A Highly Efficient Method for the Preparation of the A/B-Ring Component of Phycobilin Derivatives Starting from Bilirubin

Daisuke Sawamoto and Katsuhiko Inomata*
Department of Chemical Science, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192

(Received March 28, 2001; CL-010280)

An efficient synthetic method of the A/B-ring component of phycobilin derivatives (PΦB and PCB) was established by using bilirubin as a starting material, which allows to construct the phycobilins in a few steps.

Phytochrome is one of the major photosensory chromoproteins in plants and mediates a variety of light-responsive developmental processes in a photoreversible manner. The chromophore named phytochromobilin (PΦB, **6a**), which is covalently bound to apoproteins at C-3' position of the A-ring through a thioether, receives the photoinformation of environment by photoisomerization of the double bond at C-15.[1] Little had been 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 in synthesizing PΦB (**6a**),[2d] phycocyanobilin (PCB, **7a**)[2b,c] and the modified PCBs[2e,f] in free acid forms, which made it possible to assemble the chromophores with the apoproteins in vitro to analyze the spectral properties of the resulting holoproteins.[3]

Recently, it was reported that addition of thiobarbituric acid to biliverdin dimethyl ester proceeded to afford the C/D-ring component of PΦB (**6a**) resulting from the cleavage between the B- and C-rings,[4] and the C/D-ring component was coupled with the chemically synthesized A/B-ring to provide PΦB dimethyl ester in extremely short steps.[4a]

On the other hand, we have reported the total synthesis of (±)-PΦB (**6a**) in a free acid form starting from 4-methyl-3-[2-(tolylthio)ethyl]-2-tosylpyrrole as a precursor of the A- and D-rings and a 2-formyl pyrrole common to the B- and C-rings. Especially, the pyrromethenone derivative having a vinyl group was found to be readily converted to the A/B-ring component by reducing with Al(Hg) and the subsequent acid treatment.[2d]

In order to construct PΦB (**6a**) or PCB (**7a**) in a few steps, we attempted to obtain the isomeric pyrromethenone derivative **3** as an A/B-ring precursor along with C/D-ring component **2** of PΦB by cleaving between the B- and C-rings of bilirubin diallyl ester (**1b**), since the former product **3** was expected to be converted to the A/B-ring component (**4**) of phycobilin derivatives by reduction with Al(Hg) (Figure 1).

Commercially available bilirubin (**1a**) was converted to the diallyl ester (**1b**) in 95% yield by treating with allyl bromide and DBU in DMF. Oxidation of **1b** with DDQ afforded the biliverdin diallyl ester (**8**) in 66% yield.[5] At first, the resulting **8** was reacted with thiobarbituric acid in MeOH to cleave between the B- and C-rings to afford the C/D-ring component (**2a**) of PΦB and a precursor of the A/B-ring component (**3a**) of phycobilin derivatives along with equimolar amounts of the isomeric thiobarbituric acid adducts (**2c**, **3c**), respectively, in quantitative yields (Scheme 1).[4,6]

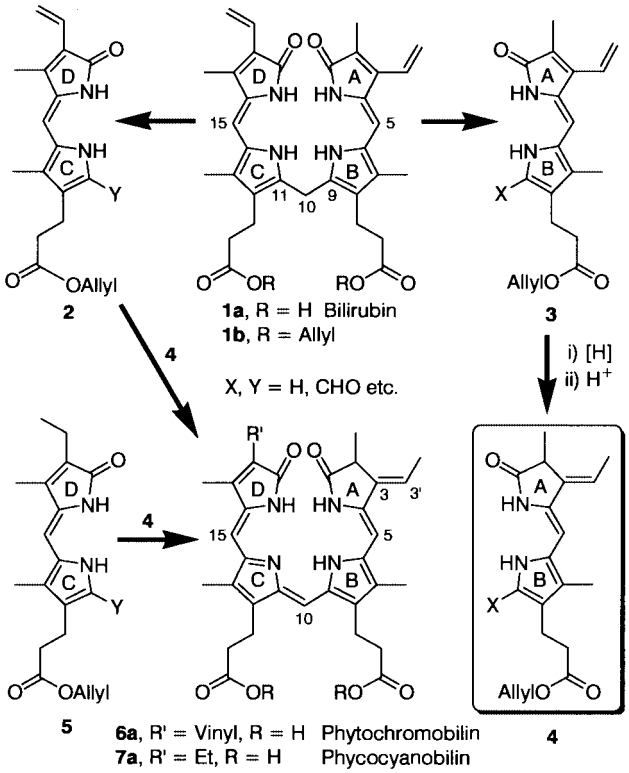
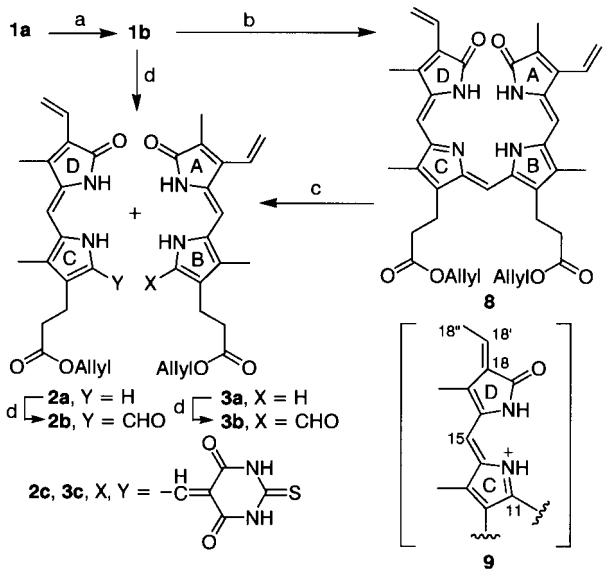


Figure 1.

The two pyrromethenone derivatives (**2a**, **3a**) were treated with trimethyl orthoformate in trifluoroacetic acid (TFA) to afford the formylated compounds, **2b** and **3b**, in 81% and 93% yields, respectively.

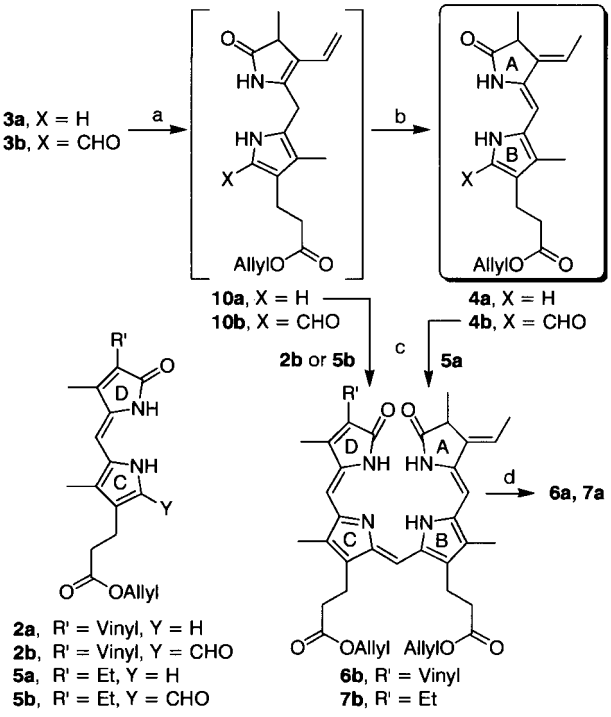
It is known that bilirubin derivatives are cleaved between the B- and C-rings in the acid solution to yield the pyrromethenone derivatives,[7] even though they further react to afford the scrambled mixture of tetrapyrrole derivatives by recondensation of the pyrromethenones, or decomposed. Thus, we next attempted the direct formation of the formylated compounds, **3b** and **2b**, from **1b** under the similar conditions used above for the preparation of **3b** and **2b** to avoid the recondensation or decomposition. When **1b** was treated with trimethyl orthoformate in TFA at 0 °C for 30 min, **3b** was obtained in 27% yield by direct formylation at C-9 accompanied by cleavage between C-9 and C-10, but not the C/D-ring component (**2b**). When the reaction was stopped after 20 min, **3b** was obtained in 21% yield along with **2b** in 4% yield. The C/D-ring part of **1b** seemed to decompose in acidic medium probably through the intermediate (**9**) protonated at C-18'', in which the electron density at C-11 is decreased to retard the desired formylation. Formylation of **1b** by Vilsmeier reaction

or introduction of ester group by Friedel–Crafts type reaction was also attempted, but the expected products could not be obtained.



a) AllylBr (5.0 equiv), DBU (2.1 equiv) in DMF, 60 °C, 4 h. **1b**, 95%. b) DDQ (1.1 equiv) in benzene, rt, 20 min. **8**, 66%. c) Thiobarbituric acid (1.5 equiv) in MeOH, rt, 1 h. **2a**, **3a**, **2c**, **3c**, quant., respectively.[6] d) $(MeO)_3CH$/TFA (1/2, v/v), 0 °C, 30 min. **2b**, 81% from **2a** (used a small amount of CH_2Cl_2). **3b**, 93% from **3a**, 27% from **1b**.

Scheme 1.



a) Al(Hg) (3.0 equiv) in THF/H_2O (10/1, v/v), rt, 4 h. b) For **10b**, TsOH·H_2O (1.0 equiv) in CH_2Cl_2, rt, 10 min. **4b**, 57% from **3b**. c) For **4b**, **5a** (1.2 equiv) in MeOH, cat. 30% HBr/AcOH, rt, 4 h. **7b**, 40% from **4b**; For **10a**, **2b** or **5b** (2.0 equiv) in MeOH, cat. H_2SO_4, rt, 20 min. **6b**, 27%, **7b**, 27% from **3a**, respectively. d) See References 2b and 2d.

Scheme 2.

The formylated A/B-ring component (**3b**) was treated with Al(Hg) in THF/H_2O to afford the reduced product **10b**, followed by acid treatment in CH_2Cl_2 to give the A/B-ring having an ethylidene group (**4b**) in 57% yield from **3b**. This product **4b** was coupled with the C/D-ring component (**5a**)[2] with an acid catalyst in methanol to yield the PCB diallyl ester (**7b**) in 40% yield as shown in Scheme 2.

On the other hand, there are two possible methods to prepare PΦB diallyl ester (**6b**), namely, by the combination of **4b** and **2a**, or **4a** and **2b**. Attempts to couple **4b** obtained above with **2a** were not successful, since **2a** decomposed prior to the expected coupling reaction under the acidic conditions. Therefore, reduction of **3a** with Al(Hg) was examined to prepare **4a**. Although preparation of **10a** by Al(Hg) reduction of **3a** was confirmed by monitoring with TLC, **4a** was not obtained after the following acid treatment. The product **10a** may be unstable under the acidic conditions. Then, the crude product **10a** was mixed with **2b** and treated with an acid catalyst in MeOH to afford the desired PΦB diallyl ester (**6b**) in 27% yield. This method using the intermediary reduction product **10a** was also applicable for the synthesis of PCB diallyl ester (**7b**) by employing **5b** instead of **2b** in 27% yield.

As we have already established the Pd(0)-catalyzed deprotection of diallyl esters of **6b** and **7b**,[2b,d] the present synthetic method of the A/B-ring component made it possible to construct phycobilin derivatives in very short steps starting from commercially available bilirubin (**1a**).

The present work was financially supported in part by Grand-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture.

References and Notes

1 W. Rüdiger and F. Thümmler, *Angew. Chem., Int. Ed. Engl.*, **30**, 1216 (1991); 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 (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. f) D. Sawamoto, H. Nakamura, H. Kinoshita, S. Fujinami, and K. Inomata, *Chem. Lett.*, **2000**, 1398. See also the references cited therein.

3 H. Hanzawa, K. Inomata, H. Kinoshita, T. Kakiuchi, K. P. Jayasundera, D. Sawamoto, A. Ohta, K. Uchida, K. Wada, and M. Furuya, *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 3612 (2001); Recently, a related paper was reported: U. Robben, I. Lindner, W. Gärtner, and K. Schaffner, *Angew. Chem. Int. Ed.*, **40**, 1048 (2001).

4 a) I. Lindner, B. Knipp, S. E. Braslavsky, W. Gärtner, and K. Schaffner, *Angew. Chem. Int. Ed.*, **37**, 1843 (1998). b) P. Manitto and D. Monti, *J. Chem. Soc., Chem. Commun.*, **1980**, 178.

5 R. Bonnett and A. F. McDonagh, *J. Chem. Soc., Sect. D*, **1970**, 238; P. Manitto and D. Monti, *Experientia*, **35**, 9 (1979) [*Chem. Abstr.*, **90**, 99638g (1979)].

6 Methanol was found to be a solvent of choice to accelerate the reaction using a smaller amount of the solvent. The quantitative reaction can produce the products **2a**, **3a**, **2c** and **3c** in 50% yields from **8**, respectively.

7 A. F. McDonagh and F. Assisi, *J. Chem. Soc., Chem. Commun.*, **1972**, 117.