Total Syntheses of (±)-Phycocyanobilin and Its Derivatives Bearing a Photoreactive Group at D-ring

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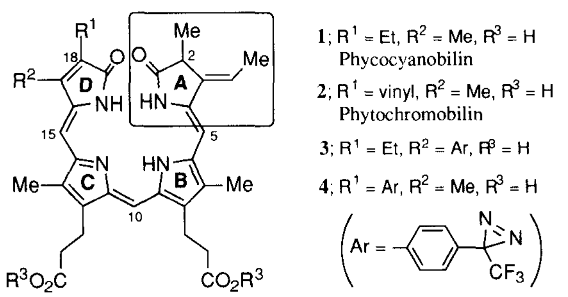
(±)-Phycocyanobilin and its derivatives bearing a photoreactive group at D-ring were first synthesized by the development of a new and convenient method for the preparation of A-ring, transesterification for propanoic acid side-chains of the pyrrole derivative common to B- and C-rings, and deprotection of allyl ester side-chains with a palladium catalyst to avoid migration of exocyclic olefin of A-ring.

Phycocyanin and phytochrome are chromoproteins and widely concerned in algal photosynthetic systems and a variety of processes in higher plants such as growth, development, and morphogenesis etc., respectively. Their chromophores named phycocyanobilin (**1**) and phytochromobilin (**2**) are linear tetrapyrrole derivatives and covalently bound to each apoprotein at A-ring. Recent developments in gene technology have made it possible to assemble the chromophores such as **1** and **2** with the apoproteins obtained by the over-expression of the corresponding cDNA in bacteria and yeast, and the photophysical and photochemical properties of wild type phytochrome are quite similar to those of the reconstituted chromoproteins.[1] On the other hand, though the total syntheses of dimethyl ester derivatives (R^3 = Me in Figure 1) of **1** and **2** have been reported by Gossauer and his co-workers,[2] to the best of our knowledge, there is no report regarding the syntheses of their acid forms applicable to assemble with the apoproteins.

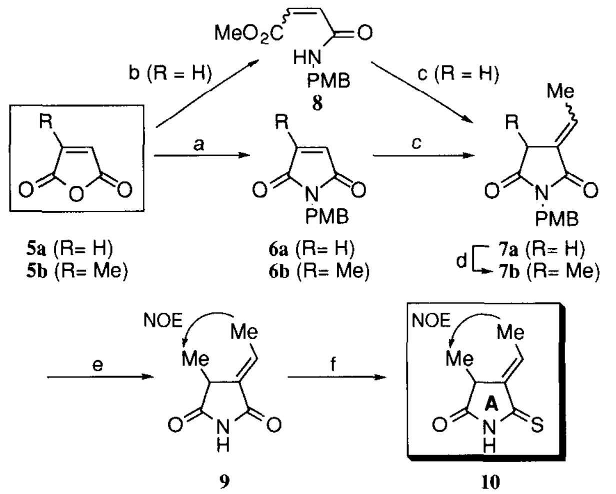
For the structure/function analysis of phytochrome, we have been studying on the syntheses of phycobilin derivatives.[3] In this paper, we wish to report the first total syntheses of (±)-phycocyanobilin (**1**) and its derivatives (**3** and **4**) bearing a photoreactive group (Ar) at D-ring for a photoaffinity study.[3c]



1; R^1 = Et, R^2 = Me, R^3 = H
Phycocyanobilin
2; R^1 = vinyl, R^2 = Me, R^3 = H
Phytochromobilin
3; R^1 = Et, R^2 = Ar, R^3 = H
4; R^1 = Ar, R^2 = Me, R^3 = H

Figure 1.

Recently, we have reported the synthesis of A-ring common to phycocyanobilin and phytochromobilin from mucochloric acid. The intermediary 2-ethyliden-3-methyl-succinimide (**9**) was found to be regioselectively monothiocarbonylated with Lawesson's reagent to give **10** as an A-ring, but the synthetic method for **9** still required many steps.[3b]



a) PMB-NH_2 (n eq.) in refluxing solvent, t h. **6a** 63% (1.2 eq., xylene, 30 h, from **5a**); **6b** 80% (1.0 eq., toluene, 13 h, from **5b**). b) (1) PMB-NH_2 (1.0 eq.) in benzene at rt, 1 h; (2) sat. HCl in MeOH at 0 °C, 1 h. **8** 92% [almost (*Z*)-isomer]. c) $EtNO_2$ (1.0 eq.), DBU (1.0 eq.) in THF at 0 °C, 5 min. **7a** [only (*E*)-isomer] 80% (from **6a**), 71% (from **8**); DBU (1.0 eq.) in $EtNO_2$ at 0 °C, **6b**, 1 h. (*E*)-**7b** 52%, (*Z*)-**7b** 25%. d) (1) MeI (3.0 eq.), HMPA (4.0 eq.) in THF; (2) LDA (1.2 eq.) was added dropwise at -78 °C. (*E*)-**7b** 76%. e) (1) CAN (2.5 eq.) in $MeCN/H_2O$ (3/1) at rt, 2 h; (2) $NH_2NH_2 \cdot H_2O$ (0.4 eq.) in MeOH, 5 min. **9** 84%. f) Lawesson's reagent (1.0 eq.) in refluxing 1,4-dioxane, 10 min. **10** 61%.

Scheme 1.

Therefore, a new and practical method for preparation of **9** was established to conduct on large scale as outlined in Scheme 1. Starting from maleic anhydride (**5a**) or citraconic anhydride (**5b**), the compound **9** was efficiently prepared via **7a,b**. In step c, slow addition of **6a,b** or **8** to a mixed solution of $EtNO_2$ and DBU was critical to get the reproducible good results.[4] Reaction of **9** with Lawesson's reagent in refluxing 1,4-dioxane[5] afforded A-ring component **10** in 61% yield.

Next, B,C-rings components were prepared according to the modified procedure of the method reported in literatures[6,7] utilizing the common pyrrole **11a**[8] as shown in Scheme 2. It was initially planned to prepare phycocyanobilin (**1**) by hydrolysis of dimethyl ester derivative (R^1 = Et, R^2 = Me, R^3 = Me in Figure 1), however, an exocyclic olefin at C-3 was found to tend to migrate to endocyclic position (C-2) under basic conditions at several synthetic stages toward **1**. Thus, methyl ester group of **11a** was transformed to other ester groups [R^3 = Bn (**11b**), CH_2CCl_3 (**11c**), or allyl (**11d**). See Methods A-C in Scheme 2] removable under neutral conditions prior to the preparation of the B,C-rings components. Ultimately, allyl ester (**11d**) was chosen since it turned out to be applicable to the syntheses of photoactivatable phycobilin derivatives (**3** and **4**).[3c,9,10]

A-ring component **10** prepared above was coupled with **12** to give A/B-ring component **14** in 84% yield, followed by decarboxylation and formylation to lead to **15**.[10] It was further

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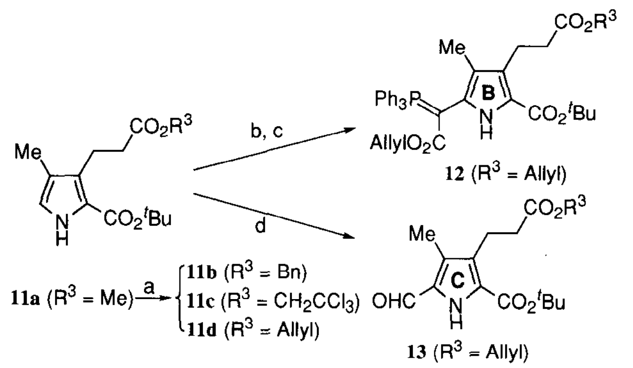
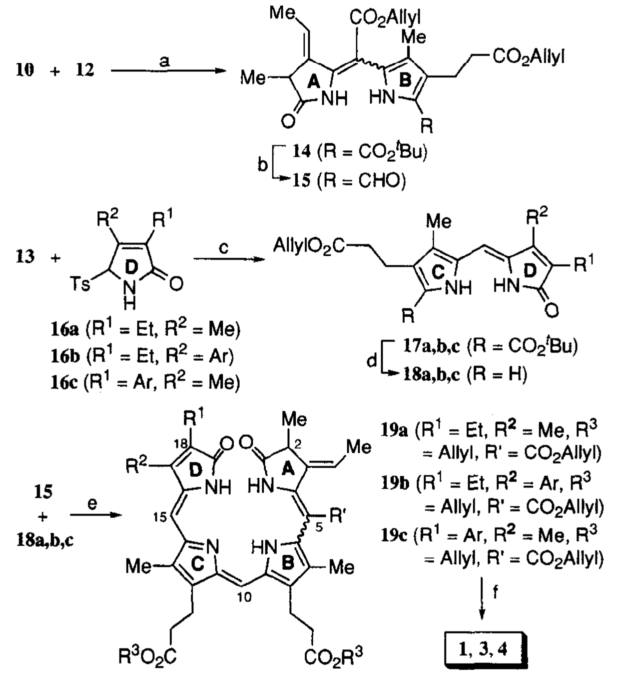


a) Method A: $TiCl_4$ (0.1 eq.), Et_3N (0.4 eq.), R^3OH (2 eq.) in refluxing benzene using a Dean Stark apparatus, 18 h. Method B: (1) 3 M KOH in MeOH at rt, 2 h; (2) DCC (1.1 eq.), DMAP (0.3-0.8 eq.), R^3OH (1.1-3.0 eq.) in CH_2Cl_2 at rt, 1-3 h. Method C: LiBr (5 eq.), DBU (0.5 eq.) in R^3OH at rt, 8 h. **11b** 90% (Method A); **11c** 89% (Method B); **11d** 74% (Method C), 95% (Method B). b) $AllylO_2CCHO$ (1.5 eq.), $ZnCl_2$ (0.1 eq.) in CH_2Cl_2 at rt, 3 h. Alcohol 96%. c) NCS (1.5 eq.), PPh_3 (4.0 eq.) in CH_2Cl_2 at rt, 3 h, then aq. Na_2CO_3. **12** 95%. d) (1) $POCl_3$ (1.6 eq.) in DMF at 65 °C, 1 h; (2) 10% aq. AcONa at 70 °C, 1 h. **13** 93%.

Scheme 2.

reacted with C/D-ring components **18a-c**, prepared via **17a-c** from **13** and **16a-c** as described previously,[3c] to construct the corresponding tetrapyrrole intermediates **19a-c**.[10,11]

Finally, three allyl ester groups (R' and CO_2R^3 of two propanoic acid residues) were deprotected all at once with a palladium catalyst in the presence of excess amounts of morpholine to avoid the migration of exocyclic olefin of A-ring, and subsequent treatment with TFA afforded the desired (±)-phycocyanobilin (**1**) and its derivatives (**3** and **4**) by decarboxylation (R' = $CO_2H \rightarrow H$) as single stereoisomers with all-*Z*, all-*syn* conformations (confirmed by NOESY). They were purified twice by silica gel column chromatography using different solvent systems ($CHCl_3$/MeOH/AcOH and AcOEt /MeOH/AcOH/TFA) and/or back-extraction procedure.



a) refluxed in toluene, 8 h. **14** 84%. b) TFA at rt, 1 h, then $HC(OMe)_3$ at rt, 2 h. **15** 83%. c) For **16a**: (1) Bu_3P (2 eq.), DBU (1.1 eq.) in THF at rt, 4 h; (2) cat. I_2 in CH_2Cl_2 at rt, 3 h. **17a** 88%. See ref. 3c for **16b** (73%) and **16c** (87%). d) TFA at rt, 1 h. **18a-c** were not isolated. e) **19a** 86% (cat. HBr/AcOH in MeOH at rt, 4 h); **19b** 60% (cat. conc. H_2SO_4 in EtOH at rt, 12 h); **19c** 65% (cat. CH_3SO_3H in EtOH at 60 °C, 6 h). f) (1) $Pd(PPh_3)_4$ (0.2 eq.), morpholine (10 eq.) in THF at rt, 1 h; (2) TFA at rt, 2-3 h. **1** 96%; **3** 80%; **4** 65%.

Scheme 3.

Now, we have phycocyanobilin (**1**) and its derivatives (**3** and **4**)[12] with acid forms in hand. Investigations on the reconstituted chromoproteins using these phycobilins are in progress for a photoaffinity study.

References and Notes

1 P. Schmidt, U. Westphal, K. Worm, S. E. Braslavsky, W. Gärtner, and K. Schaffner, *J. Photochem. Photobiol. B : Biology*, **34**, 73 (1996), and references cited therein.

2 a) A. Gossauer and R.-P. Hinze, *J. Org. Chem.*, **43**, 283 (1978). b) J.-P. Weller and A. Gossauer, *Chem. Ber.*, **113**, 1603 (1980).

3 a) K. Kohori, M. Hashimoto, H. Kinoshita, and K. Inomata, *Bull. Chem. Soc. Jpn.*, **67**, 3088 (1994). b) H. Ngwe, E. Nakayama, T. Higashi, H. Kinoshita, and K. Inomata, *Chem. Lett.*, **1995**, 713. c) T. Masukawa, H. Kato, T. Kakiuchi, K. P. Jayasundera, H. Kinoshita, and K. Inomata, *Chem. Lett.*, **1998**, 455, and references cited therein.

4 R. Ballini and A. Rinaldi, *Tetrahedron Lett.*, **35**, 9247 (1994).

5 1,4-Dioxane was better as a solvent than toluene used previously,[3b] and it was essential not to react longer than the time required.

6 A. Gossauer and D. Miehe, *Liebigs Ann. Chem.*, **1974**, 352.

7 J. E. Bishop, J. F. O'Connell, and H. Rapoport, *J. Org. Chem.*, **56**, 5079 (1991).

8 The pyrrole **11a** is readily available by our previous method.[3a] See also M. A. Drian and T. D. Lash, *J. Heterocyclic Chem.*, **31**, 255 (1994); P. A. Jacobi and R. B. DeSimone, *Tetrahedron Lett.*, **33**, 6239 (1992).

9 K. Inomata, *Kagaku (Chemistry)*, **51**, 730 (1996).

10 All new compounds were characterized by ^1H NMR spectra, IR spectra, and elemental analyses and/or MS spectra.

11 Each of **19a-c** was isolated as a mixture of (*Z,Z,Z*)- and (*E,Z,Z*)-isomers. **19a**: mp 78-79 °C (from Et_2O/heptane). Found: C, 68.75; H, 6.81; N, 7.38%. Calcd for $C_{43}H_{50}N_4O_8$: C, 68.78; H, 6.71; N,7.46%. HRMS (FAB): (M^++1), Found: m/z 751.3714. Calcd for $C_{43}H_{51}N_4O_8$: 751.3707. **19b**: mp 134-137 °C (from cyclohexane). Found: C, 64.91; H, 5.59; N, 8.83%. Calcd for $C_{50}H_{51}N_6O_8F_3$: C, 65.21; H, 5.58; N, 9.12%. HRMS (FAB): (M^++1), Found: m/z 921.3805. Calcd for $C_{50}H_{52}N_6O_8F_3$: 921.3799. **19c**: mp 108-112 °C (from Et_2O/heptane). Found: C, 64.99; H, 5.45; N, 9.07%. Calcd for $C_{49}H_{49}N_6O_8F_3$: C, 64.89; H, 5.45; N, 9.27%. HRMS (FAB): (M^++1), Found: m/z 907.3629. Calcd for $C_{49}H_{50}N_6O_8F_3$: 907.3642.

12 **1**:[13] mp >300 °C (from AcOEt/hexane). IR (KBr) 3417, 3260, 2969, 2932, 2873, 1694, 1597, 1539, 1455, 1396, 1279, 1236, 1210, 1159, 1110, 1066, 1040, 964, 896, 744, 688 cm^{-1}. ^1H NMR (pyridine-d_5) δ = 1.26 (t, J = 7.56 Hz, 3H), 1.50 (d, J = 7.56 Hz, 3H), 1.72 (d, J = 7.32 Hz, 3H), 2.03 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 2.44-2.58 (m, 2H), 2.86 (t, J = 7.32 Hz, 2H), 2.88 (t, J = 6.95 Hz, 2H), 3.12 (t, J = 7.32 Hz, 2H), 3.21 (t, J = 6.95 Hz, 2H), 3.37 (brq, J = 7.32 Hz, 1H), 5.87 (s, 1H), 6.09 (s, 1H), 6.34 (dq, J = 2.68, 7.32 Hz, 1H), 7.29 (s, 1H) ppm. UV/Vis (MeOH) λmax 364 (ε = 48,000), 621 (ε = 16,000) nm. HRMS (FAB): (M^++1), Found: m/z 587.2873. Calcd for $C_{33}H_{39}N_4O_6$: 587.2870.
3: mp (decomp.) above 230 °C (from $CHCl_3$/hexane). IR (KBr) 3400, 3208, 2920, 2851, 1702, 1613, 1589, 1456, 1441, 1408, 1385, 1343, 1312, 1231, 1186, 1156, 1050, 938, 829, 746, 693 cm^{-1}. ^1H NMR (pyridine-d_5) δ = 1.27 (t, J = 7.44 Hz, 3H), 1.50 (d, J = 7.32 Hz, 3H), 1.73 (d, J = 7.32 Hz, 3H), 1.91 (s, 3H), 1.96 (s, 3H), 2.46-2.59 (m, 2H), 2.78 (brt, 2H), 2.80 (brt, 2H), 3.07 (brt, 2H), 3.14 (brt, 2H), 3.36 (q, J = 7.07 Hz, 1H), 5.84 (s, 1H), 5.87 (s, 1H), 6.34 (dq, J = 1.95, 7.32 Hz, 1H), 7.38 (d, J = 8.05 Hz, 2H), 7.24 (s, 1H), 7.74 (d, J = 8.05 Hz, 2H) ppm. UV/Vis (MeOH) λmax 369 (ε = 32,000), 628 (ε = 13,000) nm. HRMS (FAB): (M^++1), Found: m/z 757.2969. Calcd for $C_{40}H_{40}N_6O_6F_3$: 757.2961.
4: mp (decomp.) above 180 °C (from AcOEt/hexane). IR (KBr) 3423, 3240, 2971, 2923, 2866, 1697, 1595, 1550, 1456, 1442, 1408, 1389, 1343, 1275, 1231, 1183, 1156, 1112, 1068, 1039, 997, 964, 938, 883, 828, 747, 692 cm^{-1}. ^1H NMR (pyridine-d_5) δ = 1.38 (d, J = 7.32 Hz, 3H), 1.67 (d, J = 7.32 Hz, 3H), 2.04 (s, 3H), 2.20 (s, 3H), 2.32 (s, 3H), 2.86 (t, J = 7.44 Hz, 2H), 2.90 (t, J = 7.07 Hz, 2H), 3.12 (t, J = 7.44 Hz, 2H), 3.22 (t, J = 7.07 Hz, 2H), 3.35 (brq, J = 7.32 Hz, 1H), 5.88 (s, 1H), 6.28 (s, 1H), 6.34 (dq, J = 2.22, 7.32 Hz, 1H), 7.29 (s, 1H), 7.35 (d, J = 8.30 Hz, 2H), 7.98 (d, J = 8.30 Hz, 2H) ppm. UV/Vis (MeOH) λmax 373 (ε = 41,000), 636 (ε = 13,000) nm. HRMS (FAB): (M^++1), Found: m/z 743.2792. Calcd for $C_{39}H_{38}N_6O_6F_3$: 743.2805.

13 B. Knipp, K. Kneip, J. Matysik, W. Gärtner, P. Hildebrandt, S. E. Braslavsky, and K. Schaffner, *Chem. Eur. J.*, **3**, 363 (1997).