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Synthetic Studies in Phytochrome Chemistry

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Abstract

An account is given of the author's several approaches to the synthesis of the parent chromophore of phytochrome (**1**), a protein-bound linear tetrapyrrole derivative that controls photomorphogenesis in higher plants. These studies culminated in enantioselective syntheses of both 2*R*- and 2*S*-phytochromobilin (**4**), as well as several ^{13}C-labeled derivatives designed to probe the site of *Z*,*E*-isomerization during photoexcitation. When reacted *in vitro*, synthetic 2*R*-**4** and recombinant-derived phytochrome apoprotein **N-C** produced a protein-bound chromophore with identical difference spectra to naturally occurring **1**.

Keywords

Phytochrome; Pyrroles; Lactams; Alkynes; Palladium

1. Introduction and Background

The biliproteins are a family of naturally occurring chromophores that are made up of linear tetrapyrrole derivatives covalently bonded to a protein (P). Representative examples include phytochrome (**1**), which functions as the "on-off" switch for photomorphogenesis in higher plants,[1] and the phycocyanins (**2**) and phycoerythrins (**3**) (Figure 1).[2] These latter materials are commonly found in blue-green, eucaryotic and cryptomonad algae and serve as light harvesting proteins in photosynthesis (Note: linear representations of type **1**–**3** are not meant to imply stereochemistry at the methine bridges, which may be either *E* or *Z*).

Photomorphogenesis can be divided conceptually into three stages: Reception of light by means of a pigment(s), transduction of the light signal from pigment to gene, and induction of development through genetic regulation (Figure 2).[1e] Information provided by photoactivation of **1** is crucial to the timing of seasonal phenomena, including seed germination, flowering, fruiting and chlorophyll production. However, in comparison to photosynthesis (the other major light-induced process in nature), relatively little is known about photomorphogenesis at the molecular level. Partly this is because phytochrome (**1**) is present in only very small quantities in plants and is difficult to isolate in pure form. Even in seedlings grown in the dark (etiolated), which are free of the masking pigment chlorophyll, the deep blue photoreceptor **1** is barely discernible to the naked eye. In a typical isolation procedure 4 kg of etiolated oat seedlings provide 50–60 mg of the protein complex, which must then be further processed to obtain phytochromobilipeptides in μmol quantities.[3b]

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It was demonstrated as early as 1959 that phytochrome (**1**) exists in either of two forms in living plants, a predominate red-absorbing form known as **Pr**, and an activated far red absorbing form designated as **Pfr**.[4a] In the native state these two species are readily interconverted upon irradiation at 660 and 730 nm, respectively, a photoreversible-photochromic behavior that has been the subject of intensive study for many years.[5] Most evidence suggests that **Pfr** is derived from **Pr** by photoreversible *Z*,*E*-isomerization about one of the methine bridges (C_5, C_{10} or C_{15}). In Figure 3 this is illustrated for the case of photoisomerization about C_{15}–C_{16} with retention of a "semi-extended" chromophore conformation.[4d] The concomitant red shift in **Pfr** vs **Pr** is thought to be partly due to a decrease in out-of-plane twisting in the protein-stabilized **Pfr** chromophore, allowing for more effective conjugation. Similar structural changes have been postulated for isomerization between C_4–C_5[4c,5a] and C_{10}–C_{11},[5b,c] but the precise site of activation is still not known with certainty.[5h] Irrespective of site, the most important consequence of E,Z-isomerization is that it induces a significant change in the tertiary structure of the surrounding protein shell **N-C**, thereby providing a molecular basis for transduction of the light signal to the cells genetic regulatory apparatus (cf. Figure 2). A similar mode of action is known to operate for *cis*-retinal in the vision process.

2. Early Interests

Our interest in biliprotein chemistry had its origins in a series of papers published by Rapoport et al. during the period 1979–1984, describing structural and synthetic studies relating to phytochrome (**1**) and other plant bile pigments.[3] More than a decade earlier (1966) Siegelman had shown that **1** suffered thermal cleavage in hot MeOH, liberating the parent chromophore phytochromobilin (**4**) and the apoprotein **N-C** (Figure 3).[6] In 1969 Rüdiger also obtained **4** by oxidation of **1** with chromic acid.[7] The structure of **4** was first deduced by spectroscopic analysis and later confirmed by Gossauer by total synthesis of PΦB dimethylester (**5**).[8] In common with most tetrapyrrole syntheses of this era Gossauer employed the classic "AB + CD" strategy, of which more will be said later. As applied to **5**, the AB-fragment **8** was prepared by thio-Wittig reaction of **6** and **7** followed by hydrogenolysis. Condensation of **8** with the CD-synthon **9** then gave (±)-**5** in ~45% yield (Scheme 1).

Though elegant, degradation studies of the type described above have limitations. In particular, all evidence concerning the nature of the protein-chromophore bond and the C_3,$C_{3'}$-stereocenters is lost. It remained for Lagarias and Rapoport to extend these studies to the intact phytochrome chromophore.[3b] NMR analysis of **1-Pr** itself, consisting of a protein of average molecular weight 90,000–150,000 and a single attached chromophore, was not practical. Therefore it was necessary to devise a means of obtaining intact peptide-bound chromophore of more manageable size. To accomplish this the authors employed a modification of a proteolysis procedure initially devised by Fry and Mumford,[9] involving sequential pepsin-thermolysin digestion of oat phytochrome in the **Pr** form (Scheme 2). In this way the undecapeptide **10** and four related chromopeptides were isolated in pure state and their amino acid sequence was determined. From this data, along with that obtained from truncated chromopeptides derived by Edman degradation, they were able to demonstrate that a cysteine mercaptide group anchored the protein-chromophore bond. Furthermore, NMR analysis established the general substitution pattern for ring A in **10**, and suggested a *trans* relationship between the substituents at C_2 and C_3. Finally, the relative stereochemistry at $C_{3'}$ was inferred from mechanistic studies reported by Rüdiger et al.[10] This group proposed that the *E*-ethylidene in phytochromobilin (**4**) was derived by a concerted elimination of **N-C** from **1**, presumably via an energetically favorable *anti*-periplanar transition state (cf. Figure 3).

Rapoport's stereochemical assignments for C_2 and C_3 gained additional support in 1984, following extensive NMR studies on the *cis*- and *trans* model tetrapyrroles **16c** and **16t** (Scheme 3). [3d] Even considered alone the synthesis of these materials was a *tour de force* for

the time. The key pyrromethenone **13** was prepared by KOH-induced condensation of the pyrrolin-2-one **11** with formylpyrrole **12**, followed by re-esterification. It was planned to selectively introduce the *cis* relationship at C_2–C_3 in **14c** by catalytic hydrogenation. In practice, though, this reaction produced a complex mixture of products from which **14c** could be isolated in 37% yield by HPLC. The epimeric *trans* dihydropyrromethenone **14t** was subsequently obtained in moderate yield by equilibration of **14c** with hot methanolic KOH. Both **14c** and **14t** were then converted to the respective tetrapyrroles **16c** and **16t** by condensation with the pyrromethenone **15**. As anticipated,[2b] the *trans*-tetrapyrrole **16t** exhibited excellent correlation of both chemical shifts (δ) and coupling constants (*J*) for H-2 and H-3 with the chromopeptide **10** (cf. Scheme 2). From these experiments the absolute stereochemistry at C_2, C_3 and $C_{3'}$ in **1** could be tentatively assigned as either *R,R,R* (shown) or *S,S,S*. Clearly enormous progress had been made in this area during the quarter century following the initial postulate of a **Pr** → **Pfr** photoequilibrium. However the years ahead still offered many challenges.

We saw several areas where synthetic methodology in biliprotein chemistry might be improved, including: *(a)* the highly functionalized pyrroline and pyrrole starting materials can be difficult to prepare and are frequently obtained as mixtures (cf. **6** and **7** in Scheme 1); *(b)* in many cases regio- and stereochemical control in ring A is modest (cf. **14c** and **14t** in Scheme 3); *(c)* joining rings A and B can be problematic in the presence of sensitive functionality (for example, each coupling step in Scheme 3 requires subsequent re-esterification); and *(d)* there were no means of controlling absolute stereochemistry.

3. The azomethine imine strategy: A saxitoxin connection

At the time of Rapoport's 1984 paper we had just completed a synthesis of the marine toxin saxitoxin (**20**),[11] which on first inspection bears little structural resemblance to phytochrome (**1**) or its parent chromophore (Scheme 4). However, we saw an opportunity to apply certain aspects of the methodology developed in our synthesis of **20** to the synthesis of biliprotein chromophores. In common with **1**, saxitoxin has three contiguous chiral centers, encompassing the *cis*-fused perhydropurine ring juncture (C_1 and C_5) and the pseudo-axial carbamate functionality at C_6. Since these stereocenters cannot be set employing thermodynamic control, we devised an approach where the *cis*-ring juncture of **20** was introduced by an intramolecular 1,3-dipolar cycloaddition of azomethine imine **18**, generated *in situ* by reaction of hydrazide **17** with methyl glyoxalate. The resultant adduct **19** was obtained in 75% yield on multigram scales, with exclusively the β-stereochemistry at C_6 predicted for kinetic control. However, in contrast to saxitoxin itself the [3.3.0]-azabicyclooctane ring system in **19** has well defined concave and convex faces, and the thermodynamically favored α-stereochemistry at C_6 was attained in >99:1 ratio by base-catalyzed epimerization of the carbomethoxy group. With all stereocenters intact, and following minor functional group adjustments, the labile N-N bond was cleaved employing Na/NH_3 reduction (dashed line), and the resulting diamine was elaborated to the pyrimidine ring found in **20**.

How do these studies relate to our efforts at synthesizing chiral chromophores of the type found in phytochrome (**1**)? We should first note that this was a period of great advances in acyclic stereocontrol, led by a number of groups investigating asymmetric aldol condensations (a *reagent*-controlled reaction). Much of this activity was driven by the isolation of a plethora of new natural products of the polypropionate class, many with interesting biological activity. In any event it is fair to say that a turning point had been reached, where the "Woodwardian" concept of stereoselectivity, often characterized by ingenious application of *substrate* control, was finding considerably less favor.

In this context we were intrigued by a paper by Schreiber et al.,[12b] who were investigating asymmetric variants of the Nicholas reaction (Scheme 5).[13] These authors found that Lewis acid catalyzed condensation of the Evans oxazolidinone **21** with cobalt complex **22** gave an ~80% yield of the *syn*-adduct **23***S,S*, with only minor amounts of the diastereomeric *anti*-isomer **23***S,R*. Moreover, adduct **23***S,S* was converted in two steps to the corresponding enantiomerically pure alkyne acid **24**, by oxidative decomplexation of Co followed by hydrolysis of the chiral auxilliary. Finally, one other aspect of this paper deserves special mention. The Yale group carried out exceptionally detailed mechanistic studies of this reaction, which among other findings uncovered a novel "double stereodifferentiating" process. The authors further concluded that reactions of this class should accommodate a wide range of substituents at C_2 and C_3.[12] Since the *syn*-orientation in alkyne acid **24**-*syn* corresponds to a *trans*-substitution pattern in its cyclic conformer **24**-*trans* (Scheme 5), it was natural to consider whether the Schreiber methodology might be adapted to prepare chiral, enantiomerically pure derivatives of ring-A in phytochrome (**1**). Our initial efforts in this direction had several features in common with our synthesis of saxitoxin (**20**).

In principle all of the stereo- and regiochemical features of ring-A might be incorporated in an alkyne acid of general structure **25**, prepared in homochiral form by condensation of two relatively simple fragments employing the Nicholas-Schreiber methodology (dashed line in **25**, Scheme 6). In the case of **1**, though, a chiral auxilliary enantiomeric to **21** would be required to introduce the desired 2*R*,3*R* configuration at C_2,C_3. As for ring-B, it was at this point that we succumbed to a slight bias arising from our experience with **20** (cf. Scheme 4). We chose as a precursor the N-aminopyrrole **26**, which upon condensation with **25** would give the hydrazide derivative **27** (dashed arrow). Our premise was that with X as a leaving group thermolysis of **27** would provide the azomethine imine **28**,[14] which upon intramolecular capture would generate the fused-ring target **29**. Finally, reductive cleavage of the hydrazide N-N bond would unmask the completely functionalized dihydropyrromethenone **30** (R = H or CO_2Me).

To test this plan Mr. Imad Odeh (Ph.D. Wesleyan University, 1984) synthesized a representative group of model substrates **31** and **32**, incorporating in **31** both electron-rich and electron-deficient π-systems, and in **32** a variety of protecting groups (**A**), leaving groups (**X**) and carboxyl derivatives (**R**) (Figure 4).[15] We quickly found that N-aminopyrroles **32** had to be synthesized and handled with great care. However, after several false starts we settled on the route outlined in Scheme 7, by which we were able to prepare the key N-aminopyrrole **37** on gram scales using well-established chemistry. Fortunately **37** proved to be reasonably stable and it served as a convenient precursor to numerous model systems. We first investigated the properties of hydrazides of general structure **41**, which were prepared in four steps from **37** (R = H, CO_2Me; R′ = CH_3, benzyl). Not surprisingly, the oxidative cleavage of **38** to **39** presented a significant challenge, since the lability of the pyrrole ring greatly limited our choice of reagents. Eventually it was found that the combination of $NaIO_4$ and catalytic OsO_4 afforded modest quantities of **39** (10–15%), but always accompanied by substantial decomposition. In an effort to improve this reaction Mr. Odeh carried out a series of control experiments, leading to the surprising discovery that $NaIO_4$ alone provided better results. This transformation may warrant further study. However, for the purpose at hand the yield of **39** was optimized to 35–40% employing a two phase solvent system of benzene/H_2O at rt for 10 h. While far from ideal, this procedure provided workable quantities of **39** for conversion to **41** (the purpose of $PrCl_3$ in the reduction of **39** to the corresponding alcohol **40** is discussed below).

Having now reached the "moment of truth", we were disappointed to find that hydrazides **41** showed no inclination to participate in the desired intramolecular 1,3-dipolar cycloaddition (Scheme 8). Under most conditions we obtained intractable mixtures that were difficult to characterize. However, on occasion we were able to isolate small amounts of the dimeric

species **42** (dashed line), possibly indicating that the initial 1,5-elimination of R′CO_2H had occurred as planned. To avoid dimerization we also studied the reactivity pattern of lactones **43** and oximes **45**, both of which were derivable from enol ethers **38**.[15] In the case of **43**, dipole formation would be accompanied by generation of the required propionic acid group at C_4. In similar fashion, Beckman fragmentation in **45** would produce the analogous propiononitrile group in **46**. All of these efforts proved to be futile. With hindsight the azomethine imine route to biliprotein chromophores was probably "over-engineered", fueled by our enthusiasm for extending the saxitoxin methodology. However, one final observation from these studies deserves mention, since it presaged our first successful syntheses in this area.

We alluded above to the beneficial effect of $PrCl_3$ in the $NaBH_4$ reduction of aldehydes **39** to alcohols **40**, particularly in the case of electron deficient dipolarophiles (Scheme 7). In the absence of $PrCl_3$, $NaBH_4$ catalyzed an efficient 5-*exo*-*trig* cyclization of **39** involving the hydrazide N-H group. For example, alkene ester **39a** gave a 45% overall yield of the pyrrolidinone **47** upon $NaBH_4$ reduction followed by acylation (Scheme 9). Pyrrolidone **47** was also obtained in 85% yield by NEt_3-promoted cyclization of the corresponding acetoxymethyl derivative **41a**. With ample quantities of **47** in hand, we briefly explored the possibility that the model A,B-ring precursor **49** might be prepared by S_N2' substitution, via the intermediacy of the ester enolate anion **48**. However, **47** was unstable to the strongly basic conditions necessary to generate **48**. We also examined the reactivity of the related enamide **51**, prepared in ~90% yield upon brief warming of alkyne ester **50**. Our thought was that the nitrogen free electron pair might initiate the desired transformation. In the event, **51** exhibited none of the desired reactivity and the S_N2' route was soon abandoned. On a positive note, though, the facile conversion of **50** to **51** provided the basis for a new strategy that was fruitful.

4. The sigmatropic rearrangement strategy: First success

The ready availability of N-pyrroloenamide derivatives of type **51** raised an interesting possibility. While studying somewhat related ring systems, Patterson et al. had discovered a novel photochemical rearrangement of N-allylpyrroles **52a,b** (Scheme 10).[16] Upon irradiation these materials were converted to regioisomeric mixtures of **53a,b** and **54a,b**, which in the case of R = H were further transformed to pyrroles **56** and **57** by 1,5-hydrogen transfer (pyrrole **55** arose by photodissociation). These results were interpreted in terms of competing 3,5- and 1,5-sigmatropic rearrangements, the former likely by a concerted pathway. Having what appeared to be good precedent, we felt confident that N-pyrroloenamides **58** would undergo an analogous transformation, producing dihydropyrromethenones **30**. Possibly, even, the transformation of **58** to **30** would be facilitated by the weakness of the N-N bond, as well as the favorable polarization of the pyrrole ring relative to the migrating terminus of the enamide. As in the azomethine imine strategy (vide supra), stereochemical and regiochemical features incorporated into **58** would be transposed in unequivocal fashion to **30**.

The feasibility of this strategy was initially tested by Mr. Subhas Buddhu (Ph.D. Wesleyan University, 1988), employing N-aminopyrroles **59** (R = H, CO_2Me) (Scheme 11).[17] Because of their symmetry these compounds were readily prepared following literature procedures.[18,19a,l] Pyrroles **59** were cleanly converted to the corresponding hydrazide derivatives **61** by DCC mediated coupling with the alkyne acid **60**. These last materials then underwent a facile 5-*exo*-*dig* ring closure, affording a >90% yield of the N-pyrroloenamides **62** (cf. also Schemes 7 and 9). This step completed the formation of rings A and B. Upon photolysis, **62** gave reaction mixtures which contained trace amounts of the desired products of 3,5-sigmatropic rearrangement (**63**), in addition to products corresponding to 1,3- and 1,5-sigmatropic shifts (**66, 67**), and N-N bond cleavage (**64**, **65**). After further experimentation, we found that the ratio of products **63**–**67** was strongly influenced by both triplet state quenchers and sensitizers.[19l] For example, at 300 nm **62a** gave 5–10% yields of rearrangement products **63a**, **66a** and

67a, accompanied by ~50% of cleavage products **64** and **65a**. Similar results were obtained at 253 nm. Significantly, cleavage products **64** and **65** were the sole products in the presence of triplet sensitizers. With triplet quenchers,[20] however, cleavage was reduced to trace amounts and **63a** was obtained in 40–50% yield as an ~1:1equilibrium mixture of *E*- and *Z*-isomers. Similarly, **62b** (R = H, CO_2Me) was converted in 40–50% yield to **63b** (R = CO_2Me), whose structure was confirmed by X-ray analysis.[21] These results are consistent with a reaction pathway where photodissociation occurs *via* a triplet state in competition with a singlet state 3,5-sigmatropic shift. Although the yields in the conversion of **62** to dihydropyrromethenones **63** were not as high as might be desired, we were sufficiently encouraged to pursue additional experiments with substrates bearing the natural substitution pattern.

In order for our preliminary studies to be extrapolated to the preparation of dihydropyrromethenones **70** (a logical precursor to **1**–**3**), it was necessary to devise efficient syntheses N-aminopyrroles of type **68** and highly substituted alkyne acids of type **69** (Scheme 12). Ms Guolin Cai (Ph.D. Wesleyan University, 1991), and subsequently Drs. Sundaramoorthi Rajeswari and Douglas Fry, met this challenge with great enthusiasm.

The synthesis of **68** required strict regiochemical control, which turned out to present a significant challenge. Ultimately, however, these materials were prepared by the route outlined in Scheme 13, taking advantage of a highly *ortho*-selective Diels-Alder reaction of 2-alkoxy-1,3-pentadiene derivatives **71** with 2-oxo-3-butenoate esters **72**.[19b,f] Adducts **73** were then converted in high yield to N-aminopyrroles **68** by a three step sequence consisting of ozonolysis (**73** → **74)**, Paal-Knorr cyclization with N-aminophthalimide, and hydrazinolysis. Since the ozonolysis products **74** were generally not isolated, this route constitutes a convenient three step sequence for preparing **68** from relatively simple starting materials. However, before leaving this topic, it is worth noting that R′ in **71** must be chosen with care to minimize competing Diels-Alder pathways. This is because 2-oxo-3-butenoate esters of general structure **72** are also highly reactive heterodienes (cf. transition state **B** leading to 2-alkoxypyran **75**).[20] In the case of R′ = Et the heterodiene pathway competed favorably, giving a 2.9:1 ratio of the regioisomeric adducts **73** and **75** (combined yield 54%). Fortunately, though, the ratio of **73:75** depended strongly upon the nature of R′, improving to ~99:1 following the order Et < TMS < TBDMS < TIPS (81% yield). This selectivity pattern closely parallels the size of R′, suggesting that the ratio of **73:75** mirrors steric interactions in the competing transition states **A** and **B** (Scheme 13). One would expect R′ to have little impact on the rate of cyclization leading from **A** to the desired adducts **73**. In contrast, this group introduces significant steric crowding in **B**, leading to the prediction that the rate of formation of **75** will decrease with increasing size of R′.

We expected alkyne acids **69** to be of greatest utility when Y = carboalkoxy, on the assumption that an electron deficient triple bond was required for 5-*exo*-*dig* cyclization (cf. Scheme 9). Therefore our preliminary studies focused on preparing simple dimethyl analogs of type **78a–c**, which were synthesized in analogous fashion to **23***S*,*S* but employing the chiral oxazolidinone **76** (Scheme 14). In this case, however, we were disappointed to find that *syn*-selectivity in the reaction **76** + **77** → **78** was only ~3:1. This result is in general accord with the observations of Schreiber *et al.*, who noted that selectivity increases with increasing size of the alkyne substituent.[12] Equally disappointing, we were unable to selectively remove the chiral auxiliary in **78** without concomitant hydrolysis of the acetylenic ester to afford diacid **79**.[23] This lack of differentiation was a serious complication, since all attempts at mono-functionalization of **79** invariably led to complex mixtures of products.

In contrast to the poor selectivity profile of cobalt complexes **77** (Scheme 14), trimethylsilyl derivative **22** and oxazolidinone **76** gave a 90–95% yield of Nicholas adduct **80** with >98% *syn-(R,R)*-selectivity (Scheme 15). Oxazolidinone **80** was then converted in high yield to the

alkyne hydrazide **81b** (Y = H), employing the Evans' protocol for hydrolysis to the alkyne acid *ent*-**24** (concomitant removal of TMS group),[23] followed by EDCI mediated coupling with N-aminopyrrole **59b**. At this juncture, however, we experienced difficulty effecting the 5-*exo-dig* cyclization leading from **81b** (Y = H) to enamide **82**. Not surprisingly **81b** was unreactive to cyclization under thermal conditions and rapidly decomposed upon attempted acid or base catalysis. Eventually we achieved some measure of success employing the reagent system $PdCl_2(MeCN)_2$/NaOAc, which afforded 60–70% yields of the desired enamide **82b**.[24,25] However, by far the most useful procedure was discovered serendipitously upon attempted cleavage of the TMS group from alkyne hydrazide **81a** (Y = TMS). This material was obtained from adduct **80** by hydrolysis and amidation under carefully controlled conditions. Upon warming with *n*-Bu_4NF (TBAF), **81a** afforded none of the expected terminal alkyne **81b**, but rather was directly converted to the identical N-pyrroloenamide **82b** obtained from Pd (II) catalyzed cyclization of **81b**. The same conditions, when applied to terminal alkyne **81b**, afforded enamide **82b** in 70–90% yield. The precise mechanism by which fluoride ion catalyzes the cyclization of **81b** to **82b** is not known with certainty, but it presumably involves a strong hydrogen bond between F^- and the hydrazide N-H group, with an attendant increase in N-nucleophilicity.[26] Interestingly, however, the active catalytic species in these and related cyclizations may be a decomposition product of TBAF (vide infra).[27] In any event we utilized an identical two step sequence to convert alkyne acid **24** to the enantiomeric hydrazide *ent*-**81b** (*ent* = mirror image of structure shown), which upon warming with TBAF gave an excellent yield of enamide *ent*-**82b** (not shown). Both materials were obtained as single enantiomers within the limits of detection. Finally, photochemical rearrangement of **82b** and *ent*-**82b** as described in Scheme 11 afforded 40–50% yields of the corresponding model A,B-ring dihydropyrromethenones **83** and *ent*-**83**, having optical rotations of essentially equal magnitude but opposite sign.

Drs. Fry and Rajeswari now took on the task of preparing alkyne acids of proper constitution for correlation with **1**–**3**. In phytochrome (**1**) the absolute stereochemistry at C_2,C_3 is believed to be *R*, but it was important to maintain as much flexibility as possible in our synthesis to confirm this assignment. As above we planned to control both relative and absolute stereochemistry at C_2,C_3 employing the Nicholas-Schreiber methodology, while stereochemistry at $C_{3'}$ (also believed to be *R*) would be established utilizing an appropriate aldehyde **84** from the "chiral pool" (Scheme 16; **84** → **85** → **69**).

In principle the aldehyde chosen could incorporate a sulfur ligand of proper absolute configuration at the start (X = S-R). Alternatively, the desired configuration could be attained by nucleophilic displacement with inversion of an activated hydroxyl group at a later stage of the synthesis (X = O-R).[28] The second approach offered a greater degree of flexibility and it also had the advantage that both *R*- and *S*-α-hydroxyaldehyde derivatives of the required composition are readily available from *R*- and *S*-lactic acid, respectively.[29] We therefore opted to explore this strategy first, although more will be said of the "direct" approach later.

The synthesis of the desired alkyne acids **88** and *ent*-**88** followed closely according to plan (Scheme 17).[19c,l] Thus, condensation of lithium trimethylsilylacetylide (LiTMSA) with aldehydes *S*-**86b,c** cleanly afforded the corresponding alkyne alcohols,[29] which without isolation were methylated (DMS) to give the methyl propargyl ethers *S*-**87b,c** in excellent overall yield (unknown configuration at C_3). These materials were then converted to the desired homochiral alkyne acids **88b,c** following the by-now well established sequence of cobalt complexation, Nicholas-Schreiber condensation and hydrolysis (60–70% overall yields from *S*-**86b,c**). The structure of **88c** was confirmed by X-ray analysis.[21] For both compounds we employed the chiral oxazolidinone **76** to form the crucial C_2–C_3 bond, which gave *syn*-selectivity of >98%. Subsequently, however, we found that comparable levels of diastereoselectivity were obtained employing achiral oxazolidinones due to the strong directing

effect of the chiral center at $C_{3'}$. In analogous fashion we prepared the enantiomeric 2*S*,3*S*,3′*R*-alkyne acids *ent*-**88b,c** with >98% stereoselectivity, but in these examples employing boron enolate **21**.

From all indications we were close to our goal of synthesizing homochiral analogs of phytochrome (**1**). Building on our model studies, alkyne acid **88b** and N-aminopyrrole **68** were coupled to give the pyrrolohydrazide **90b** (88%), which upon TBAF-induced ring closure afforded the corresponding pyrroloenamide **91b** (not shown) (Scheme 18). This material, upon 3,5-sigmatropic rearrangement, then gave a 46% yield of the desired dihydropyrromethenone **92b** as an *E,Z*-mixture. In principle **92b** has all of the structural features necessary for eventual conversion to biliprotein chromophores related to **1**, requiring only inversion of configuration at $C_{3'}$ with a thiol nucleophile (**92b** → **93** → **70**).[28] A prerequisite to such a displacement was cleavage of the methyl ether in **92b** to produce an alcohol **93** suitable for activation. Unfortunately, though, this transformation could not be selectively achieved in the presence of the two ester groups.

In contrast to methyl ethers **92b**, there was good precedent for selectively cleaving O-benzyl groups in pyrromethenone ring systems. We therefore synthesized the benzyl protected N-pyrroloenamide **91c** by an analogous sequence to that outlined in Scheme 18. However, the photochemical behavior of pyrroloenamides **91b** and **91c** turned out to be markedly different (Scheme 19). While **91b** underwent rapid photoisomerization to **92b** (Scheme 18), **91c** afforded mainly **94** and **95**, the products of hydrazide cleavage. This result was not entirely unexpected based upon our experience with model systems **62** (Scheme 11), since **91c** contains a phenyl group that is capable of internal triplet sensitization.[30] Despite some success we had reached a point where the photochemical strategy no longer appeared practical. It was at this point that we took our first steps into the realm of transition metal chemistry.

5. Regrouping with Pd chemistry

Considering all of the transformations described in Section 4, the TBAF-catalyzed cyclization of non-activated alkyne hydrazides **81** had the greatest impact (cf. Scheme 15). This reaction provided the basis for a more direct strategy for synthesizing dihydropyrromethenones incorporating the substitution pattern found in phytochrome (**1**). The basic premise of this strategy was that alkyne amides **98** would undergo analogous 5-*exo-dig* cyclization leading to dihydropyrromethenones **99** (Scheme 20). Alkyne amides **98**, in turn, were in principle available by Sonogashira coupling of halopyrroles **97** and alkyne amides **96**.[31] A significant advantage to this approach is that bond connectivity between C_5 and C_6 is established directly, thereby eliminating the need for subsequent 3,5-sigmatropic rearrangement. Also, we expected that kinetic control in the amide addition to the alkyne triple bond would lead directly to the naturally occurring *Z*-configuration at C_5–C_6.

At the time we began this work only scattered reports had appeared describing the coupling of alkynes with halopyrroles,[31] and few provided experimental details. Dr. Rajeswari now turned her considerable skills to investigating this reaction, employing iodopyrrole **102** and phenylacetylene as a model system (Scheme 21). Iodopyrrole **102** was prepared by iodination of the cyclo-hexylpyrrole **101**, itself derived in 75% yield from 1-nitrocyclohexene (**100**) and methyl isocyanoacetate using the methodology of Zard *et al*.[32] After some effort we developed reproducible conditions for the Sonogashira coupling of **102** and phenylacetylene. By far the most important parameter in optimizing this reaction was to remove all traces of oxygen. At least three freeze-thaw cycles are necessary for optimum yields of **103** and to minimize alkyne dimerization.[34] The best results were obtained with a 10% molar excess of phenylacetylene, employing DMF as solvent under rigorously degassed conditions. This protocol consistently afforded **103** in >85% yield with little or no dimer formation.

These experiments were readily extrapolated to the use of more complicated alkyne amides of type **96** (Scheme 22). In every case, Pd(0) mediated coupling of iodopyrrole **102** with alkyne amides **96a–e** and *ent*-**96b** was accomplished in yields of >88%. Significantly, there was no need to protect either the amide or pyrrole components. Furthermore, cyclization of the pyrroloalkynes **104a–e** and *ent*-**104b** took place under nearly identical conditions to those employed with hydrazide **91c**, affording *Z*-dihydropyrromethenones **105a–e** and *ent*-**105b** in 73–83% yield. Little or no formation of the corresponding *E*-isomers was detected, except for the case of **105e** (R = benzyl), where steric crowding causes partial *Z,E*-isomerization. The materials thus obtained were identical in both physical properties and optical rotation to the corresponding *Z*-isomers prepared using the photochemical strategy (vide supra). As a point of interest, all of these cyclizations exhibited a brief induction period prior to the onset of reaction. This characteristic led us to suspect that the actual catalytic species in these reactions is the thermodynamically stable *n*-$Bu_4N^+FHF^-$ complex ("tetra-*n*-butylammonium bifluoride"), which forms rapidly upon heating THF solutions of TBAF, or upon attempted drying of *n*-$Bu_4NF.3H_20$ at 40–70 °C.[35a] Supporting this hypothesis, commercially available *n*-$Bu_4N^+FHF^-$ exhibited similar catalytic activity with no induction period. Although not conclusive, this evidence suggests that *n*-$Bu_4N^+FHF^-$ might be involved in other F^--catalyzed reactions that specify the use of TBAF at T >40 °C.[35b,c]

Before extending these studies to the synthesis of dihydropyrromethenones related to phytochrome (**1**), it was necessary to devise an efficient preparation of the iodopyrrole **109**. This was accomplished using either of the routes outlined in Scheme 23, the details of which can be found in the papers describing this work.[19h,m] Most importantly, iodopyrrole **109** proved to be an excellent precursor for dihydropyrromethenones related to **1** (Scheme 24). Thus, in a very efficient two-step sequence, Pd(0) catalyzed coupling of **109** with the enantiomerically pure amide **96d** gave a virtually quantitative yield of the alkyne pyrrole **111**, which upon TBAF-promoted cyclization gave the ring-A,B precursor **112** in 87% yield. In identical fashion but beginning with alkyne amide *ent*-**96d**, we synthesized enantiomer *ent*-**112** as a single isomer, having optical rotation of essentially equal magnitude but opposite sign (the small difference is due to different purities of the commercial *R*- and *S*-lactic acids). Clearly this methodology has significant advantages over the photochemical strategy.

We now explored the possibility that 3′*R*-benzyl mercaptide derivatives of type **114** could be prepared using an analogous Nicholas-Schreiber reaction (Scheme 25). It was our good fortune that Mr. Wanjun Zheng (Ph.D. Wesleyan University 1993) was able to join these studies, having recently completed an unrelated project. However, summarizing many months effort, all attempts at condensing boron enolate *ent*-**21** with the *R*-cobalt complex **113a** (X = S) were unsuccessful. At low temperatures little or no reaction occurred, while more forcing conditions brought about rapid decomposition. The analogous condensation of *ent*-**21** with the *R*-benzyl ether **113b** (X = O) also proved to be problematic. These compounds reacted in a *mis*-matched fashion, affording *anti*-adduct **115** with 12:1 diastereoselectivity. The structure of **115** was established by a three step sequence consisting of Curtius rearrangement, followed by oxidative cleavage and DCC-catalyzed cyclization to afford the known β-lactam **116**.[36] We believe this to be the first example of a Nicholas-Schreiber condensation exhibiting *anti*-selectivity, again testifying to the powerful directing influence of the chiral substituent at $C_{3'}$ (cf. also Scheme 17).

Subsequently we found that a $C_{3'}$-*R*-mercaptide group could be introduced by thia-Mitsunobu inversion after condensation to form the C_2–C_3 bond. In early deprotection studies Mr. Robert DeSimone (Ph.D. Wesleyan University, 1992) observed a novel reaction upon treatment of benzyl ether **88** with P_4S_{10} (Scheme 26). Our intent was to convert **88** to the corresponding thioacid, which based upon hard-soft-acid-base theory (HSAB) might undergo intramolecular transfer of the O-benzyl group. In practice this reaction gave an excellent yield of lactone

117 following much the same mechanism as postulated. Details of this transformation have been described elsewhere.[37] Dr. Jiasheng Guo followed up on this observation, finding that $LiAlH_4$ reduction of **117** afforded a quantitative yield of the expected diol, which upon selective protection with TBDMSCl gave an 82% yield of the secondary alcohol **118**. This last material then underwent thia-Mitsunobu inversion with the reagent combination ZIRAM (**119**)–DEAD-Ph_3P,[38] affording a 39% yield of the desired 2*R*,3*R*,3′*R* mercaptide **120**. Finally, mercaptide **120** was converted in 49% overall yield to the alkyne amide **121** by the three step sequence of deprotection, oxidation of the 1°-alcohol and amidation. Although circuitous, this route was suitable for preparing gram quantities of **121** with excellent stereocontrol.

At this point, though, we were disappointed to find that **121** gave only modest yields of the alkyne pyrrole **122** upon Pd(0) mediated coupling with iodopyrrole **109** (Scheme 27). Presumably this is because sulfur interferes by poisoning the Pd catalyst. Equally discouraging, all attempts at effecting cyclization of **122** to the corresponding dihydropyrromethenone **123** failed.

By now we had concluded that thia-Mitsunobu inversion at $C_{3'}$ could only be accomplished *after* formation of the dihydropyrromethenone ring. Initially this presented a problem, since all attempts at cleaving protected hydroxyl derivatives of type **112** had thus far failed (*cf.* Scheme 24). Eventually this obstacle was overcome by taking advantage of the fact that lactone **117** underwent facile ring opening with amines **124** (R = H, *p*-methoxybenzyl), affording alkyne amides **125** in excellent yield (Scheme 28). Alkynes **125a,b** then afforded 80–95% yields of the corresponding pyrroloalkynes **126a,b** upon Pd(0) catalyzed coupling with iodopyrrole **109**. With a reliable source of both **126a** (R = H) and **126b** (R = PMB) secured, we turned our attention to the remaining steps necessary to complete the synthesis of the A,B-ring system **128** (Scheme 28). Unexpectedly the cyclization of **126a** turned out to be relatively slow, giving a 43% yield of **127a** after 48 h heating with excess TBAF. Of more pressing concern, **127a** failed to undergo the desired thia-Mitsunobu inversion. After further experimentation this problem was traced to interference by the lactam N-H group in ring A, which undergoes competitive reaction with DEAD. In any event much more satisfactory results were obtained with **126b** (R = PMB), which gave an 80% yield of dihydropyrromethenone **127b** upon brief warming with TBAF (*Z*-isomer exclusively). More will be said of the rate-enhancing effect of N-substitution on alkyne amide cyclizations in Section 7.[19j] Finally, **127b** gave a 62% overall yield of the desired A,B-ring precursor **128b** upon thia-Mitsunobu inversion followed by acid catalyzed decarboxylative formylation.[28,39] These results constitute the first enantioselective synthesis of the core A,B-dihydropyrromethenone of **1**.

6. Constructing the C,D-ring pyrromethenone of phytochrome (1)

Thus far we have had little to say of the C,D-ring pyrromethenones of phytochrome (**1**) and related chromophores, partly because the synthetic challenges in this area are less stringent. This is reflected by the fact that a number of syntheses of these ring systems have been reported, in particular those found in **1** and phycocyanin (**2**) (cf. **131** and **132** below). Nearly all published syntheses employ some variant of the Gossauer strategy,[8] typified by the KOH promoted condensation of formylpyrrole **129** with unsaturated lactams of type **130** (Scheme 29; C + D → CD). This approach is appealing because of its highly convergent nature and in principle it offers a wide range of flexibility. For example, condensation of **129** with **130a** (X,G = H) gave dihydropyrromethenone **131** in good yield following re-esterification.[40] In some instances, though, the strongly alkaline conditions of this reaction are incompatible with sensitive functionality. This is particularly an issue for lactams **130** where X = leaving group, which are logical precursors to Δ-21 derivatives related to phytochrome (**1**). In such cases activation is typically delayed until after condensation, which can cause complications.[8,41] For this reason,

and others that will become clear later, we felt it necessary to develop independent syntheses of **132**, two of which are outlined below. A third is described in Section 8.

Our first experiments in this area were conducted with the imide derivative **133** (Scheme 30), itself derived by acylation of 2-oxazolidinone with 4-bromobutyryl chloride.[19e] Imide **133** underwent clean condensation with the cobalt complex **22**, affording an ~80% overall yield of adduct **134** following Co-decomplexation (*syn*-isomer exclusively). Adduct **134** contains all of the features necessary for eventual conversion to ring D of **1**. Interestingly, hydrolysis of the imide group in **134** led directly to the formation of the alkyne lactone **135**, which was complete in <5 min at 0 °C. Although not planned this transformation was put to good use. Lactone **135** was now cleanly converted to the ring-opened alkyne amide **136** by initial S_N2 displacement with sodium *p*-chlorophenylselenide (72%),[42] followed by amidation with *i*-butyl-chloroformate/NH_3 (93%). Finally, amide **136** gave an ~50% overall yield of dihydropyrromethenone **138** upon Pd(0)-mediated coupling with iodopyrrole **109** followed by 5-*exo*-*dig* cyclization and DDQ oxidation (*Z*-isomer only). Selenide **138** was obtained as a stable, crystalline solid, that had identical spectral and physical properties to those reported in the literature.[8,43] This material has previously been converted to **132** by oxidative elimination with H_2O_2.[8] Although successful, a number of considerations led us to seek a more direct synthesis of **132**. Foremost of these was the issue of efficiency, since by this route we were unable to prepare **132** in the quantities necessary for our studies. Partly this was a consequence of employing a strategy based upon the Nicholas-Schreiber condensation (*i.e.* **22** + **133** → **134**), which is an excellent method for preparing *syn*-adducts of type **134** in enantiomerically pure form. In the present case, however, this strategy incorporates an unnecessary level of complexity, since the ultimate target **132** is devoid of stereochemical features.

As an alternative approach we set out to develop a stereoselective synthesis of the unsaturated alkyne amide **143**, which is in the proper oxidation state for final cyclization (Scheme 31). Control of double bond geometry at C_{17} was accomplished beginning with commercially available 2-acetylbutyrolactone (**139**), which upon enolization at −78 °C afforded nearly exclusively the *Z*-lithioenolate. This species, upon quenching with triflic anhydride (Tf_2O), then gave *Z*-enoltriflate **140** as the only detectable isomer (yields represent a range from many experiments). Selectivity in this case presumably is due to Li^+ coordination with the enolate anion derived from **139**, in analogy to the work of Brückner et al. with 2-formylbutyrolactone.[44] Sonogashira coupling of **140** with trimethylsilylacetylene then gave an excellent yield of the alkyne lactone **141**,[31] which correctly sets the double bond geometry as *Z*. Next, **141** was directly converted to the alkyne amide **142** by initial S_N2 ring opening with sodium *p*-chlorophenylselenide[42] followed by *in situ* amidation. Amide **142** is a stable, crystalline compound that was routinely prepared on multigram scales. Finally, oxidation of **142** with H_2O_2 effected smooth selenoxide elimination, producing the alkene derivative **143** as an easily polymerized solid.

Both **142** and **143** were excellent substrates for Pd(0)-mediated coupling with the iodopyrrole **109** (Scheme 32). On relatively small scales (<1 g) Sonogashira coupling of **109** with the unsaturated alkyne amide **143** gave a 90–95% yield of the pyrroloalkyne **145**. This pathway has the advantage of being highly convergent. However, the success of this reaction depends critically upon employing only freshly prepared **143**, since this material polymerizes rapidly even when stored at 0 °C. For larger scale reactions (>1g) it was generally more convenient to delay oxidative elimination until a later step of the synthesis. Thus, Pd(0)-catalyzed coupling of alkyne amide **142** with **109** gave an 85–90% yield of the stable alkyne pyrrole **144**, which upon treatment with H_2O_2 was cleanly converted to the identical unsaturated derivative **145**. Finally, we investigated many conditions for effecting the requisite 5-*exo*-*dig* cyclization leading from **145** to **132**, which proved to be unusually slow with TBAF. By far the best results were obtained with the reagent system CsF/$Si(OMe)_4$ initially introduced by Corriu and Perz

as a catalyst for Michael additions.[45a] Although less convenient, this reagent is generally more effective than TBAF for sluggish cyclizations.

7. Instability issues: The "BC + D + A" strategy

To this point we had been reasonably successful in our synthetic efforts, developing practical syntheses of enantiomerically pure A,B-dihydropyrromethenones including **112**, **127a,b** and **128**, and also C,D-pyrromethenones **9** and **132** (Scheme 33). However, an unpleasant surprise lay ahead, as for example we obtained only trace amounts of tetrapyrrole **146** upon attempted acid catalyzed coupling of **112** and **9**. Similarly, dihydropyrromethenones **127** and **128** were unstable to the strongly acidic conditions required for condensation with **9** to afford tetrapyrroles.

Stability issues of this nature might be overcome with sufficient study, and we eventually solved this problem in closely related systems.[46] Concomitantly, though, we decided to explore a new strategy where the most sensitive portion of the tetrapyrrole would be introduced last (Scheme 34). We also planned to take advantage of the symmetrical relationship of rings B and C in most biliprotein chromophores. As in our earlier work we would employ a Nicholas-Schreiber reaction for preparing ring A in homochiral form and with unambiguous control over regiochemistry. However, in our new approach alkyne amides **96** and **148** would be joined to the B,C-diododipyrrin **147**, which represents the most stable part of the tetrapyrrole nucleus. This operation would be simplified by the fact that **147** has a pseudo plane of symmetry (dotted line), in that the pyrrole rings freely interconvert *via* tautomerization (upon protonation, rings B and C are equivalent, as determined by NMR studies at low pH). Therefore, for the purpose of regiochemical control the alkyne fragments **96** and **148** can be coupled in either order. For practical purposes, though, it would generally be best to add the less stable ring-A synthon last (BC + D + A --> ABCD).

To explore this route Dr. Lisa Coutts developed an efficient synthesis of the *bis*-iodo derivative **147**, building upon the work of Rapoport[47a] Johnson,[47b] and others (Scheme 35).[47c,d] The details of this work are provided in reference [19n]. Dr. Sam Leung, a newly arrived postdoctoral, then teamed with Dr. Guo on cyclization studies with the symmetrical *bis*-alkyne amide **154**, prepared in 77% yield by Pd(0) mediated coupling of **147** with excess homochiral alkyne amide **153a** (Scheme 36). As expected, **154** underwent facile cyclization on catalysis with TBAF,[19l] giving an 89% yield of *mono*-cyclized intermediate **155** after 1.5 h at 25 °C. Interestingly, though, the subsequent cyclization of **155** to **156** was markedly slower, requiring 5 h at 65 °C to complete reaction. This step is probably inhibited by the electron donating nature of the ring D enamide functionality, a point that is discussed further below. In any event we were sufficiently encouraged by these results to apply this same approach to unsymmetrical tetrapyrroles.

According to plan, the unsymmetrical *bis*-alkyne amide **158a** (R = H) was derived by sequential coupling of **147** first with ring-D precursor **153a**, followed by the less stable ring-A precursor **125a** (Scheme 37). As with **154** above, *mono*-cyclization of **158a** was fast, affording a mixture of tripyrroles **159** after 1.5 h at 25 °C (not shown). However, the subsequent ring closure leading to tetrapyrrole **160a** turned out to be exceptionally slow, affording only trace amounts of the desired product after 7 h at 65 °C. This reaction was also accompanied by substantial decomposition. Much better results were obtained with the *bis*-alkyne amide **158b** (R = *p*-methoxybenzyl), which was prepared in good overall yield by sequential Pd(0) mediated coupling of **147** with the *p*-methoxybenzyl amides **153b** and **125b**. In Scheme 28 we commented that N-substitution can dramatically accelerate the rate of alkyne amide cyclizations.[19j] This was especially true for **158b**, which gave a 57% yield of tetrapyrrole **160b** after 1.5 h at 0 °C. Finally, thia-Mitsunobu inversion at $C_{3'}$ in **160b** was accomplished

without difficulty,[28] affording a 53% yield of tetrapyrrole **161b** having the desired (2*R*,3*R*,3′*R*)-configuration found in **1**–**3**. This route avoids the stability problems associated with the classical AB + CD strategy (Scheme 33), and constitutes an efficient route to tetrapyrroles where *both* rings A and D are saturated.

It was now of interest to explore the effect of ring D oxidation state on the rate of cyclization leading to ring A, since the natural tetrapyrroles **1**–**3** are unsaturated at C_{17}–C_{18}. Dr. Guo carried out these studies with alkyne amides **155** and **162**, which differ only in oxidation state at ring D (Scheme 38; **162** was generated *in situ* by DDQ oxidation of **155** and utilized as an ~50:50 mixture). One would expect that cyclization of **162** (ΔC_{17}–C_{18}) would be fast relative to **155**, since the alkyne amide in **162** (but not in **155**) is conjugated with the electron withdrawing carbonyl group in ring D. Therefore it should be more susceptible to internal nucleophilic attack. This turned out to be the case. Thus, in competition experiments, alkyne-amide **155** (saturated C_{17}–C_{18}) was completely unreactive toward cyclization to tetrapyrrole **156** employing TBAF at 40 °C (cf. also Scheme 36). Under the same conditions, however, alkyne-amide **162** (ΔC_{17}–C_{18}) was rapidly converted to tetrapyrrole **163** having the natural oxidation state of phycocyanin (**2**).

We were optimistic now that B,C,D-ring systems of type **165** might be versatile precursors to tetrapyrroles related to phytochrome (**1**) (Scheme 39). The requisite precursor **164** was prepared in good yield by Pd(0) mediated coupling of alkyne amide **142** with the dipyrrin **147**. Disappointingly, though, **164** suffered rapid decomposition upon attempted 5-*exo*-*dig* ring closure with TBAF. This outcome probably reflects the lability of the phenylselenide group to TBAF.

More promising results were obtained with the conjugated derivative **166** (Scheme 40), prepared in 70% yield by peracid induced elimination of *p*-Cl-Ph-SeOH from **164**. Though unstable to TBAF, **166** underwent rapid cyclization upon acid catalysis, affording a 32% yield of a product that had the analytical and spectroscopic properties expected for the tricyclic lactam **167a** (X = NH, Y = O). The presumed **167a** was then coupled with the ring-A alkyne amide **125a**, which afforded a 63% yield of a tetracycle having the spectral properties anticipated for the phytochrome precursor **168** (R = H). Upon acylation this last material gave an O-acyl derivative presumed to be **169** (R = Ac) in excellent yield. However, the product obtained had different chromatographic behavior and NMR spectra from authentic tetrapyrrole **169**, synthesized in very low yield by decarboxylative condensation of pyrromethenones **171** and **9**. After further studies, culminating in X-ray analysis,[48] this discrepancy was traced to the fact that the initial acid catalyzed cyclization of alkyne amide **166** had produced the isomeric iminolactone **167b** (X = O; Y = NH). Furthermore, Pd(0) induced coupling/cyclization of ring A also occurred with *oxa*-selectivity, affording tetrapyrrole **170** as the only isolable product The structure of **170** was confirmed by hydrolysis to the corresponding *bis*-lactone, which was identical with an authentic sample (vide infra).

This was the first occasion we observed *oxa*-selectivity in an alkyne-amide cyclization, and it appears to result from extended conjugation (Scheme 41). For example, this reaction pathway is not observed with tetrahydro substrates **157A** or dihydro substrates **164** (cf. Schemes 37 and 39). These observations are consistent with a pathway involving protonation of **166** to give the relatively stable delocalized cation **166**-H^+, followed by capture with the “hard” amide oxygen.[49]

Dr. Leung devoted much effort to altering the chemoselectivity in the cyclization of **166** to **167**, mostly without success. We also explored two routes for converting iminolactone **167b** to the desired lactam **167a** (Scheme 42). The first of these was based upon a Dimroth rearrangement, involving ring opening of **167b** to the keto-amide **172** followed by

cyclodehydration. Alternatively, acid catalyzed hydrolysis of **167b** could lead directly to the tricyclic lactone **173**. Were that the case, reaction of **173** with NH_3 should give the keto-amide **172**, which upon cyclodehydration would afford the desired lactam **167a**. Precedent for both pathways existed in the group.[50] In practice, though, all attempts at effecting the Dimroth rearrangement of **167b** gave only trace amounts of the tricyclic lactam **167a**, producing mainly lactone **173**. The structure of **173** was confirmed by Pd(0) mediated coupling of dipyrrin **147** with the alkyne acid **174** corresponding to ring D, followed by acid catalyzed cyclization.[51] Unfortunately, though, all attempts at reacting **173** with NH_3 led to extensive decomposition, and no keto-amide **172** could be detected.

Ms Sheila Hauck (Ph.D. Wesleyan University, 1998) found a relatively simple solution to this problem, delaying aminolysis until after formation of the tetrapyrrole (Scheme 43).[52] Earlier Ms Hauck observed that Pd(0) catalyzed coupling of **173** with alkyne acid **88c** led directly to the *bis*-lactone **175**, which was obtained in 45–65% yield exclusively as the 5-*Z*,10-*Z*,15-*Z*-isomer. In contrast to **173** (Scheme 42), **175** reacted cleanly with liquid NH_3 at −78 °C and gave the ring-A lactam **176** in high yield. In this case aminolysis was accompanied by spontaneous cyclodehydration. While aminolysis at ring D in **176** was somewhat slower, this reaction gave 40–50% yields of **146** following acid catalyzed dehydration. Although not yet optimized this route to **146** has clear advantages over the original AB + CD approach (*cf.* Scheme 33), where the instability of the starting dihydropyrromethenone **112** precluded decarboxylative condensation. Tetrapyrrole **146** was an attractive precursor to phytochromobilin (**4**), the importance of which is described in Section 8.

8. Enantioselective syntheses of 2*R*- and 2*S*-phytochromobilin (4)

It was now certain that Rapoport had correctly assigned the relative stereochemistry at C_2, C_3 and $C_{3'}$ in **1**,[3d] but the question of absolute configuration remained. Generally this was assumed to be 2*R*,3*R*,3′*R* by analogy to the closely related chromophore phycocyanin (**2**) (cf. Figure 1). However, direct evidence for this assignment was sparse. In this section we describe experiments that confirmed the postulated stereochemistry in **1**, but the story requires some background information.

Upon thermolysis phytochrome (**1**) undergoes a concerted E2-elimination, expelling as a "leaving group" the intact apoprotein **NC-1** (known as apophytochrome; cf. Figure 3). The residual chromophore phytochromobilin (**4**) retains only the C_2 chiral center in **1**, those at C_3–$C_{3'}$ being lost to the ethylidene group. In analogous fashion phycocyanin (**2**) produces apophycocyanin (**NC-2**) and phycocyanobilin (**PCB**) (Figure 5). **PCB** differs from **4** only in oxidation state at $C_{21,22}$ (bold). Beginning in 1989, reports began to appear suggesting that under certain conditions this process could be reversed *in vitro*.[53] These early studies were carried out with phycocyanin (**2**) due to the relative abundance of this light harvesting biliprotein. However, attempted reconstitution of phycocyanobilin (**PCB**) with apophycocyanin (**NC-2**) afforded only un-natural adducts containing a C_2–C_3 double bond.[54] These results suggested that assembly of phycocyanin (**2**) from **PCB** and **NC-2** requires additional enzymes and/or cofactors. Interestingly, however, it was subsequently found that **PCB** *does* combine with apo*phytochrome* (**NC-1**) to afford photochemically active adducts.[53b] Although the degree of incorporation was low (<10%), this experiment demonstrated that **NC-1** is an enzyme that promotes autocatalytic bilin attachment and holoprotein assembly, even with un-natural substrates. This activity appears to be unique among chromoproteins having linear tetrapyrrole prosthetic groups.[53] In 1991–92, Lagarias *et. al* described the first reconstitution studies employing recombinant apophytochrome (**NC-1**) with its native substrate phytochromobilin (**4**), the reverse of the process illustrated in Figure 3.[55] The results were dramatic in that the *in vitro* adducts displayed a difference spectrum identical to that of native oat phytochrome (**1**). This experiment suggests that the recombinant apophytochromes,

expressed in both *Saccharomyces cerevisiae* (yeast) and *E. coli* (bacteria), adopt a structure similar to that of the apoprotein biosynthesized *in vivo*. The ability **1** to self-assemble in the dark undoubtedly reflects its role in etiolated plants as a first light sensor.

By chance I attended the Gordon Research Conference where Professor Lagarias described these results, and their significance was readily apparent. If one could achieve enantioselective syntheses of both 2*R*- and 2*S*-phytochromobilin (**4**) then each enantiomer could be compared directly to naturally derived **4**, examining both their optical properties and their ability to reconstitute with **N-C**. In principle these studies could unequivocally establish the absolute stereochemistry at all three chiral centers in **1**. Of equal importance, experiments of his type might provide a direct means for studying the molecular events involved in the **Pr → Pfr** interconversion (vide infra). In any event, following his lecture I discussed these thoughts with Professor Lagarias, and that was the beginning of a long and fruitful collaboration.

Initially we planned to prepare 2*R*- and 2*S*-**4** by elimination of BnOH from tetrapyrroles **146** and *ent*-**146**, followed by ester hydrolysis (Scheme 44). This approach had precedent from studies by Rüdiger et al. with phycocyanin derived chromophores.[10] However, we were unable to effect the desired elimination. Prolonged thermolysis of **146** provided only trace amounts of phytochromobilin dimethyl ester (2*R*-**5**) accompanied by many products of decomposition. Moreover, all attempts at catalyzing this elimination at ambient temperature failed. At this impasse, though, serendipity again intervened in our favor. While recording NMR data, Dr. Leung had prepared a $CDCl_3$ solution of dihydropyrromethenone **112** and allowed it to stand overnight. The surprising outcome was that **112** was cleanly converted to the known ethylidene derivative **177**, previously employed by Gossauer et al. in a synthesis of racemic **5**. This observation paved the way for our own successful efforts.

The Gossauer synthesis of (±)-**5** followed the classic AB + CD strategy, involving condensation of (±)-**177** with the previously described pyrromethenone **9** (*path a*, Scheme 45).[8] Over the years a number of related strategies have been described, as well as improvements to the syntheses of (±)-**177**, **9**, and analogs of these materials.[56] Despite these improvements, though, we had concerns about employing *path a* for the synthesis of enantiomerically pure, ^{13}C-labeled derivatives of **5**. One of these pertained to the strongly acidic conditions required for decarboxylative condensation (neat TFA), which might cause racemization of **177**. Also, *path a* offered little flexibility for introducing ^{13}C at the requisite *meso*-positions. Finally, we hoped to attain higher yields for the condensation of relatively expensive ^{13}C-labeled precursors. For this purpose *path b* appeared to hold greater promise. The successful realization of this strategy is outlined below.

Mr. Douglas Pippin (Ph.D. Dartmouth College, 2001) was the last of my students who made the move from Wesleyan, and he took responsibility for seeing the project through to a successful conclusion. This entailed considerable skill, enthusiasm, and above all perseverance. Our first attempt at synthesizing **178** closely mirrored our earlier synthesis of **112**, initiated by Pd(0)-catalyzed coupling of iodopyrrole **180** with alkyne amide **96d** (Scheme 46). This step gave good-excellent yields of the expected pyrroloalkyne **181**. However, despite much effort we were unable to effect the cyclization of **181** to the corresponding A,B-dihydropyrromethenone **182**. Mainly this failure was due to the unstable nature of **181**, which underwent competitive decomposition. Eventually this problem was resolved employing the alkyne *acid* **88c**, which participated in a highly efficient Pd(0)-initiated coupling-cyclization reaction with iodopyrrole **180**. In this way we obtained a high yield of enelactone **183** as a single stereo- and geometric isomer.[19p] The structure of **183** was assigned based upon its highly characteristic NMR and IR spectra and confirmed by its eventual conversion to pyrromethenone **178** and finally phytochromobilin dimethyl ester (**5**). The first step in elaborating **183** involved aminolysis at −33 °C, the same conditions that caused decomposition

with *bis*-lactone **173** (cf. Scheme 42). In the present case, though, lactone **183** underwent rapid ring opening followed by keto-amide cyclization to give an essentially quantitative yield of hemiamidal **184**. We evaluated many conditions for effecting the elimination of H_2O and BnOH from **184** without concomitant racemization or isomerization (ethylidenes of type **178** are known to undergo facile *exo*-*endo* double bond migration). In the end the most convenient conditions were modeled on Dr. Leung's observation with **112**, where a similar elimination had taken place in an NMR tube (cf. Scheme 44). On a preparative scale the conversion of **184** to **178** was carried out in a two phase system of $CHCl_3$ and 1 eq 12 N HCl, wherein the desired eliminations took place at RT in >95% overall yield. Compound **178** was obtained as a single enantiomer having exclusively the 3*E*,5*Z*-double bond geometry. In identical fashion, but beginning with alkyne acid *ent*-**88c**, we prepared dihydropyrromethenone *ent*-**178** as a single isomer (not shown).

Our proposed C,D-ring precursor to **5** was the pyrromethenone **179** (cf. Scheme 45), a known compound that we could derive in modest yield by decarboxylation of the corresponding *tert*-butylester **132**. However, we chose to develop a new synthesis of **179** that would be more amenable to subsequent ^{13}C-labeling studies. An additional priority was to avoid the strongly alkaline conditions typically employed in aldol-like condensations with formylpyrrole **12** (cf. Scheme 3). A convenient solution to this problem was realized in the form of the silyloxypyrrole **185**, which Mr. Indranath Ghosh (Ph.D. Dartmouth College, 2003) synthesized on multigram scales from simple starting materials (Scheme 47). The details of this synthesis are found in reference [19o].[19o] Pyrrole **185** is an excellent surrogate for ring D, since it displays strongly nucleophilic properties under mild Lewis acid conditions. With $TiCl_4$, for example, condensation of **12** and **185** took place rapidly at −78 °C and gave a >95% yield of the aldol products **186** (R = OH, TBS). Initially these adducts were isolated and characterized by analytical and spectral data. However, it proved advantageous to carry this mixture forward to the next step. Dissolution of **186** in TFA at ambient temperature initiated a remarkably clean series of transformations. The first of these was dehydration to generate the *meso*-double bond, followed by cleavage of both the Boc protecting group and the *tert*-butyl ester. The resulting pyrromethenone carboxylic acid could be isolated if desired but upon decarboxylation gave a virtually quantitative yield of **187**. The final step to **179** called for selenide oxidation, which we expected would effect spontaneous elimination. In our first experiments this transformation was capricious, in that the liberated *p*-chlorophenylselenous acid reacted rapidly with the unsubstituted pyrrole ring in **179** to give variable yields of adduct **188**. However, this side reaction was minimized by carrying out the elimination step in a two phase system consisting of CH_2Cl_2 and pH 12 buffer. Under these conditions the selenous acid was rapidly removed from the organic layer, and the formation of **188** was limited to trace amounts.

With gram quantities of both **178** and **179** in hand, we were confident that sufficiently mild conditions could be found to effect their coupling to give phytochromobilin dimethyl ester (**5**). However, this turned out not to be the case, since pyrromethenone **179** decomposed rapidly under even mildly acidic conditions (Scheme 48). Fortunately this problem was circumvented by reversing the order of selenoxide elimination and ring coupling. Thus, employing the reagent combination of HCl/Et_2O/MeOH, pyrromethenones **178** and **187** gave a 76% yield of tetrapyrrole **189** with little or no decomposition. Oxidation of selenide **189** was then effected at −78 °C, followed by elimination using the same two phase system described above for pyrromethenone **179**. Phytochromobilin dimethyl ester (**5**) thus prepared was obtained as the 2*R*-enantiomer exclusively, constituting the first synthesis of this material in its naturally derived form. Ms Lixia Shang in Professor Lagarias's lab determined that our synthetic 2*R*-**5** had identical CD spectra to authentic **5**, thereby confirming the absolute configuration at C_2 in phytochrome (**1**) as *R* (Figure 6).[57]

In identical fashion, but employing *ent*-**88c**, we synthesized the optical antipode of phytochromobilin (*ent*-**5**), whose CD spectra displayed the expected mirror image relationship to that of 2*R*-**5** (Figure 7).[19p]

Finally, upon hydrolysis of 2*R*-**5** to 2*R*-**4,** and reconstitution with recombinant-derived **N-C**, Dr. Lagarias' laboratory produced totally synthetic phytochrome (**1**) having identical difference spectra to native **1** (Figure 8).[57]

9. Probing the site of Pr photoisomerization

Various spectroscopic techniques have been used in attempts to confirm the site of *E,Z*-isomerization in phytochrome (**1**). Among these the most powerful tool is Resonance Raman Spectroscopy, which is well suited for studying prosthetic groups *in vivo*.[58] In 1988, Mathies and Lagarias reported the first Resonance Raman study of the **Pr** form of phytochrome,[4e] using 752 nm excitation to avoid interference from fluorescence. Vibrational assignments were made for **Pr** by comparison with spectra derived from model compounds and by nitrogen deuteration experiments. In 1990 these studies were extended to the **Pfr** form of phytochrome, which has frequencies and intensities that are distinctly different from those of **Pr**.[4g] In particular the absorption at 803 cm^{-1} in **Pr** is replaced by an unusually intense peak at 814 cm^{-1} in **Pfr**. Calculations on model tetrapyrrole chromophores suggest that these low-energy absorptions are due to hydrogen out-of-plane (HOOP) wagging vibrations involving the C_{15}-methine hydrogen.[4g] These calculations lend support to the hypothesis that the **Pr** chromophore has a C_{15}-*Z*, sy*n*-configuration, while the **Pfr** form adopts an *E*, *anti*-configuration (*cf.* Figure 3).[12e]

Prior to the reconstitution experiments described above this hypothesis would be difficult to test. However, the capability of reconstituting native phytochrome from recombinant **N-C** and **4** changes this situation, particularly if the appropriate ^{13}C-phytochromobilin derivatives are available. In principle this combination would provide a means for definitively assigning the pertinent Raman bands for both **Pr** and **Pfr**. For example, reconstitution of recombinant apophytochrome (**NC-1**) with $^{13}C_{15}$-labeled phytochromobilin ($^{13}C_{15}$-**4**) would produce native phytochrome specifically labeled at C_{15} (*refer to* Figure 5 for visualization). Resonance Raman analysis of $^{13}C_{15}$-**Pr** would then enable assignment of individual vibrational transitions to specific chromophore bonds. Specifically, were the absorption at 803 cm^{-1} in **Pr** due to the C_{15}-HOOP wagging vibration,[4g] this peak would be shifted by ~5 cm^{-1} to lower frequencies in $^{13}C_{15}$-**Pr**.[59] Upon photoisomerization, $^{13}C_{15}$-**Pr** would produce $^{13}C_{15}$-**Pfr** also specifically labeled at C_{15}. In $^{13}C_{15}$-**Pfr** the intense absorption at 814 cm^{-1} in non-labeled **Pfr** would be shifted to approximately 809 cm^{-1}. These results could be corroborated with phytochromobilin specifically deuterated at C_{15} but *unlabeled* with ^{13}C. In this case the shift in absorption should be even larger for both **Pr** (C_{15}-D) and **Pfr** (C_{15}-D).[60] In turn, repetition of these experiments with $^{13}C_{4,5}$- and $^{13}C_{10}$-labeled phytochromobilin would permit identification of the H_5- and H_{10}-wagging vibrations, respectfully. If the postulated mechanism of *E,Z*-isomerization about C_{15} were correct, these species would exhibit no absorption shift upon photoisomerization to **Pfr**. Our collaboration with Professor Lagarias afforded a unique opportunity to test these assumptions.

Mr. Pippin set out to prepare the requisite ^{13}C-labeled 2*R*- and 2*S*-phytochromobilin ester derivatives (**5** and *ent*-**5**), focusing on ^{13}C-incorporation at $C_{4–5}$, C_{10} and C_{15} (Figure 9; we included *ent*-**5** to determine if chirality at C_2 had a significant impact on reconstitution). With only slight modifications this was accomplished employing the same methodology as for the non-labeled compounds, but incorporating the appropriate ^{13}C-labeled precursors. For example, pyrroles ^{13}C-**180** and ^{13}C-**12** were synthesized in analogous fashion to the parent compounds, but substituting ^{13}C-DMF as the source of ^{13}C. In like manner we prepared enantiomerically pure alkyne acids ^{13}C-**88c** and ^{13}C-*ent*-**88c**, beginning with *S*and *R*-**86c** and

bis-^{13}C-labeled trimethylsilylacetylene (TMSA). Details of these syntheses can be found in the Supporting Information of reference [19p]. Following this approach we synthesized all permutations of ^{13}C-**2***R*- and ^{13}C-**2***S*-phytochromobilin (**4**), specifically labeled at the positions most suitable for study by surface enhanced Resonance Raman Spectroscopy (Figure 7). Reconstitution studies with these materials are ongoing in Professor Lagarias's laboratory and should provide definitive information regarding the photoactivation of **Pr** (**1**) and the role of **Pfr** (**2**) in inducing photomorphogenesis.

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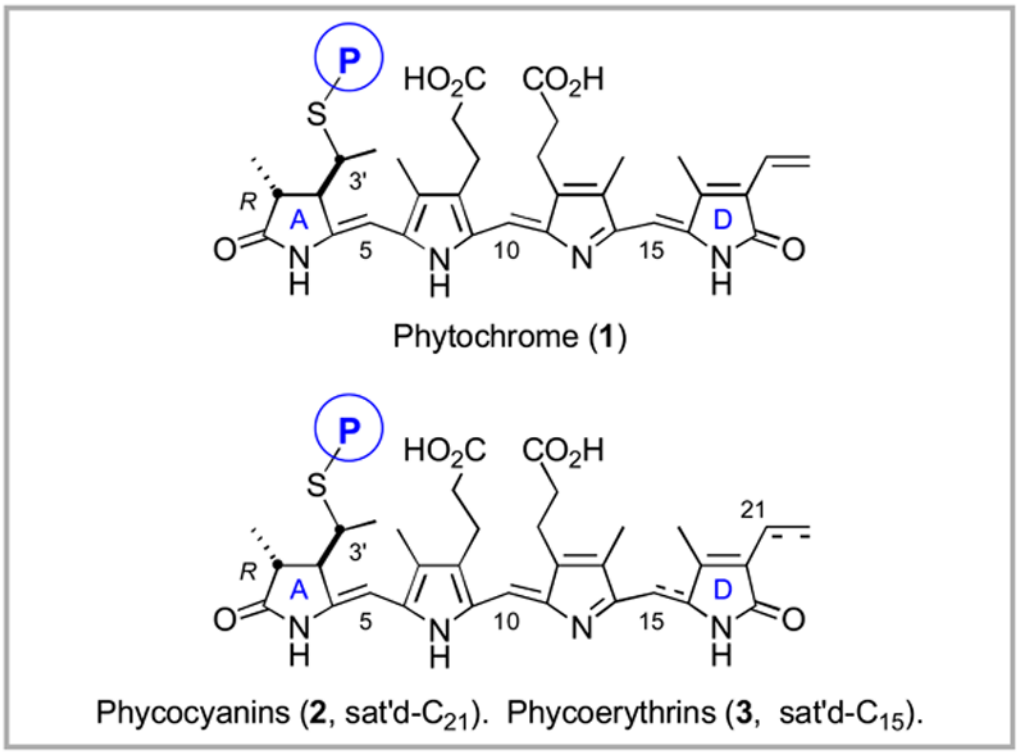


Figure 1.
Phytochrome (**1**) controls photomorphogenesis in higher plants. The phycocyanins (**2**) and phycoerythrins (**3**) are auxiliary pigments in photosynthesis.

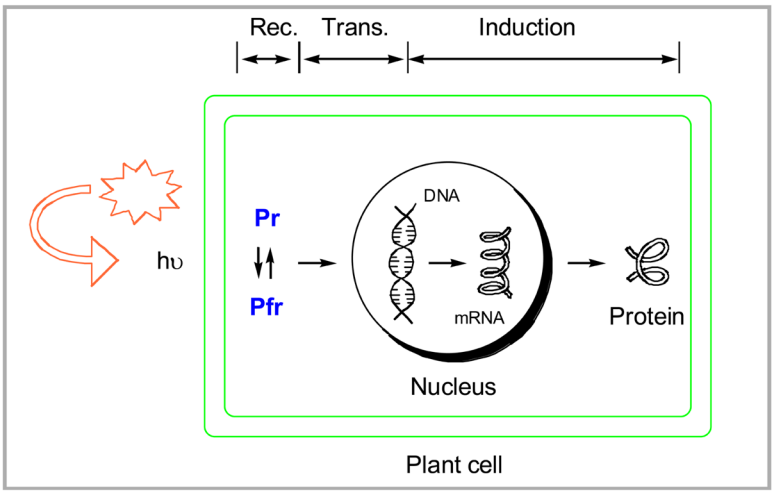


Figure 2.
Schematic representation of photomorphogenesis: (a) **Pr** undergoes photoisomerization to a mixture of **Pr** and **Pfr** (reception); (b) the formation of **Pfr** initiates transduction, where the light signal is communicated to plant genes in the cell nucleus; and (c) certain activated (induced) genes undergo transcription to mRNA and translation to proteins that govern plant growth cycles.[1e]

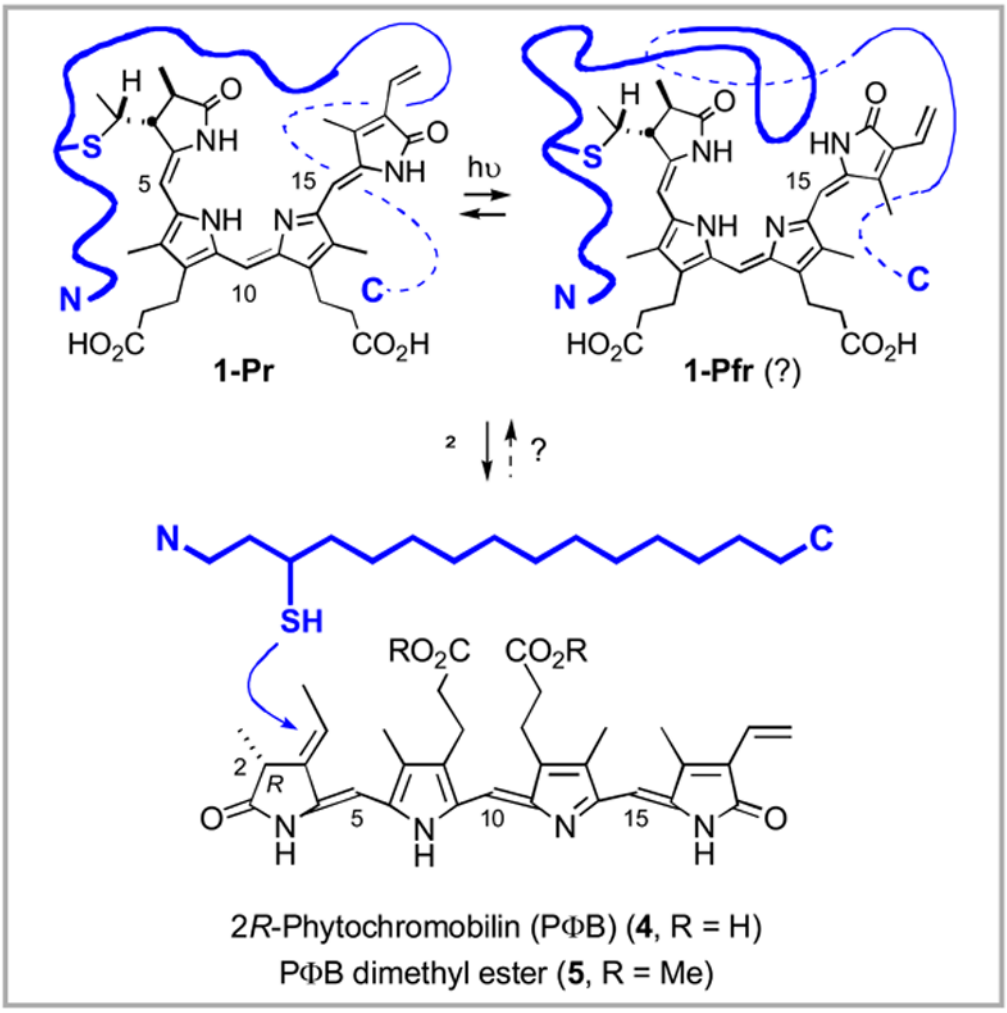


Figure 3.
(a) One proposal for the photochemical interconversion of **Pr** and **Pfr**. (b) Thermal elimination of the phytochrome apoprotein **N-C** to give phytochromobilin (**PΦB**, **4**).

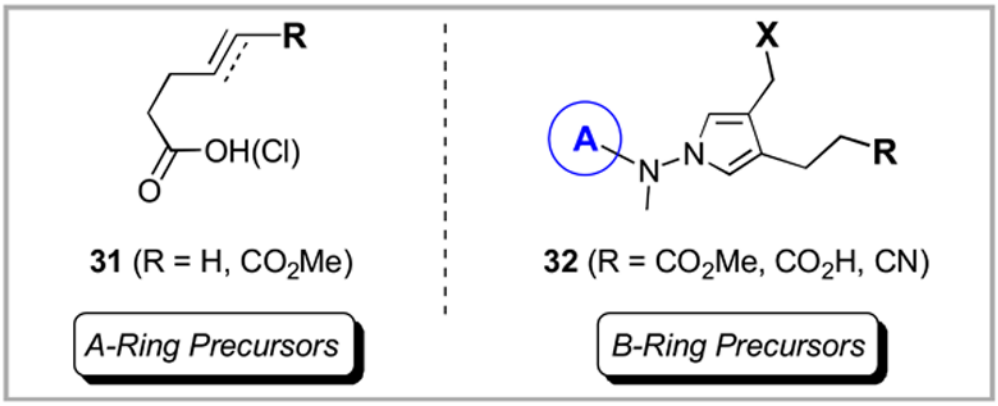


Figure 4.
A- and B-ring precursors for model studies.

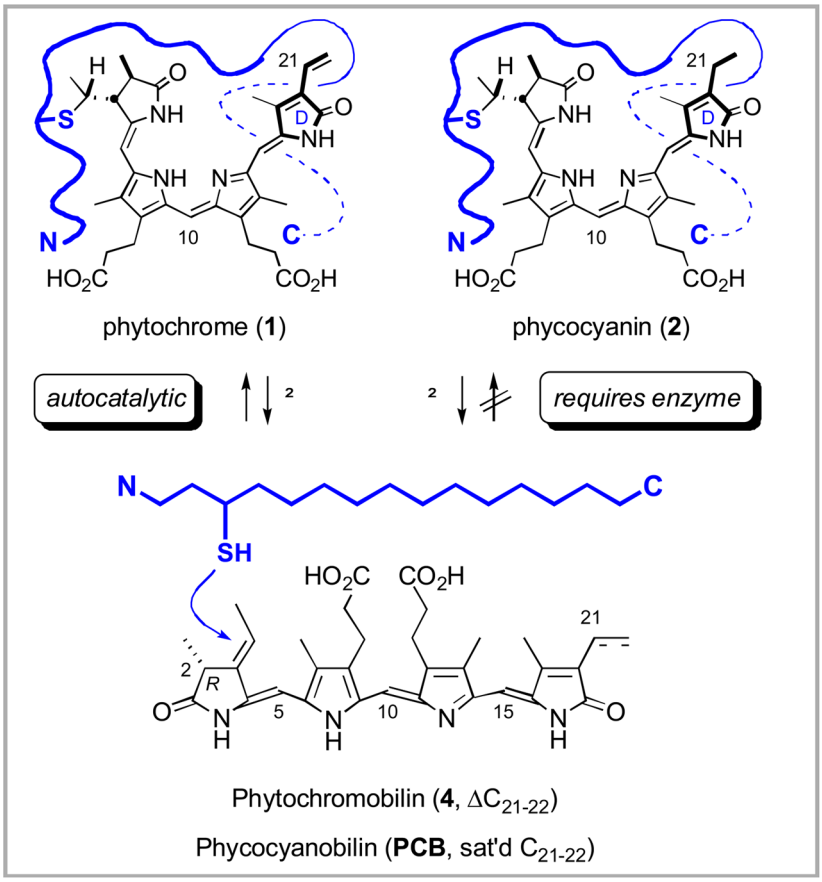


Figure 5.
Phycocyanobilin reassembly requires enzymes. Phytochromobilin reassembly is autocatalytic.

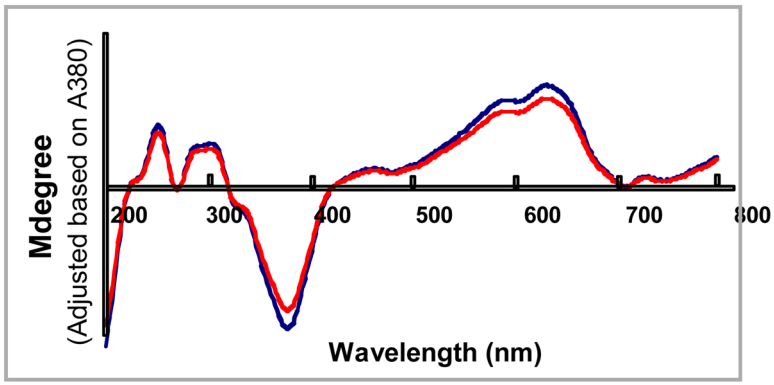


Figure 6.
CD-Spectra of synthetic 2*R*-(blue) and natural (red) phytochromobilin dimethyl ester (**5**).

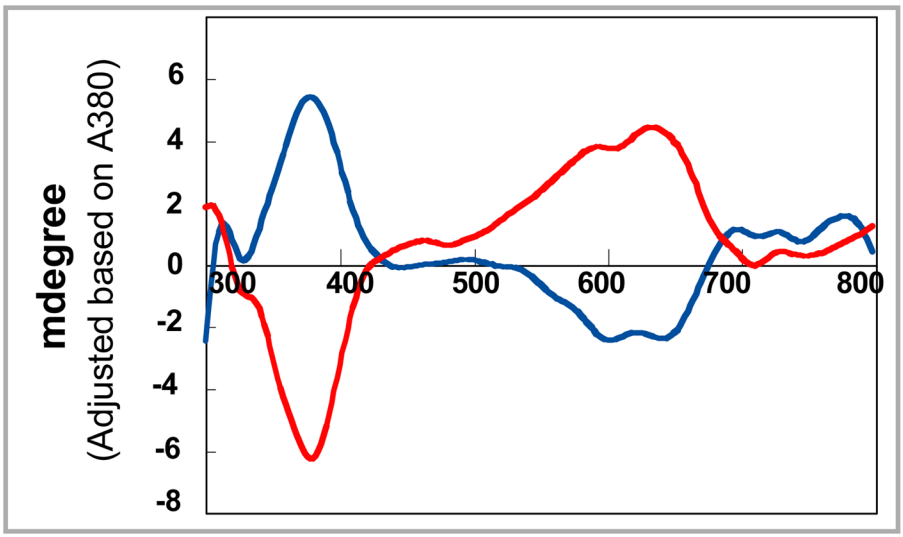


Figure 7.
CD-Spectra of synthetic 2*R*-(red) and 2*S*-(blue)-phytochromobilin dimethyl ester (**5**)

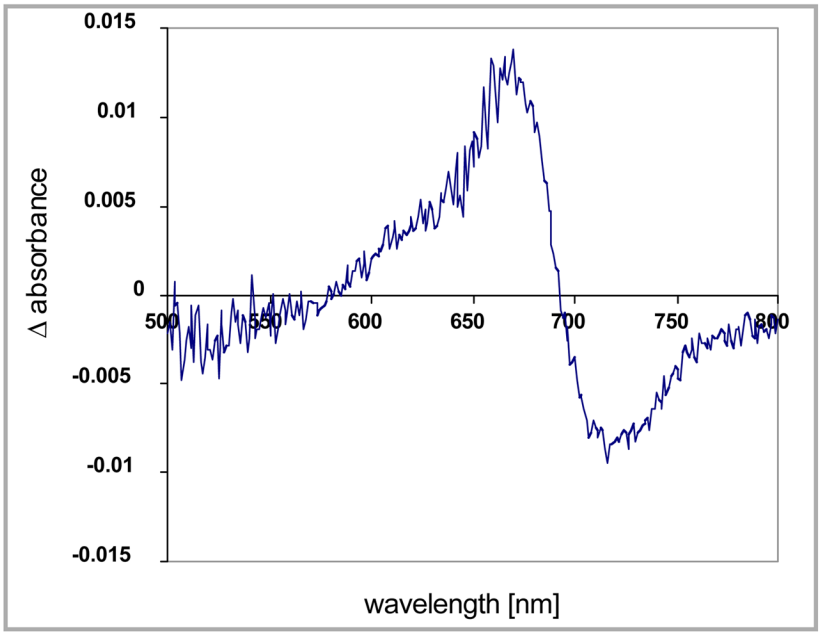


Figure 8.
Difference Spectra obtained after incubation of recombinant Cph 1 **N-C** with synthetic 2*R*-phytochromobilin (**4**).

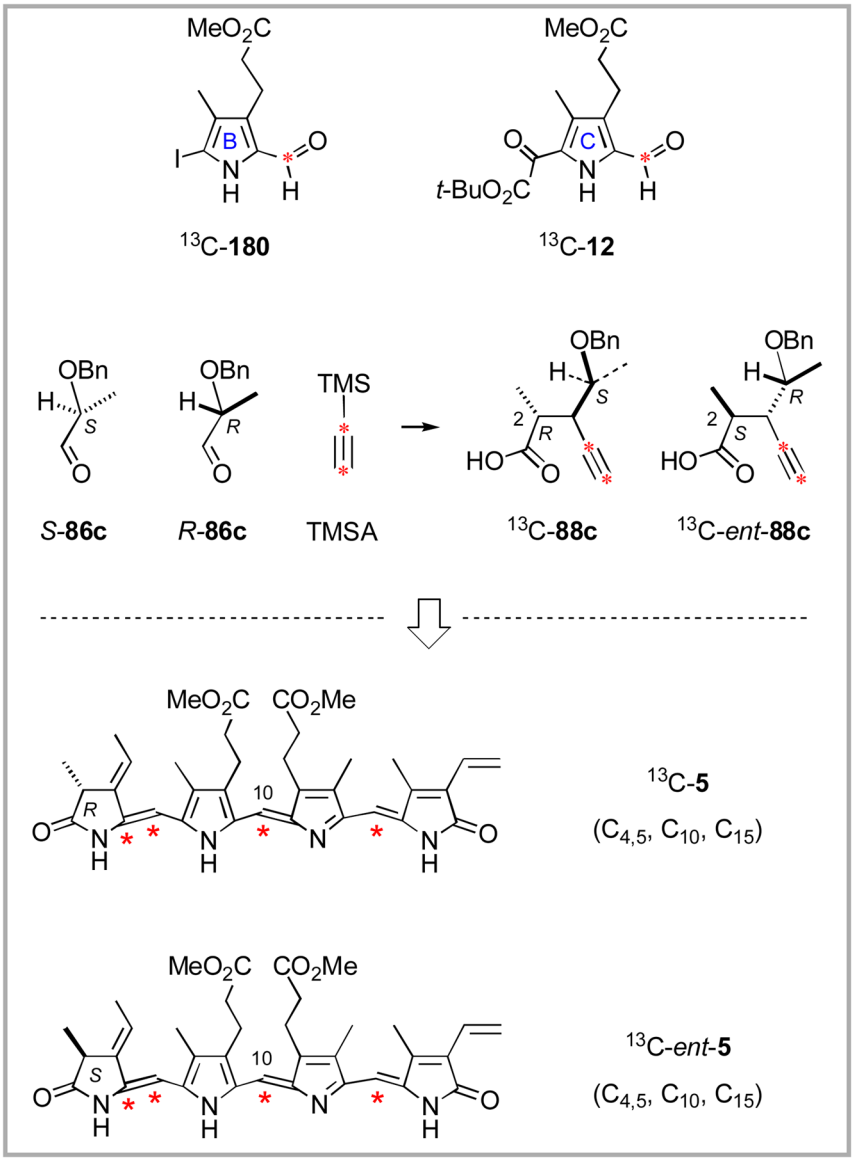
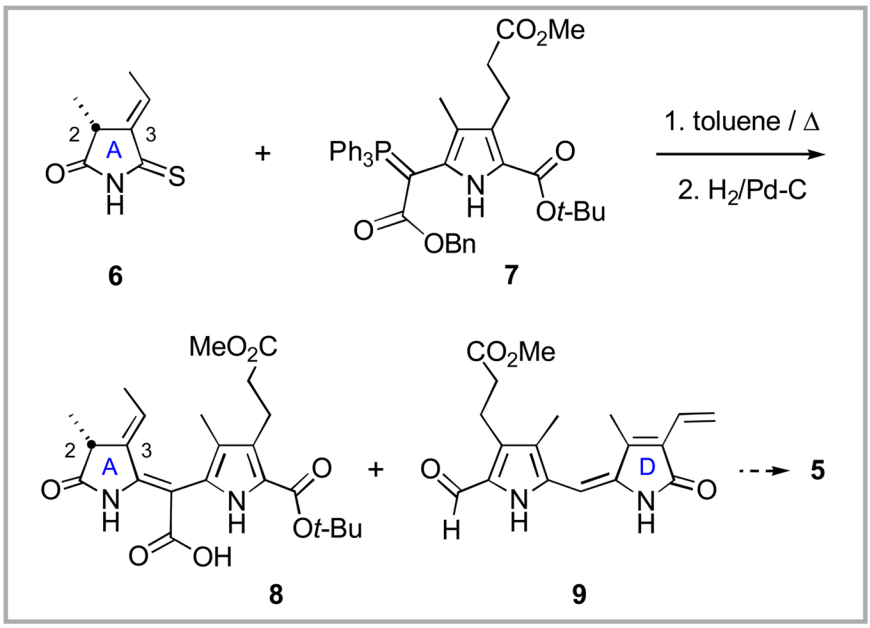
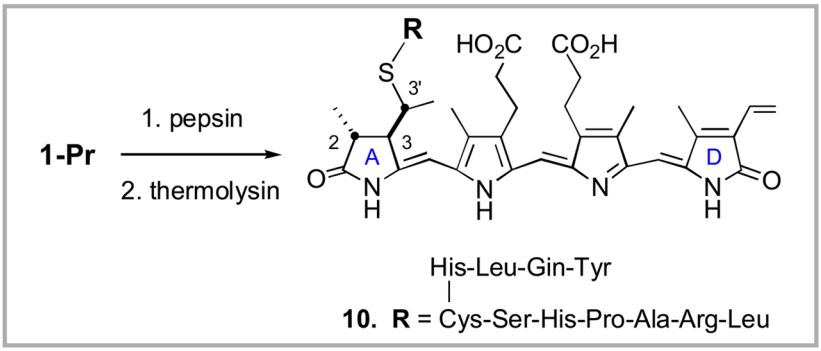


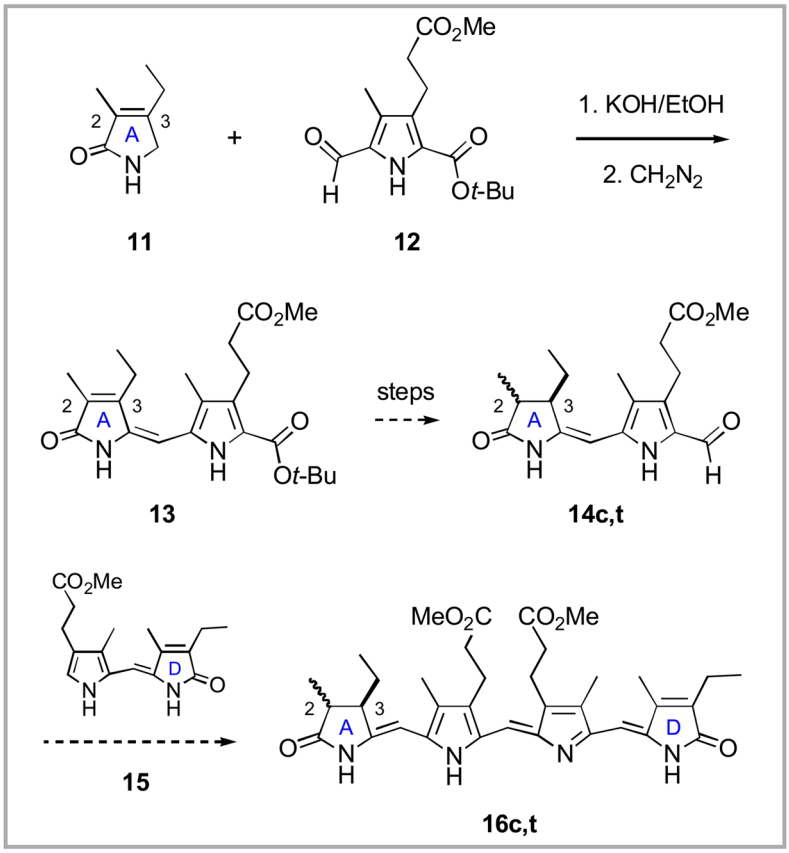
Figure 9.
Synthesis of ^{13}C-labeled 2*R*- and 2S-phytochromobiln esters



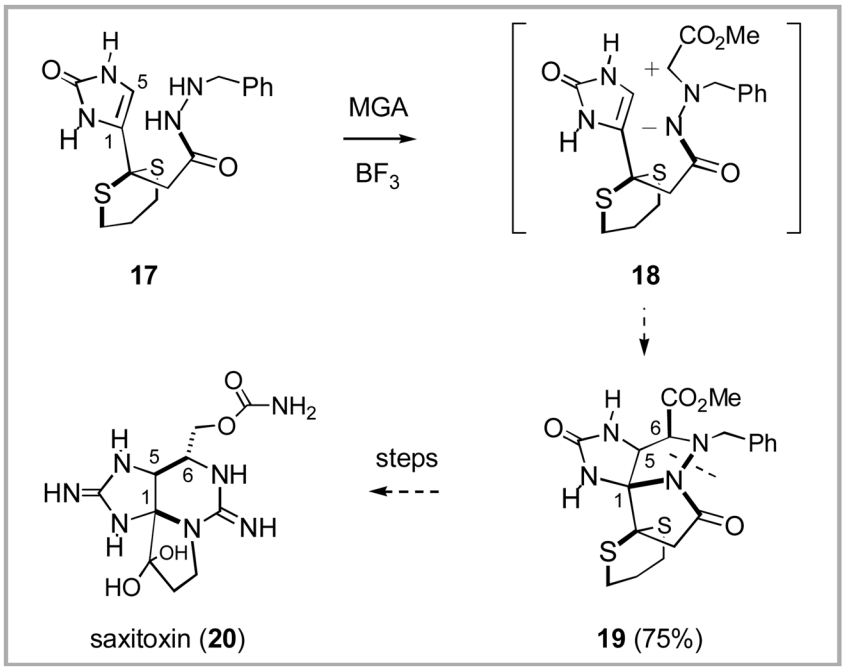
Scheme 1.
Synthesis of (±)-phytochromobilin dimethylester (**5**) by the AB + CD approach (Ref. 8e).



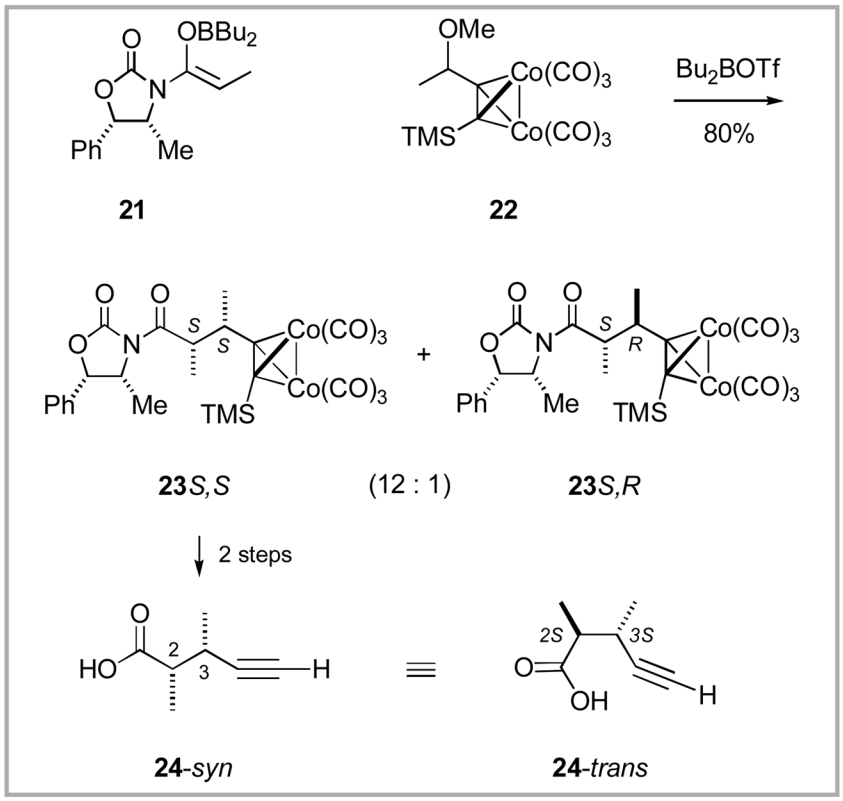
Scheme 2.
Sequential pepsin-thermolysin digestion of the **Pr** form of **1** yields phytochromobilipeptides suitable for NMR analysis.



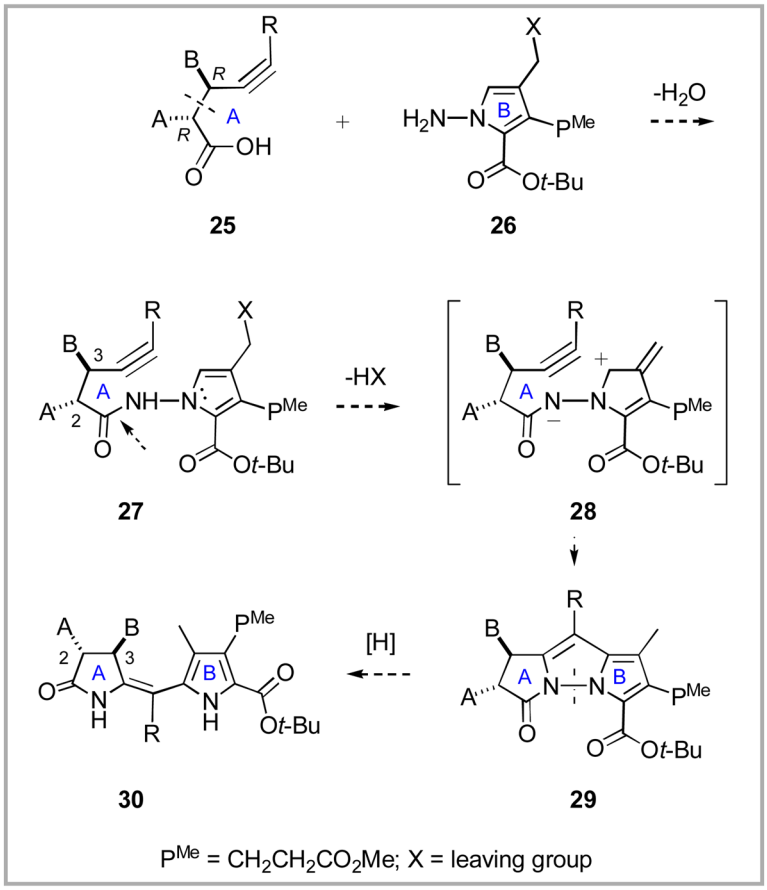
Scheme 3.
Synthesis of model chromophores to establish the relative stereochemistry between C_2 and C_3 (Ref. 8a).



Scheme 4.
The azomethine imine route to saxitoxin (**20**).

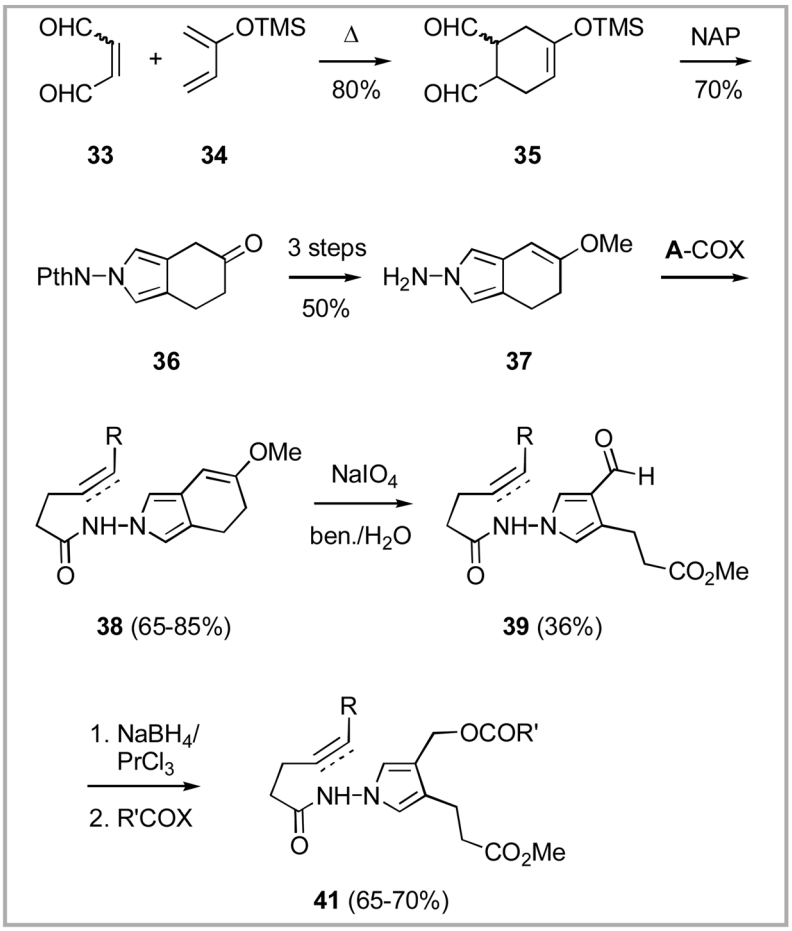


Scheme 5.
Schreiber's asymmetric synthesis of alkyne-acid synthons.



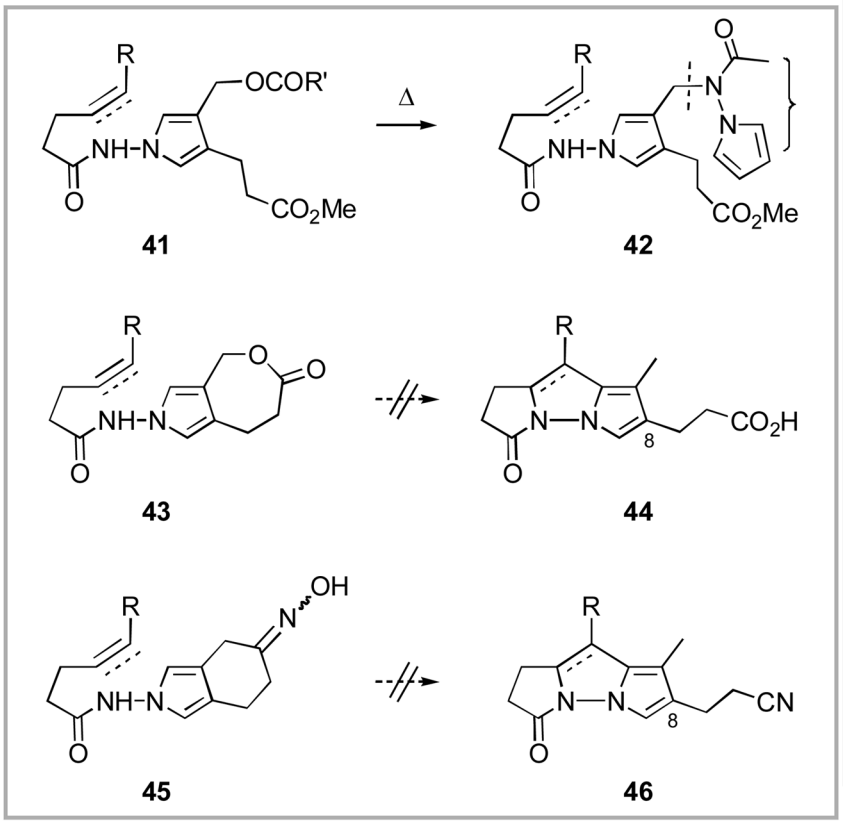
Scheme 6.

The azomethine imine strategy had precedent from our synthesis of saxitoxin (**20**).



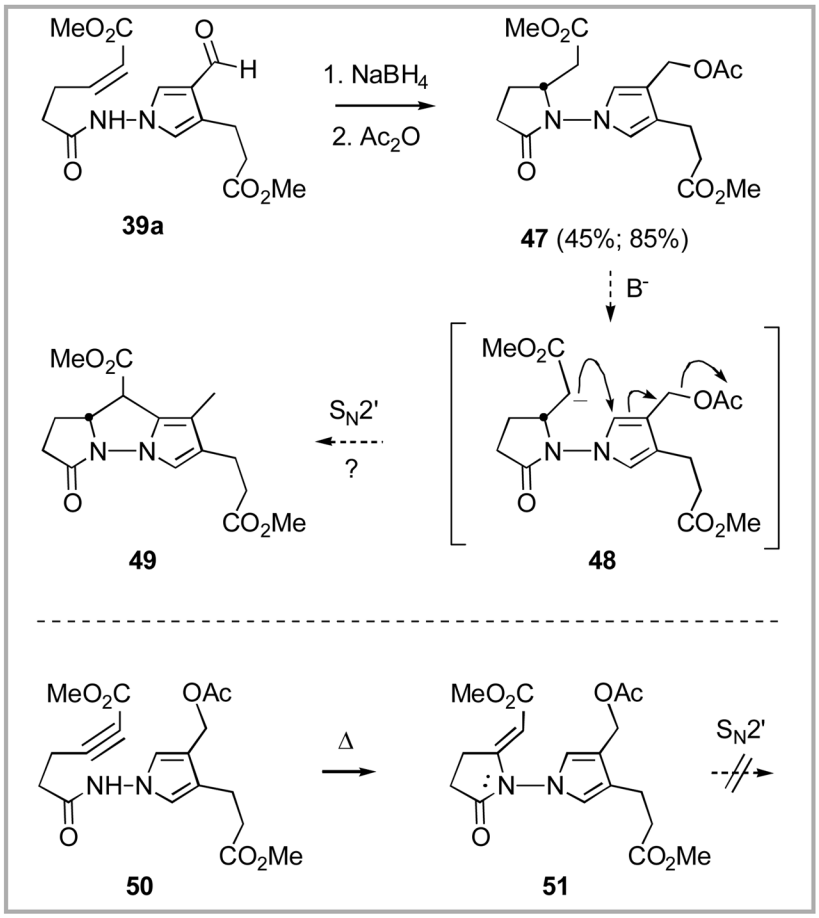
Scheme 7.

Synthesis of the key N-aminopyrrole **37** and its elaboration to the first model hydrazides **41** (NAP = N-aminophthalimide).

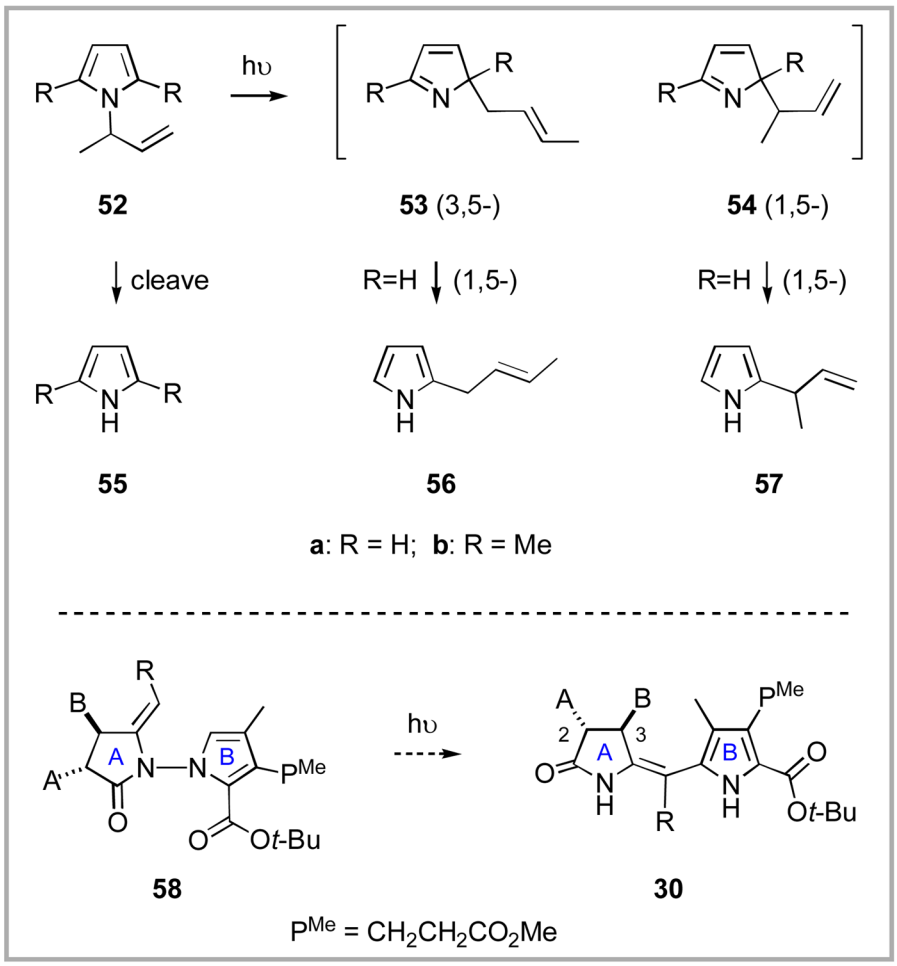


Scheme 8.

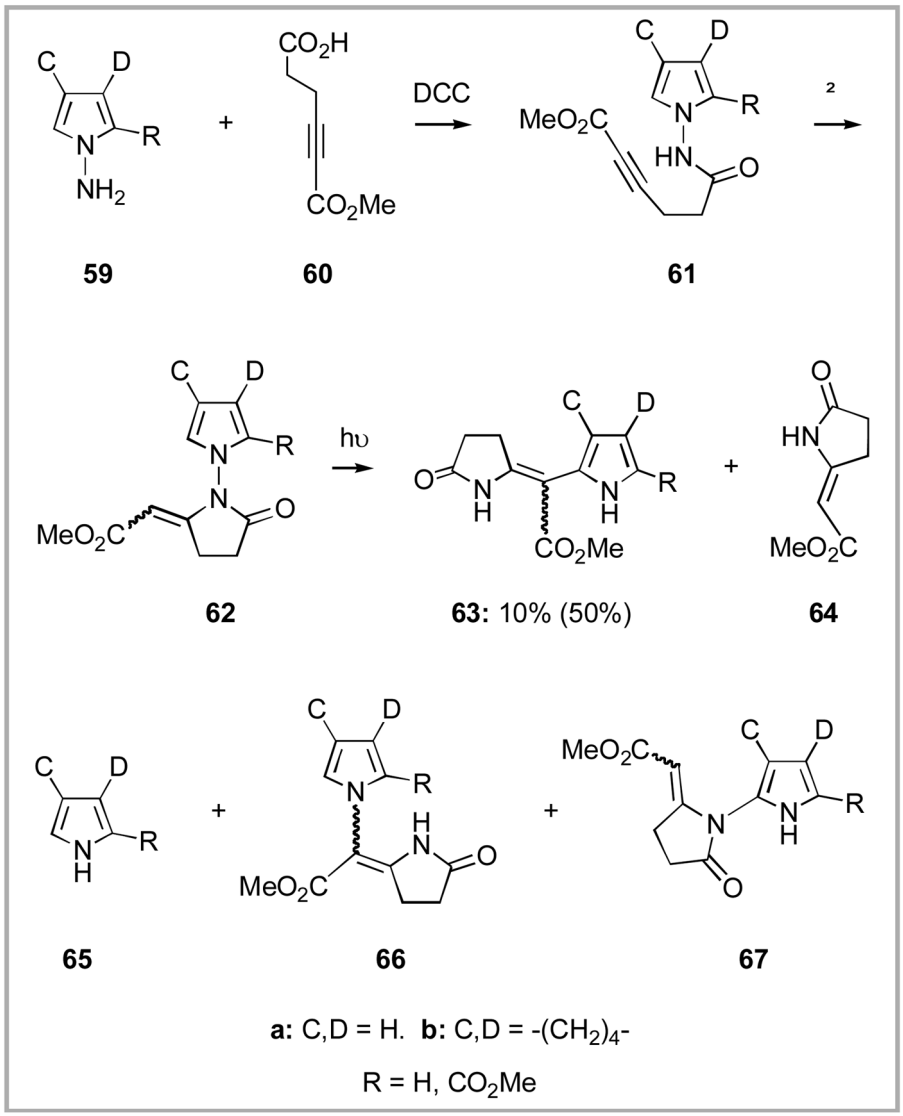
Attempted azomethine imine generation with substrates **41**, **43** and **45**. In no case were the desired cycloadducts observed.



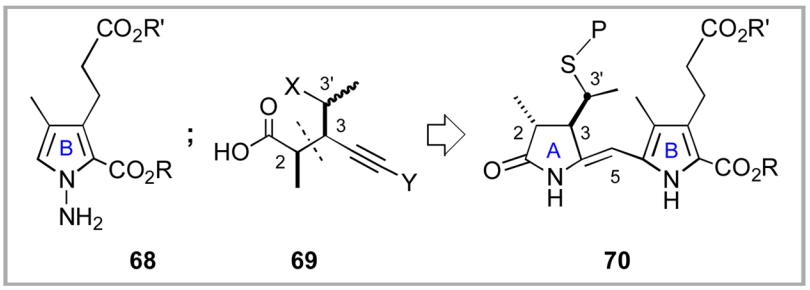
Scheme 9.
A brief S_N2' interlude.



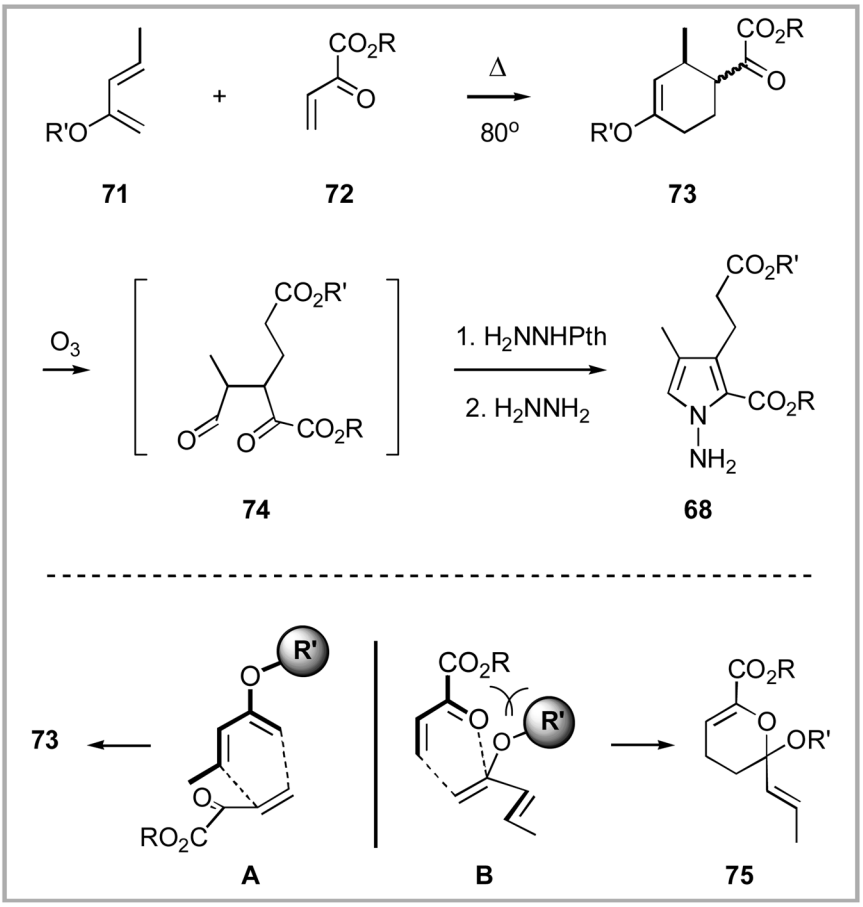
Scheme 10.
Dihydropyrromethenones by 3,5-sigmatropic rearrangement of N-pyrroloenamides.



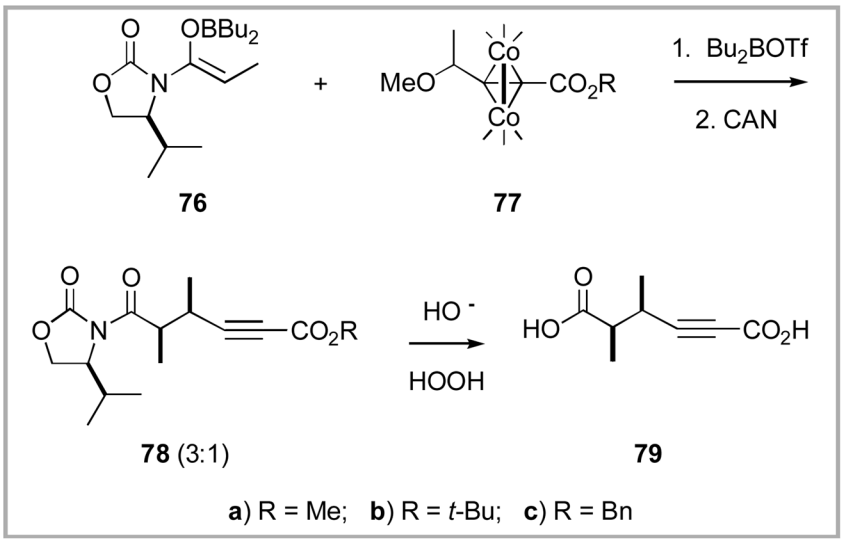
Scheme 11.
Photochemical rearrangement of N-pyrroloenamides **62**. Piperylene (triplet quencher) significantly increases the yield of **63**.



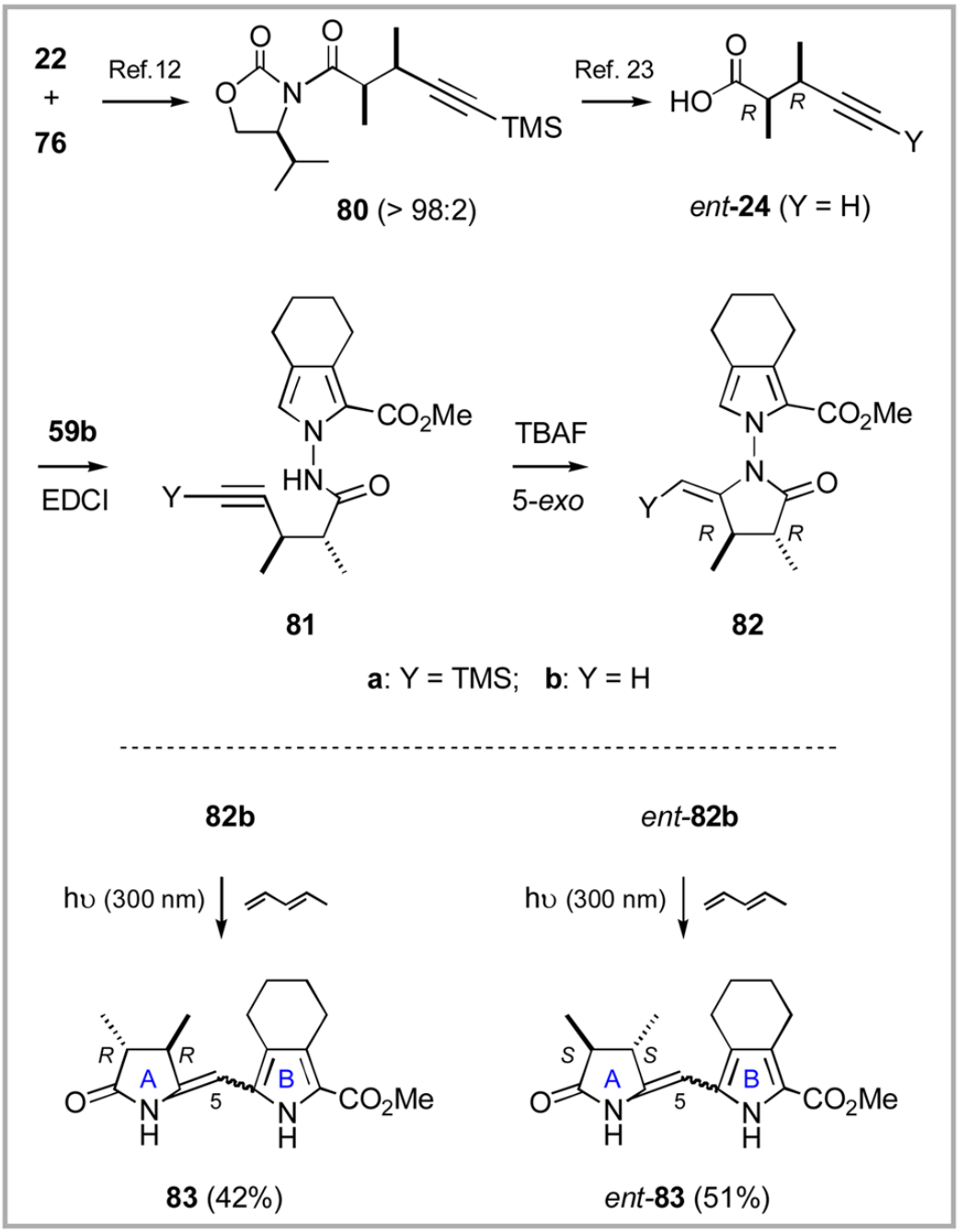
Scheme 12.
Precursors for the A,B-ring of phytochrome (**1**).



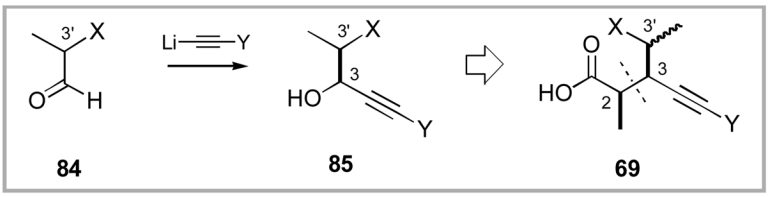
Scheme 13.
Regiospecific synthesis of N-aminopyrroles **68**.



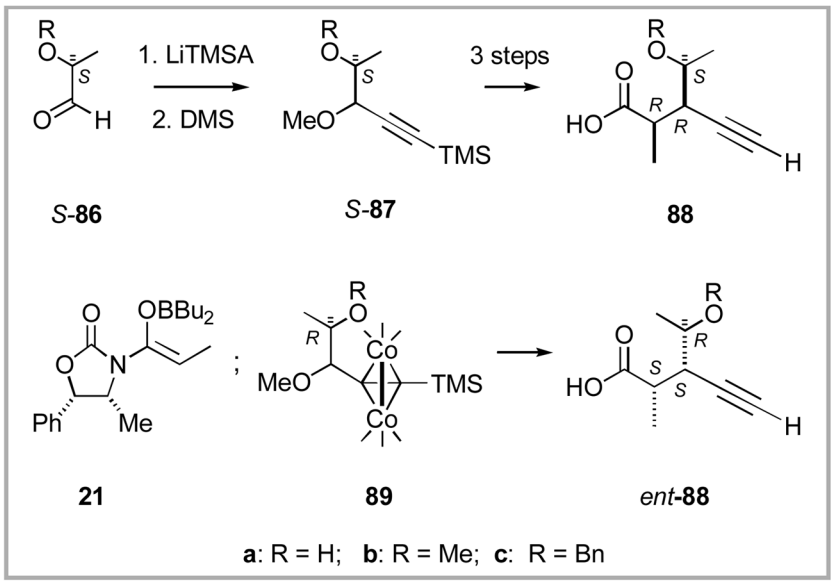
Scheme 14.
Syn-selectivity is influenced by the alkyne substituent.



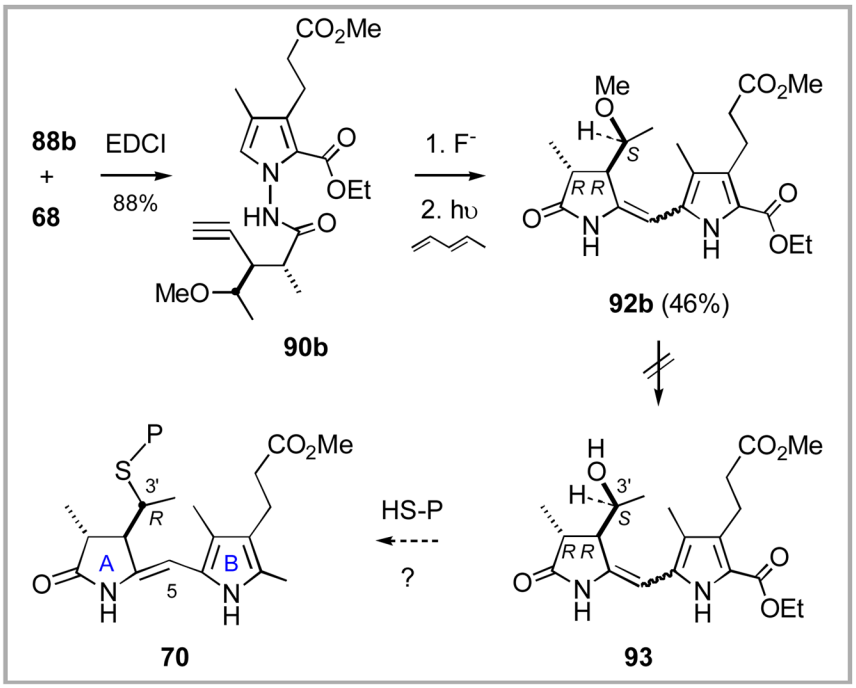
Scheme 15.
TBAF-catalyzed cyclization of alkyne hydrazides.



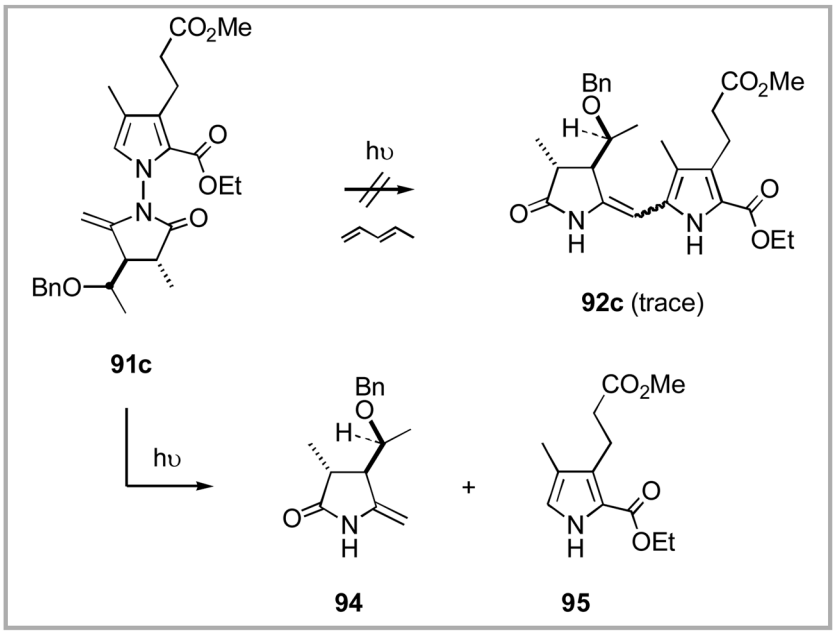
Scheme 16.
Strategy for synthesizing homochiral ring-A precursors.



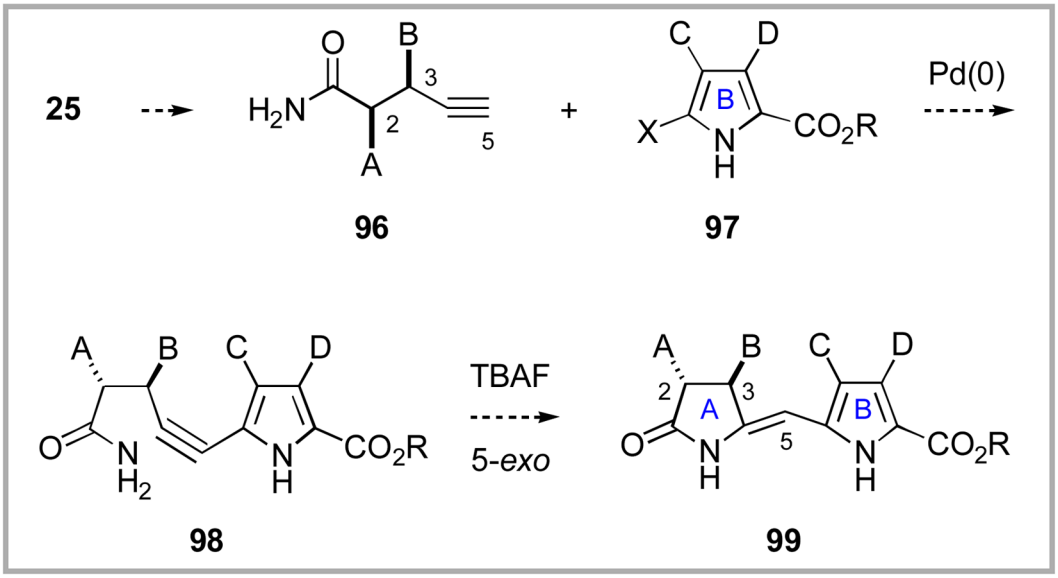
Scheme 17.
Synthesis of the key alkyne acid precursors to ring-A.



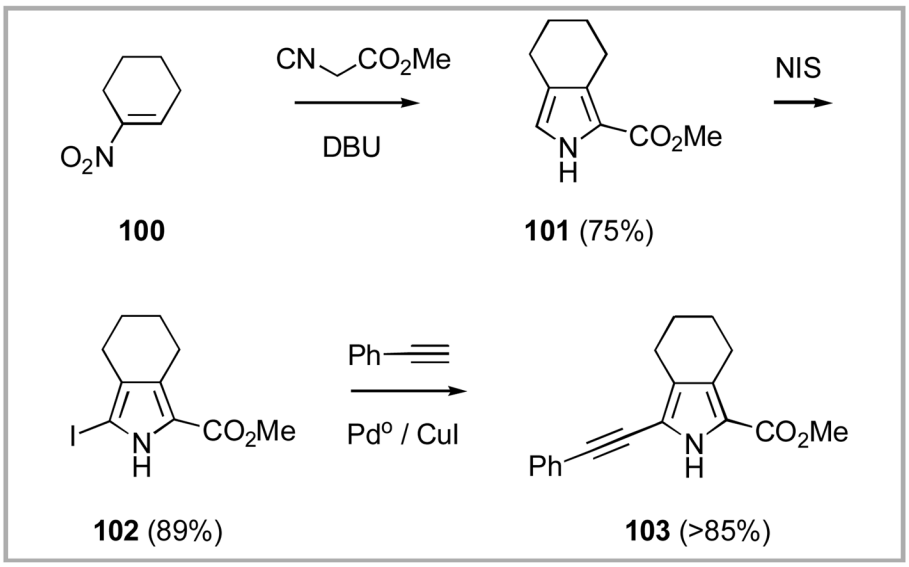
Scheme 18.
Introducing the three chiral centers at C_2, C_3 and C_3'.



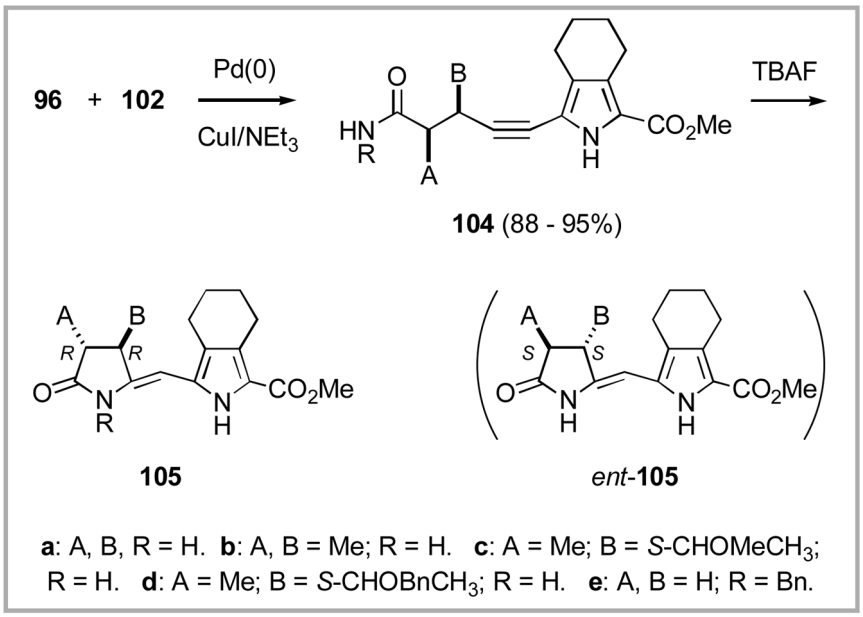
Scheme 19.
Benzyl ethers fail to undergo the desired rearrangement.



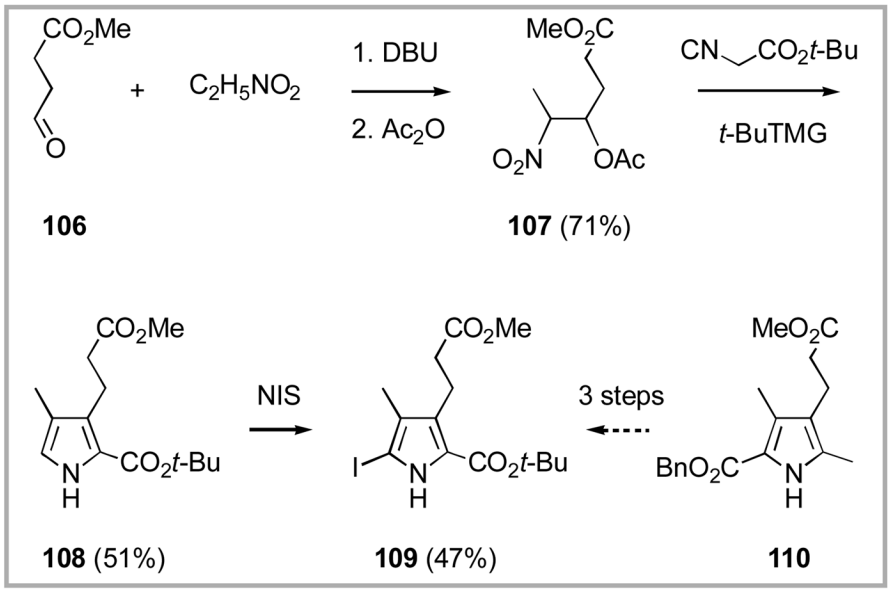
Scheme 20.
General features of the Pd(0)-coupling strategy.



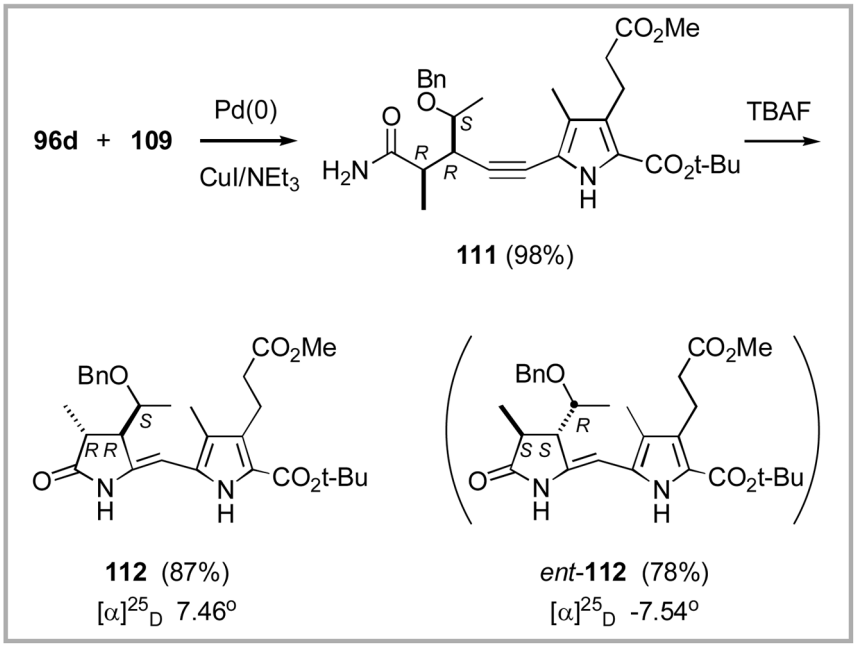
Scheme 21.
Optimizing the Sonogashira coupling.



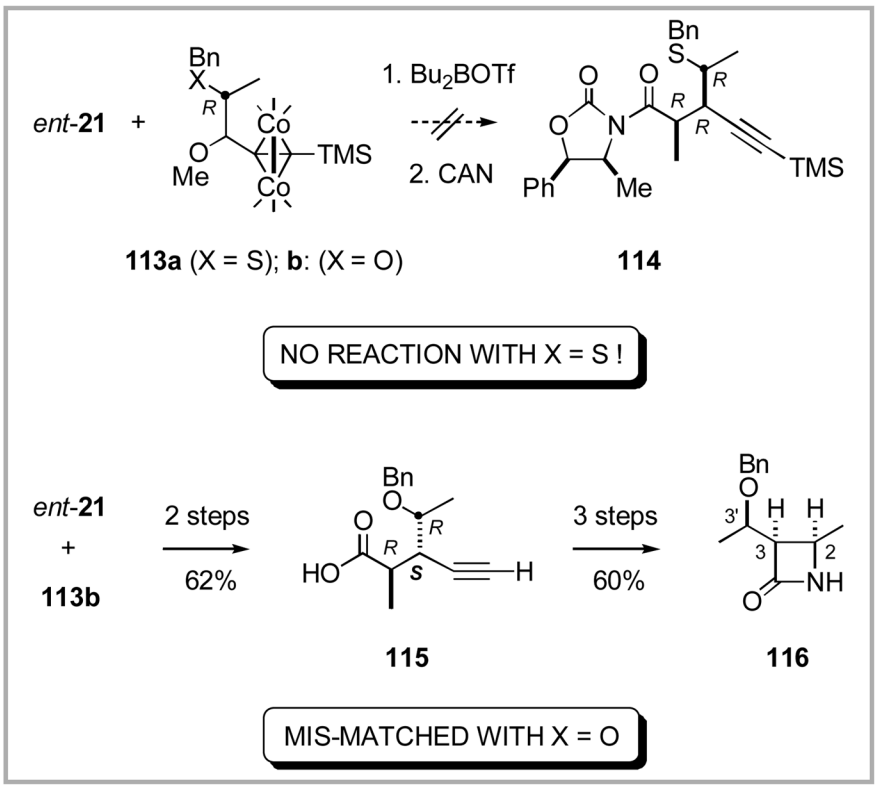
Scheme 22.
Model studies for the “Pd” strategy.



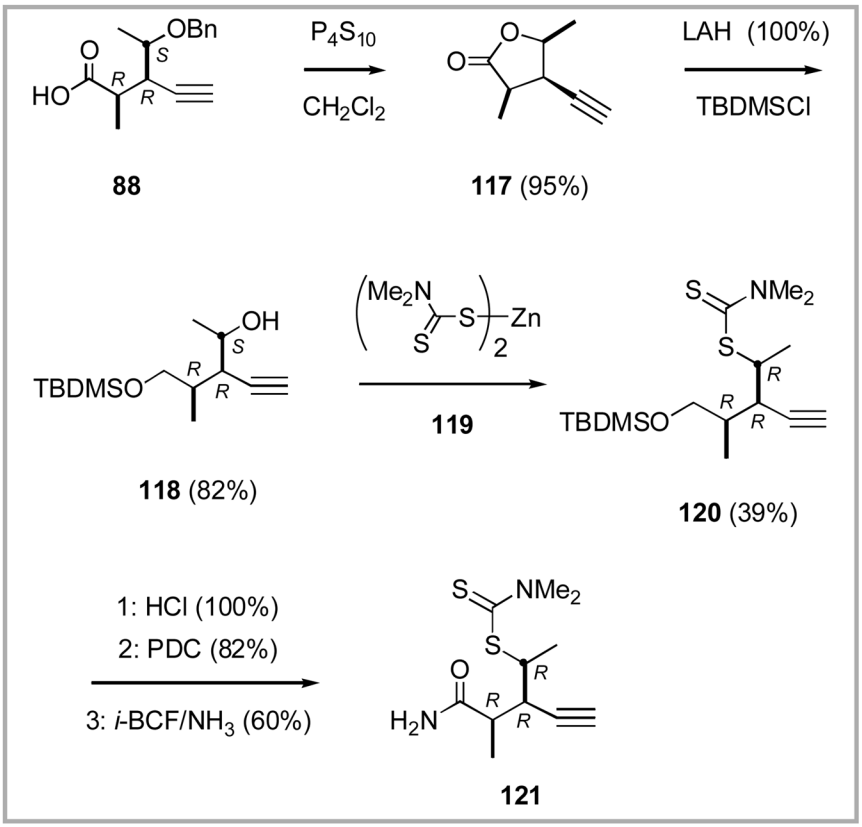
Scheme 23.
Synthesis of the “real” ring-B of phytochrome (**1**).



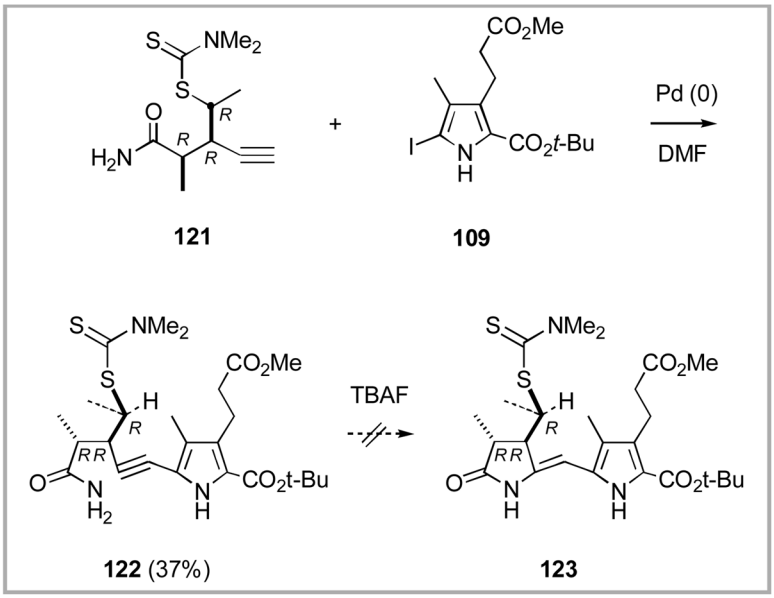
Scheme 24.
Synthesis of the “real” ring-A of phytochrome (**1**).



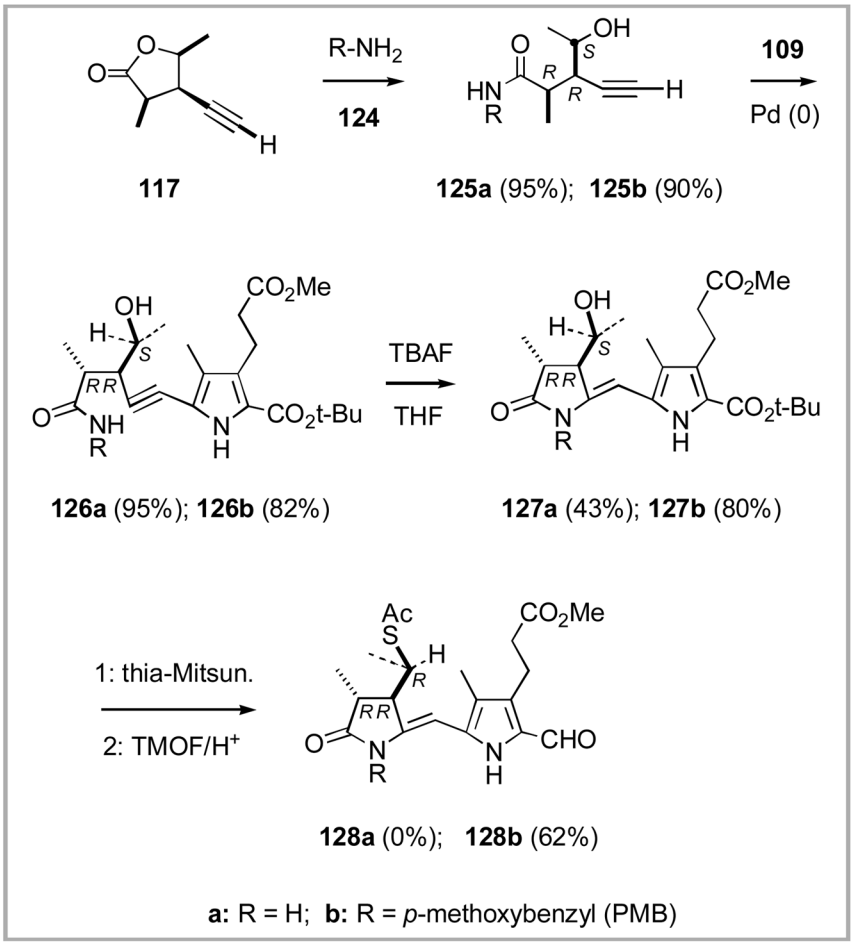
Scheme 25.
Complications introducing the $C_{3'}$-mercaptide group.



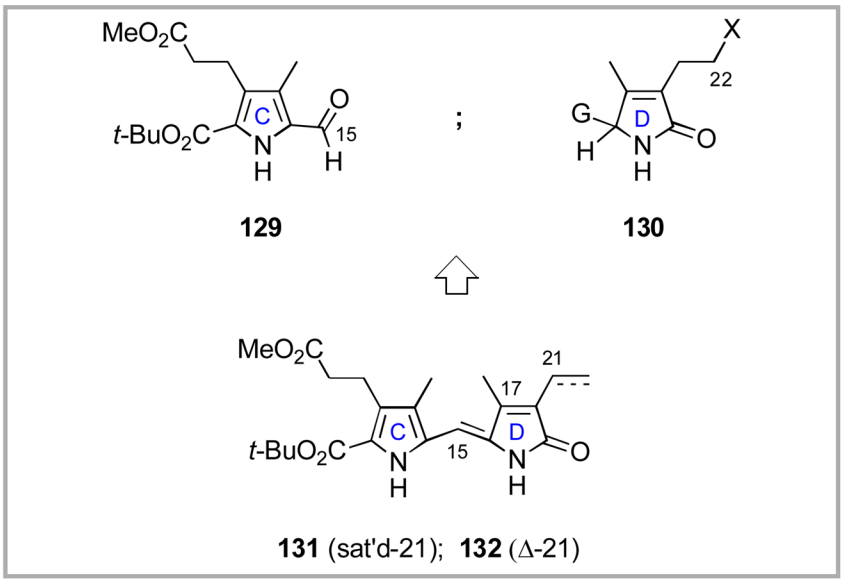
Scheme 26.
First successful introduction of a C_3'-R mercaptide.



Scheme 27.
Sulfur interferes with both key steps.

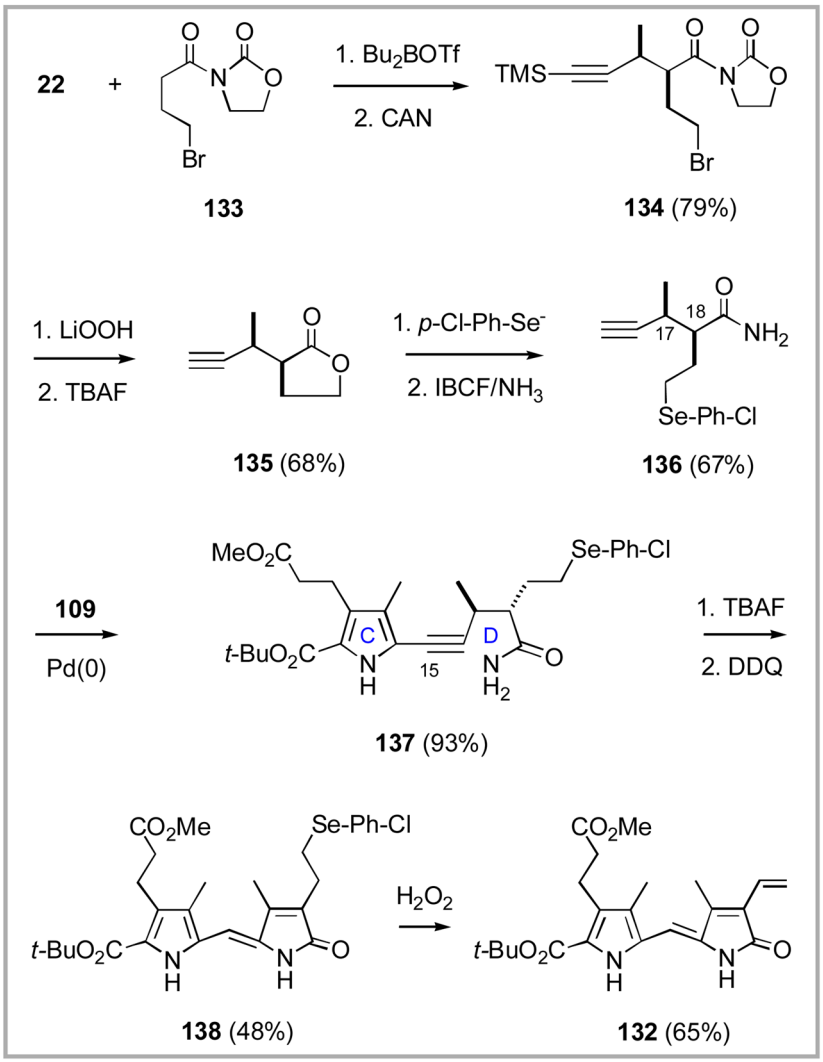


Scheme 28.
First enantioselective synthesis of the core A,B-ring pyrromethenone of phytochrome (**1**).



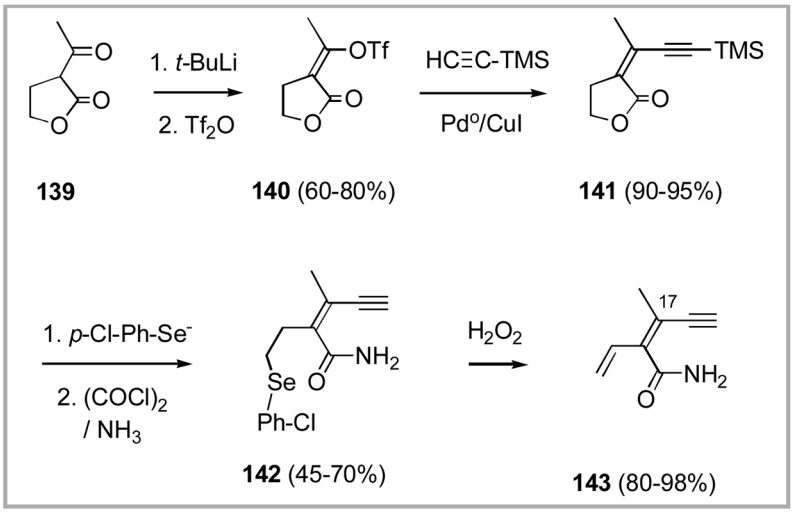
Scheme 29.

The “Gossauer strategy” for synthesizing the C,D-ring pyrromethenone of biliprotein chromophores.

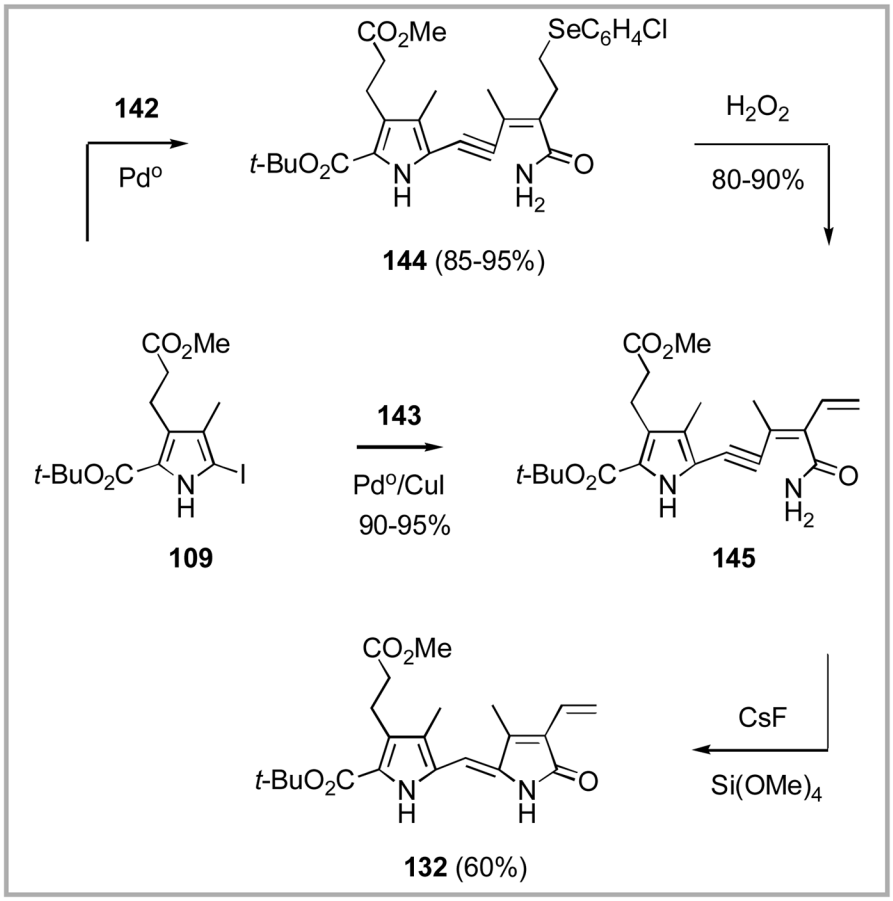


Scheme 30.

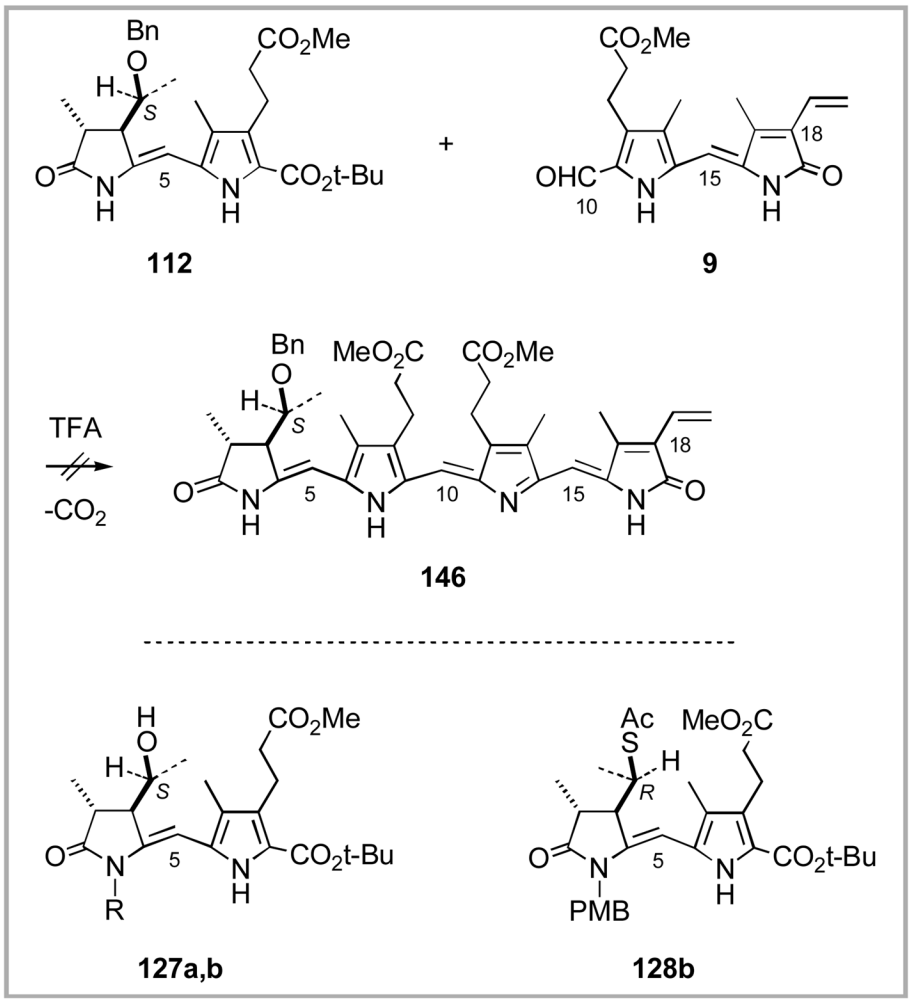
The Nicholas-Schreiber route to C,D-pyrromethenone **132**. Another example of "over-engineering"?



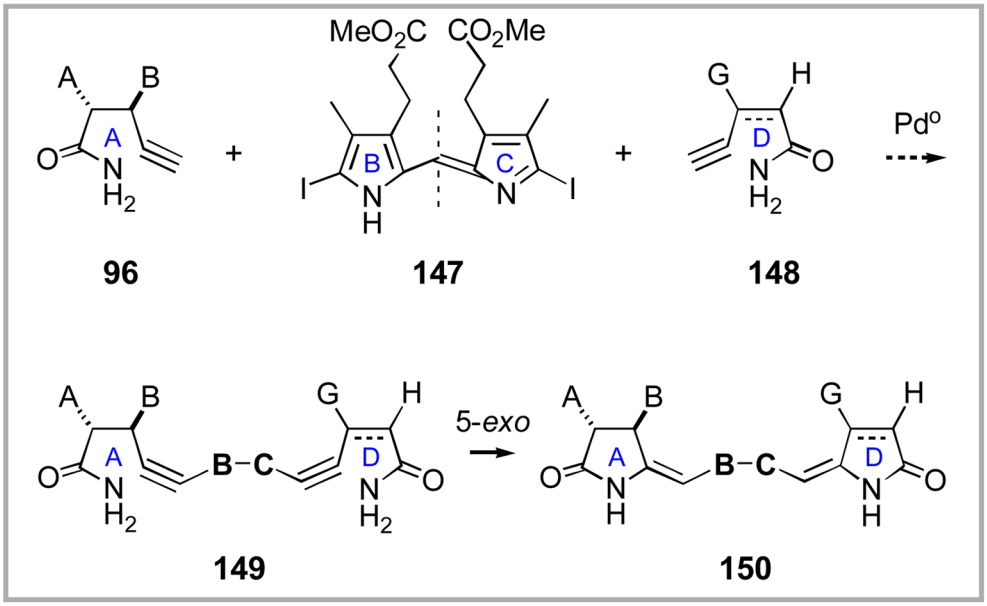
Scheme 31.
Synthesis of the ring-D precursor 143 of proper oxidation state and geometry.



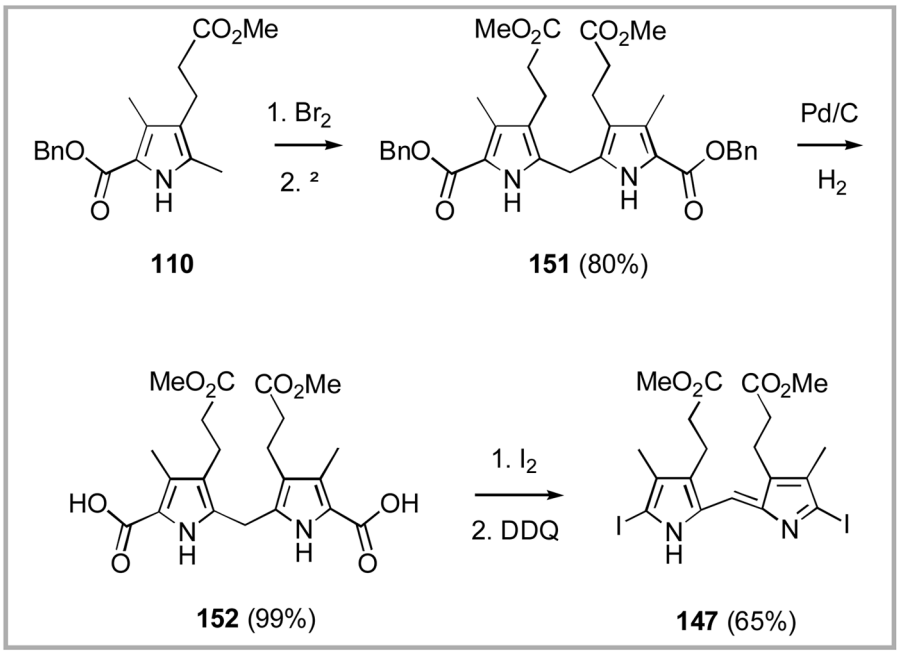
Scheme 32.
Second generation synthesis of pyrromethenone **132**.



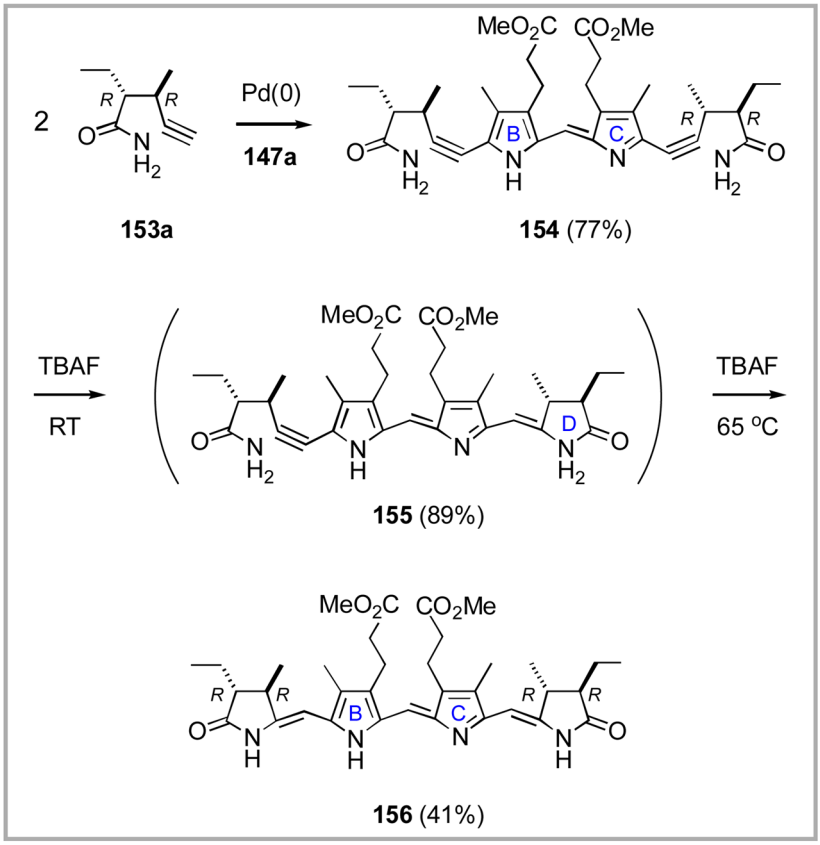
Scheme 33.
The AB + CD strategy fails with sensitive substrates.



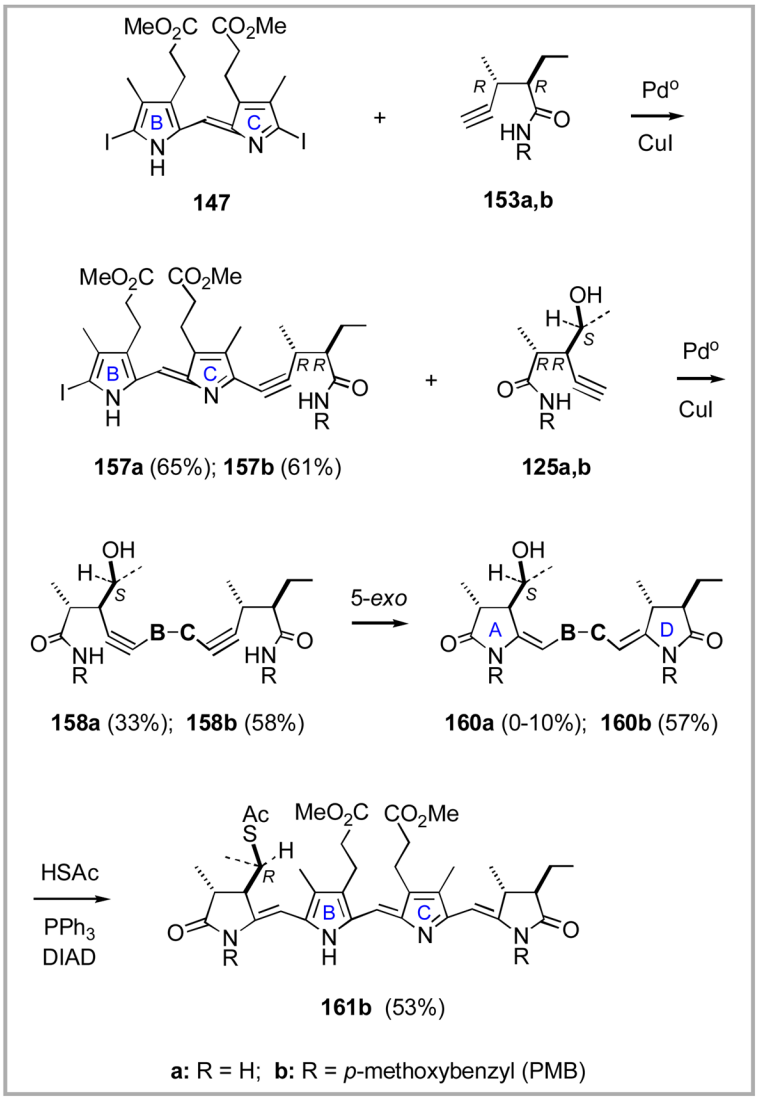
Scheme 34.
The BC + D + A strategy



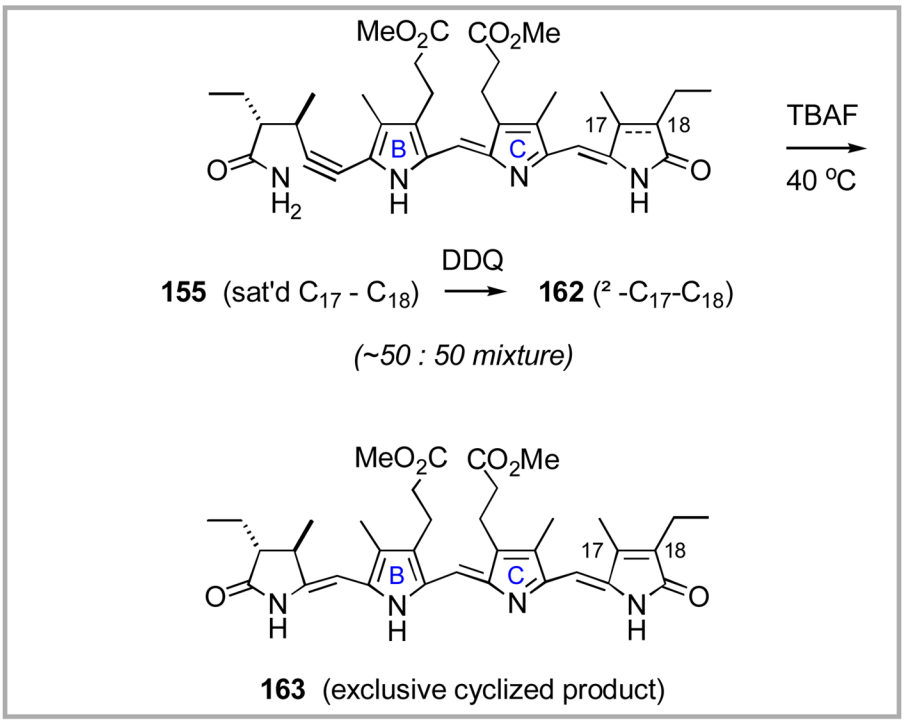
Scheme 35.
Synthesis of the key B,C-dipyrrin component



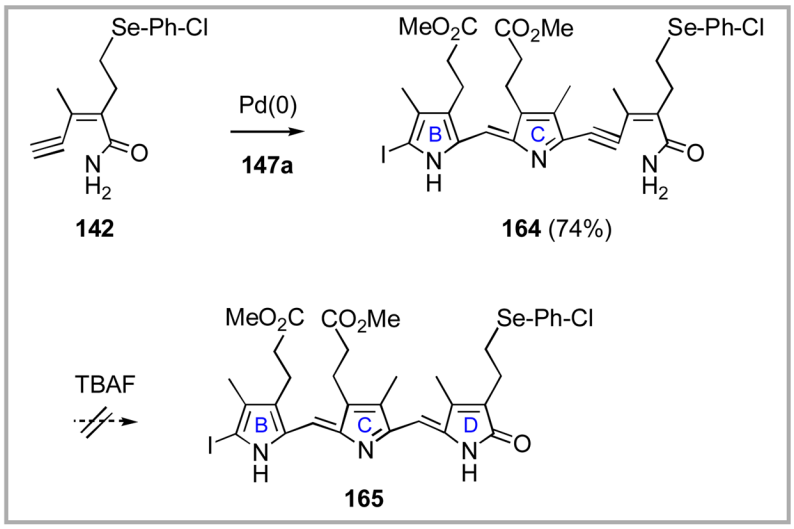
Scheme 36.
Symmetrical tetrapyrroles by the BC + D + A strategy



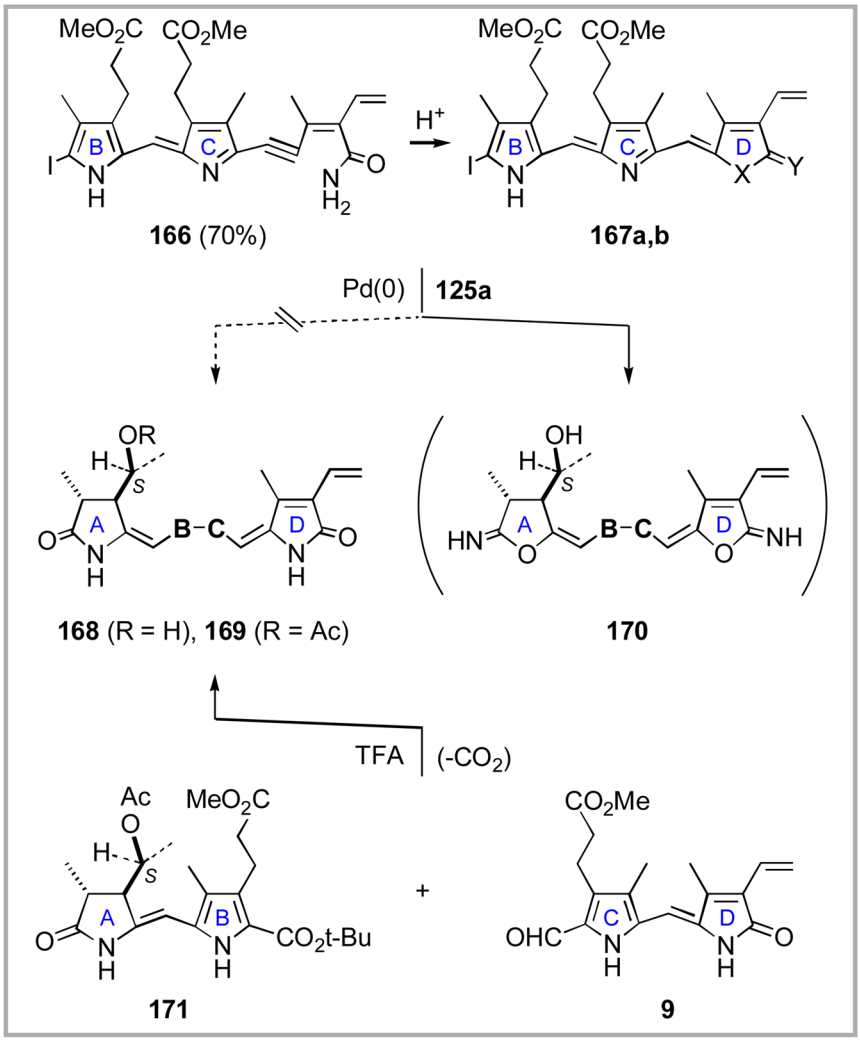
Scheme 37.
Unsymmetrical tetrapyrroles by the BC + D + A strategy



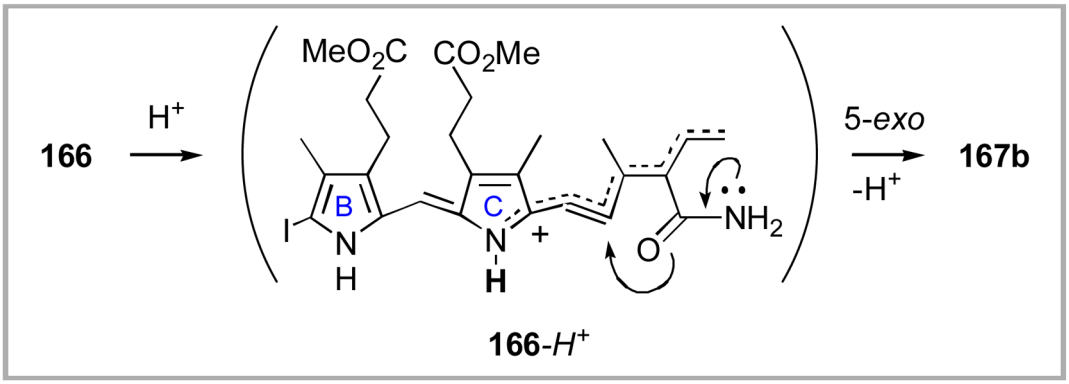
Scheme 38.
Probing the effect of unsaturation on ring D cyclization.



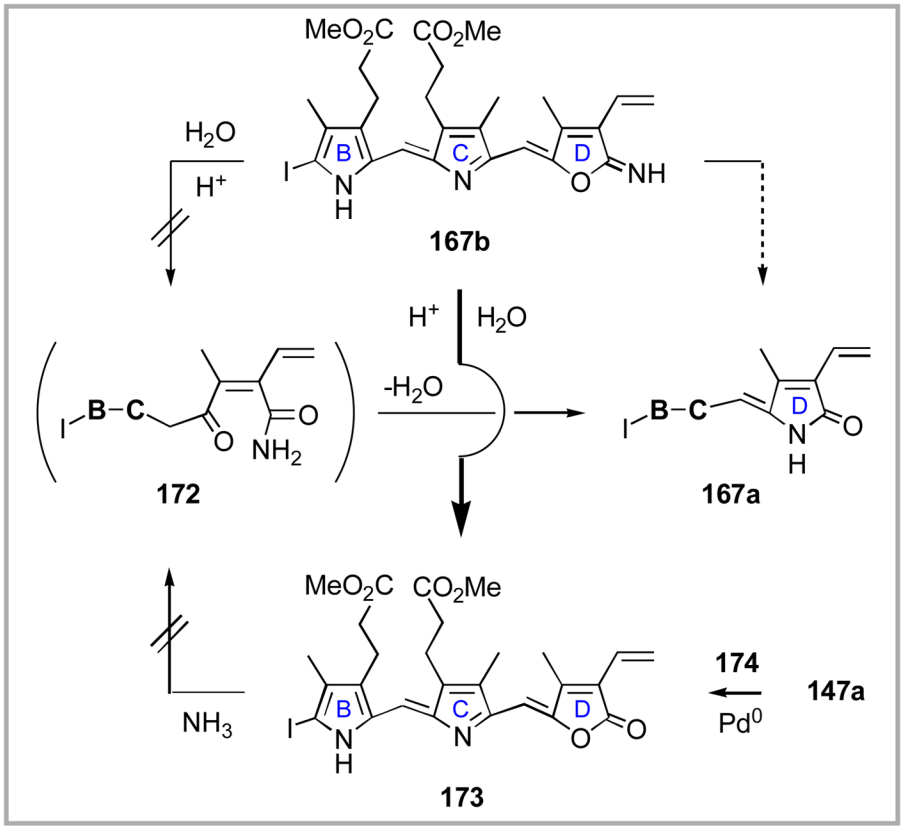
Scheme 39.
Attempted synthesis of a B,C,D-precursor to phytochrome



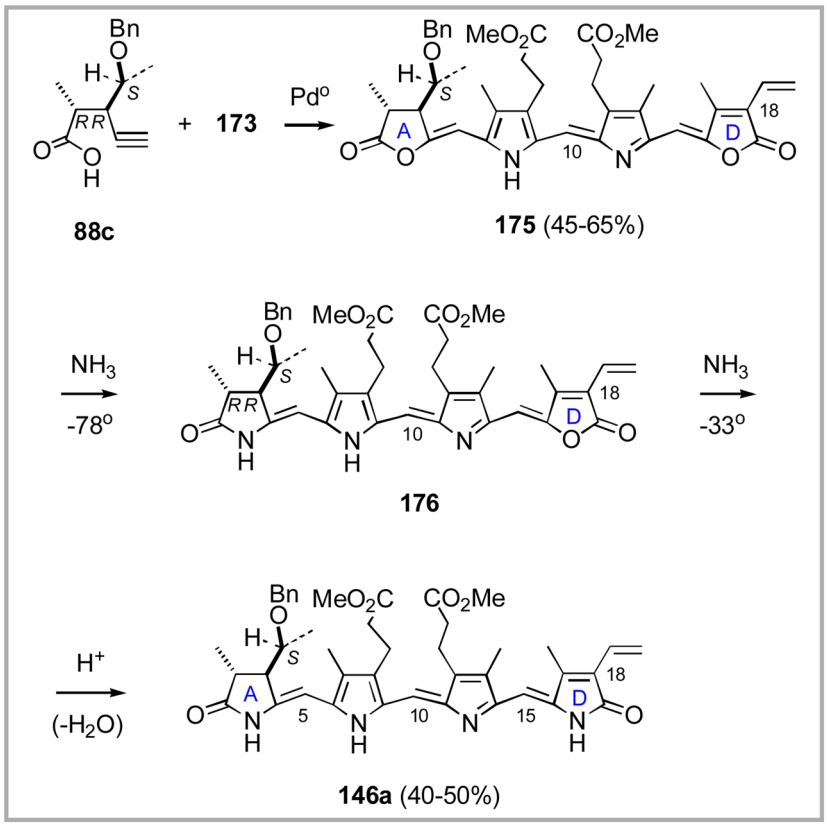
Scheme 40.
An unexpected *oxa*-selective alkyne-amide cyclization



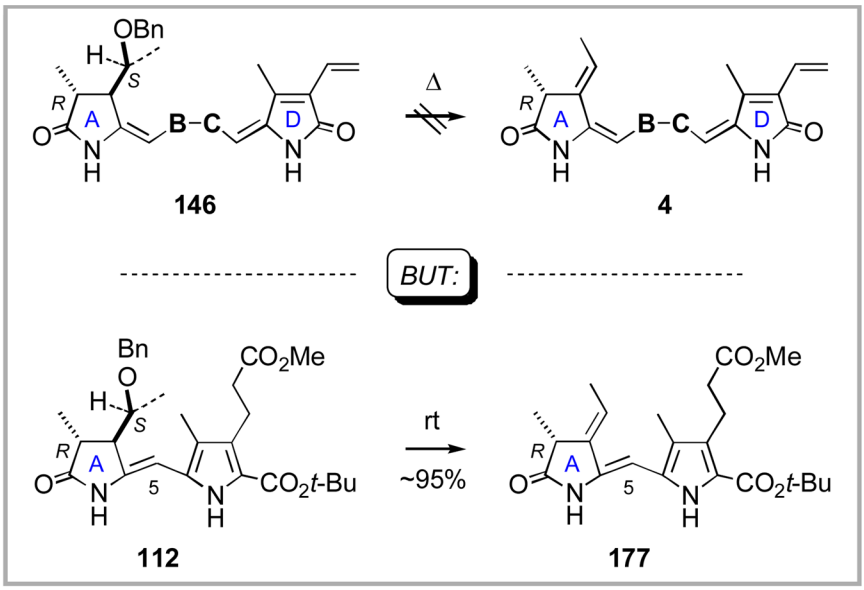
Scheme 41.
Proposed pathway for *oxa*-selectivity



Scheme 42.
Possible alternative routes to the desired lactam **167a**.

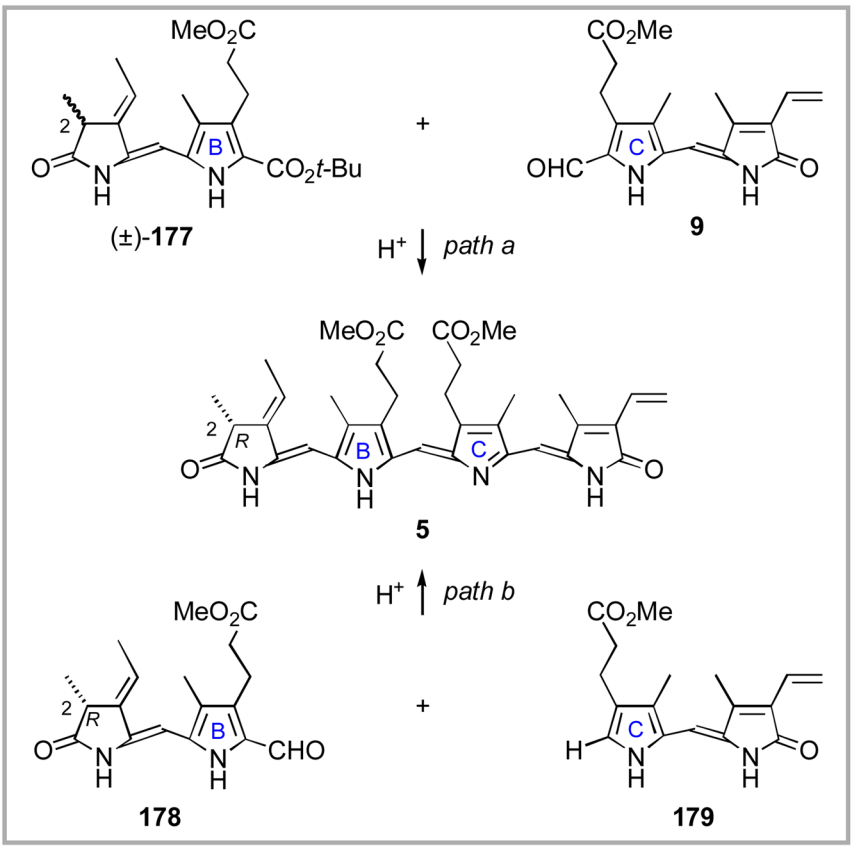


Scheme 43.
Synthesis of a potential precursor to phytochromobilin (**4**)

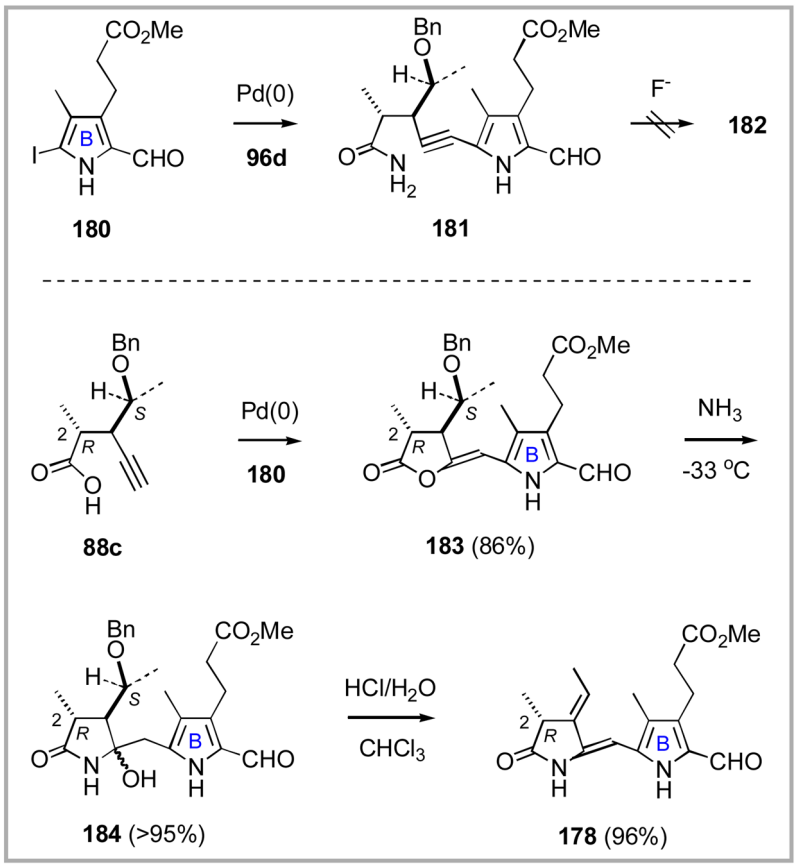


Scheme 44.

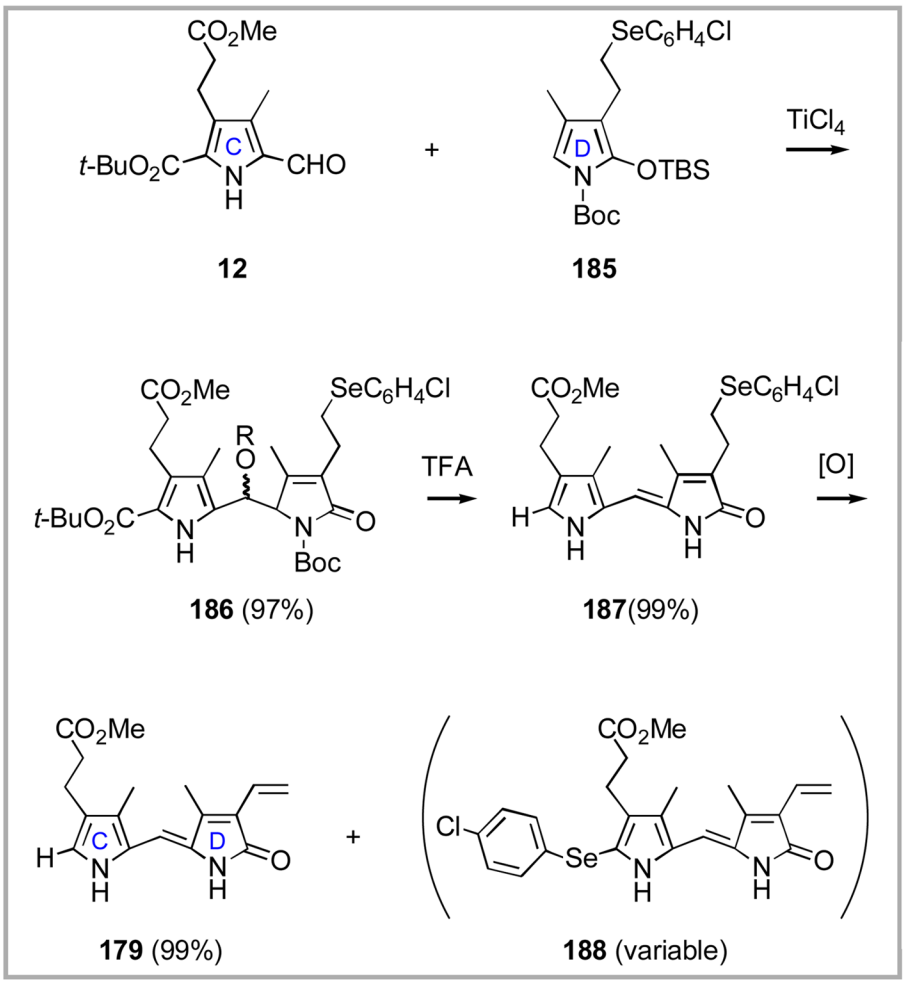
Thermal elimination of BnOH from **146** fails. The same elimination from **112** occurs at rt in $CDCl_3$



Scheme 45.
AB + CD routes to phytochromobilin ester (**5**)

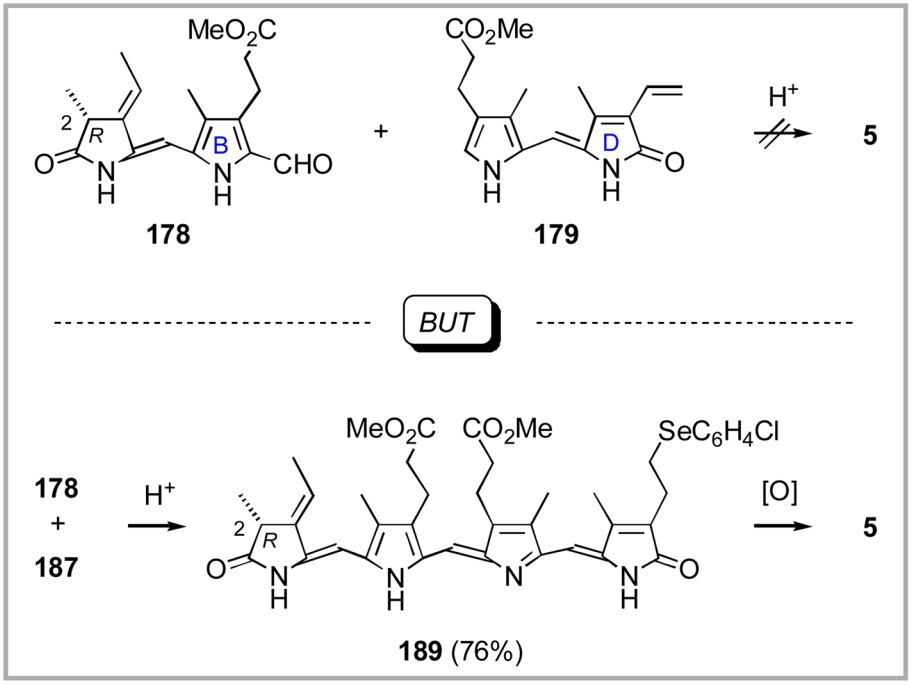


Scheme 46.
Enantioselective synthesis of phytochrome precursor **178**



Scheme 47.

Synthesis of the C,D-pyrromethenone **179** by a route suitable for introducing ^{13}C.



Scheme 48.
Successful synthesis of 2*R*-phytochromobiln dimethyl ester (**5**)