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Enantioselective Syntheses of ^{13}C-Labeled (2*R*)- and (2*S*)-Phytochromobilin Dimethyl Ester

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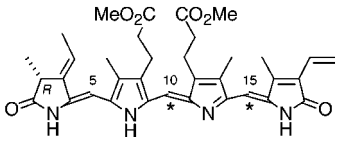
ABSTRACT



2*R*-Phytochromobilin (PΦB), Dimethyl Ester

(2*R*)- and (2*S*)-phytochromobilin dimethyl ester have been prepared in enantiomerically pure form, specifically ^{13}C-labeled at C_{10} or C_{15}.

Phytochrome (Pr; **1**) is a member of the biliprotein family of chromophores, which are made up of linear tetrapyrrole derivatives covalently bonded to the protein **N**−**C** (Figure 1).[1] In green plants, Pr functions as the "on-off" switch for photomorphogenesis, the process by which light transmits growth regulatory information to a cell's genetic apparatus. Information of this type is crucial to the timing of seasonal phenomena, such as seed germination, flowering and fruiting, and chlorophyll production.[1] In comparison to photosynthesis (the other major light-induced process in nature), relatively little is known about photomorphogenesis at the molecular level. In part this is due to the difficulty of isolating **1** from natural sources, where it is present in only trace amounts.

In the native state **1** can adopt at least two distinct forms: an inactive red-absorbing species (Pr) and an active, far red absorbing form designated as Pfr.[1] These species are readily interconverted upon irradiation at 665 and 730 nm, a photoreversible/photochromic behavior that reflects the stabilization of select geometries within the microenvironment of the protein **N**-**C**. At present, only the structure of the inactive Pr form of phytochrome is known with some degree of certainty. Among other theories, it has been suggested that Pfr (**2**) might be derived from Pr by photoisomerization about the C_{15}−C_{16} bond, with retention of a "semiextended" conformation.[2] According to this model, *Z*,*E*-

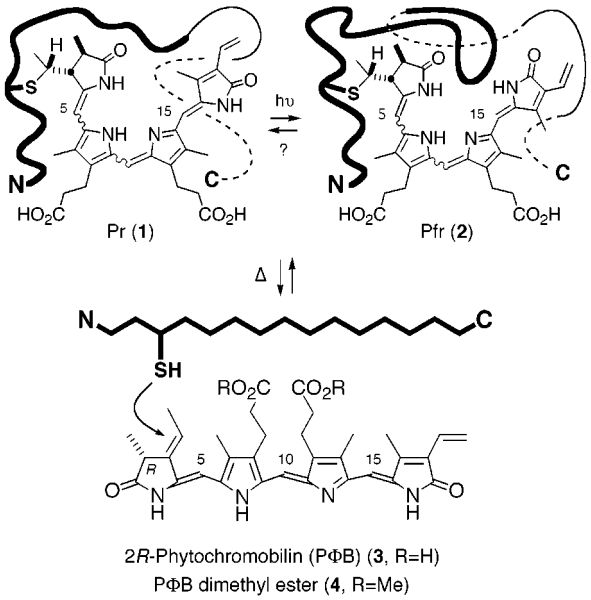


Figure 1. Interconversion of Pr (**1**), Pfr (**2**), and PΦB (**3**).

(1) (a) Statter, R. L.; Galston, A. W. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: New York, 1976; Vol. 1, p 680. (b) *Phytochrome and Photoregulation in Plants*; Furuya, M., Ed.; Academic Press: New York, 1987. (c) Rüdiger, W.; Thümmler, F. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1216.

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isomerization induces a change in the tertiary structure of the surrounding protein **N-C**, providing a molecular basis for transduction of the light signal to the cell's genetic regulatory apparatus. Similar theories have been postulated involving isomerization about C_4-C_5 and $C_{10}-C_{11}$.

Thermolysis of phytochrome (**1**) affords the tetrapyrrole chromophore phytochromobilin (PΦB, **3**), along with the apoprotein **N**−**C** (Figure 1). Cleavage is believed to take place via a concerted E2 elimination to selectively generate the 3*E*-double bond in ring A. Remarkably, the reverse of this process has also been demonstrated in vitro, employing recombinant apophytochrome (**N**−**C**) and its native substrate **3**.[3] The in vitro adduct **1** exhibited a difference spectrum identical to that of native oat phytochrome (Pr, **1**). The ability of phytochrome (**1**) to self-assemble in the dark may be related to its role in etiolated plants as a first light sensor. In principle, this process also provides a means for studying the molecular events involved in the Pr ↔ Pfr interconversion.

Various spectroscopic techniques have been employed in attempts to confirm the site of *Z*,*E*-isomerization. Perhaps the most powerful tool is resonance Raman spectroscopy (RS), which is readily adaptable to the study of prosthetic groups in vivo.[4] Using this technique it is possible to directly observe the hydrogen out-of-plane (HOOP) wagging vibrations of the methine hydrogens at C_5, C_{10}, and C_{15}, for both Pr and Pfr. Significant differences in these absorptions, consistent with *Z*,*E*-isomerization, were noted as early as 1990 by Mathies and Lagarias. However, thus far it has proven difficult to confirm specific assignments. One means of accomplishing this would be by employing ^{13}C-isotopic substitution, which induces a characteristic shift in the HOOP vibration frequency. In this paper we describe enantioselective syntheses of the ^{13}C-labeled PΦB esters **5**, **6**, and *ent*-**5**,**6**, as a first step toward achieving this goal (Figure 2).

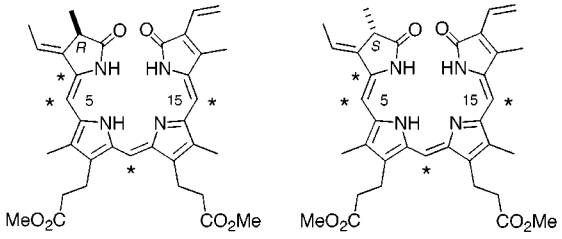
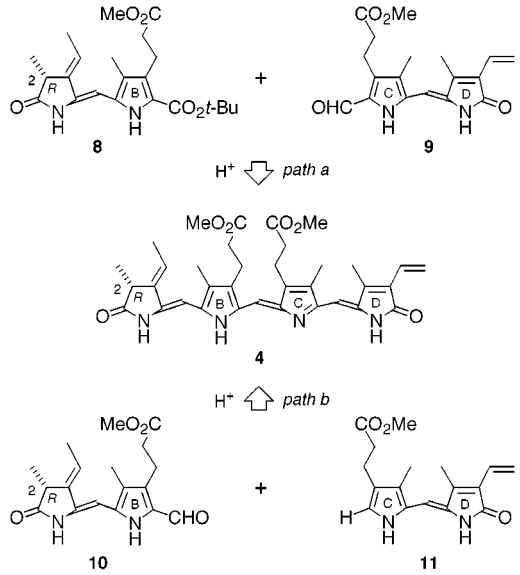


Figure 2. ^{13}C-Labeled Me esters of PΦB and *ent*-PΦB.

Incorporation studies with both the 2*R*- and 2*S*-enantiomers of PΦB should also serve to confirm the assigned absolute stereochemistry in Pr.

Racemic PΦB dimethyl ester (*rac*-**4**) was first prepared in 1980 by Gossauer et al.,[5a] who obtained a ~40% yield of *rac*-**4** by decarboxylative condensation of pyrromethenones *rac*-**8** and **9** (*path a*, Scheme 1).[5] Several closely related syntheses of *rac*-**4** have appeared since. The versatility of

Scheme 1



this "AB + CD" approach to tetrapyrroles derives from its highly convergent nature, although the precursor pyrromethenones can be difficult to prepare. A number of alternative strategies have recently been reported,[5b,c,h] in addition to improvements on the syntheses of both *rac*-**8** and **9** and analogues.[5d−g] Despite these improvements, however, we had several concerns about employing *path a* for the synthesis of enantiomerically pure, ^{13}C-labeled PΦB derivatives. One of these pertained to the strongly acidic conditions required for decarboxylative condensation (neat TFA), which might cause epimerization at the labile C_2-position in **8**. Also, *path a* offered little flexibility for the introduction of ^{13}C at the requisite *meso*-positions (vide infra). Finally, we hoped

(2) (a) Thümmler, F.; Rüdiger, W. *Tetrahedron* **1983**, *39*, 1943. (b) Rüdiger, W.; Thümmler, F.; Cmiel, E.; Schneider, S. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6244. (c) Farrens, D. L.; Holt, R. E.; Rospendowski, B. N.; Song, P.-S.; Cotton, T. M. *J. Am. Chem. Soc.* **1989**, *111*, 9162. (d) Fodor, S. P. A.; Lagarias, J. C.; Mathies, R. A. *Biochemistry* **1990**, *29*, 11141. (e) Mizutani, Y.; Tokutomi, S.; Kitagawa, T. *Biochemistry* **1994**, *33*, 153. (f) Bischoff, M.; Hermann, G.; Rentsch, S.; Strehlow, D.; Winter, S.; Chosrowjan, H. *J. Phys. Chem. B* **2000**, *104*, 1810. (g) Andel, F., III.; Murphy, J. T.; Haas, J. A.; McDowell, M. T.; van der Hoef, I.; Lugtenburg, J.; Lagarias. J. C.; Mathies, R. A. *Biochemistry* **2000**, *39*, 2667 and references therein.

(3) (a) Lagarias, J. C.; Lagarias, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5778. (b) Deforce, L.; Tomizawa, K.-I.; Ito, N.; Farrens, D.; Song, P.-S.; Furuya, M. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10392. (c) Wahleithner, J. A.; Li, L.; Lagarias, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10387. (d) Li, L.; Lagarias, J. C. *J. Biol. Chem.* **1992**, *267*, 19204.

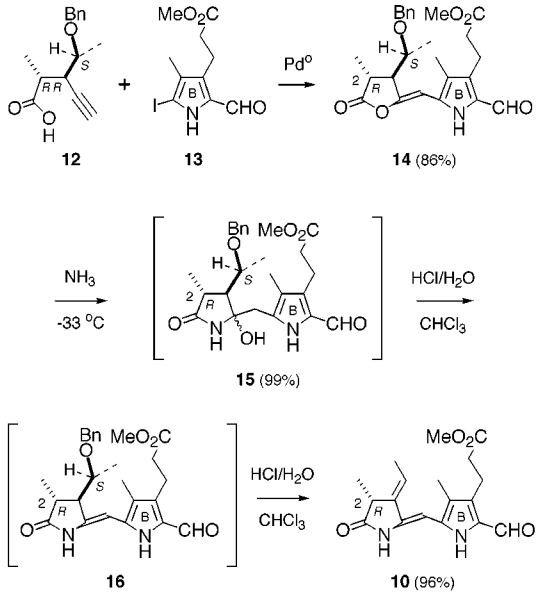
(4) *Biological Applications of Raman Spectroscopy*; Spiro, T. G., Ed.; Wiley-Interscience: New York, 1987. See also refs 2c−e.

(5) (a) Weller, J.-P.; Gossauer, A. *Chem. Ber.* **1980**, *113*, 1603. (b) Jacobi, P. A.; Guo, J.; Rajeswari, S.; Zheng, W. *J. Org. Chem.* **1997**, *62*, 2907. (c) Jacobi, P. A.; Buddhu, S. C.; Fry, D.; Rajeswari, S. *J. Org. Chem.* **1997**, *62*, 2894. (d) Lindner, I.; Knipp, B.; Braslavsky, S. E.; Gärtner, W.; Schaffer, K. *Angew. Chem., Int. Ed.* **1998**, *37*, 1843. (e) Jayasundera, K. P.; Kinoshita, H.; Inomata, K. *Chem. Lett.* **1998**, 1227. (f) Takashi, K.; Kinoshita, H.; Inomata, K. *Synlett* **1999**, *S1*, 901. (g) Jayasundera, K. P.; Kinoshita, H.; Inomata, K. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 497. (h) Jacobi, P. A.; Coutts, L. D.; Guo, J.; Hauck, S. I.; Leung, S. *J. Org. Chem.* **2000**, *65*, 205.

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.

Our synthesis of enantiomerically pure pyrromethenone **10** took advantage of the ready availability of chiral alkyne acids **12** and *ent*-**12** (Scheme 2).[6] Recently we employed

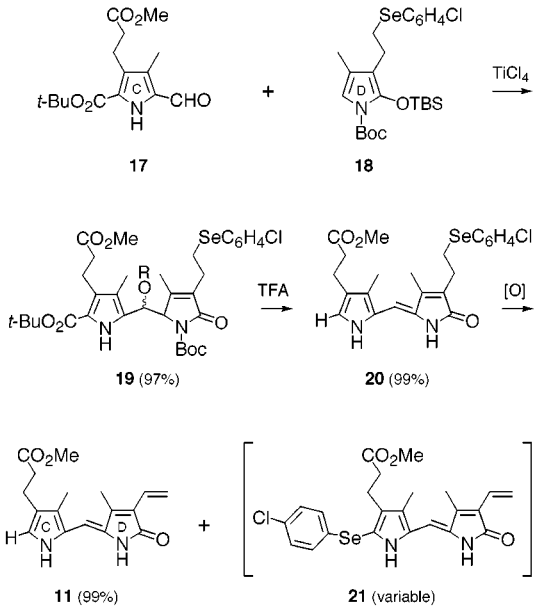
Scheme 2



these compounds in an asymmetric synthesis of the ring-A,B portion of phytochrome (**1**),[5c] where it was necessary to control the relative and absolute stereochemistry at C_2, C_3, and $C_{3'}$. In the case of **10**, alkyne acids **12** and *ent*-**12** proved to be equally efficient precursors for ring-A. The joining of rings A and B was accomplished by a Pd^0-initiated coupling−cyclization reaction with iodopyrrole **13**.[7] This reaction afforded an 86% yield of the enelactone **14** as a single stereo- and geometric isomer.[8b] The structure of **14** was evident from its highly characteristic spectral data (cf. Supporting Information) and confirmed by its eventual conversion to both **10** and PΦB dimethyl ester (**4**). The first step in this sequence involved aminolysis at −33 °C, a procedure that we and others have employed for the conversion of enelactones to cyclic enamides.[8] Under these conditions the lactone ring suffers rapid opening, followed by keto-amide cyclization to give an essentially quantitative yield of the amidoalcohols **15** as a mixture of diastereomers. Normally this mixture was not separated, but rather was directly carried on to the dehydration and benzyl ether elimination steps. We explored many conditions for effecting this transformation without concomitant racemization or isomerization (ethylidenes of type **10** are known to undergo facile *exo-endo* double bond migration). In most cases we observed either no reaction or under forcing conditions, significant decomposition. However, we eventually found that a two-phase system consisting of $CHCl_3$ and 1 equiv of 12 N HCl accomplished the desired transformations at rtand in >95% overall yield. Compound **10** thus prepared was obtained as a single enantiomer, and with exclusively the 3*E*,5*Z*-double bond geometry. In identical fashion, but beginning with alkyne acid *ent*-**12**, we also prepared pyrromethenone *ent*-**10** as a single isomer.

We have also developed an efficient synthesis of the C,D-ring pyrromethenone **11** (Scheme 3). Here it was desirable

Scheme 3



to avoid the strongly alkaline conditions typically employed in aldol-like condensations with the formylpyrrole **17**. A convenient solution to this problem was realized in the form of the silyloxypyrrole **18**, which can be prepared on a multigram scale from inexpensive starting materials.[9] Pyrrole **18** is an excellent surrogate for ring D, since it exhibits strong nucleophilic properties under mild Lewis acid catalysis. Employing $TiCl_4$, for example, condensation of **17** and **18** occurred rapidly at −78 °C, to afford a >95% yield of the aldol products **19** (R = OH, TBS). Initially these adducts were isolated and characterized by analytical and spectral data. However, once again it proved advantageous to carry this mixture forward to the next step. This involved dissolution of **19** in TFA at rt, which initiated a remarkably clean series of transformations. The first of these involved dehydration to generate the *meso*-double bond, followed by

(6) Schreiber, S. L.; Klimas, M. T.; Sammakia, T. *J. Am. Chem. Soc.* **1987**, *109*, 5749. See also refs 5b,c,h.

(7) (a) Bishop, J. E.; O'Connell, J. F.; Rapoport, H. *J. Org. Chem.* **1991**, *56*, 5079. (b) Jacobi, P. A.; DeSimone, R. W.; *Tetrahedron Lett.* **1992**, *33*, 6239.

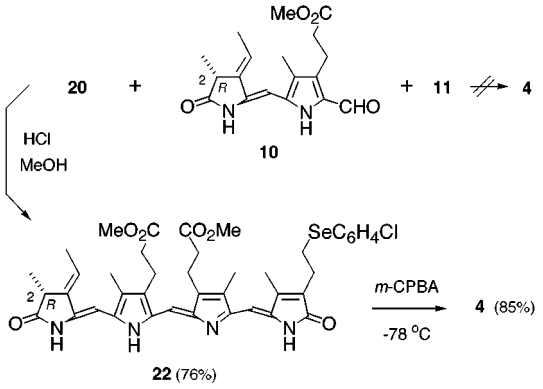
(8) (a) Micklefield, J.; Mackman, R. L.; Aucken, C. J.; Beckmann, M.; Block, M. H.; Leeper, F. J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun* **1993**, 275 and references therein. (b) Jacobi, P. A.; Liu, H. *J. Org. Chem.* **1999**, *64*, 1778. See also ref 5h.

(9) Jacobi, P. A.; DeSimone, R. W.; Ghosh, I.; Guo, J.; Leung, S. H.; Pippin, D. *J. Org. Chem.* **2000**, *65*, 8478.

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 **20**. Finally it was required to carry out the oxidative elimination of selenide **20** to give the desired C,D-ring pyrromethenone **11**. This presented some initial difficulties, since the liberated *p*-chlorophenylselenous acid reacted rapidly with the unsubstituted pyrrole ring in **11** to give variable yields of adduct **21**. However, these difficulties were resolved 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 production of **21** was limited to trace amounts.

With ample quantities of both **10** and **11** in hand, we were confident that sufficiently mild conditions could be developed to effect their coupling to give PΦB dimethyl ester (**4**) (Scheme 4; see also *path b*, Scheme 1). However, this was

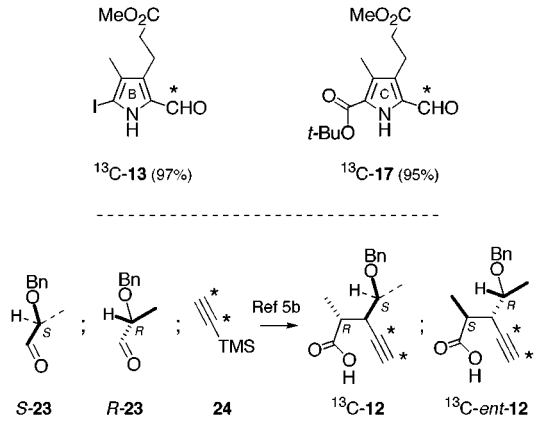
Scheme 4



not the case, since pyrromethenone **11** decomposed rapidly under even mildly acidic conditions. Eventually this problem was circumvented by reversing the order of selenoxide elimination and coupling. Thus, pyrromethenones **10** and **20** underwent clean condensation to afford the tetrapyrrole **22** (76%), under conditions which cause little or no decomposition ($HCl/Et_2O/MeOH$). Finally, selenoxide formation occurred cleanly at −78 °C, followed by elimination using the two-phase system described above for pyrromethenone **11** (cf. Scheme 3). PΦB dimethyl ester (**4**) thus synthesized was obtained as the 2*R*-enantiomer exclusively, which we believe is the first time this material has been prepared in its naturally derived form. In identical fashion, but beginning with *ent*-**10**, we have also synthesized the optical antipode *ent*-**4**.

For the purpose of preparing ^{13}C-labeled PΦB derivatives **5** and **6** (and *ent*-**5**,**6**) (cf. Figure 2), it was necessary to synthesize the appropriate B- and C-ring pyrroles ^{13}C-**13** and ^{13}C-**17** (Scheme 5). With only slight modifications, this was

Scheme 5



accomplished by following the same procedures as for the nonlabeled species **13** and **17**, but employing ^{13}C-DMF (*) as the source of ^{13}C (Supporting Information).[10] The syntheses of **5**, **6**, and *ent*-**5**,**6** were then carried out as previously described for PΦB (**4**) (Schemes 2−4).

Currently we are synthesizing the $C_{4,5}$-labeled phytochrome derivatives **7** and *ent*-**7** (cf. Figure 2), beginning with the A-ring precursors ^{13}C-**12** (^{13}C-*ent*-**12**) (Scheme 5). Also, we are investigating the hydrolysis of **5**−**7** to the corresponding free carboxylic acids, which will be reconstituted with recombinant apoprotein **N**−**C**. Studies employing these ^{13}C-labeled phytochrome derivatives should help clarify the role of **1** in photomorphogenesis.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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