Efficient Synthesis of B- and C-Rings Components of Phycobilin Derivatives for Structure/Function Analysis of Phytochrome

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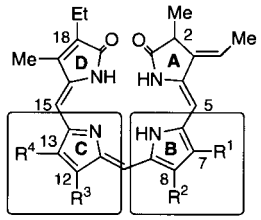
B- and C-Rings components of phycobilin derivatives were efficiently prepared starting from the corresponding lactones. This synthetic method made it possible to prepare not only phycocyanobilin (PCB), but also PCB derivatives having butanoic acid side chain(s) instead of propanoic one(s), regioselectively monoesterified PCB derivatives at C8 or C12, and regioisomers of PCB with respect to methyl and propanoic acid substituents of B- and C-rings for the first time toward the structure/function analysis of phytochrome.

Phycocyanin and phytochrome are chromoproteins widely concerned with the photosynthetic light-harvesting systems of cyanobacteria or red algae and the regulation of plant gene expression by light, respectively. The chromophores named phycocyanobilin (PCB, **1**) and phytochromobilin (PΦB, ethyl group at C18 of **1** is replaced to vinyl group) are linear tetrapyrrole derivatives and covalently bound to their apoproteins at A-ring.[1] For the structure/function analysis of phytochrome, we have been studying on the synthesis of phycobilin derivatives,[2] and have succeeded for the first time in syntheses of free acid forms of PCB (**1**)[2b,c] and PΦB[2d] possible to assemble with the apoproteins.

In this paper, we wish to report the extremely efficient method for the preparation of B- and C-rings components of phycobilin derivatives and its application to the syntheses of several phycobilins (Figure 1) useful to investigate the substituent effect

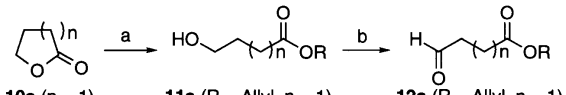


1 (R^1, R^4 = Me; R^2, R^3 = $(CH_2)_2CO_2H$) (Phycocyanobilin)
2 (R^1, R^4 = Me; R^2 = $(CH_2)_2CO_2H$; R^3 = $(CH_2)_3CO_2H$)
3 (R^1, R^4 = Me; R^2 = $(CH_2)_3CO_2H$; R^3 = $(CH_2)_2CO_2H$)
4 (R^1, R^4 = Me; R^2 , R^3 = $(CH_2)_3CO_2H$)
5 (R^1, R^4 = Me; R^2 = $(CH_2)_2CO_2Me$; R^3 = $(CH_2)_2CO_2H$)
6 (R^1, R^4 = Me; R^2 = $(CH_2)_2CO_2H$; R^3 = $(CH_2)_2CO_2Me$)
7 (R^1, R^3 = Me; R^2, R^4 = $(CH_2)_2CO_2H$)
8 (R^1, R^3 = $(CH_2)_2CO_2H$; R^2, R^4 = Me)
9 (R^1, R^4 = $(CH_2)_2CO_2H$; R^2, R^3 = Me)

Figure 1.

of chromophores on the function of the reconstituted holoproteins,[1] namely, PCB (**1**) and its derivatives having butanoic acid side chain(s) instead of propanoic one(s) (**2**-**4**), regioselectively monoesterified PCB derivatives at C8 or C12 (**5**,**6**),[3] and regioisomers of PCB with respect to methyl and propanoic acid substituents of B- and C-rings (**7**-**9**) for the structure/function analysis of phytochrome.

Previously we reported the convenient method for the preparation of a common pyrrole precursor to B- and C-rings starting from methyl 4-oxobutanoate (**12c**) readily available by rhodium(I) catalyzed hydroformylation of methyl acrylate.[2a] Compound **12c** is also available from methyl 4-nitrobutanoate by Nef reaction.[4] On the other hand, we have found that allyl ester is the most suitable protecting group for the carboxylic acid moiety at C8 and C12 of phycobilin derivatives to synthesize their free acid forms possible to assemble with the apoproteins.[2b] However, since it was not easy to directly prepare allyl 4-oxopropanoate (**12a**) by the above methods, we developed a new and extremely convenient method which can provide not only the desired **12a** but also other derivatives such as **12b**-**d** useful for production of a variety of phycobilins depending upon the using alcohols (ROH) and the starting lactones (**10**) as shown in Scheme 1.



10a (n = 1)
10b (n = 2)

11a (R = Allyl, n = 1)
11b (R = Allyl, n = 2)
11c (R = Me, n = 1)
11d (R = Me, n = 2)

12a (R = Allyl, n = 1)
12b (R = Allyl, n = 2)
12c (R = Me, n = 1)
12d (R = Me, n = 2)

a) RONa (X equiv. See Table 1) in ROH, rt, 1.5 h, then 6 M HCl in H_2O or MeOH. b) PCC (1.5 equiv.), MS 3A powder in CH_2Cl_2, rt, 1.5 h.

Scheme 1.

After treating the lactones (**10a**,**b**) with sodium allyloxide or methoxide in the corresponding alcohols at room temperature, the resulting crude ω-hydroxy esters (**11a**-**d**) were oxidized by PCC in CH_2Cl_2 at room temperature to afford the corresponding 4-oxobutanoates (**12a**,**c**) or 5-oxopentanoates (**12b**,**d**) as listed in Table 1. In the case of 5-membered lactone **10a**, it was necessary to use excess amounts of alkoxide to obtain **11a**,**c** in reproducible good yields (Runs 2 and 5), probably due to the existence of equilibrium going back to the starting lactone **10a**.

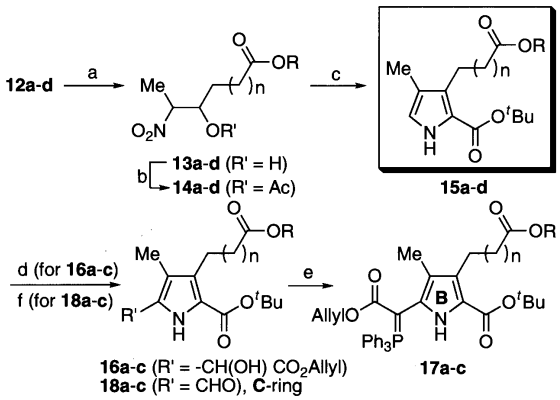
Table 1.

Run	R	n	X	Yield of **11**[a] / %	Yield of **12** / %
1	Allyl	1	1	**a**, 25 (25)	**a**, —[b]
2	Allyl	1	2	**a**, 69 (trace)	**a**, 73
3	Allyl	2	1	**b**, 95 (trace)	**b**, 73
4	Me	1	1	**c**, 20 (25)	**c**, —[b]
5	Me	1	2	**c**, 85 (trace)	**c**, 77
6	Me	2	1	**d**, 84 (trace)	**d**, 71

[a]Numbers in parentheses show the recovered yields of **10a,b**. [b]See runs 2 and 5, respectively.

The aldehydes **12a**-**d** thus prepared were next reacted with nitroethane in the presence of a base to afford the nitroalcohols **13a**-**d**, which were further converted to the corresponding pyrroles **15a**-**d**. The pyrroles **15a**-**d** were then converted to ylides **17a**-**c** as B-ring via **16a**-**c** or α-formyl derivatives **18a**-**c** as C-ring by Vilsmeier-Haack reaction in high yields as report-

ed previously (Scheme 2).[2b]



a) 1 M KOH/MeOH (0.2 equiv.) in $EtNO_2$ (1.5 equiv.), rt, 2 h. b) Ac_2O (1 equiv.), DMAP (0.2 equiv.) in THF, 0 °C - rt, 1 h . c) $CNCH_2CO_2{}^tBu$ (1.1 equiv.), DBU (2 equiv.) in MeCN, 0 °C - rt, ca. 3 h (-30 °C - rt, overnight when n = 2). **15a** (R = Allyl, n = 1), 31% from **12a**; **15b** (R = Allyl, n = 2), 56% from **12b**; **15c** (R = Me, n = 1), 51% from **12c**; **15d** (R = Me, n = 2), 50% from **12d**. d) $AllylO_2CCHO$ (1.5 equiv.), $ZnCl_2$ (0.1 equiv.) in CH_2Cl_2, rt, 5 h. **16a** (R = Allyl, n = 1), 96%; **16b** (R = Allyl, n = 2), 97%; **16c** (R = Me, n = 1), 99%. e) NCS (1.5 equiv.), PPh_3 (4 equiv.) in CH_2Cl_2, 0 °C - rt, 5 h, then sat. aq. Na_2CO_3 (excess). **17a** (R = Allyl, n = 1), 95%; **17b** (R = Allyl, n = 2), 92%; **17c** (R = Me, n = 1), 78%. f) $POCl_3$ (1.5 equiv.) in DMF, 0°C - 65°C, 1 h, then 10% aq. AcONa (excess). **18a** (R = Allyl, n = 1), 95%; **18b** (R = Allyl, n = 2), 83%; **18c** (R = Me, n = 1), 94%.

Scheme 2.

In a similar manner, regioisomers (**17d** and **18d**) of **17a** and **18a** were prepared starting from allyl 4-nitrobutanoate and acetaldehyde through pyrrole **19** (Figure 2).

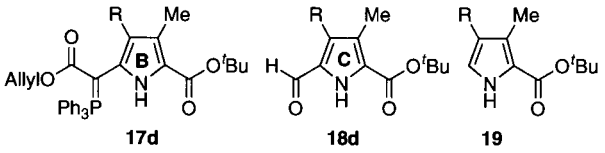
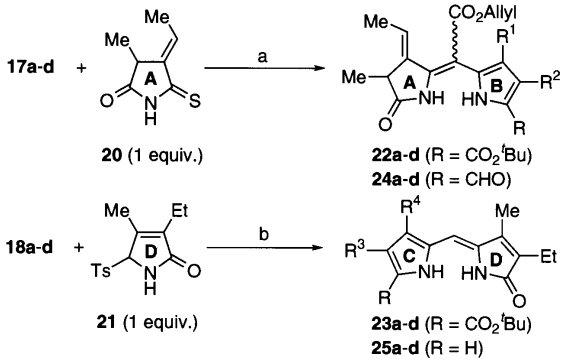


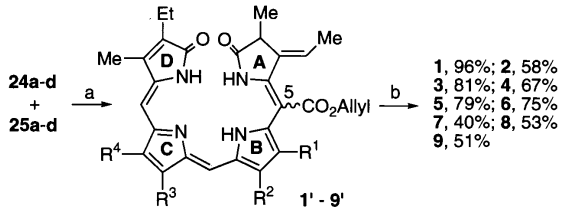
Figure 2. [R = $(CH_2)_2CO_2$Allyl].

B- and C-Rings (**17a-d** and **18a-d**) were coupled with A-ring (**20**) and D-ring (**21**), respectively, to afford the corresponding A/B- and C/D-ring components (**22a-d** and **23a-d**) according to our previous methods[2b] in good yields as shown in Scheme 3. A/B-Rings (**22a-d**) were converted to formyl derivatives (**24a-d**) by treatment with methyl orthoformate in TFA, followed by coupling



a) In toluene, reflux, 14 h. **22a** (R^1 = Me, R^2 = $(CH_2)_2CO_2$Allyl), 80%: **22b** (R^1 = Me, R^2 = $(CH_2)_3CO_2$Allyl), 73%; **22c** (R^1 = Me, R^2 = $(CH_2)_2CO_2$Me), 88%; **22d** (R^1 = $(CH_2)_2CO_2$Allyl, R^2 = Me), 54%. b) (1) $^nBu_3P$ (2-3 equiv.), DBU (1.0-1.2 equiv.) in THF, 0 °C - rt, 4 h; (2) cat. I_2 in CH_2Cl_2, rt, 3 h - overnight. **23a** (R^3 = $(CH_2)_2CO_2$Allyl, R^4 = Me), 83%; **23b** (R^3 = $(CH_2)_3CO_2$Allyl, R^4 = Me), 65%; **23c** (R^3 = $(CH_2)_2CO_2$Me, R^4 = Me), 92%: **23d** (R^3 = Me, R^4 = $(CH_2)_2CO_2$Allyl), 86%.

Scheme 3.



a) **24** (1.2 equiv.), **25** (1 equiv.) in MeOH, cat. 30% HBr/AcOH, rt, 2 h. b) cat. $Pd(PPh_3)_4$, morpholine (10 equiv.) in THF, rt, 0.5-1.0 h, then TFA, rt, 3 h. not yet optimized (R^1 - R^4 of **1'** - **9'** correspond to those of **1** - **9**). **1'** 86%; **2'** 72%; **3'** 62%; **4'** 61%; **5'** 78%; **6'** 79%; **7'** 70%; **8'** 68%; **9'** 70%.

Scheme 4.

with decarboxylated C/D-rings (**25a-d**), available from **23a-d** by treating with TFA, to construct the corresponding tetrapyrrole intermediates **1'**–**9'**, whose structures were fully characterized by spectroscopic methods.

Finally, three or two (for **5'**, **6'**) allyl ester groups of **1'**–**9'** were deprotected all at once with excess amounts of morpholine in the presence of palladium catalyst, followed by treatment with TFA to afford PCB (**1**) and its derivatives **2-9** by decarboxylation at C5 as single isomers with all-*Z*, all-*syn* conformations[5] (confirmed by NOESY).

Now, we could have various kinds of PCB derivatives with free acid forms in hand. Investigations on the reconstituted chromoproteins using these phycobilins are in progress to reveal the substituent effect on the function of the holoproteins.

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References and Notes

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3 When both carboxyl groups were methylated, the chromophore ligation and photochromism were inhibited. Although it was found that PCB monomethyl ester formed the covalent adduct with apoprotein, but without photochromism, regiochemistry of the PCB monomethyl ester used there was ambiguous: S. H. Bhoo, T. Hirano, H.-Y. Jeong, J.-G. Lee, M. Furuya, and P.-S. Song, *J. Am. Chem. Soc.*, **119**, 11717 (1997).

4 M. A. Drian and T. D. Lash, *J. Heterocycl. Chem.*, **31**, 255 (1994).

5 Spectral data of the final products **1-9** are given for UV/Vis (MeOH) λmax and HRMS (FAB) (M^++1) in the following. **1**: 364 (49500), 619 (16100) nm; Found: *m/z* 587.2873. Calcd for $C_{33}H_{39}N_4O_6$: 587.2870. **2**: 364 (46500), 622 (15700) nm; Found: *m/z* 601.3026. Calcd for $C_{34}H_{41}N_4O_6$: 601.3026. **3**: 364 (45600), 624 (14900) nm; Found: *m/z* 601.3026. Calcd for $C_{34}H_{41}N_4O_6$: 601.3026. **4**: 365 (41200), 623 (13400) nm; Found: *m/z* 615.3184. Calcd for $C_{35}H_{43}N_4O_6$: 615.3185. **5**: 363 (44800), 619 (14800) nm; Found: *m/z* 601.3026. Calcd for $C_{34}H_{41}N_4O_6$: 601.3026. **6**: 364 (51,400), 620 (16900) nm; Found: *m/z* 601.3019. Calcd for $C_{34}H_{41}N_4O_6$: 601.3026. **7**: 364 (38100), 624 (12700) nm; Found: *m/z* 587.2876. Calcd for $C_{33}H_{39}N_4O_6$: 587.2870. **8**: 363 (47600), 622 (16300) nm; Found: *m/z* 587.2862. Calcd for $C_{33}H_{39}N_4O_6$: 587.2870. **9**: 363 (37700), 622 (13300) nm; Found: *m/z* 587.2859. Calcd for $C_{33}H_{39}N_4O_6$: 587.2870.