Total Syntheses of Phycocyanobilin Derivatives Bearing a Modified A-Ring toward the Structure/Function Analysis of Phytochrome

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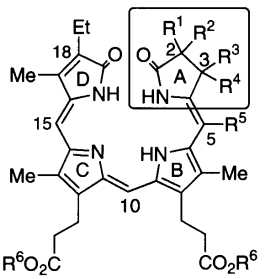
Phycocyanobilin (PCB) derivatives bearing a modified A-ring, including 3,3'-dihydrogenated PCB derivatives, were efficiently synthesized in free acid forms by applying our original methods for the preparation of A-, A/B-, and C/D-ring components toward the structure/function analysis of phytochrome.

Phytochrome, the best-characterized light-sensing photoreceptor in plants, mediates a variety of light-responsive developmental processes in photoreversible manner. The chromophores named phytochromobilin (PΦB) and its substitute phycocyanobilin (PCB, **2** in Figure 1), that differs from PΦB only by substitution of the vinyl group at C-18 with an ethyl group, are linear tetrapyrrole derivatives and covalently bound to their apoproteins at A-ring.[1]

Little is known about the structural requirements of the chromophore for holophytochrome functions, because of the difficulty of chemical synthesis of the chromophore. Therefore, we have been studying on the syntheses of phycobilin derivatives,[2] and succeeded for the first time in synthesizing PCB (**2**)[2b,c] and PΦB[2d] in free acid forms.

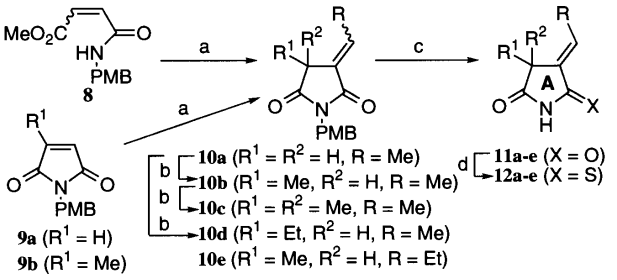
In this paper, we wish to report the syntheses of PCB derivatives bearing a modified A-ring, such as 2-norPCB (**1**), 2-methylPCB (**3**), 2-homoPCB (**4**), and 3-homoPCB (**5**), for the structure/function analysis (Figure 1). Further, 3,3'-dihydrogenated PCB derivatives (**6** and **7**) were also synthesized to investigate their non-covalent interaction with apoprotein toward the development of affinity chromatography to purify apoprotein.



1 ($R^1 = R^2 = H$; $R^3,R^4 = CHMe$; $R^5 = R^6 = H$)
2 ($R^1 = Me$; $R^2 = H$; $R^3,R^4 = CHMe$; $R^5 = R^6 = H$) (PCB)
3 ($R^1 = R^2 = Me$; $R^3,R^4 = CHMe$; $R^5 = R^6 = H$)
4 ($R^1 = Et$; $R^2 = H$; $R^3,R^4 = CHMe$; $R^5 = R^6 = H$)
5 ($R^1 = Me$; $R^2 = H$; $R^3,R^4 = CHEt$; $R^5 = R^6 = H$)
6 ($R^1 = R^2 = Me$; $R^3 = Et$; $R^4 = R^5 = R^6 = H$)
7 ($R^1 = Me$; $R^2 = H$; $R^3 = Et$; $R^4 = R^5 = R^6 = H$)

Figure 1.

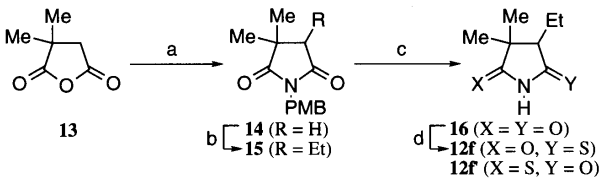
A-rings (**12a–e**) for PCB and/or its analogs (**1–5**) were prepared according to our method starting from compound **8**, maleic or citraconic imide (**9a**,**b**) as shown in Scheme 1.[2b]



a) RCH_2NO_2, DBU, 0 °C. b) MeI or EtI, LDA, HMPA in THF, -78 °C. c) CAN in $MeCN/H_2O$ at rt. d) Lawesson's reagent in refluxing THF or dioxane.

Scheme 1.

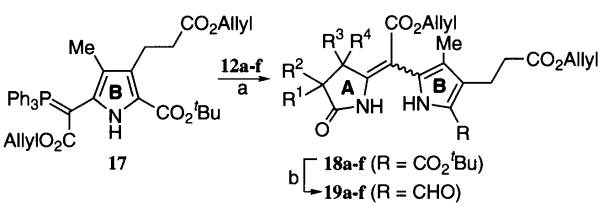
On the other hand, A-ring **12f** for 3,3'-dihydrogenated PCB derivative **6** was prepared starting from 2,2-dimethylsuccinic anhydride (**13**) as shown in Scheme 2. However, the method was not applicable to the synthesis of A-ring of PCB analog **7**, as it was difficult to discriminate between ethyl and methyl groups for monothiocarbonylation with Lawesson's reagent. Therefore, A/B-ring component of **7** was constructed in an alternative manner as will be described later.



a) PMB-NH_2 (1.0 eq.) in refluxing toluene, 11 h. **14** quant. b) EtI (3.0 eq.), HMPA (4.0 eq.) in THF, then LDA (2.0 eq.) at -78 °C, 40 min. **15** 71%. c) (1) CAN (2.5 eq.) in $MeCN/H_2O$ (3/1, v/v) at rt, 22 h; (2) $NH_2NH_2{\cdot}H_2O$ (0.4 eq.) in MeOH at rt, 5 min. **16** 83%. d) Lawesson's reagent (0.5 molar eq.) in refluxing dioxane, 1 h. **12f/12f'** (ca. 2/1) 64%.

Scheme 2.

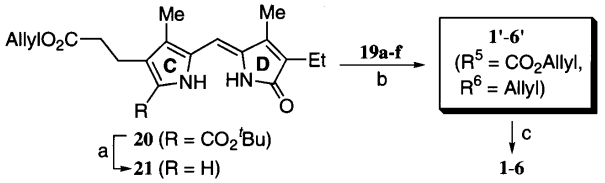
A-rings **12a–f** thus prepared were coupled with ylide **17** as B-ring to afford A/B-rings **18a–f** (Scheme 3). It was essential to add DBU to couple **12f** with **17**, probably due to the poor nucleophilicity of the thiocarbonyl group of **12f** as compared to those of **12a–e** by lack of conjugation with the exo-olefinic bond.



a) **17** (1.5 eq.), **12** (1.0 eq.) in refluxing toluene, 10-13 h. **18a** 56%; **18b** 88%; **18c** 88%; **18d** 60%; **18e** 49%; **18f** 66% (based on **12f**. 0.5 eq. of DBU and **12f/12f'** mixture were used). b) TFA at rt, 1 h, then $HC(OMe)_3$ at rt, 1-2 h. **19a** 96%; **19b** 83%; **19c** 93%; **19d** 86%; **19e** 70%; **19f** 79%.

Scheme 3.

A/B-rings **18a–f** were formylated with methyl orthoformate in TFA accompanying decarboxylation to afford **19a–f**, which were coupled with C/D-ring **21** to give triallyl ester derivatives **1'–6'** of PCB and its analogs (Scheme 4).



a) TFA at rt, 1 h. **21** (not isolated). b) **1'** 89% (cat. 30% HBr/AcOH in MeOH, rt, 3 h); **2'** 86% (cat. 30% HBr/AcOH in MeOH, rt, 4 h); **3'** 84% (cat. H_2SO_4 in MeOH, rt, 3 h); **4'** 78% (cat. H_2SO_4 in MeOH, rt, 3 h); **5'** 74% (cat. H_2SO_4 in MeOH, rt, 3 h); **6'** 77% (cat. H_2SO_4 in MeOH, rt, 3 h). c) $[Pd(PPh_3)_4]$ (0.2 eq.), morpholine (10 eq.) in THF, rt, 0.5 h, then TFA, rt, 3 h. **1** 31%; **2** 96%; **3** 79%; **4** 22%; **5** 16%; **6** 25% (TFA, 60 °C, 4 h).

Scheme 4.

Interestingly, two methyl groups at C-2 of **3'** were not identical on ^{1}H NMR spectrum. Thus, the structure of the analog **3"** (R^6 = Me) was determined by X-ray crystallography as shown in Figure 2, which revealed that A-ring cannot conjugate with B~D-rings by steric demand by an ester group at C-5.[3]

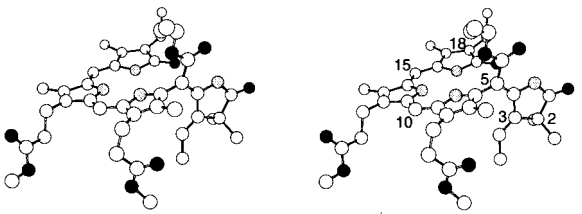
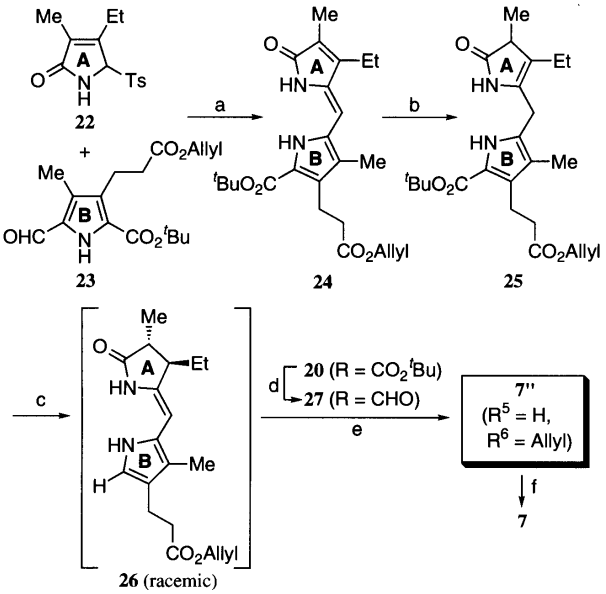


Figure 2. Stereoscopic view of **3"** (R^1 = R^2 = Me, R^3,R^4 = CHMe, R^5 = CO_2Allyl, R^6 = Me).

Finally, triallyl ester groups of **1'–6"** were deprotected all at once by morpholine with Pd-catalyst, followed by decarboxylation at C-5 by treating with TFA to afford free acid forms **1–6**[4] with all-*Z*, all-*syn* conformations (confirmed by NOESY). In the case of **6'**, decarboxylation proceeded only when heated in TFA. It looks to be due to the lack of stabilization of the cation at C-4, produced by initial protonation to C-5, by the neighboring exo-olefin. Under the same conditions, compound **7'** (R^5 = CO_2Allyl, R^6 = Allyl; prepared in another way) was decomposed. Such situation also prompted us to develop an alternative method, which does not require decarboxylation at C-5, for the construction of A/B-ring of **7**.

A precursor of A/B-ring **24** was obtained by the Wittig-type coupling reaction of 5-tosylpyrrolinone **22** with a formylpyrrole **23** in high yield as previously reported.[2a] Compound **24** was then reduced with aluminum amalgam to **25**, followed by isomerization in TFA accompanying decarboxylation to afford the unstable A/B-ring **26**.

C/D-ring **20** was formylated contrary to the cases of the construction of **1'–6'**, in which A/B-rings were formylated, and the resulting C/D-ring **27** was coupled with **26** to afford the tetrapyrrole **7"** in good yield. Allyl ester groups of **7"** were deprotected in a similar manner described for triallyl esters **1'–6'** to yield the racemic **7**[4] as all-*Z*, all-*syn* conformations. It was confirmed that methyl and ethyl groups of the A-ring are in *trans* configuration from the coupling constant (5.86 Hz) between methine protons at C-2 and C-3 of the final product **7**.



a) (1) **22** (1.1 eq.), **23** (1.0 eq.), ^{n}Bu$_3$P (2.0 eq.), DBU (1.0 eq.) in THF, 0 °C - rt, 3 h. (2) cat. I_2 in CH_2Cl_2, rt, 2 d. **24** 84%. b) Al(Hg) (3.0 eq.) in THF/H_2O (10/1, v/v), rt, 2 h. **25** (isolable, but not isolated). c) TFA, rt, 20 min. **26** (not isolated). d) TFA, $HC(OMe)_3$, rt, 2 h. **27** 87%. e) **26** (from 2.0 eq. of **24**), **27** (1.0 eq.) in MeOH, cat. H_2SO_4, rt, 1 h. **7"** 61%. f) $[Pd(PPh_3)_4]$ (0.2 eq.), morpholine (4.0 eq.) in THF, rt, 1 h. **7** 59%.

Scheme 5.

Now, we could have various kinds of PCB derivatives bearing a modified A-ring in free acid forms in hand. Investigations on the in vitro assembly of these PCB derivatives with recombinant apophytochrome are in progress.

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References and Notes

1 M. Furuya and P.-S. Song, "Assembly and Properties of Holophytochrome," in "Photomorphogenesis in Plants," 2nd ed., ed. by R. E. Kendrick, G. H. M. Kronenberg, Kluwer Academic Publishers, Dordrecht, The Netherlands (1994), Chap. 4.3, pp. 105–140; M. Stanek and K. Grubmayr, *Chem. Eur J.*, **4**, 1653 and 1660 (1998). See also the references cited therein.

2 a) K. Kohori, M. Hashimoto, H. Kinoshita, and K. Inomata, *Bull. Chem. Soc. Jpn.*, **67**, 3088 (1994). b) T. Kakiuchi, H. Kato, K. P. Jayasundera, T. Higashi, K. Watabe, D. Sawamoto, H. Kinoshita, and K. Inomata, *Chem. Lett.*, **1998**, 1001. c) K. P. Jayasundera, H. Kinoshita, and K. Inomata, *Chem. Lett.*, **1998**, 1227. d) T. Kakiuchi, H. Kinoshita, and K. Inomata, *Synlett*, **1999**, 901. e) A. Ohta, D. Sawamoto, K. P. Jayasundera, H. Kinoshita, and K. Inomata, *Chem. Lett.*, **2000**, 492. See also the references cited therein.

3 Crystal data for $C_{40}H_{48}N_4O_8$ at rt. Dark violet prism, fw = 712.84, triclinic, space group $P\bar{1}$ with Z = 4, a = 16.595(3), b = 17.552(3), c = 13.445(7) Å, α = 91.20(2), β = 94.52(3), γ = 86.565(9)°, V = 3896(2) Å^3, D_c = 1.215 g cm^{-3}, R = 0.080 and R_w = 0.129 for 7934 data.

4 Spectral data of the final products **1-7** are given for UV/vis (MeOH) λ_{max} and HRMS (FAB) (M^++1) in the following. **1**: 364 (41700), 622 (13700) nm; Found: *m/z* 573.2708. Calcd for $C_{32}H_{37}N_4O_6$: 573.2715. **2**: see ref. 2b. **3**: 364 (58300), 615 (20600) nm; Found: *m/z* 601.3023. Calcd for $C_{34}H_{41}N_4O_6$: 601.3028. **4**: 364 (53400), 595 (17700) nm; Found: *m/z* 601.3036. Calcd for $C_{34}H_{41}N_4O_6$: 601.3028. **5**: 365 (49700), 597 (16100) nm; Found: *m/z* 601.3018. Calcd for $C_{34}H_{41}N_4O_6$: 601.3028. **6**: 345 (30500), 584 (13700) nm; Found: *m/z* 603.3166. Calcd for $C_{34}H_{43}N_4O_6$: 603.3185. **7**: 345 (33000), 580 (14000) nm; Found: *m/z* 589.3017. Calcd for $C_{33}H_{41}N_4O_6$: 589.3028.