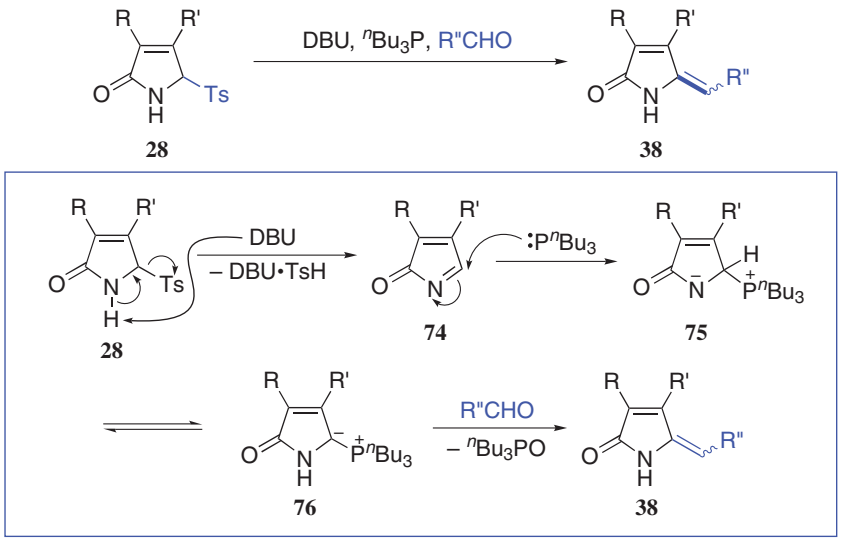
2.7 Coupling of the A- and B-Rings. Although there have been reports on the syntheses of bilin ester derivatives, most of the published studies in this area for the synthesis of the AB-ring component have been carried out by utilizing either the Eschenmoser's sulfide contraction,[41] the thio-Wittig coupling,[42,43,89] or the photochemical rearrangement of *N*-pyrroloenamide.[111,112] We first tried the thio-Wittig coupling reaction between A-ring **11** and ylide **25a**, as the B-ring precursor, in refluxing toluene to afford the AB-ring component **72** of natural type of PΦB and PCB as shown in Scheme 18. AB-ring component **72** was then formylated with methyl orthoformate in TFA, accompanied by decarboxylation to afford **73**, which was coupled with the CD-ring component to give PCB derivative as will be described later.[51]

This method provides a viable route to the AB-ring component; however, it requires removing a carboxylic acid moiety at the meso-position under acidic conditions, which sometimes brought about difficulty toward the total synthesis of bilin chromophores. For example, when we applied this method to the synthesis of PΦB bearing an acid labile vinyl group at the C18 position, a tetrapyrrole intermediate obtained by coupling AB-ring component **73** with the CD-ring component decomposed at the final stage to remove the carboxylic acid moiety at the C5 meso-position after deprotection of the allyl ester group by Pd-catalyzed reaction together with other two



11 25a 72, 84% 73, 83%

Scheme 18. Synthesis of the AB-ring component by thio-Wittig coupling reaction.



28 38 74 75 76

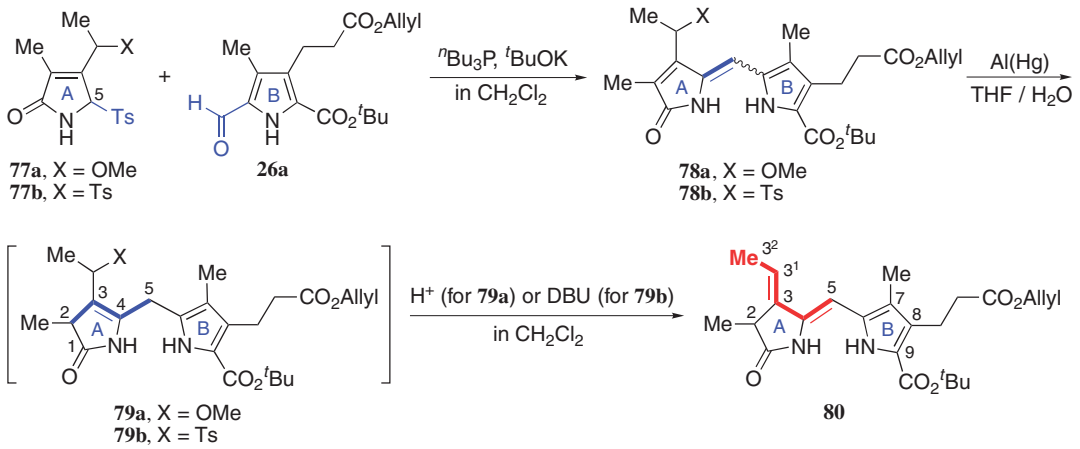
Scheme 19. A plausible reaction mechanism of the Wittig-type coupling of 5-tosylpyrrolinone **28** and aldehyde in the presence of DBU and tributylphosphine.

allyl esters of the side chains at the C8 and C12 positions of the PΦB precursor.

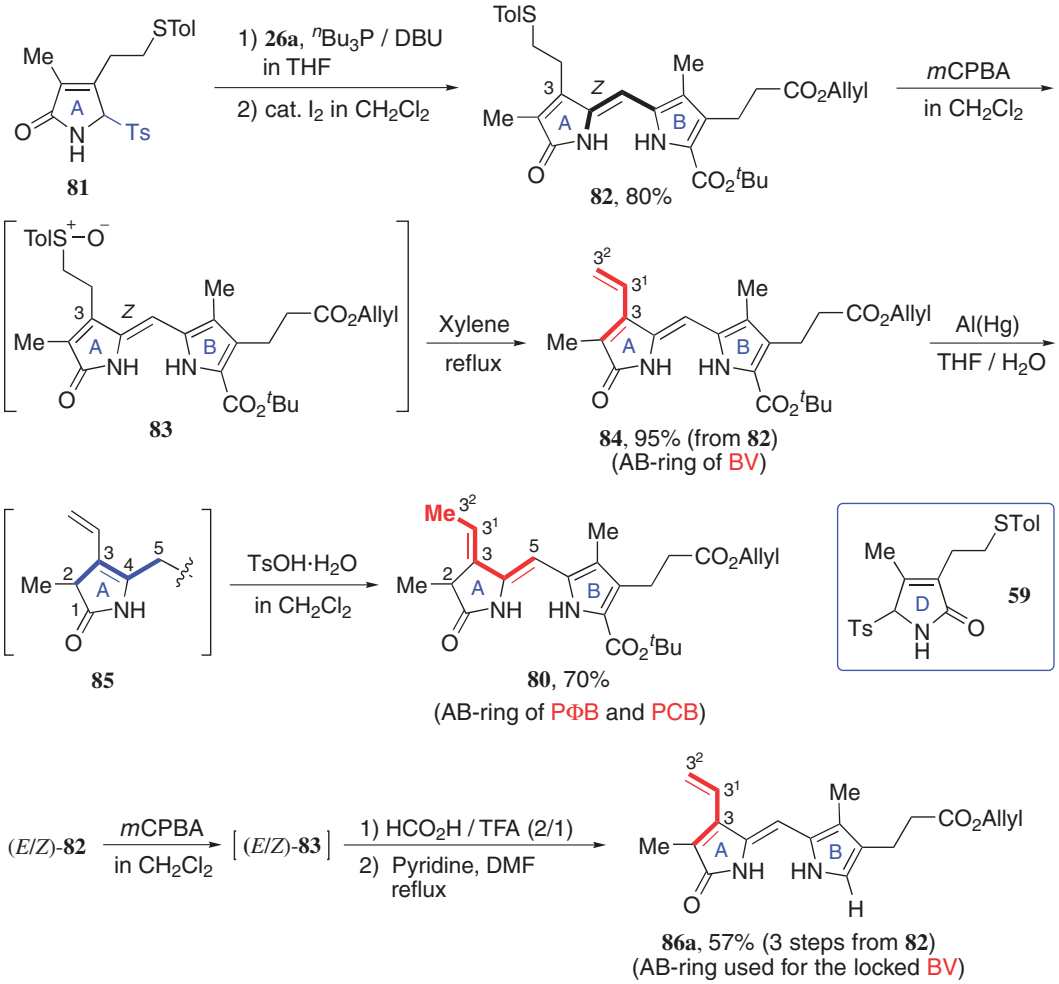
On the other hand, 5-tosylpyrrolinones **28** were found to undergo the Wittig-type coupling reaction with various aldehydes (R″CHO) in the presence of DBU and two molar amounts of tributylphosphine to give the corresponding 5-exomethylene derivatives **38**, including a pyrromethenone derivative (R″ = 2-pyrrolyl), as illustrated in Schemes 9 and 19. This reaction, which was originally developed to prepare the CD-ring component, seems to proceed through the mechanism shown in Scheme 19.[44–46]

By applying this Wittig-type coupling reaction, we developed an alternative method for the synthesis of the AB-ring component, which was applicable to the synthesis of PΦB, due to the absence of an ester group at the meso-position. When 4-(1-methoxyethyl or 1-tosylethyl)-3-methyl-5-tosyl-1,5-dihydro-2*H*-pyrrol-2-one **77a** (X = OMe) or **77b** (X = Ts), as the A-ring precursor, was reacted with a 2-formylpyrrole derivative **26a**, as the B-ring precursor, in the presence of $^{n}Bu_3P$ and $^{t}BuOK$ in CH_2Cl_2, the corresponding coupling product **78a** (X = OMe) or **78b** (X = Ts) was obtained as a mixture of *E*- and *Z*-isomers in good yield. Reduction of the coupling products **78a** and **78b** with aluminum amalgam [Al(Hg)] gave intermediates **79a** and **79b**, respectively.[113] Intermediates **79a** and **79b** were treated with pyridinium *p*-toluenesulfonate (PPTS, for **79a**) or with DBU (for **79b**) without further purification to give the desired AB-ring component **80** as a single *Z*-isomer (confirmed by NOE measurement) via the elimination of methanol or *p*-toluenesulfinic acid, respectively, in good yields as shown in Scheme 20.[48,53]

However, the preparation of **77a** and **77b** required many steps. Therefore, we employed **81**, which is a regioisomer of D-ring precursor **59**, instead of **77a** and **77b** as the A-ring precursor to establish a more efficient and flexible method for the preparation of not only AB-ring component **80** of PΦB and PCB, but also AB-ring component **84** of BV, which is used as a chromophore in bacteriophytochromes (Scheme 21). When 5-tosylpyrrolinone **81** was coupled with formylpyrrole **26a** in the presence of tributylphosphine and DBU in THF at room temperature, a mixture of (*E*)- and (*Z*)-pyrromethenone intermediate **82** was obtained in 80% yield. The *E*-isomer of **82** was readily converted to the thermodynamically favored

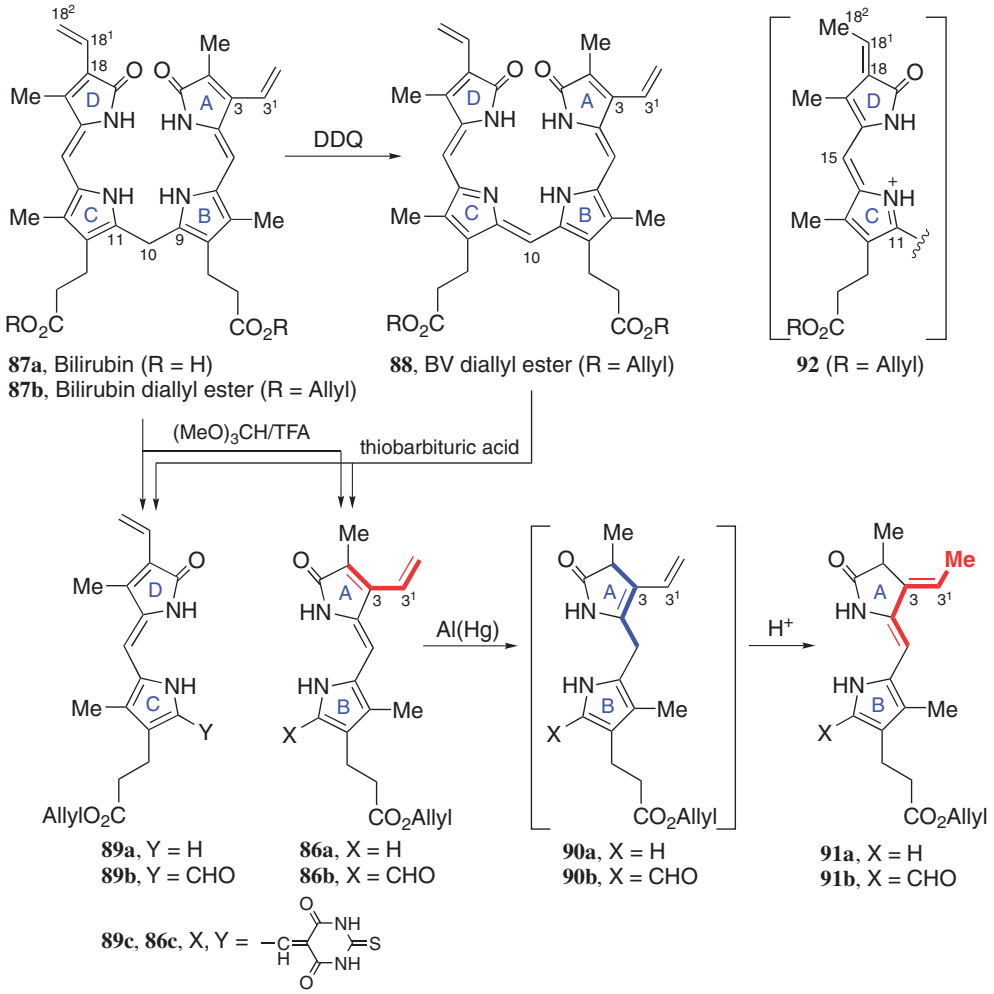


Scheme 20. An alternative method for the preparation of AB-ring component **80** by Wittig-type coupling reaction between 5-tosylpyrrolinones **77a** and **77b** and formylpyrrole **26a**.



Scheme 21. An efficient and flexible method for the preparation of the AB-ring components of BV, PΦB, and PCB starting from 5-tosylpyrrolinone **81** and formylpyrrole **26a**.

Z-isomer by treatment with a catalytic amount of iodine in CH_2Cl_2 at room temperature in quantitative yield. The 2-(tolylthio)ethyl group of the coupling product was oxidized to the corresponding sulfoxide **83** with *m*CPBA in CH_2Cl_2 and converted to a vinyl group by heating in xylene to afford **84**, which is regarded as the AB-ring component of BV. Pyrromethenone derivative **84** having a vinyl group on the A-ring was then reduced with Al(Hg) to the reduced intermediate **85**,[113] followed by acid treatment, without further purification to obtain AB-ring component **80** via rearrangement of a proton at the C5 meso-position as a single *Z*-isomer (confirmed by NOE measurement) in good yield as shown in



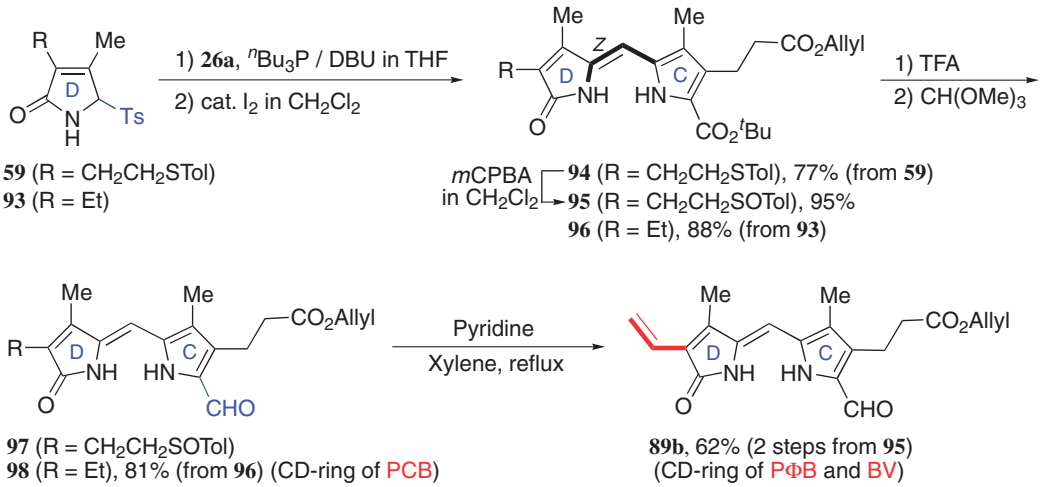
Scheme 22. Efficient preparation of the AB-ring components (**86a** and **86b** for BV and **91a** and **91b** for PΦB and PCB) and CD-ring components (**89a** and **89b** for PΦB) via cleavage between the B- and C-rings of bilirubin or BV diallyl esters, respectively.

Scheme 21.[48,49,114] Transformation of pyrromethenone derivative **84** having a vinyl group to AB-ring component **80** of PΦB and PCB through the reduced intermediate **85** is of interest in connection with the mostly speculative biosynthetic pathway for the transformation of biliverdin IXα (BV) to PΦB through "dihydrobiliverdin."[16]

Decarboxylated AB-ring component **86a**, which was used for the synthesis of the locked BV derivatives, was prepared from (*E*/*Z*)-**82** via oxidation to the sulfoxide (*E*/*Z*)-**83** and subsequent treatment with a mixture of formic acid and TFA for decarboxylation, followed by refluxing in DMF, to introduce a vinyl group in the presence of pyridine as only *Z*-isomer in 57% yield in three steps.[115] Compound **86a** was also available by treating BV diallyl ester with thiobarbituric acid.[116,117]

It has been reported that addition of thiobarbituric acid to BV dimethyl ester proceeded to afford the CD-ring component of PΦB resulting from the cleavage at the C10 position between the B- and C-rings,[118,119] and the CD-ring component obtained by the cleavage was coupled with the chemically synthesized AB-ring component to provide PΦB dimethyl ester in extremely short steps.[119] On the other hand, we found that the pyrromethenone derivative having a vinyl group was readily converted to the AB-ring component of PΦB and PCB by reducing with Al(Hg) and subsequent acid treatment as described above.[49] Based on these facts, we next attempted to obtain pyrromethenone derivatives **86a** and **86b** as an AB-ring precursor along with CD-ring components **89a** and **89b** of PΦB by cleaving between the B- and C-rings of bilirubin diallyl ester **87b** (R = Allyl, Scheme 22) and BV diallyl ester **88**, since the former products **86a** and **86b** were expected to be converted to AB-ring components **91a** and **91b** of PΦB and PCB derivatives by reduction with Al(Hg).

Commercially available bilirubin **87a** (R = H) was converted to the diallyl ester **87b** (R = Allyl) in excellent yield by treating with allyl bromide and DBU in DMF. Oxidation of **87b** with DDQ afforded biliverdin (BV) diallyl ester **88** in good yield.[120,121] Compound **88** was reacted with thiobarbituric acid in MeOH to cleave between the B- and C-rings to afford CD-ring component **89a** of PΦB and AB-ring precursor **86a** of the PΦB and PCB derivatives along with equimolar amounts of the isomeric thiobarbituric acid adducts **89c** and **86c**, respectively, in quantitative yields (Scheme 22).[118,119] Two pyrromethenone derivatives (**89a** and **86a**) were treated with methyl orthoformate in TFA to afford the formylated compounds (**89b** and **86b**) in high yields, respectively.[117]



Scheme 23. An efficient method for preparation of CD-ring components **89b** and **98** of PΦB/BV and PCB, respectively, starting from 5-tosylpyrrolinones **59** or **93** and formylpyrrole **26a**.

It is known that bilirubin derivatives are cleaved between the B- and C-rings in an acid solution to yield pyrromethenone derivatives,[116] even though they further react to afford a mixture of tetrapyrrole derivatives by recondensation of the pyrromethenones or decompose. Thus, we next attempted the direct synthesis of the formylated compounds **89b** and **86b** from **87b** under conditions similar to those used above for the preparation of **89b** and **86b** to avoid the recondensation or decomposition. When **87b** was treated with methyl orthoformate in TFA at 0 °C for 30 min, **86b** was obtained in 27% yield by direct formylation at the C9 position accompanied by cleavage between the C9 and C10, but not CD-ring component **89b**. When the reaction was stopped after 20 min, **86b** was obtained in 21% yield along with **89b** in 4% yield. The CD-ring part of **87b** seems to decompose in acidic medium, TFA, probably through intermediate **92** formed by protonation at a vinyl group ($C18^2$ position) of the D-ring of **87b**, in which the electron density at C11 is decreased to retard the desired formylation reaction. Formylation of **87b** by Vilsmeier reaction or introduction of an ester group by Friedel–Crafts type reaction to cleave between the B- and C-rings was also attempted, but the expected products could not be obtained.[117]

Formylated AB-ring component **86b** was treated with Al(Hg) in THF/H_2O to afford reduced intermediate **90b**, followed by acid treatment in CH_2Cl_2 to give AB-ring **91b** having an ethylidene group at the A-ring in 57% yield from **86b**. The present synthetic method of the AB-ring component made it possible to construct bilin chromophores in short steps starting from commercially available bilirubin **87a**.

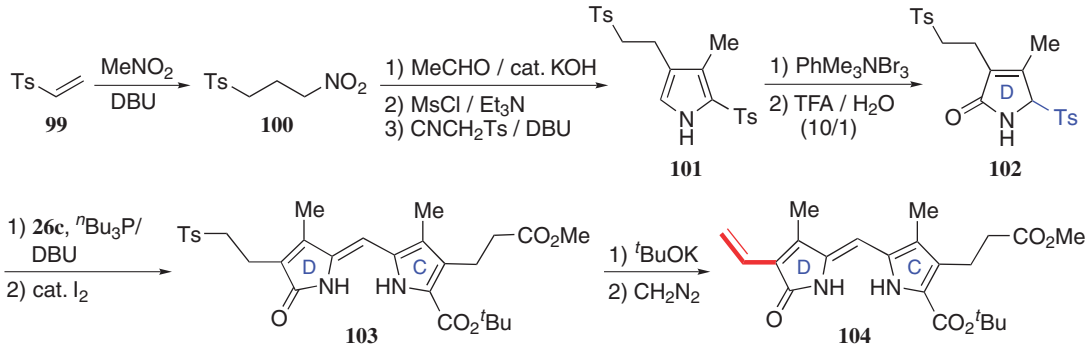
2.8 Coupling of the C- and D-Rings. A base-catalyzed Knoevenagel-type reaction has so far been employed for condensation between an α-unsubstituted pyrrolinone **27** as the D-ring and a formylpyrrole as the C-ring bearing a methoxycarbonylethyl group to construct a pyrromethenone framework as the CD-ring component of bilin chromophores. The condensation reaction, however, requires a subsequent reesterification of the propanoic acid side chain of the resulting coupling product with diazomethane. Therefore, we exploited the new Wittig-type coupling reaction between 5-tosylpyrrolinone **28** and a formylpyrrole under mild conditions as shown in Scheme 19, for the preparation of the AB-ring component as described above.[45,46,77]

In a similar manner, CD-ring components **89b** of PΦB/BV and **98** of PCB were prepared from 5-tosylpyrrolinones **59**, which is a regioisomer of A-ring precursor **81**, and **93** as the D-ring precursor of PCB, by coupling with formylpyrrole **26a** by using our original Wittig-type coupling reaction. The resulting *E*/*Z*-mixture of **94** and **96** were treated with a catalytic amount of iodine to give only *Z*-isomers in good yields. A thioether bond of the coupling product **94** was oxidized with *m*CPBA to afford sulfoxide **95**. Pyrromethenone derivatives **95** and **96** were treated with TFA for decarboxylation, followed by formylation with methyl orthoformate, to afford the corresponding formylated products **97** and **98**, the latter of which is the CD-ring component of PCB. CD-ring component **89b** of PΦB/BV was obtained by heating **97** in xylene in the presence of pyridine to introduce a vinyl group on the D-ring as shown in Scheme 23.[49]

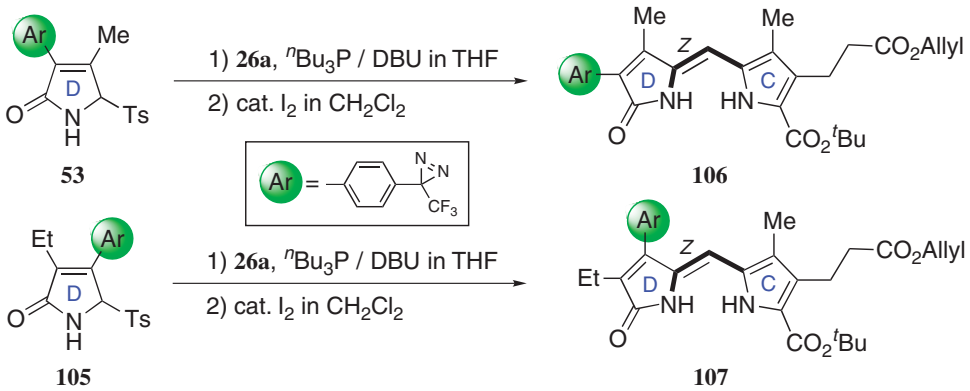
CD-ring component **104** of PΦB and BV was also available by using pyrrolinone **102**, which has a 2-tosylethyl group as a potential equivalent of a vinyl group and prepared from tosylethene **99** via **100** and **101** as shown in Scheme 24 as the D-ring precursor, whereas it required the use of a strong base, *t*-BuOK, to convert the 2-tosylethyl group of coupled compound **103** into a vinyl group by β-elimination of *p*-toluenesulfinate. The free carboxylic acid, which was partially formed by the attack of *p*-toluenesulfinate toward the methyl ester, was reesterified with diazomethane to afford methyl ester derivative **104**.[46]

The Wittig-type coupling reaction allowed us to prepare CD-ring components **106** and **107** bearing a photoreactive 4-[3-(trifluoromethyl)diaziren-3-yl]phenyl (Ar) group[122,123] on the D-ring starting from **53** and **105**, as outlined in Scheme 25, to investigate the structural relationship between the chromophore and the apoprotein in the reconstituted phytochrome for both Pr and Pfr forms. Pyrrolinone **53** was available via rearrangement of the tosyl group of 2-tosylpyrrole **50** (Scheme 13).[87]

2.9 Construction of Tetrapyrrole Framework of Bilin Chromophores. As described above, the AB- and CD-rings



Scheme 24. An alternative method for the preparation of CD-ring component **104** of PΦB and BV starting from tosylethene (**99**).



Scheme 25. CD-ring components **106** and **107** bearing a photoreactive 4-[3-(trifluoromethyl)diaziren-3-yl]phenyl (Ar) group.

components were in hand. Thus, they were coupled in alcohol in the presence of acid catalyst to construct the corresponding tetrapyrrole derivatives.

Scheme 26 shows the coupling between the AB-ring component bearing an ester group at meso-position and the CD-ring component. AB-ring component **110** was formylated with methyl orthoformate in TFA accompanying decarboxylation to afford **111**, which was coupled with CD-rings component **109** obtained from **108** by treating with TFA, to afford triallyl ester derivatives of PCB and its analogs.

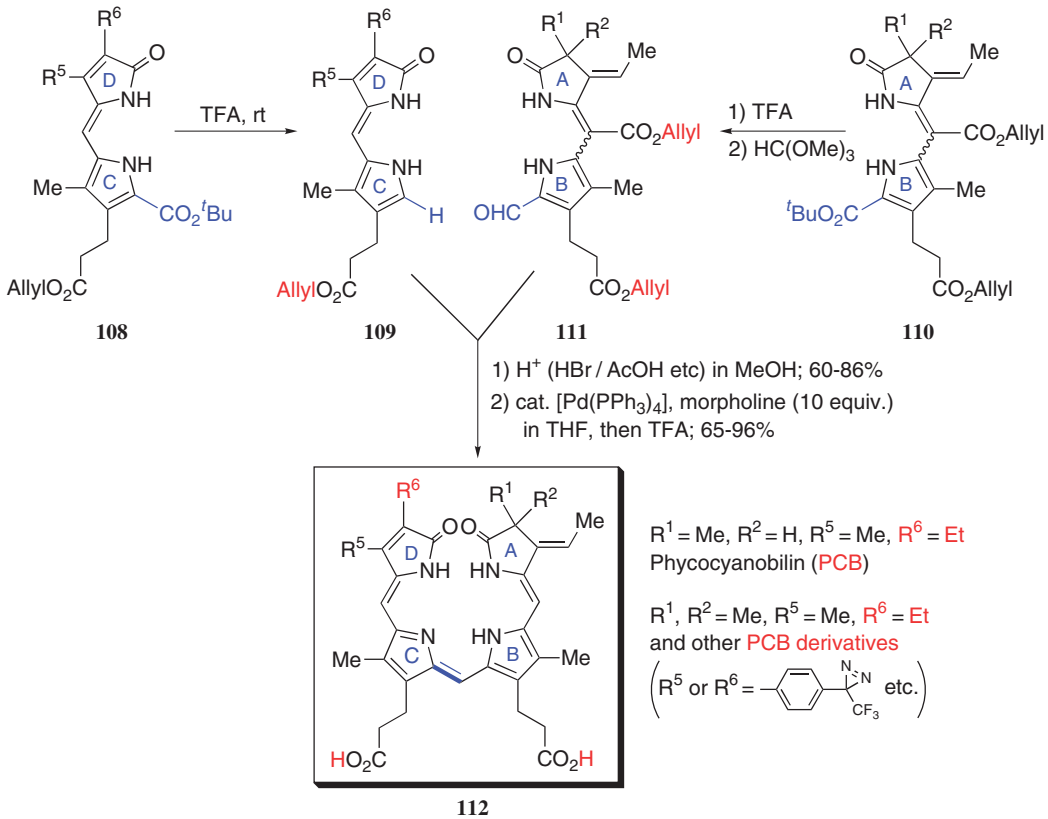
Finally, three allyl ester groups of the coupling products were deprotected all at once with excess amounts of morpholine in the presence of Pd^0-catalyst,[124] avoiding the migration of exocyclic olefin of the A-ring to the endocyclic position, followed by decarboxylation at the C5 position by treating with TFA to afford free acid form **112**, as well as the PCB derivatives bearing a photoreactive group on the D-ring for a photoaffinity study.[47,48,53,87] It was confirmed by NOESY that all of the synthesized bilin chromophores have an all-*Z* configuration and an all-*syn* conformation, respectively.

Various PCB derivatives with a modified A-ring, such as 2-norPCB, 2-methylPCB, and 2- or 3-homoPCB, could be prepared by applying this method for the structure/function analysis. 3,3[1]-Dihydrogenated PCB derivatives were also synthesized to investigate their non-covalent interaction with phytochrome apoprotein toward the development of affinity chromatography to purify apoprotein.[47,51]

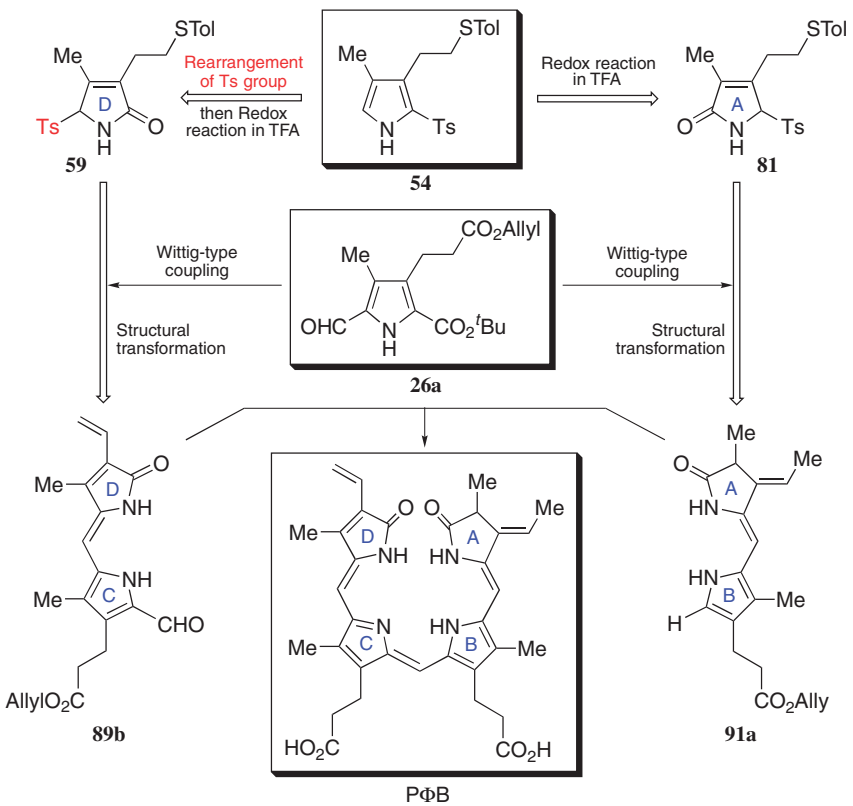
Furthermore, PCB and its derivatives were synthesized by coupling CD-ring components **109** with the AB-ring components, which do not have an ester group at meso-position, such as **91b** instead of **111**, under acidic conditions in a similar manner described above. These method also allowed us to prepare various kinds of PCB derivatives with the modified A-, B-, C-, and D-rings including PCB derivatives with a photoreactive 4-[3-(trifluoromethyl)diaziren-3-yl]phenyl group[122,123] on the D-ring for a photoaffinity study.[47,48,53,87]

On the other hand, the total synthesis of PΦB was achieved starting from only two pyrroles, 4-methyl-3-[2-(tolylthio)-ethyl]-2-tosylpyrrole (**54**) as a precursor of the A- and D-rings and 2-formylpyrrole **26a** common to the B- and C-rings,[49] employing the novel synthetic reactions described above: (1) rearrangement of the tosyl group of 2-tosylpyrrole to the 5-position under acidic conditions, (2) efficient transformation of 2-bromo-5-tosylpyrrole to the corresponding 5-tosylpyrrolinone under anhydrous redox conditions, (3) Wittig-type coupling reaction between 5-tosylpyrrolinone and 2-formylpyrrole, followed by reductive transformation of the AB-ring component, and (4) protection and palladium-catalyzed deprotection of propanoic acid side chains at the C8 and C12 positions via allyl esters (Scheme 27).[49] It is also possible to introduce a formyl group to the AB-ring component instead of the CD-ring; however, it looked better to introduce the electron-withdrawing formyl group to the CD-ring component than to the AB-ring, due to the existence of an acid labile vinyl group at the D-ring of PΦB, as discussed for the cleavage between the B- and C-rings of bilirubin diallyl ester (see **92** in Scheme 22).

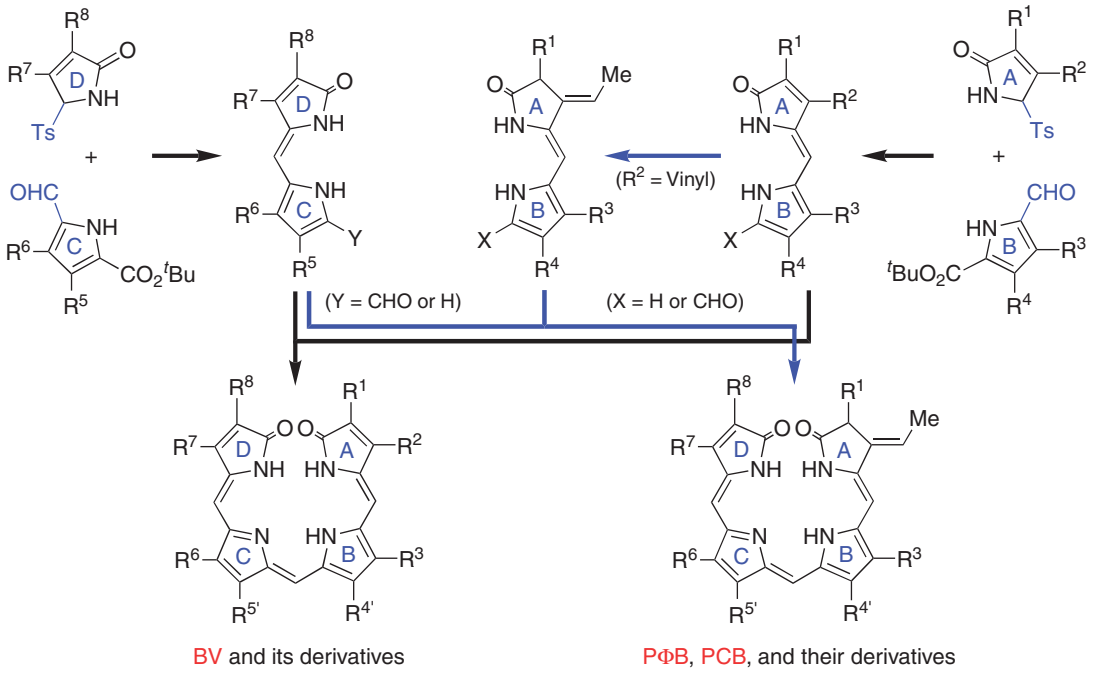
In the course of the investigation, it was found that PΦB was extremely unstable in free acid form compared to PCB except the solid state after purification.



Scheme 26. Construction of tetrapyrrole framework of bilin chromophores by coupling the AB-ring component bearing an ester group at meso-position and the CD-ring component.



Scheme 27. An efficient total synthesis of PΦB starting from only two pyrroles, **54** as a precursor of the A- and D-rings and **26a** as a common precursor of the B- and C-rings, respectively.



Scheme 28. A general and efficient synthetic method of BV, PΦB, PCB, and their derivatives.

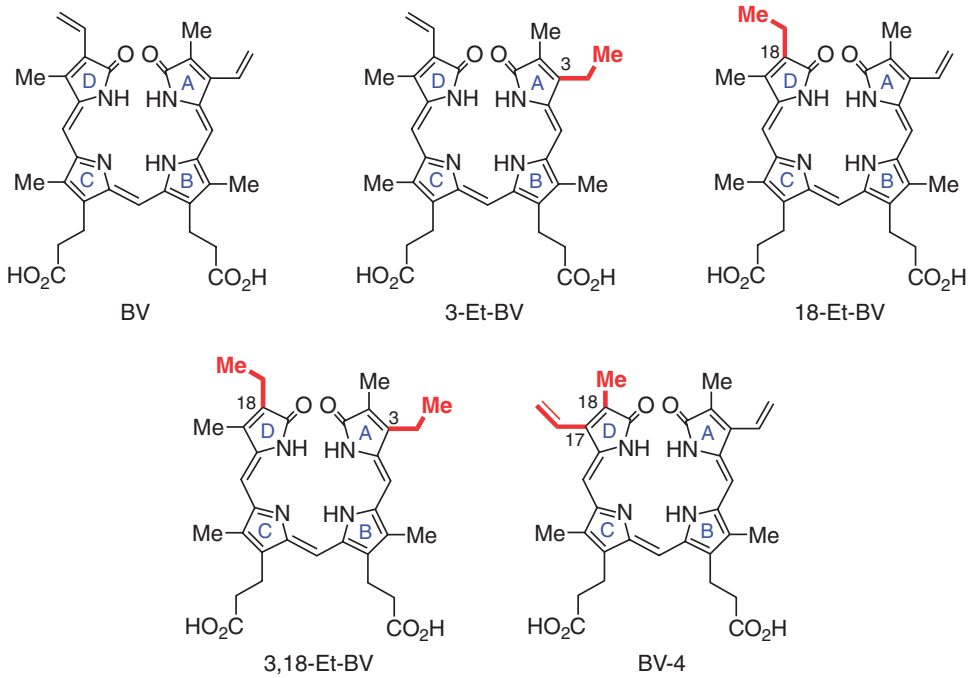


Fig. 8. BV and its derivatives synthesized by using our general method for the preparation of bilin chromophores.

This synthetic method was applicable not only for the preparation of PCB, PΦB, and their derivatives, but also for the preparation of BV and its derivatives (Scheme 28). Actually we could prepare BV and its derivatives, 3-Et-BV, 18-Et-BV, 3,18-Et-BV, and BV-4 shown in Fig. 8, for assembly experiment with *Agrobacterium* phytochrome Agp1 as will be discussed later.

2.10 Synthesis of the Sterically Locked BV Derivatives. As mentioned above, we established an efficient and flexible synthetic method of various kinds of bilin chromophores,[46–51] including PΦB,[49] PCB,[47,48] and more than 20 kinds of PCB derivatives,[50,51] in free acid forms, which made it possible to assemble them with the apoproteins not only in vitro to analyze the spectral properties of the resulting holoproteins,[125] but also in vivo to observe their physiological functions[126] as will be seen later.

On the other hand, for plant phytochrome, it has been shown that the first step of the Pr to Pfr photoconversion is a *Z* to *E* isomerization of the chromophore around the C15=C16 double bond.[28] In principle, each exocyclic single bond can adopt

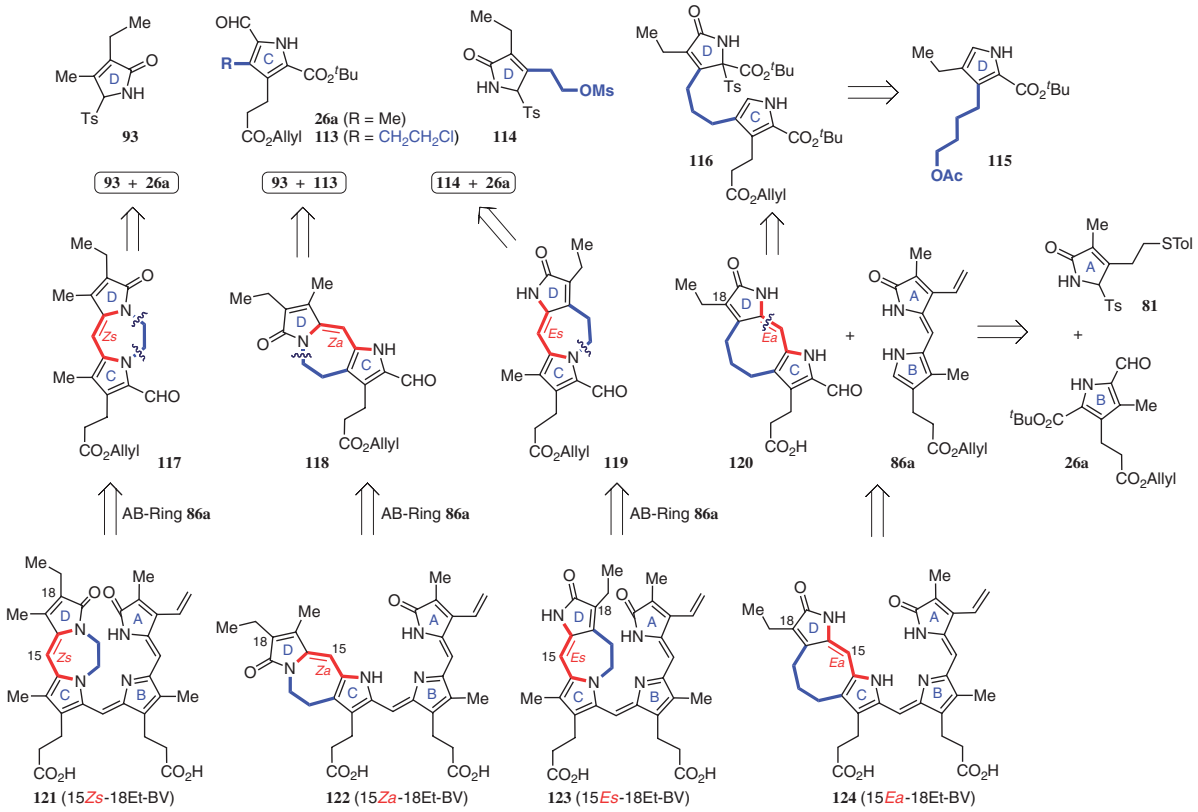


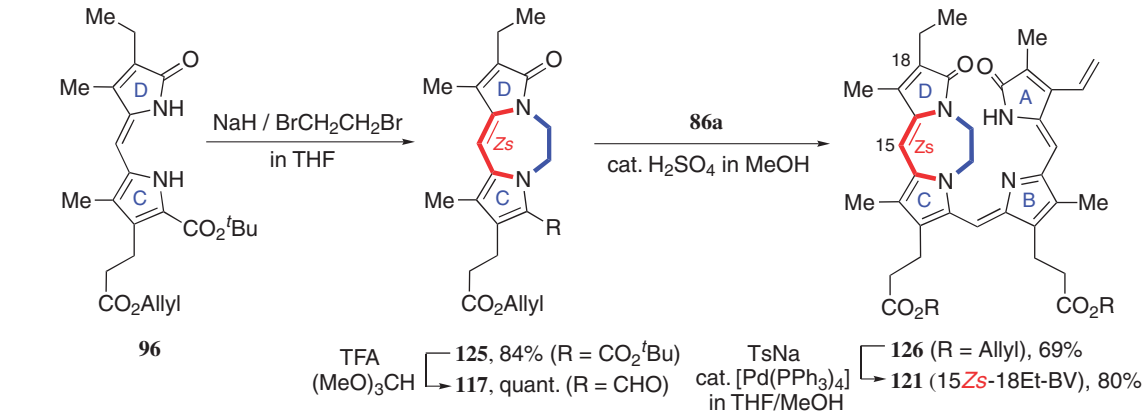
Fig. 9. Retrosynthetic analysis of the sterically locked BV derivatives.

either the *syn* or *anti* conformation during photoconversion.[16] Vibrational spectroscopy gained indirect insight into the conformation of the phytochrome chromophore in the Pr, Pfr, and intermediate states, but the data were ambiguous and have been interpreted in different ways.[29–32] For example, it has been proposed that the formation of Pfr is accompanied by a *syn*/*anti* rotation around the C14–C15 single bond.[31] More recently, interpretation of resonance Raman spectra by using density functional theory (DFT) calculations indicated that the C14–C15 single bond is in the *anti* conformation throughout the entire photocycle and that the C5–C6 single bond rotates from *anti* to *syn* upon conversion from Pr to Pfr as shown in Fig. 2.[32] Therefore, we attempted to synthesize the sterically locked bilin chromophores to directly confirm the stereochemistry of the chromophore in phytochrome around the C15 position for both Pr- and Pfr-forms by applying the above general synthetic method of bilin chromophores.

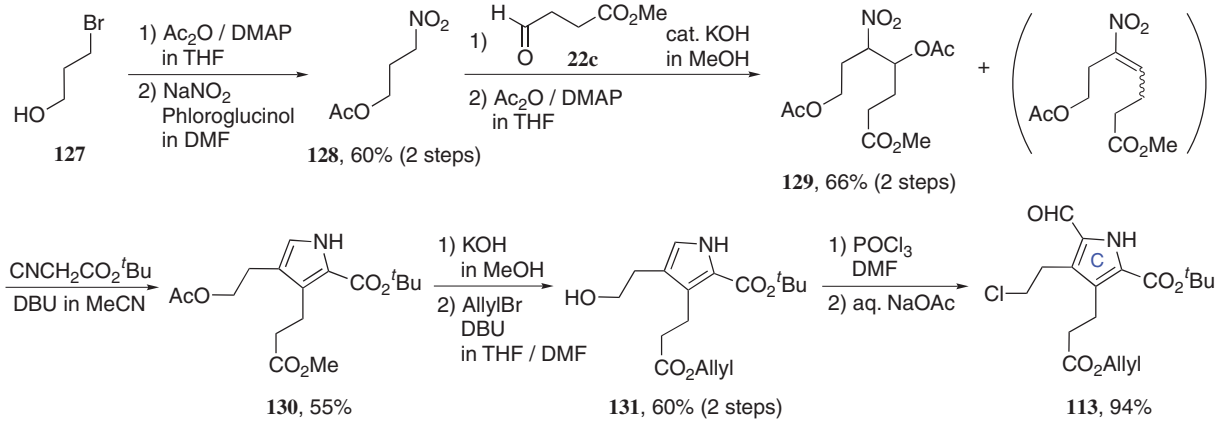
The recent discovery of phytochrome-related proteins in photosynthetic cyanobacteria and nonphotosynthetic eubacteria has opened new avenues for investigating biliprotein photosensory function.[127,128] The latter phytochromes use BV as a chromophore. Thus, we planned to prepare the sterically locked BV derivatives at first, since it required fewer steps than the case of the synthesis of the corresponding PCB or PΦB derivatives as obviously shown in Scheme 28. AB-ring component **86a** common to the locked BV derivatives were prepared as shown in Scheme 21 from (*E*/*Z*)-**82**.[115] Thus, total syntheses of the four different types of BV derivatives (**121**–**124**), in which the stereochemistry between the C- and D-rings are locked in *Z-syn*, *Z-anti*, *E-syn*, and *E-anti* configuration and conformation, respectively, were accomplished via establishment of new and efficient methods for the construction of sterically locked CD-ring components based on the retrosynthetic analysis depicted in Fig. 9.

2.10.1 Synthesis of the *Z-syn* CD-Ring Component and 15*Zs*-18Et-BV: CD-ring precursor **125**, which is sterically locked in *Z-syn* configuration and conformation, was prepared by treating (*Z*)-**96** with 1,2-dibromoethane in the presence of NaH in 84% yield. The *t*-butoxycarbonyl group of **125** was converted to a formyl group by treating with TFA followed by addition of methyl orthoformate at room temperature to give the formylated CD-ring component **117** in quantitative yield, as shown in Scheme 29. Coupling reactions between CD-ring component **117** and AB-ring component **86a** were carried out in methanol under acidic conditions to afford 15*Zs*-18Et-BV diallyl ester **126** in 69% yield. The two allyl ester groups were deprotected by using a Pd^0-catalyzed reaction with sodium *p*-toluenesulfinate (NaTs) as a nucleophile in THF/MeOH to give 15*Zs*-18Et-BV **121** in free acid form in 80% yield.[54]

2.10.2 Synthesis of the *Z-anti* CD-Ring Component and 15*Za*-18Et-BV: *Z-anti* CD-ring component **118** was prepared from D-ring **93**[52] and C-ring **113** with a leaving group for cyclization, as shown in Scheme 30.[115] Commercially available 3-bromo-1-propanol (**127**) was acetylated, followed by nitration using sodium nitrite in the presence of phloroglucinol in DMF, to give 3-nitropropyl acetate (**128**) in 60% yield. Compound **128** was coupled with oxoester **22c** in a similar manner for the preparation of the B- and C-rings,[46,50] followed by acetylation of the resulting alcohol to give nitro-acetate **129** in



Scheme 29. Synthesis of 15*Zs*-18Et-BV **121**.



Scheme 30. Synthesis of C-ring **113** bearing a 2-chloroethyl group useful for cyclization.

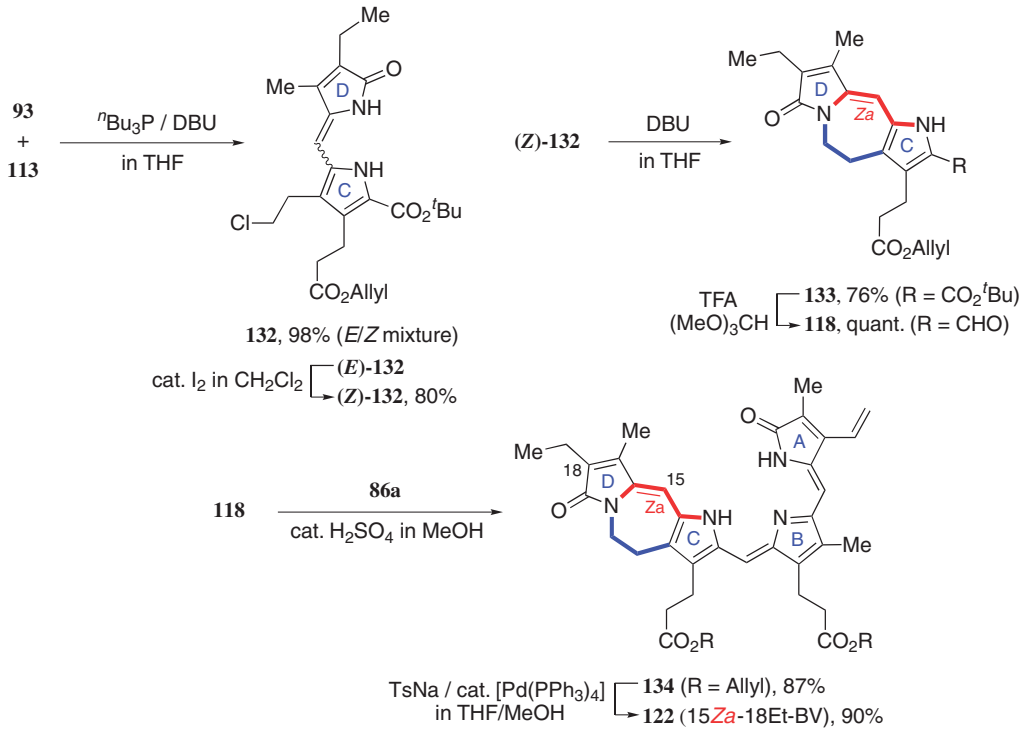
66% yield, along with a small amount of nitroolefin. When compound **129** was treated with *t*-butyl isocyanoacetate and DBU according to Barton's method[83] in acetonitrile, pyrrole derivative **130** was obtained in 55% yield. Hydrolysis of **130** with KOH in MeOH, followed by allylation with allyl bromide in the presence of DBU in THF/DMF, gave pyrrole **131** in 60% yield. When compound **131** was formylated by the Vilsmeier reaction to give the formylated product, chlorination of the hydroxy group proceeded simultaneously to afford formylpyrrole **113** with a 2-chloroethyl group in 94% yield.

As shown in Scheme 31, Wittig-type coupling reaction between 5-tosylpyrrolinone **93** and formylpyrrole **113** proceeded satisfactorily using tributylphosphine in the presence of DBU in THF at 0 °C to room temperature to afford CD-ring components **132** as a mixture of *E*- and *Z*-isomers in 98% yield. It was found that compound (*E*)-**132** had to be converted to the *Z*-isomer by treating with a catalytic amount of iodine in CH_2Cl_2 prior to the cyclization. Compound (*Z*)-**132** was cyclized at 50 °C in the presence of DBU in THF affording the desired cyclized product **133** in 76% yield. Subsequent formylation was accomplished by treating with methyl orthoformate in TFA to give formylated CD-ring component **118** in quantitative yield.[54,115]

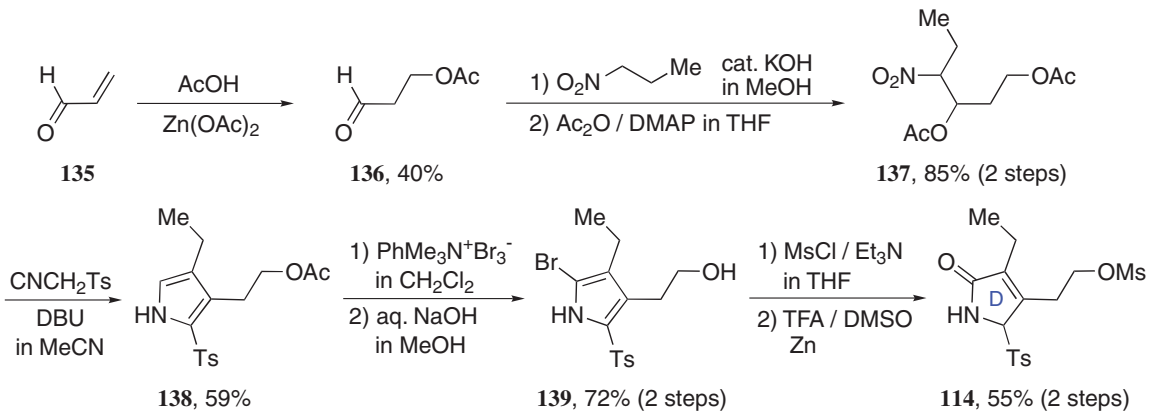
Coupling reaction between CD-ring **118** and AB-ring **86a** was carried out under acidic conditions to afford the sterically locked 15*Za*-18Et-BV diallyl ester derivative **134** in 87% yield. Finally, the deprotection of the allyl ester moiety of **134** was achieved by Pd^0-catalyzed reaction[47,49] using sodium *p*-toluenesulfinate as a nucleophile in THF/MeOH to give the desired locked chromophore **122** (15*Za*-18Et-BV) in 90% yield.[54,115]

2.10.3 Synthesis of the *E-syn* CD-Ring Component and 15*Es*-18Et-BV: 5-Tosylpyrrolinone derivative **114** was prepared starting from propenal (**135**) as shown in Scheme 32. Treatment of **135** with acetic acid in the presence of a catalytic amount of zinc acetate afforded 3-acetoxypropanal (**136**) in 40% yield.[129,130] Compound **136** was then coupled with 1-nitropropane according to Henry reaction in the presence of a catalytic amount of KOH in MeOH. The resulting nitro-alcohol was then acetylated using acetic anhydride in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in THF to give the nitro-diacetate **137** in 85% yield in two steps. Compound **137** was reacted with tosylmethyl isocyanide in the presence of DBU to afford 2-tosylpyrrole derivative **138** in 59% yield. Compound **138** was then brominated with trimethylphenylammonium tribromide in CH_2Cl_2, followed by hydrolysis of the acetoxy group with aq NaOH in MeOH, to give compound **139** in 72% yield in two steps. Mesylation and subsequent redox-type reaction[44,52] using DMSO and zinc in TFA afforded the 5-tosylpyrrolinone derivative **114** in 55% yield in two steps.[54]

Compound **114** and **26a** were coupled by Wittig-type reaction using tributylphosphine and DBU in THF to directly afford the cyclized CD-ring component **140** as *E-syn* isomer in



Scheme 31. Synthesis of 15*Za*-18Et-BV **122**.



Scheme 32. Synthesis of D-ring **114** carrying a leaving group useful for cyclization.

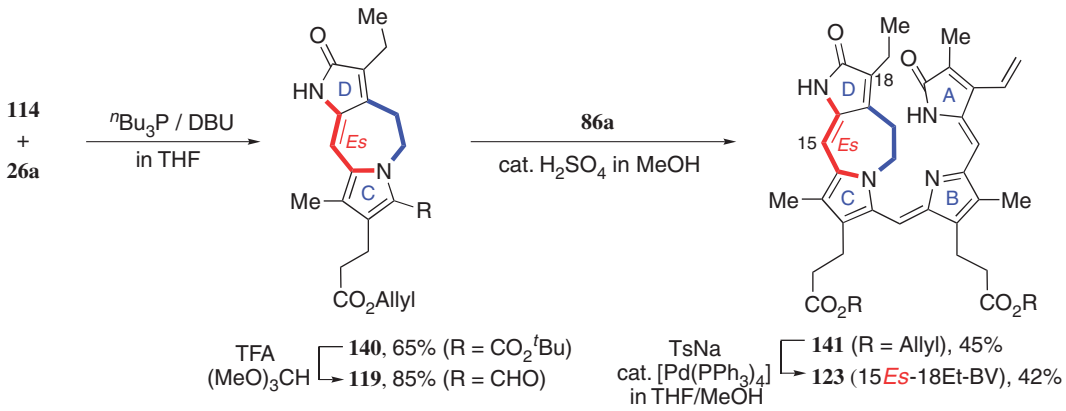
65% yield. Formylation reaction was carried out by treating **140** with TFA and subsequent addition of methyl orthoformate to give the formylated CD-ring component **119** in 85% yield.

Coupling reaction between CD-ring **119** and AB-ring **86a** was carried out under acidic conditions in a similar manner as in the case of other locked chromophores described above to afford the sterically locked 15*Es*-18Et-BV diallyl ester derivative **141** in 45% yield. The deprotection of the allyl ester moiety of **141** by Pd^0-catalyzed reaction[47,49] with sodium *p*-toluenesulfinate as a nucleophile in THF/MeOH gave locked chromophore **123** (15*Es*-18Et-BV) in 42% yield (Scheme 33).[54,115]

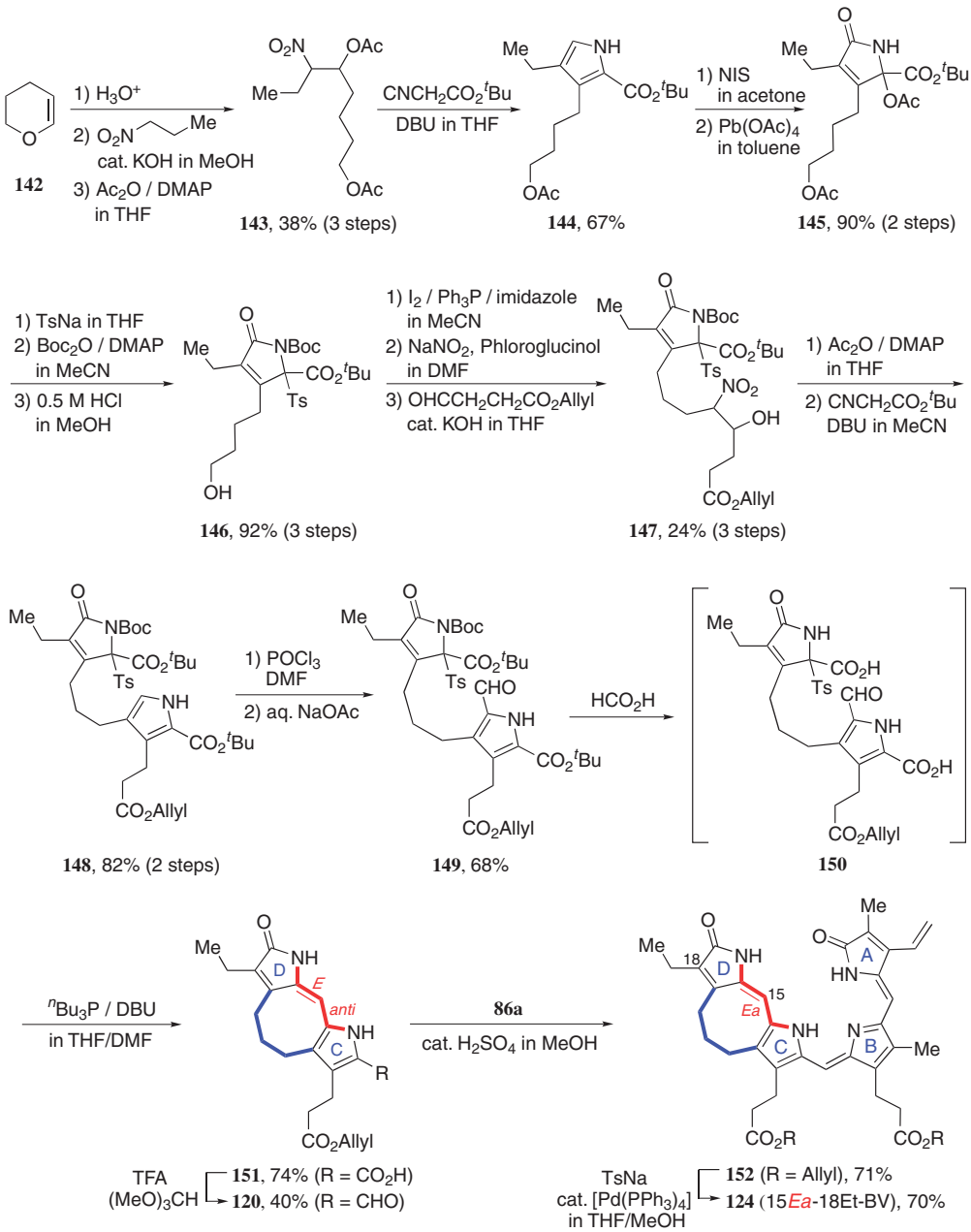
2.10.4 Synthesis of the *E-anti* CD-Ring Component and 15*Ea*-18Et-BV: Biliverdin (BV) derivative **124** (15*Ea*-18Et-BV) bearing an *E-anti* CD-ring component was synthesized by fixing with 8-membered ring as shown in Scheme 34.[131]

CD-ring component **120** was prepared starting from the commercially available 3,4-dihydro-2*H*-pyran (**142**). 5-Hydroxypentanal obtained by acid hydrolysis of **142** was reacted with 1-nitropropane in the presence of a base according to the Henry reaction, followed by acetylation in the presence of DMAP to give nitro-diacetate **143** in 38% yield in three steps. Compound **143** was reacted with *t*-butyl isocyanoacetate in the presence of DBU in THF applying Barton's method[83] to give pyrrole derivative **144** in 67% yield.

Iodination of α-position of pyrrole **144** with *N*-iodosuccinimide (NIS), followed by oxidation utilizing Pb(OAc)$_4$ in toluene, gave pyrrolinone derivative **145** in 90% yield. The α-acetoxy group of the pyrrolinone derivative **145** was replaced by Ts group using anhydrous sodium *p*-toluenesulfinate (TsNa) in refluxing THF, followed by protection of the pyrrolinone-NH using di-*t*-butyl dicarbonate (Boc$_2$O) in MeCN. Then, hydrolysis of the acetate group in 0.5 M methanolic HCl afforded N-protected tosylpyrrolinone derivative **146** in 92% yield. Io-



Scheme 33. Synthesis of 15*Es*-18Et-BV **123**.



Scheme 34. Synthesis of 15*Ea*-18Et-BV **124**.

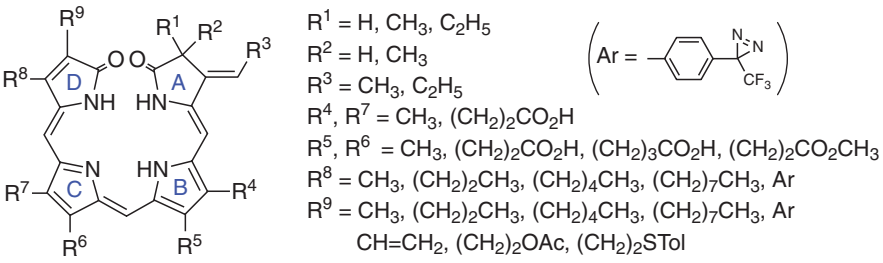


Fig. 10. Chemical structures of synthesized bilin chromophores, which were used for assembly experiment with PHYB.

dination of the resulting alcohol using iodine and triphenylphosphine in the presence of imidazole in MeCN, followed by nitration using sodium nitrite in the presence of phloroglucinol in DMF, gave the tosylpyrrolinone derivative bearing a nitro group in its side chain in 38% yield. The resulting nitro compound was reacted with allyl 4-oxobutanoate to construct tosylpyrrolinone derivative **147** with a nitroalcohol side chain in 64% yield (24% yield from **146** in three steps). Acetylation of the resulting alcohol **147**, followed by reaction with *t*-butyl isocyanoacetate, gave dipyrrole derivative **148** in 82% yield from **147** in two steps.

Compound **148** was subjected to the Vilsmeier reaction to afford formylated dipyrrole derivative **149** in 68% yield. Compound **149** was then treated with 99% formic acid to cleave the Boc and *t*-butyl esters giving dicarboxylic acid derivative **150**, which was subjected to Wittig-type coupling reaction using tributylphosphine in the presence of DBU in THF/DMF at $-75\,^{\circ}$C–rt to give the pyrromethenone derivative **151** in 74% yield. Compound **151** was formylated by treating with methyl orthoformate in TFA to give the desired formylated *E-anti* CD-ring component **120** in 40% yield.

The coupling reaction between CD-ring **120** and AB-ring **86a** was carried out under acidic conditions to afford 15*Ea*-18Et-BV diallyl ester derivative **152** in 71% yield. Finally, deprotection of the allyl esters was achieved by using Pd^0-catalyzed reaction in the presence of sodium *p*-toluenesulfinate as nucleophile in THF/MeOH to give 15*Ea*-18Et-BV **124** in 70% yield.[131]

3. Assembly of the Synthetic Bilin Chromophores with Phytochrome Apoproteins

3.1 In Vitro Assembly of PΦB, PCB, and PCB Analogs with Phytochrome B Apoprotein. Now various kinds of PCB, PΦB, BV, and their derivatives have become available in free acid forms for us to study on the reconstituted phytochromes. Phytochromes have long been believed to be the photoreceptors for red/far-red reversible reactions, but it has become evident that phytochrome A and B (PhyA and PhyB) recognize light in essentially different manners.[132] PhyB photoreversibly switches responses on or off upon irradiation with red/far-red light, whereas PhyA triggers very low-fluence or high-irradiance responses.[133] The spectral properties of PhyA have been well characterized, because of the relatively high abundance of this photoreceptor in dark-grown tissue. In contrast, PhyB has not been well characterized, because of its low abundance in native tissue. Several groups have reported in vitro assembly of PhyB and have shown that it has similar spectral properties to PhyA.[134–136]

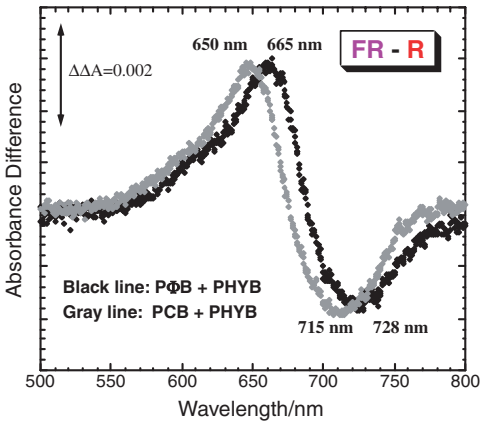


Fig. 11. Difference absorption spectra of the PHYB adducts with the chemically synthesized PΦB and PCB.[125] The difference spectra were obtained by subtracting the absorption spectra recorded after red light irradiation from that recorded after far-red light irradiation. Black and gray dotted lines indicate the difference spectra of PΦB and PCB adducts, respectively. The vertical bar represents 0.002 absorbance unit.

To analyze the structural requirements of the chromophore for the spectral properties of PhyB, we designed and chemically synthesized more than 20 bilin chromophore derivatives (Fig. 10), including natural PΦB and PCB, and reconstituted them with recombinant *Arabidopsis* PhyB apoprotein (PHYB) through collaborative study with biologists.[125]

The difference absorption spectra of the in vitro-assembled adducts of the synthetic bilin chromophores with PHYB were measured on actinic far-red and red light irradiations. The adduct with PCB showed a similar difference spectrum to that of the PΦB adduct, although its peaks were slightly blue shifted due to the difference of the length of π-conjugation system when compared with the PΦB adduct corresponding to the natural phytochrome,[137–139] as shown in Fig. 11.[125]

Some of the difference absorption spectra of PHYB adducts with the synthesized PCB analogs modified at the A-, B-, C-, and D-rings, respectively, are shown in Fig. 12. Binding efficiency of each analog by using zinc blot analysis was reported in our original report.[125]

The A-ring acts mainly as the anchor for ligation to PHYB, because the modification of the side chains at the C2 and C3 positions did not significantly influence the formation (based on the results from zinc blot analysis) or difference spectra of adducts (Figs. 12a and 12b). The results indicated that a hydrophobic environment and open space accommodate the A-

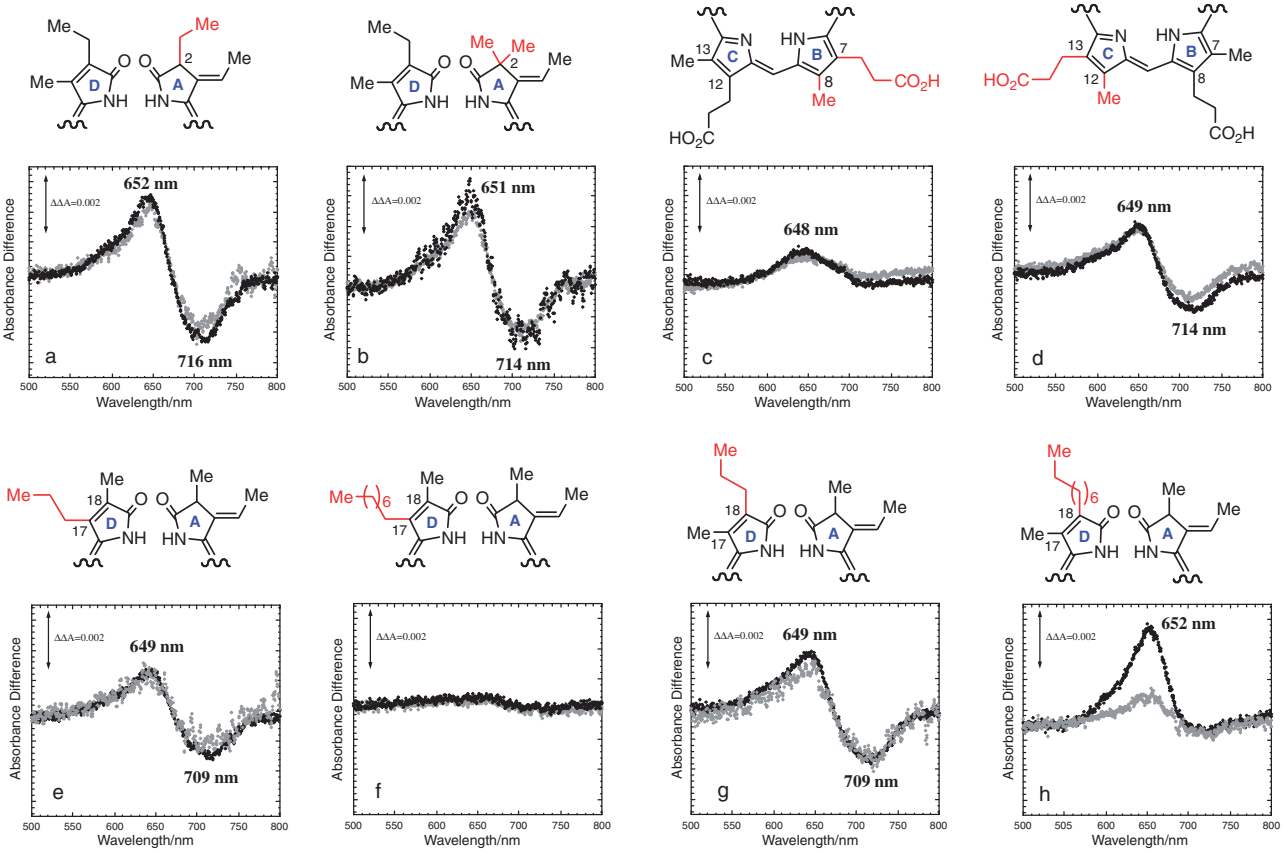


Fig. 12. Difference absorption spectra of the PHYB adducts with some of the chemically synthesized PCB analogs (a–h).[125] The difference spectra were obtained by subtracting the absorption spectra recorded after red light irradiation from that recorded after far-red light irradiation. Difference spectra were recorded after two consecutive red/far-red cycles. Black and gray dotted lines indicate the difference spectra obtained after the first and second cycles, respectively. The vertical bars represent 0.002 absorbance units.

ring of the bilin chromophore at the binding site of PHYB, and that stereochemistry at the C2 position is not crucial, since the analog dimethylated at the C2 position showed similar difference spectra (Fig. 12b).

In contrast, the side chains of the B- and C-rings are crucial to position the chromophore properly in the chromophore pocket of PHYB and for photoreversible spectral changes. Typical examples are shown in Figs. 12c and 12d. Exchanging the methyl groups and the propanoic acid side chains of the B- and C-rings decreased the binding efficiency remarkably (by zinc blot analysis),[125] suggesting that free-acid elements are required for chromophore binding, probably dictated by the electrostatic interaction between propanoic acid side chain and basic amino acid residues, such as Arg or Lys of the phytochrome apoprotein. When PHYB adducts retained a propanoic acid side chain at the C8 position of the B-ring, they displayed photochromism (Fig. 12d). Thus, propanoic acid side chain at the C8 (Fig. 12d) was found to be more crucial for photoreversible conversion of phytochrome than that at the C12 position (Fig. 12c).

Modification of the D-ring was essential for analysis of photochromism, because the D-ring (the C15 position) is the site at which isomerization occurs during photoconversion of phytochrome. Substituents at the C17 and C18 positions of the D-ring were varied as vinyl (corresponds to PΦB), methyl, propyl, pentyl, or octyl groups in a regioselective manner for structure/function analysis. In addition, acetoxy and tolylthio groups were introduced at the β-position of the ethyl group at the C18 position. The side-chain structure of the D-ring was required for the photoreversible spectral change of the adducts.[125] Some difference absorption spectra of the adducts are shown in Figs. 12e–12h. When methyl and ethyl groups at the C17 and C18 positions are replaced with a propyl or octyl group, respectively, the photoreversible spectral change of the adducts depended on the length of the side chains. Comparison of the difference absorption spectra of the adducts replaced with an octyl group (Figs. 12f and 12h) suggested that the environment around the C18 position of the chromophore within the chromophore-biding pocket of PHYB is hydrophobic and more flexible than that of the C17 position. This suggestion was supported by the results from other two analogs, which have the 2-(acetoxy)ethyl or 2-(tolylthio)ethyl groups at the C18 position.[125]

The results of these studies allowed us to outline the order of events during in vitro assembly of PHYB with its chromophore. First, the ligation of chromophore to PHYB is initiated by an electrostatic interaction between the propanoic acid side chains of the B- and C-rings and a basic amino acid residue such as Arg or Lys. Second, the A-ring falls into the hydrophobic site close to the Cys of PHYB, followed by nucleophilic

attack of the thiol group at the C3[1] position of the ethylidene substituent of the A-ring to form a thioether bond through a sort of Michael-type addition reaction. Finally, the D-ring also falls into a hydrophobic pocket where open space exists at least around the C18 position of the chromophore. Rotation around the C14–C15 single bond axis probably occurs at this stage, forming the extended structure of the chromophore in PHYB.

It was possible to replace a side chain at the C17 or C18 position of the D-ring with a bulky substituent without loss of photoconversion. Thus, these positions seemed to be suitable to be labeled with a photoreactive group for photoaffinity studies. However, PCB analogs carrying a photoreactive 4-[3-(trifluoromethyl)diaziren-3-yl]phenyl group at the C17 or C18 position of the D-ring were not incorporated into PHYB, probably due to the bulkiness and the lack of flexibility of the employed photoreactive group.

From these studies, we proposed that each pyrrole ring of the linear tetrapyrrole (bilin) chromophore plays a different role in chromophores assembly and the photochromic properties of PhyB. This collaborative study with biologists has opened an avenue of research toward understanding the nature of holophytochrome assembly and the chromophore pocket in vitro.

3.2 In Vivo Assembly of PΦB, PCB, and PCB Analogs with Phytochromes A and B Apoproteins. Phytochromes are encoded by a small gene family, and five distinct phytochromes (A to E) have been identified in the model plant species *Arabidopsis thaliana*,[140,141] in which phytochrome A (PhyA) and B (PhyB) are most abundant, principally working throughout the life cycle. Photobiological analyses have established that, under continuous irradiation, phytochrome A is primarily responsible for plant's sensitivity to far-red light, whereas the other phytochromes respond mainly to red light, using various phytochrome-deficient mutants.[4] To elucidate a question whether different bilin structures affect the photobiological activities of phytochromes in vivo, we have collaborated with biologists to incorporate synthetic bilin chromophores into apopytochromes A (PHYA) and B (PHYB) in *Arabidopsis hy1* and *hy2* mutants, which are deficient in PΦB biosynthesis.[142–144] Parks and Quail have restored photomorphogenesis in these mutants with exogenously supplied biliverdin IXα

Fig. 13. Difference of the growth phenotype of FR irradiated 7-day-old *hy2* seedlings, which were treated with PΦB (left) or PCB (right), respectively.[126]

(BV), a direct precursor of PΦB.[145] By using an analogous approach, synthetic bilins were fed exogenously to *hy1* and *hy2* seedlings to test whether they restore the photobiological functions of PhyA and PhyB in vivo.[126] When these seedlings were intermittently irradiated with far-red (FR) light for 48 h, the PΦB-treated *hy1* and *hy2* seedlings showed a significantly different growth phenotype compared with PCB-treated mutant in terms of hypocotyls length, cotyledon opening, and cotyledon expansion (Fig. 13). FR-irradiated PCB-treated seedlings resembled dark-grown seedlings, whereas the PΦB-treated seedlings showed a characteristic de-etiolated phenotype, hypocotyl growth inhibition, similar to that of the FR-irradiated wild type (WT) seedlings.[126]

Figure 14 shows effect of exogenously supplied PΦB and PCB to *hy2* mutant seedlings in terms of hypocotyl length.[126] The far-red light sensitivity of PhyA was found to depend on the structure of the bilin chromophore. By reconstitution of holophytochrome in vivo through feeding various synthetic bilins to chromophore-deficient mutants of *Arabidopsis*, the requirement for a double bond on the D-ring of PΦB for restoring PhyA function has been established (Fig. 14A). In contrast, PhyB function was restored with both PΦB and PCB with a saturated D-ring substituent (Fig. 14B).[126] These results were confirmed further by using *hy1/phyA* and *hy1/*

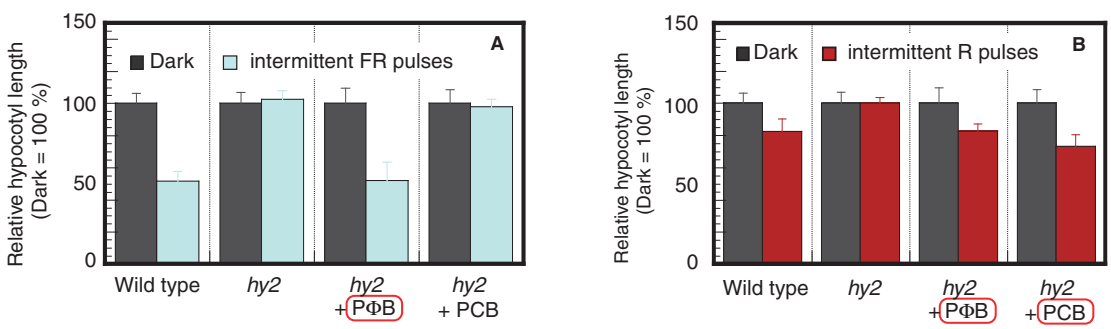


Fig. 14. Effect of exogenously supplied PΦB and PCB to *hy2* mutant seedlings on hypocotyls growth.[126] (A) The seedlings were irradiated with intermittent far-red (FR) light pulses to observe the photosensory function of PhyA. D (black), kept in darkness; FR (bluish gray) at 3 min cycles for 2 days. The PhyA-mediated responses occurred only when PΦB was supplied. (B) The seedlings were irradiated intermittent red (R) light pulses to observe the function of PhyB. D (black), kept in darkness; R (red) at 4 h cycles for 2 days. The PhyB-dependent responses were restored by both PΦB and PCB.[126] Error bars represent SE.

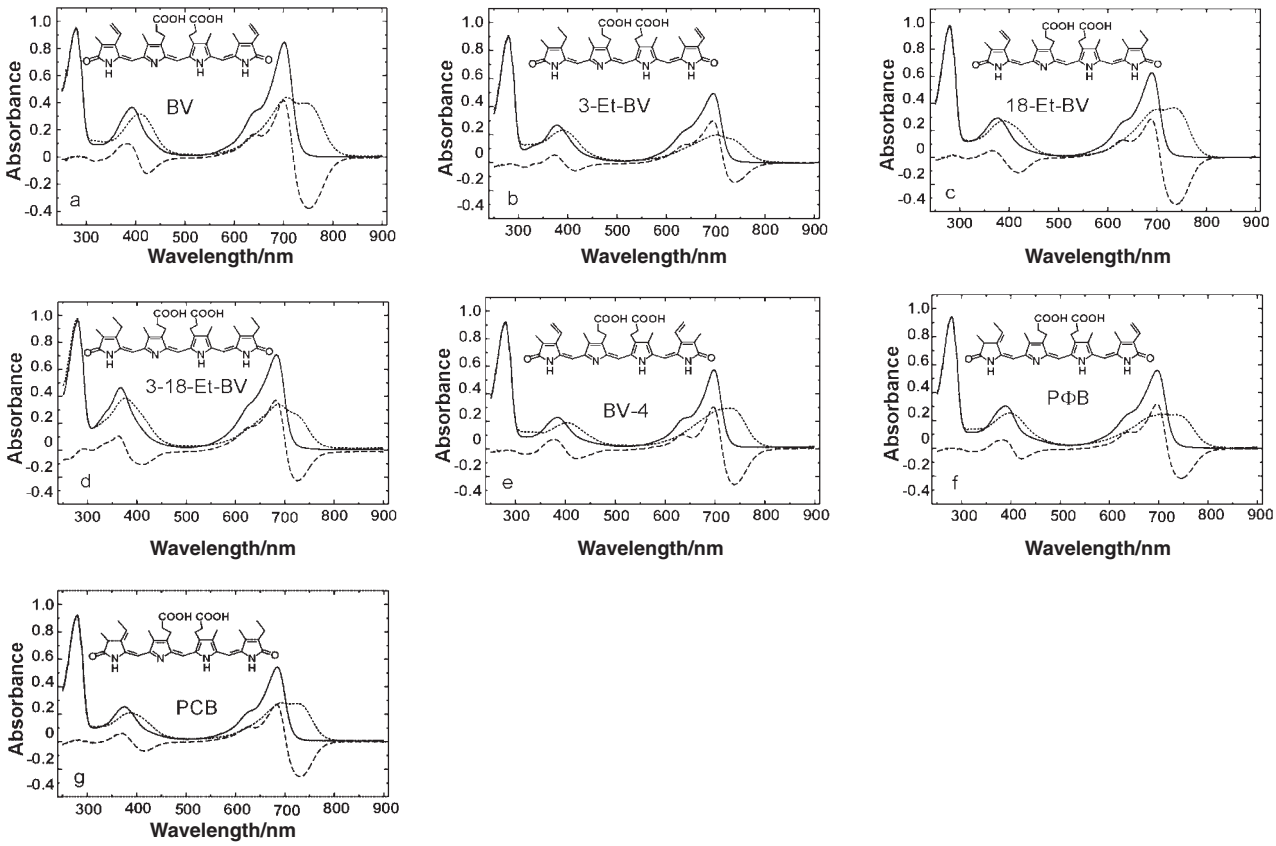


Fig. 15. Chromophore assembly with Agp1 (a–g).[147] Agp1 was mixed with BV and BV derivatives. Each panel shows the Pr spectrum after mixing chromophore and protein (solid line), the spectrum after photoconversion by red light (dotted line), and the Pr minus Pfr difference spectrum (dashed line).

phyB double mutant seedlings in the presence or absence of PΦB and PCB followed by intermittent R, R/FR, FR, and FR/R light treatments.[126]

The loss of PhyA-mediated response to FR light in PCB-supplied seedlings might be caused by reasons other than the spectrophotometric properties of the PhyA adduct with PCB. One possible explanation is that PΦB (not PCB) might have some direct role as signaling molecules in PhyA signaling pathway. Another possibility is that loss of PhyA function in PCB-supplied chromophore-deficient mutants is caused by a lack of interaction between chromophore and phytochrome apoprotein.

This collaborative work with biologists demonstrated a quite suggestive discovery that a double bond in the vinyl side chain of D-ring of PΦB is crucial for the photosensory function of PhyA. Perhaps some amino acid(s) in the N-terminal domain of PHYA interacting directly have an essential role in the direct interaction with this vinyl side chain. It is likely that tight binding with PHYA through the vinyl group at the hydrophobic cavity prevents free movement of PΦB from the binding site even when PhyA is irradiated with FR light. This binding process might be required for PhyA-specific biological activity for photomorphogenesis.

3.3 In Vitro Assembly of BV and Its Analogs with *Agrobacterium* Phytochrome Agp1. In plant phytochromes, the natural chromophore, phytochromobilin (PΦB), is coupled by a thioether bond between its A-ring ethylidene side chain and a conserved cysteine residue within the so-called GAF domain of the protein.[1] Many bacterial phytochromes carry biliverdin (BV) as a natural chromophore, which is coupled in a different manner to the protein, because BV has a vinyl side chain on the A-ring instead of an ethylidene side chain of PΦB. In phytochrome Agp1 of *Agrobacterium tumefaciens*, BV is covalently attached to a cysteine residue close to the N-terminus (position 20). It has been proposed that BV binds via its D-ring to the protein,[146] but the assumption has not been confirmed by chemical studies.

Therefore, we used different natural and synthetic BV derivatives prepared above (Fig. 8), three BV-derivatives with a reduced A-ring and/or D-ring side chains, namely 3-Et-BV, 18-Et-BV, 3,18-Et-BV, and BV-4, in which the D-ring vinyl group of the C18 position was exchanged with the methyl group of the C17 position, to test whether the A-ring or D-ring vinyl side chain is used for covalent BV attachment in Agp1.[147] Chromophore assembly was carried out by German biologists as our collaborative work and resulted in the formation of the Pr form, which is determined by its unambiguous spectral features; upon incorporation into the protein, the maximum of the red absorption band of the chromophore shifted to approximately 700 nm. At the same time, absorbance in this spectral region increased and exceeded that of the blue absorption band. This behavior was found for all chromophores tested, indicating that they all formed adducts with the Agp1 protein (Figs. 15a–15g). In addition, all adducts showed typical

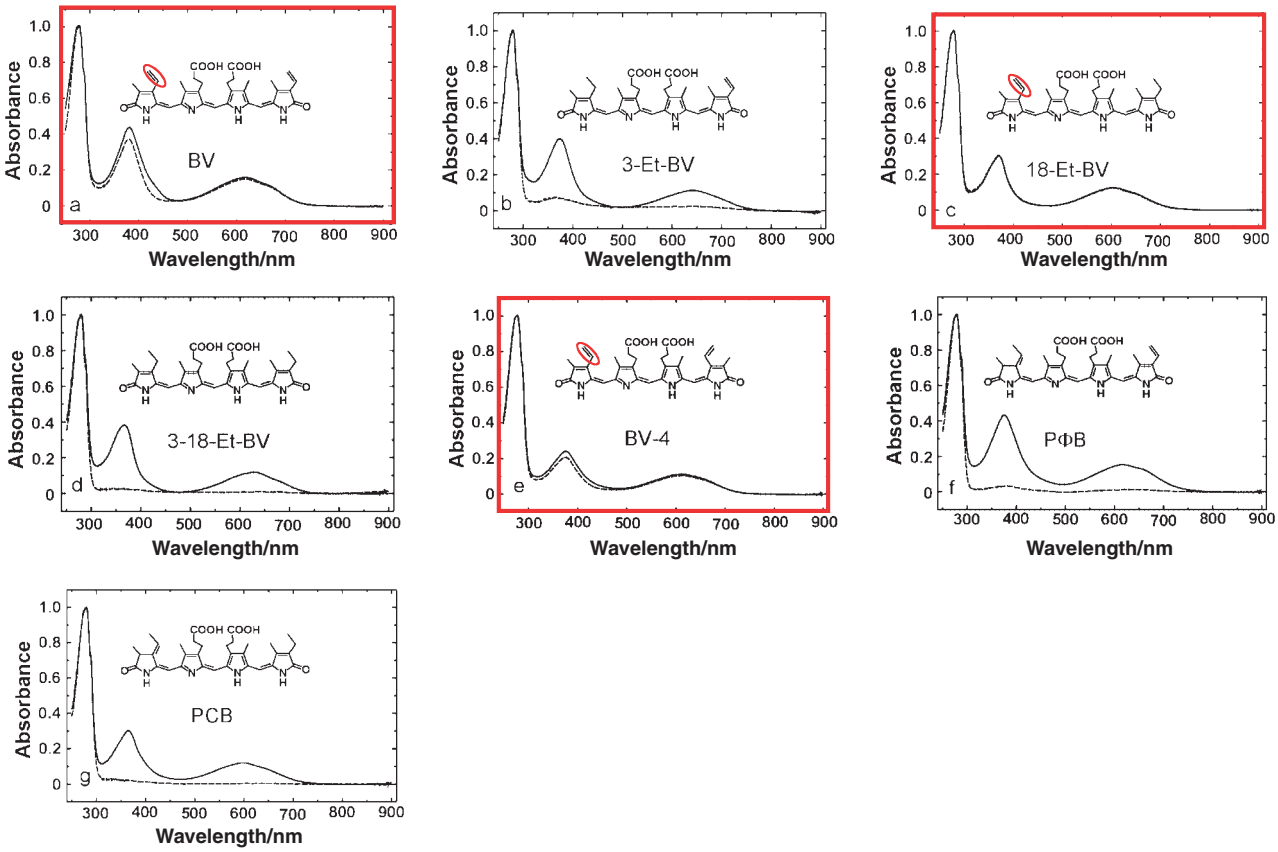


Fig. 16. Tests for covalent bilin attachment with Agp1 (a–g).[147] Spectra after SDS-denaturation before (solid line) and after (dashed line) separation of protein and free chromophore on NAP-5 desalting column. The spectra are normalized at 280 nm.

photoconversion into the longer wavelength Pfr form upon irradiation with red light.[147]

Covalent attachment of the chromophores was tested by SDS-denaturation/dissociation followed by separation of protein and the free bilin on desalting columns. If the chromophore is covalently bound, it elutes together with the protein from the desalting column, whereas noncovalently bound chromophore is separated from the protein by using SDS and remains almost entirely inside the column. Spectra that were recorded before and after the column separation are shown in Fig. 16.[147] The spectra were normalized at the protein absorption band (280 nm) to compensate for dilution effects that occur during the separation. The assay showed that most if not all of the BV was covalently attached to the protein (Fig. 16a). In the case of 18-Et-BV, both spectra were almost identical; thus, 100% of the chromophore was covalently bound (Fig. 16c). From the spectra of the BV-4 adducts, it can be judged that approximately 90% of the chromophore were covalently attached (Fig. 16e). All three covalently bound chromophores have an A-ring vinyl side chain but differ by their D-ring side chains. Those chromophores that contain an A-ring ethyl or ethylidene side chain, namely 3-Et-BV, 3,18-Et-BV, PΦB, PCB, were noncovalently bound to the protein. In all those cases, the absorbance in the visible range was very small after the column separation (Figs. 16b, 16d, 16f, and 16g). Because ≈5% of free bilins eluted from the desalting columns under the test conditions, minor traces of chromophore absorbance might be attributed to co-eluted free bilin.

From these results, we could conclude that the A-ring vinyl group forms a thioether bond with the cysteine 20 residue of Agp1.[147] Recently, this result was confirmed by X-ray crystallography of a truncated phytochrome from *Deinococcus radiodurans* consisting of the N-terminal 321 amino acids.[148]

3.4 In Vitro Assembly of Sterically Locked Synthetic BV Derivatives with *Agrobacterium* Phytochrome Agp1 and Agp2. 3.4.1 Formation of Photoinsensitive Pr- and Pfr-Like Adducts: For plant phytochromes, it has been shown that the first step of the Pr to Pfr photoconversion is a *Z* to *E* isomerization of the chromophore around the C15=C16 double bond,[28] which occurs on the picosecond time scale.[149–151] Isomerization is followed by spectral changes in the microsecond and millisecond time scale.[152–154] During photoconversion, the chromophore also undergoes movement around the exocyclic single bonds. In principle, each single bond can adopt either the *syn* or *anti* conformation.[16] Vibrational spectroscopy gained indirect insight into the conformation of the phytochrome chromophore in the Pr, Pfr, and intermediate states, but the data were not unambiguous and have been interpreted in different ways.[29–32] For example, it has been proposed that the formation of Pfr is accompanied by a *syn*/*anti* rotation around the C14–C15 single bond.[31] More recently, interpretation of resonance Raman spectra of plant phytochromes by using density functional theory (DFT) calculations indicated that the C14–C15 single bond is in the *anti* conformation throughout the entire photocycle and that the C5–C6

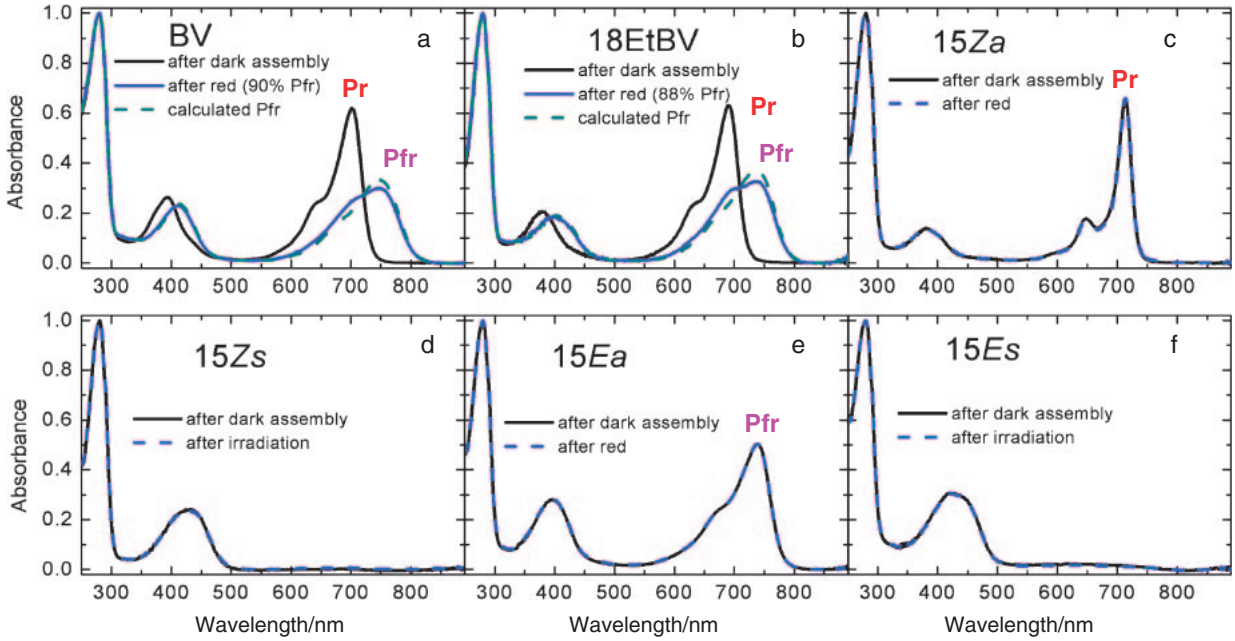


Fig. 17. Effect of irradiation on Agp1 adducts (a–f).[159] The BV, 18EtBV, 15*Za*, 15*Ea* adducts were irradiated with red light and the 15*Zs* and 15*Es* adducts with white light. The spectrum before and after irradiation is given in each panel. Only the BV and 18EtBV adducts underwent photoconversion (a, b); adducts with locked chromophores were stable in the light (c–f). In the case of the BV and 18EtBV adducts, the Pfr levels after saturating red irradiation were estimated to be 90 and 88%, respectively. The spectra of pure Pfr were calculated and also presented in the panels (a, b). The spectra were normalized at 280 nm.

single bond rotates from *anti* to *syn* upon conversion from Pr to Pfr as shown in Fig. 2.[32]

The Pr/Pfr photocycle is associated with conformational change of the protein that have been measured by using size exclusion chromatography (SEC), CD spectroscopy, or limited proteolysis.[155–157] Most bacterial phytochromes are histidine kinases, in which kinase activity is modulated by light-induced conformational changes of the protein. Kinase activity is generally stronger in the Pr and weaker in the Pfr form, although other phosphorylation patterns have also been found.[11,14,146,158]

To understand better how the structure of the chromophore is linked with the conformation of the protein, we prepared four synthetic biliverdin (BV) derivatives in which the C- and D-rings are sterically locked by cyclizing with an additional carbon chain as described above. In these chromophores, which are termed 15*Zs* (**121**), 15*Za* (**122**), 15*Es* (**123**), and 15*Ea* (**124**) in this section, the C15=C16 double bond is in either the *Z* or *E* configuration and the C14–C15 single bond in either the *syn* or *anti* conformation. Biliverdin (BV), the natural chromophore of Agp1, and 18EtBV (Fig. 8) were used as controls. These six different chromophores were first assembled with *Agrobacterium* phytochrome Agp1 by German biologists as collaborative work. Both BV and 18EtBV chromophores completely formed a covalent bond between 1 and 5 min and produced adducts that show typical red/far-red reversible photoconversion (Figs. 17a and 17b).[159]

All locked BV derivatives also bound covalently to the protein, which was confirmed by using SDS-denaturation/dissociation, followed by separation of protein and the free bilin on desalting columns as described above, and the binding was completed between 1 and 5 min except 15*Ea* that required about 140 min, producing adducts with characteristic spectral properties. The 15*Za* adduct was spectrally similar to the Pr form (Fig. 17c), and the 15*Ea* adduct was similar to the Pfr form of the BV adduct (Fig. 17e). Thus, the chromophore of Agp1 adopts a C15=C16 *Z* configuration and a C14–C15 *anti* conformation in the Pr form and a C15=C16 *E* configuration and a C14–C15 *anti* conformation in the Pfr form. Both the 15*Zs* and the 15*Es* adducts absorbed only in the blue region of the visible spectra (Figs. 17d and 17f).[159]

The results with the 15*Zs* and 15*Es* chromophores show that the Agp1 protein is rather tolerant for bilins that have the "wrong" conformation. Both locked *syn* bilins might adopt a helical conformation within the Agp1 chromophores pocket as compared with the stretched conformation of other bilins. Another possibility could be that the conjugation system of tetrapyrrole chromophore is broken by a nucleophilic attack of an amino acid side chain to the C10 position. However, the addition reaction must be reversible, because the red absorption band appeared again upon SDS treatment of the holoprotein, as found during our tests for covalent binding.

Why is the covalent bond with 15*Ea* formed rather slowly as compared with the other chromophores? It could be that after the rapid incorporation of 15*Ea* into the Agp1 chromophore pocket, the distance between the Cys-20 thiol group and the A-ring vinyl side chain of 15*Ea* is rather large and that the likelihood for covalent bond formation is thereby decreased. In this model, thermal conformational motions of the protein and/or the chromophore would be required for covalent bond formation. For the chromophore, a *syn*/*anti* rotation around the C5–C6 single bond is the most likely option. Once the *E*-isomer is present, the protein adopts its conformation in a proper manner.

It has been shown that the first step in the Pr to Pfr photo-

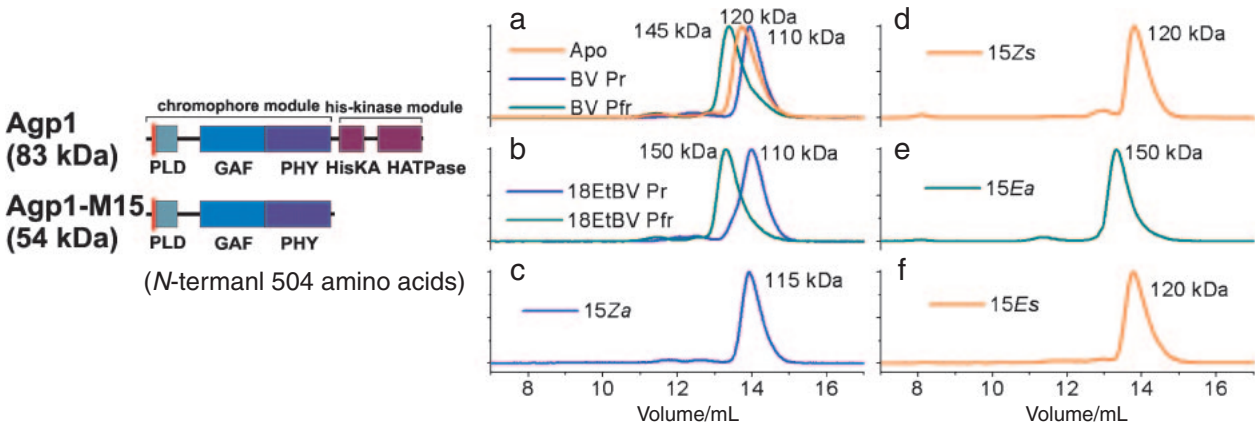


Fig. 18. Size exclusion chromatography (SEC) with Agp1-M15 (the N-terminal 504 amino acids of Agp1) apoprotein and chromophore adducts (a–f).[159] a. apoprotein and BV adducts; b. 18EtBV adducts; c, 15*Za* adduct; d, 15*Zs* adduct; e, 15*Ea* adduct; f, 15*Es* adduct. Pr, after dark incubation; Pfr, after saturating red irradiation. The apparent molecular mass in kDa is given above each trace.

conversion of plant phytochromes is a *Z* to *E* isomerization around the C15=C16 double bond.[28] Because of the spectral similarities, this mechanism was most likely universal for all phytochromes, although direct experimental evidence was lacking. If Agp1 follows the same mechanism, all adducts with locked chromophores should remain stable in the light. Indeed, we did not observe any spectral changes upon irradiation with either red or white light (Figs. 17c–17f) under conditions that induced saturating conversions of the BV and 18EtBV adducts.

Mobility of proteins subjected to size exclusion chromatography (SEC) was dependent on the shape and quaternary structure of the molecules. All chromophore adducts with Agp1-M15 (the N-terminal 504 amino acids of Agp1) apoprotein were analyzed by using SEC (Fig. 18), and also histidine kinase activity to probe for protein conformation (Fig. 19).[159] In either case, the 15*Za* adduct behaved like the Pr, and the 15*Ea* adduct behaved like the Pfr form of Agp1. Replacing the natural chromophore with a locked 15*Ea* derivative can thus bring phytochrome holoprotein in the Pfr form in the dark. In this way, physiological action of Pfr can be studied in vivo and separated from Pr/Pfr cycling and other light effects.

Agp1 and Agp2 have antagonistic properties: in the dark, Agp1 converts slowly from Pfr to Pr, whereas Agp2 converts slowly from Pr to Pfr. To see whether and how locked chromophores are incorporated into Agp2, the set of the above mentioned chromophores was also tested with purified recombinant Agp2.[160] Interesting differences between Agp1 and Agp2 and between different chromophores were found for the assembly rates. The slowest assembly with Agp1 was found for the 15*Ea* chromophore. In the case of Agp2, the 15*Ea* assembly rate is faster than that of 15*Za*. The assembly rates for 15*Ea*/15*Za* for both phytochromes are in line with the finding that Agp1 adopts a Pr form and Agp2 adopts a Pfr form in the dark.[160]

For analyses on the stereochemistry of the C5–C6 single bond, new locked chromophores 5*Zs* and 5*Za*, in which C4=C5 double bond is fixed in the *Z* configuration and the C5–C6 single bond is fixed in either the *syn* or *anti* conformation (Fig. 20), were recently synthesized in a similar

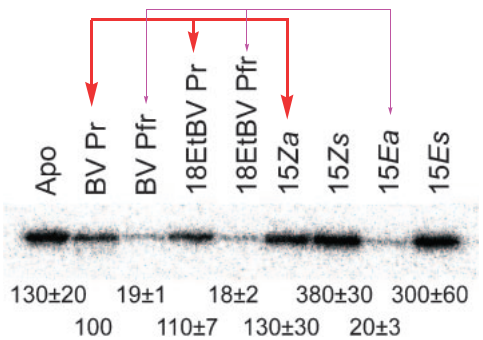


Fig. 19. Autophosphorylation of Agp1 apoprotein and chromophore adducts.[159] The BV and 18EtBV adducts were used unirradiated (Pr) or after saturating red irradiation (Pfr). Following incubation in the presence of [γ-^{32}P]ATP for 5 min, the samples were loaded onto SDS-PAGE, blotted, and analyzed by using phosphor-imaging. The area around the 85-kDa Agp1 band is shown. The relative intensities (mean ± S.E. of four experiments) are given below the blot.

manner described for the chromophores locked at the C15 position. They were also used for assembly with Agp1 and Agp2[160] and provided deeper insight into the similarities and differences between a more conventional phytochrome Agp1 and a phytochrome Agp2, which converts to Pfr in the dark, giving further clues for the understanding of chromophore stereochemistry in the ground state and during photoconversion.

Results with the 5*Zs* and 5*Za* chromophores are summarized as follows:[160] (1) Both chromophores form covalent bonds with either Agp1 or Agp2. (2) The spectra and the absorption maxima of the 5*Zs* adducts are similar to those of the Pr form of the 18EtBV control. (3) The absorption spectra of the 5*Za* adducts are also similar to Pr, but their maxima are shifted to shorter wavelengths. (4) Photoconversion shifted the absorption maxima of the 5*Zs* adducts to shorter wavelengths, whereas the 5*Za* adducts were shifted to longer wavelengths. Thus, the C5–C6 single bond of the Pfr chromophore is in an *anti* conformation, supporting the previous suggestion that

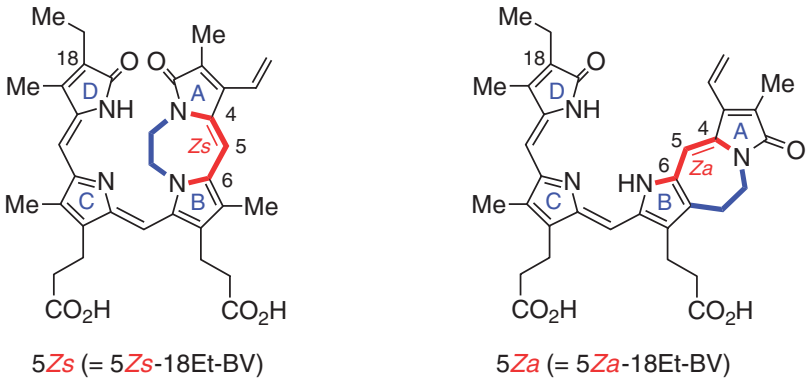


Fig. 20. New locked chromophores 5*Zs* and 5*Za*.

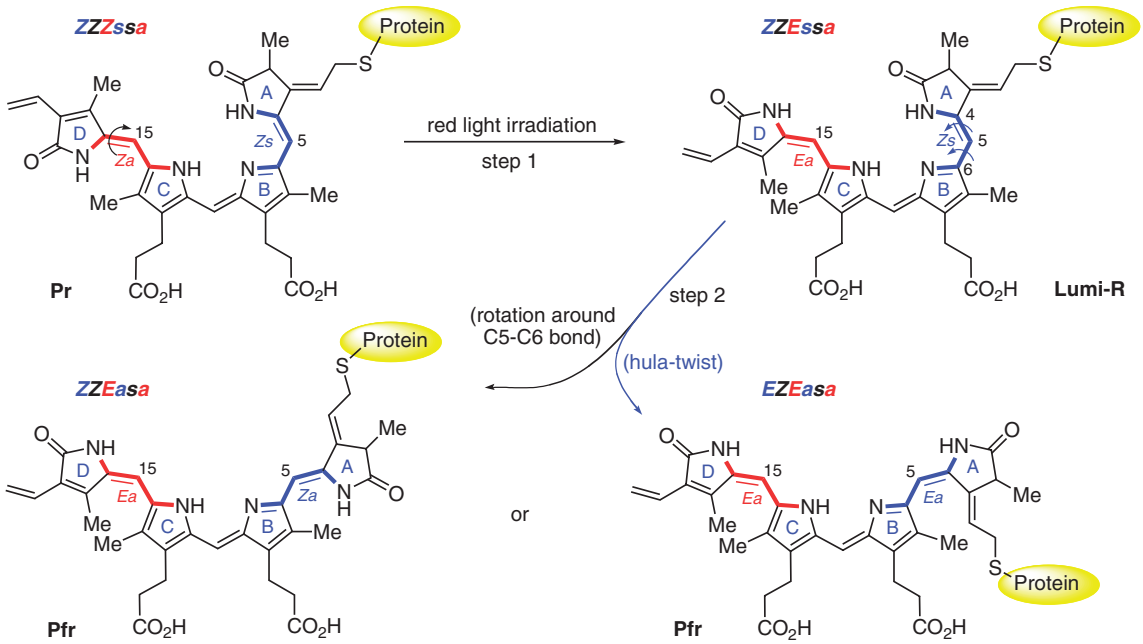


Fig. 21. Proposed stereochemistry of the Agp1 and Agp2 chromophores during conversion of Pr to Pfr.[160] Step 1 is the rapid C15=C16 *Z* to *E* isomerization, leading to the lumi-R photoproducts. During the conversion from lumi-R to Pfr (step 2), the stereochemistry of the C5 methine bridge is changed. This can involve either a rotation around the C5–C6 single bond (from *syn* to *anti*) and/or a *Z* to *E* isomerization of the C4=C5 double bond (a hula-twist mechanism).[161] Two different possibilities are drawn here.

during photoconversion of phytochromes, a rotation around the A- and B-rings connecting single bond occurs,[32] though direction of the rotation was opposite, *anti* to *syn* conformation, and the 5*Za* photoproducts are spectrally not exactly identical with Pfr. All of the data that were obtained with these chromophores lead to the conclusion that a flexibility around the A- and B-rings connecting methine bridge is essential for proper photoconversion from Pr to Pfr and for Pr to Pfr dark conversion of Agp2, although the role of C15=C16 *Z* to *E* isomerization as the initial step is beyond doubt from our results.

The spectral analogy between Pr and the 5*Zs* adducts is in line with the data from the crystal structure of the chromophore-binding domain of *Deinococcus* phytochrome.[148] In the structure, the configuration/conformation of the BV chromophore is 5*Zs*/10*Zs*/15*Za*, i.e., the single bond between the A- and B-rings, is in the *syn* conformation.

To our knowledge, there is as yet no evidence of a C4=C5 *E* configuration of the Pfr chromophore. However, such configuration might have been overlooked for chemical or technical reasons. In the next step, we intend to synthesize the locked 5*Ea* and 5*Es* chromophores and test these compounds for assembly with apophytochrome. Consequently based on the above results, we proposed that the stereochemistry of the Agp1 and Agp2 chromophores is 5*Zs*/10*Zs*/15*Za* in the Pr and 5*Za*/10*Zs*/15*Ea* or 5*Ea*/10*Zs*/15*Ea* in the Pfr form.[160] A schematic presentation of the proposed stereochemistry of Pr and Pfr is given in Fig. 21,[160] taking into account the most recent data from the crystal structure of the chromophore-binding domain of the two bacteriophytochromes.[148,162,163]

3.4.2 Crystallization and Preliminary X-ray Crystallographic Analysis: As mention above, assembly of the sterically locked chromophores with apophytochrome could afford photostable Pr- and Pfr-like adducts. This fact prompted us to use the adducts of the locked chromophores with Agp1 for

crystallization analysis carried out with German collaborators.[164]

Because of the difficulty to grow X-ray suitable crystals of full-length Agp1, our studies were focused on the truncated N-terminal photosensory chromophore module of Agp1, termed Agp1-M15 for the first crystallization experiments and preliminary X-ray analysis.[164] This 54 kDa-fragment consists of the N-terminal 504 amino acids of Agp1 and contains the PAS-like domain (PLD), GAF domain and PHY domain but lacks the C-terminal histidine-kinase module (see Fig. 18).

The protein Agp1-M15 was either assembled with the natural chromophore biliverdin (BV) or a sterically locked synthetic BV derivative 15*Za*. Both adducts could be crystallized, but the resolution was largely improved by the use of 15*Za*. Crystals of BV-Agp1-M15 diffract to 0.6 nm resolution and belong to the tetragonal space group $I422$ with unit cell dimensions $a = b = 17.1$ nm, $c = 8.1$ nm, $\alpha = \beta = \gamma = 90^\circ$ at $T = 100$ K, crystals of 15*Za*-Agp1-M15 belong to the same space group with similar unit cell dimensions $a = b = 17.4$ nm, $c = 8.0$ nm, but diffract to 0.34 nm resolution. Assuming the asymmetric unit to be occupied by one monomer of 55 kDa, the unit cell contains 54–55% solvent with a crystal volume per protein mass, V_m, of 2.7×10^{-3} nm^3 Da^{-1}.[164]

In general, for crystal inspection under the microscope, light was passed through an interference filter with a maximum transmission at 520 nm. To obtain information about the color of the crystals and to test for stability against light, some crystals were inspected under white light. The crystals of BV-Agp1-M15, which were obtained by assembling natural BV chromophore with Agp1-M15, appeared dark green, indicating that they are not salts or artifacts. During irradiation with white light at 293 K, the crystals cracked, and the green color gradually disappeared over time, showing that the holoprotein inside the crystal still undergoes light-induced conformational changes.

On the other hand, a locked chromophore 15*Za* adduct of Agp1-M15 did not undergo light-induced conformational changes, because the first step of the photocycle, the *Z–E* isomerization around the C15=C16 double bond, is not possible. The sharper bands of the vis spectrum of 15*Za*-Agp1-M15 than that of BV-Agp1-M15 suggested that 15*Za* is less flexible within the chromophore pocket as compared to BV.

Thus, by using 15*Za*-Agp1-M15 for crystallization, we could both improve the quality of the crystals and allow for continuous work under laboratory light. The rigidity of the chromophore pocket might be one reason for the improved resolution that was obtained for these crystals. Interestingly, crystals of both the BV adduct and the 15*Za* adduct were obtained under similar conditions, belong to the same space group, and have similar unit cell parameters, indicating similar crystal packing and comparable overall conformations of the different adducts. The structural similarities would facilitate straightforward structure determination by molecular replacement once the phase problem has been solved for only one of the crystal forms.

After the review of our article,[164] the crystal structure of the chromophore-binding domain of *Deinococcus* phytochrome DrBphP consisting of the N-terminal 321 amino acids was published.[148] Most information on the configuration and conformation of the chromophore is now available for the Pr form from this crystal structure; however, detailed knowledge of configurational and conformational changes of the chromophore that are reflected by spectral changes is lacking.

4. Concluding Remarks

As described above, we established an efficient, flexible, and general method for the preparation of the linear tetrapyrrole (bilin) chromophores to investigate the structure and function of phytochrome chromophores by developing several new reactions: (1) efficient synthetic reactions for the synthesis of each pyrrole ring including 2-tosylpyrroles, (2) rearrangement of a tosyl group of the 2-tosylpyrroles to the 5-position under acidic conditions, (3) efficient transformation of 2-bromo-5-tosylpyrroles to the corresponding 5-tosylpyrrolinones under anhydrous redox conditions, (4) Wittig-type coupling reaction between 5-tosylpyrrolinone and 2-formylpyrrole for the construction of the AB- and CD-ring components, (5) reductive transformation of the AB-ring component of BV to that of PΦB and PCB, (6) protection and palladium-catalyzed deprotection of propanoic acid side chains at the C8 and C12 positions through allyl esters. Actually, the method is so efficient that were able to synthesize PΦB starting from only two pyrroles and to synthesize not only PΦB and PCB as natural chromophores used in plants and cyanobacteria, respectively, but also BV used in other bacteria. Furthermore, various unnatural types of bilin chromophores bearing modified side chain(s) and sterically locked BV derivatives were prepared in free acid forms by applying the present synthetic method.

Assembly experiments of the synthesized chromophores with phytochrome apoproteins in vitro and in vivo provided us insights into the structure and function of phytochromes: (1) the different role of each substituents on four pyrrole rings of the bilin chromophore in plant phytochrome, (2) structural requirement of bilin chromophore for the photosensory specificity of phytochromes A and B, (3) BV chromophore in *Agrobacterium* phytochrome Agp1 covalently binds to the apoprotein via its A-ring vinyl side chain, (4) the stereochemistry at the C15 position of the chromophore in Pr- and Pfr-forms of Agp1 and Agp2 are 15*Z-anti* and 15*E-anti*, respectively. Furthermore, (5) photoinsensitive single crystals of Pr with a 15*Za*-locked chromophore were obtained for X-ray crystallographic analysis of the N-terminal photosensory module of phytochrome Agp1. From these results, it is obvious that an approach based on the synthetic organic chemistry toward the elucidation of the structure and function of phytochromes is very effective and necessary. Especially, sterically locked chromophores will open the new avenues for investigation of phytochrome chromophores both in vitro and in vivo in near future.

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References

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1 W. Rüdiger, F. Thümmler, in *Photomorphogenesis in Plants*, 2nd ed., ed. by R. E. Kendrick, G. H. M. Kronenberg, Kluwer Academic Publishers, Dordrecht, The Netherlands, **1994**.

2 M. M. Neff, C. Frankhauser, J. Chory, *Genes Dev.* **2000**, *14*, 257.

3 C. Fankhauser, *J. Biol. Chem.* **2001**, *276*, 11453.

4 M. Wada, K. Shimazaki, M. Iino, *Light Sensing in Plants*, Springer-Verlag, Tokyo, **2005**.

5 N. C. Rockwell, Y.-S. Su, J. C. Lagarias, *Annu. Rev. Plant Biol.* **2006**, *57*, 837.

6 M. Ahmad, A. R. Cashmore, *Nature* **1993**, *366*, 162.

7 C. Lin, *Trends Plant Sci.* **2000**, *5*, 337.

8 W. R. Briggs, C. F. Beck, A. R. Cashmore, J. M. Christie, J. Hughes, J. A. Jarillo, T. Kagawa, H. Kanegae, E. Liscum, A. Nagatani, K. Okada, M. Salomon, W. Rüdiger, T. Sakai, M. Takano, M. Wada, J. C. Watson, *Plant Cell* **2001**, *13*, 993.

9 H. A. Borthwick, S. B. Hendricks, M. W. Parker, E. H. Toole, V. K. Toole, *Proc. Natl. Acad. Sci. U.S.A.* **1952**, *38*, 662.

10 W. L. Butler, K. H. Norris, H. W. Siegelman, S. B. Hendricks, *Proc. Natl. Acad. Sci. U.S.A.* **1959**, *45*, 1703.

11 K. C. Yeh, S. H. Wu, J. T. Murphy, J. C. Lagarias, *Science* **1997**, *277*, 1505.

12 T. Lamparter, F. Mittmann, W. Gärtner, T. Börner, E. Hartmann, J. Hughes, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11792.

13 S. J. Davis, A. V. Vener, R. D. Vierstra, *Science* **1999**, *286*, 2517.

14 S. H. Bhoo, S. J. Davis, J. Walker, B. Karniol, R. D. Vierstra, *Nature* **2001**, *414*, 776.

15 C. Starostzik, W. Marwan, *FEBS Lett.* **1995**, *370*, 146.

16 H. Falk, *The Chemistry of Linear Oligopyrrole and Bile Pigment*, Springer Verlag, New York, **1989**.

17 W. Rüdiger, F. Thümmler, *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1216.

18 M. Furuya, P.-S. Song, *Assembly and Properties of Holophytochrome* in *Photomorphogenesis in Plants*, 2nd ed., ed. by R. E. Kendrick, G. H. M. Kronenberg, Kluwer Academic Publishers, Dordrecht/Boston/London, **1994**, Chap. 4.3, pp. 105–140.

19 M. Stanek, K. Grubmayr, *Chem. Eur. J.* **1998**, *4*, 1653; **1998**, *4*, 1660.

20 T. Lamparter, *FEBS Lett.* **2004**, *573*, 1.

21 N. C. Rockwell, J. C. Lagarias, *Plant Cell* **2006**, *18*, 4.

22 J. C. Lagarias, H. Rapoport, *J. Am. Chem. Soc.* **1980**, *102*, 4821.

23 N. Frankenberg, K. Mukougawa, T. Kohchi, J. C. Lagarias, *Plant Cell* **2001**, *13*, 965.

24 T. Hübschmann, T. Börner, E. Hartmann, T. Lamparter, *Eur. J. Biochem.* **2001**, *268*, 2055.

25 S. H. Wu, M. T. McDowell, J. C. Lagarias, *J. Biol. Chem.* **1997**, *272*, 25700.

26 A. Blumenstein, K. Vienken, R. Tasler, J. Purschwitz, D. Veith, N. Frankenberg-Dinkel, R. Fischer, *Curr. Biol.* **2005**, *15*, 1833.

27 I. Oberpichler, I. Molina, O. Neubauer, T. Lamparter, *FEBS Lett.* **2006**, *580*, 437.

28 W. Rudiger, F. Thummler, E. Cmiel, S. Schneider, *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6244.

29 Y. Mizutani, S. Tokutomi, T. Kitagawa, *Biochemistry* **1994**, *33*, 153.

30 C. Kneip, P. Hildebrandt, W. Schlamann, S. E. Braslavsky, F. Mark, K. Schaffner, *Biochemistry* **1999**, *38*, 15185.

31 F. Andel, III, J. T. Murphy, J. A. Haas, M. T. McDowell, I. van der Hoef, J. Lugtenburg, J. C. Lagarias, R. A. Mathies, *Biochemistry* **2000**, *39*, 2667.

32 M. A. Mroginski, D. H. Murgida, D. von Stetten, C. Kneip, F. Mark, P. Hildebrandt, *J. Am. Chem. Soc.* **2004**, *126*, 16734.

33 J. R. Wagner, J. Zhang, J. S. Brunzelle, R. D. Vierstra, K. T. Forest, *J. Biol. Chem.* **2007**, *282*, 12298.

34 L. Li, J. C. Lagarias, *J. Biol. Chem.* **1992**, *267*, 19204.

35 P. Schmidt, U. H. Westphal, K. Worm, S. E. Braslavsky, W. Gärtner, K. Schaffner, *J. Photochem. Photobiol., B* **1996**, *34*, 73, and references cited therein.

36 J. T. Murphy, J. C. Lagarias, *Photochem. Photobiol.* **1997**, *65*, 750.

37 L. Deforce, M. Furuya, P.-S. Song, *Biochemistry* **1993**, *32*, 14165.

38 S. H. Bhoo, T. Hirano, H.-Y. Jeong, J.-G. Lee, M. Furuya, P.-S. Song, *J. Am. Chem. Soc.* **1997**, *119*, 11717.

39 A. Remberg, P. Schmidt, S. E. Braslavsky, W. Gärtner, K. Schaffner, *Eur. J. Biochem.* **1999**, *266*, 201.

40 R. S. H. Liu, T. Mirzadegan, *J. Am. Chem. Soc.* **1988**, *110*, 8617.

41 A. Gossauer, W. Hirsch, *Justus Liebigs Ann. Chem.* **1974**, 1496.

42 A. Gossauer, R.-P. Hinze, *J. Org. Chem.* **1978**, *43*, 283.

43 J.-P. Weller, A. Gossauer, *Chem. Ber.* **1980**, *113*, 1603.

44 H. Kinoshita, Y. Hayashi, Y. Murata, K. Inomata, *Chem. Lett.* **1993**, 1437.

45 H. Kinoshita, H. Ngwe, K. Kohori, K. Inomata, *Chem. Lett.* **1993**, 1441.

46 K. Kohori, M. Hashimoto, H. Kinoshita, K. Inomata, *Bull. Chem. Soc. Jpn.* **1994**, *67*, 3088.

47 T. Kakiuchi, H. Kato, K. P. Jayasundera, T. Higashi, K. Watabe, D. Sawamoto, H. Kinoshita, K. Inomata, *Chem. Lett.* **1998**, 1001.

48 K. P. Jayasundera, H. Kinoshita, K. Inomata, *Chem. Lett.* **1998**, 1227.

49 T. Kakiuchi, H. Kinoshita, K. Inomata, *Synlett* **1999**, 901.

50 A. Ohta, D. Sawamoto, K. P. Jayasundera, H. Kinoshita, K. Inomata, *Chem. Lett.* **2000**, 492.

51 D. Sawamoto, H. Nakamura, H. Kinoshita, S. Fujinami, K. Inomata, *Chem. Lett.* **2000**, 1398, see also the references cited therein.

52 S. Takeda, K. P. Jayasundera, T. Kakiuchi, H. Kinoshita, K. Inomata, *Chem. Lett.* **2001**, 590.

53 K. P. Jayasundera, H. Kinoshita, K. Inomata, *Bull. Chem. Soc. Jpn.* **2000**, *73*, 497.

54 M. A. S. Hammam, H. Nakamura, Y. Hirata, H. Khawn, Y. Murata, H. Kinoshita, K. Inomata, *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1561.

55 J. Cornejo, R. D. Willows, S. I. Beale, *Plant J.* **1998**, *15*, 99.

56 C. T. Migita, X. Zhang, T. Yoshida, *Eur. J. Biochem.* **2003**, *270*, 687.

57 S. I. Beale, *Chem. Rev.* **1993**, *93*, 785.

58 T. Kohchi, K. Mukougawa, N. Frankenberg, M. Masuda, A. Yokota, J. C. Lagarias, *Plant Cell* **2001**, *13*, 425.
59 N. Frankenberg, J. C. Lagarias, *J. Biol. Chem.* **2003**, *278*, 9219.
60 Y. Hagiwara, M. Sugishima, Y. Takahashi, K. Fukuyama, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 27.
61 T. Mukaiyama, H. Ishihara, K. Inomata, *Chem. Lett.* **1975**, 527.
62 H. Kotake, K. Inomata, S. Aoyama, H. Kinoshita, *Chem. Lett.* **1977**, 73.
63 K. Inomata, S. Aoyama, H. Kotake, *Bull. Chem. Soc. Jpn.* **1978**, *51*, 930.
64 H. Kotake, K. Inomata, H. Kinoshita, S. Aoyama, Y. Sakamoto, *Heterocycles* **1978**, *10*, 105.
65 K. Inomata, M. Sumita, H. Kotake, *Chem. Lett.* **1979**, 709.
66 K. Inomata, Y. Nakayama, M. Tsutsumi, H. Kotake, *Heterocycles* **1979**, *12*, 1467.
67 E. Watanabe, N. Imai, K. Inomata, H. Kinoshita, H. Kotake, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3225.
68 S. Igarashi, Y. Haruta, M. Ozawa, Y. Nishide, H. Kinoshita, K. Inomata, *Chem. Lett.* **1989**, 737.
69 A. Gossauer, *Die Chemie der Pyrrole*, Springer-Verlag, Berlin, **1974**.
70 R. A. Jones, G. P. Bean, *The Chemistry of Pyrroles*, Academic Press, New York, **1977**.
71 M. Ono, R. Lattmann, K. Inomata, C. Lehmann, T. Früh, A. Eschenmoser, *Croat. Chem. Acta* **1985**, *58*, 627.
72 G. Ksander, G. Bold, R. Lattmann, C. Lehmann, T. Früh, Y.-B. Xiang, K. Inomata, H.-P. Buser, J. Schreiber, E. Zass, A. Eschenmoser, *Helv. Chim. Acta* **1987**, *70*, 1115.
73 H. Kinoshita, K. Inomata, M. Hayashi, T. Kondoh, H. Kotake, *Chem. Lett.* **1986**, 1033.
74 K. Inomata, H. Suhara, H. Kinoshita, H. Kotake, *Chem. Lett.* **1988**, 813.
75 H. Kinoshita, S. Tanaka, K. Inomata, *Chem. Lett.* **1989**, 1107.
76 H. Kinoshita, S. Tanaka, N. Nishimori, H. Dejima, K. Inomata, *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2660.
77 H. Ngwe, E. Nakayama, T. Higashi, H. Kinoshita, K. Inomata, *Chem. Lett.* **1995**, 713.
78 J. E. Bishop, S. A. Dagan, H. Rapoport, *J. Org. Chem.* **1989**, *54*, 1876.
79 A. M. Trzeciak, J. J. Ziólkowski, *J. Mol. Catal.* **1987**, *43*, 15.
80 M. A. Drian, T. D. Lash, *J. Heterocycl. Chem.* **1994**, *31*, 255.
81 P. Knochel, D. Seebach, *Synthesis* **1982**, 1017.
82 I. Ugi, W. Betz, U. Fetzer, K. Offermann, *Chem. Ber.* **1961**, *94*, 2814.
83 D. H. R. Barton, J. Kervogoret, S. Z. Zard, *Tetrahedron* **1990**, *46*, 7587.
84 A. H. Jackson, G. W. Kenner, K. M. Smith, *J. Chem. Soc. C* **1971**, 502.
85 P. A. Jacobi, R. W. DeSimone, *Tetrahedron Lett.* **1992**, *33*, 6239.
86 M. A. Drinan, T. D. Lash, *J. Heterocycl. Chem.* **1994**, *31*, 255.
87 T. Masukawa, H. Kato, T. Kakiuchi, K. P. Jayasundera, H. Kinoshita, K. Inomata, *Chem. Lett.* **1998**, 455.
88 A. Gossauer, D. Miehe, *Justus Liebigs Ann. Chem.* **1974**, 352.
89 J. E. Bishop, J. F. O'Connell, H. Rapoport, *J. Org. Chem.* **1991**, *56*, 5079.
90 A. R. Battersby, C. J. Dutton, C. J. R. Fooks, S. P. D. Turner, *J. Chem. Soc., Perkin Trans. 1* **1988**, 1557.
91 R. W. Schoenleber, Y. Kim, H. Rapoport, *J. Am. Chem. Soc.* **1984**, *106*, 2645.
92 D. P. Shrout, D. A. Lightner, *Synthesis* **1990**, 1062.
93 G. Puzicha, D. P. Shrout, D. A. Lightner, *J. Heterocycl. Chem.* **1990**, *27*, 2117.
94 B. R. Baker, R. E. Shaub, J. H. Williams, *J. Org. Chem.* **1952**, *17*, 116.
95 G. Muller, G. Amiard, J. Mathieu, *Bull. Soc. Chim. Fr.* **1949**, 533.
96 T. N. Ghosh, S. Dutta, *J. Indian Chem. Soc.* **1955**, *32*, 791.
97 H. Plieninger, M. Decker, *Liebigs Ann. Chem.* **1956**, *598*, 198.
98 A. Huni, F. Frank, *Hoppe Seyler's Z. Physiol. Chem.* **1947**, *282*, 96.
99 A. Eschenmoser, K. Inomata, **1983**, unpublished work.
100 A. M. Van. Leusen, J. Strating, *Org. Synth.* **1977**, *57*, 95.
101 T. Kobayashi, N. Ishida, T. Hiraoka, *J. Chem. Soc., Chem. Commun.* **1980**, 736.
102 D. S. Brown, P. Charreau, T. Hansson, S. V. Ley, *Tetrahedron* **1991**, *47*, 1311.
103 D. S. Brown, S. V. Ley, S. Vile, M. Thompson, *Tetrahedron* **1991**, *47*, 1329.
104 B. E. Hoogenboon, O. H. Oldenziel, A. M. van Leusen, *Org. Synth.* **1988**, Coll. Vol. *VI*, 987.
105 K. Kohori, H. Kinoshita, K. Inomata, *Chem. Lett.* **1995**, 799.
106 A. Hiyoshi, K. Kohori, H. Kato, H. Kinoshita, K. Inomata, **1997**, unpublished work.
107 Direct rearrangement of tosyl group of **54** did not proceed well. When *p*-toluenesulfinic acid was added to accelerate the rearrangement, sulfoxide **55** was isolated.
108 G. A. Olah, R. Malhotra, S. C. Narang, *Synthesis* **1979**, 58, direct bromination of the sulfoxide **56** produced not only the desired product, but also the reduced compound **58** in low yield.
109 Y. Murata, H. Kinoshita, K. Inomata, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3339.
110 H. Ngwe, H. Kinoshita, K. Inomata, *Bull. Chem. Soc. Jpn.* **1994**, *67*, 3320.
111 P. A. Jacobi, J. Guo, S. Rajesweri, W. Zheng, *J. Org. Chem.* **1997**, *62*, 2907.
112 P. A. Jacobi, I. M. A. Odeh, S. C. Buddhu, G. Cai, S. Rajeswari, D. Fry, W. Zheng, R. D. W. DeSimone, J. Guo, L. D. Coutts, S. I. Hauck, S. H. Leung, I. Ghosh, D. Pippin, *Synlett* **2005**, 2861, see also the references cited therein.
113 A. Gossauer, M. Blacha-Puller, *Liebigs Ann. Chem.* **1981**, 1492.
114 A. Gossauer, F. Nydegger, E. Benedikt, H.-P. Köst, *Helv. Chim. Acta* **1989**, *72*, 518.
115 M. A. S. Hammam, Y. Murata, H. Kinoshita, K. Inomata, *Chem. Lett.* **2004**, *33*, 1258.
116 A. F. McDonagh, F. Assisi, *J. Chem. Soc., Chem. Commun.* **1972**, 117.
117 D. Sawamoto, K. Inomata, *Chem. Lett.* **2001**, 588.
118 P. Manitto, D. Monti, *J. Chem. Soc., Chem. Commun.* **1980**, 178.
119 I. Lindner, B. Knipp, S. E. Braslavsky, W. Gärtner, K. Schaffner, *Angew. Chem., Int. Ed.* **1998**, *37*, 1843.
120 R. Bonnett, A. F. McDonagh, *J. Chem. Soc. D* **1970**, 238.
121 P. Manitto, D. Monti, *Experientia* **1979**, *35*, 9; *Chem.*

Abstr. **1979**, *90*, 99638g.
122 M. Nassal, *Liebigs Ann. Chem.* **1983**, 1510.
123 M. Nassal, *J. Am. Chem. Soc.* **1984**, *106*, 7540.
124 H. Kunz, C. Unverzagt, *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 436.
125 H. Hanzawa, K. Inomata, H. Kinoshita, T. Kakiuchi, K. P. Jayasundera, D. Sawamoto, A. Ohta, K. Uchida, K. Wada, M. Furuya, *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3612; a related paper was reported: U. Robben, I. Lindner, W. Gärtner, K. Schaffner, *Angew. Chem., Int. Ed.* **2001**, *40*, 1048.
126 H. Hanzawa, T. Shinomura, K. Inomata, T. Kakiuchi, H. Kinoshita, K. Wada, M. Furuya, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4725.
127 J. Hughes, T. Lamparter, *Plant Physiol.* **1999**, *121*, 1059.
128 R. D. Vierstra, S. J. Davis, *Semin. Cell Dev. Biol.* **2000**, *11*, 511.
129 R. Tsumura, M. Kanemaru, N. Ishi, Japan Kokai Tokyo Koho 7505315, **1975**; *Chem. Abstr.* **1975**, *83*, 27573q.
130 T. Urasaki, W. Funakoshi, Japan Kokai Tokyo Koho 76131817, **1976**; *Chem. Abstr.* **1977**, *86*, 120786h.
131 H. Kinoshita, M. A. S. Hammam, K. Inomata, *Chem. Lett.* **2005**, *34*, 800.
132 T. Shinomura, A. Nagatani, H. Hanzawa, M. Kubota, M. Watanabe, M. Furuya, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8129.
133 T. Shinomura, K. Uchida, M. Furuya, *Plant Physiol.* **2000**, *122*, 147.
134 T. Kunkel, K. Tomizawa, R. Kern, M. Furuya, N. H. Chua, E. Schäfer, *Eur. J. Biochem.* **1993**, *215*, 587.
135 W. Gärtner, C. Hill, K. Worm, S. E. Braslavsky, K. Schaffner, *Eur. J. Biochem.* **1996**, *236*, 978.
136 A. Ruddat, P. Schmidt, C. Gatz, S. E. Braslavsky, W. Gärtner, K. Schaffner, *Biochemistry* **1997**, *36*, 103.
137 T. D. Elich, J. C. Lagarias, *J. Biol. Chem.* **1989**, *264*, 12902.
138 J. A. Wahleithner, L. M. Li, J. C. Lagarias, *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10387.
139 L. Deforce, K. Tomizawa, N. Ito, D. Farrens, P.-S. Song, M. Furuya, *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10392.
140 R. A. Sharrock, P. H. Quail, *Genes Dev.* **1989**, *3*, 1745.
141 T. Clack, S. Mathews, R. A. Sharrock, *Plant Mol. Biol.* **1994**, *25*, 413.
142 S. J. Davis, J. Kurepa, R. D. Vierstra, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6541.
143 T. Muramoto, T. Kohchi, A. Yokota, I. Hwang, H. M. Goodman, *Plant Cell* **1999**, *11*, 335.
144 T. Kohchi, K. Mukougawa, N. Frankenberg, M. Masuda, A. Yokota, J. C. Lagarias, *Plant Cell* **2001**, *13*, 425.
145 B. M. Parks, P. H. Quail, *Plant Cell* **1991**, *3*, 1177.
146 T. Lamparter, N. Michael, F. Mittmann, B. Esteban, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11628.
147 T. Lamparter, N. Michael, O. Caspani, T. Miyata, K. Shirai, K. Inomata, *J. Biol. Chem.* **2003**, *278*, 33786.
148 J. R. Wagner, J. S. Brunzelle, K. T. Forest, R. D. Vierstra, *Nature* **2005**, *438*, 325.
149 A. R. Holzwarth, E. Venuti, S. E. Braslavsky, K. Schaffner, *Biochim. Biophys. Acta* **1992**, *1140*, 59.
150 S. Rentsch, G. Hermann, M. Bischoff, D. Strehlow, M. Rentsch, *Photochem. Photobilol.* **1997**, *66*, 585.
151 K. Heyne, J. Herbst, D. Stehlik, B. Esteban, T. Lamparter, J. Hughes, R. Diller, *Biophys. J.* **2002**, *82*, 1004.
152 C. F. Zhang, D. L. Farrens, S. C. Bjorling, P. S. Song, D. S. Kliger, *J. Am. Chem. Soc.* **1992**, *114*, 4569.
153 J. J. van Thor, B. Borucki, W. Crielaard, H. Otto, T. Lamparter, J. Hughes, K. J. Hellingwerf, M. P. Heyn, *Biochemistry* **2001**, *40*, 11460.
154 B. Borucki, D. von Stetten, S. Seibeck, T. Lamparter, N. Michel, M. A. Mroginski, H. Otto, D. H. Murgida, M. P. Heyn, P. Hildebrandt, *J. Biol. Chem.* **2005**, *280*, 34358.
155 J. C. Lagarias, F. M. Mercurio, *J. Biol. Chem.* **1985**, *260*, 2415.
156 E. Chen, V. N. Lapko, P. S. Song, D. S. Kliger, *Biochemistry* **1997**, *36*, 4903.
157 B. Esteban, M. Carrascal, J. Abian, T. Lamparter, *Biochemistry* **2005**, *44*, 450.
158 T. Hübschmann, H. J. M. M. Jorissen, T. Börner, W. Gärtner, N. Tandeau de Marsac, *Eur. J. Biochem.* **2001**, *268*, 3383.
159 K. Inomata, M. A. S. Hammam, H. Kinoshita, Y. Murata, H. Khawn, S. Noack, N. Michael, T. Lamparter, *J. Biol. Chem.* **2005**, *280*, 24491.
160 K. Inomata, S. Noack, M. A. S. Hammam, H. Khawn, H. Kinoshita, Y. Murata, N. Michael, P. Scheerer, N. Krauss, T. Lamparter, *J. Biol. Chem.* **2006**, *281*, 28162.
161 C. Dugave, L. Demange, *Chem. Rev.* **2003**, *103*, 2475.
162 J. R. Wagner, J. Zhang, J. S. Brunzelle, R. D. Vierstra, K. T. Forest, *J. Biol. Chem.* **2007**, *282*, 12298.
163 X. Yang, E. A. Stojkovic, J. Kuk, K. Moffat, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12571.
164 P. Scheerer, N. Michael, J. H. Park, S. Noack, C. Förster, M. A. S. Hammam, K. Inomata, H.-W. Choe, T. Lamparter, N. Krauß, *J. Struct. Biol.* **2006**, *153*, 97.

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