Synthesis of the Sterically Fixed Biliverdin Derivative Bearing the *Z-anti* C/D-Ring Component

Mostafa A. S. Hammam, Yasue Murata, Hideki Kinoshita, and Katsuhiko Inomata*
Division of Material Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192

(Received June 28, 2004; CL-040749)

A sterically locked biliverdin derivative was synthesized by developing an efficient method for the preparation of *Z-anti* C/D-ring component toward investigation of the stereochemistry and function of the phytochrome chromophores.

Phytochromes are biliprotein photoreceptors that play an essential role in the various phases of plant development and growth. The recent discovery of phytochrome-related proteins in photosynthetic cyanobacteria and nonphotosynthetic eubacteria has opened new avenues for investigating biliprotein photosensory function.[1] Plant phytochromes carry either phytochromobilin (PΦB) or phycocyanobilin (PCB) as chromophore which binds covalently to the protein by a thioether bond through the A-ring ethylidene side chain, and respond to red/far-red light which interchanges between *Z*- and *E*-forms at C-15 position of the chromophores. This double-bond photoisomerization converts the physiologically inactive red-light absorbing P_r form into the active far red-light absorbing P_{fr} form and vise versa. We have been studying on the syntheses of phycobilin derivatives,[2] and succeeded in synthesizing PΦB,[2d] PCB,[2b,c] and modified PCBs[2e,f] in free acid forms, which made it possible to assemble the chromophores with the apoproteins not only in vitro to analyze the spectral properties of the resulting holoproteins,[3] but also in vivo to observe their physiological functions.[4]

On the other hand, it was found that some bacterial phytochromes carry biliverdin (BV) as natural chromophore. Recently, we reported that BV binds covalently to *Agrobacterium* phytochrome Agp1 via its A-ring vinyl side chain.[5] Herein, we attempted to construct the sterically fixed BV derivative **1** bearing the *Z-anti* C/D-ring component according to the retrosynthetic analysis shown in Figure 1 toward investigation of the stereochemistry and function of phytochrome chromophores.

We have reported the total synthesis of PΦB starting from 4-methyl-3-[2-(*p*-tolylthio)ethyl]-2-tosylpyrrole as a precursor of the A- and D-rings and a 2-formylpyrrole common to the B- and C-rings.[2d] In order to construct the BV derivative, we applied this procedure to prepare the A/B-ring component **3** bearing a vinyl group at A-ring as shown in Scheme 1. Pyrromethenone derivative **7** carrying the *p*-tolylthioethyl side chain was converted to the corresponding sulfoxide by treating with *m*CPBA in CH_2Cl_2, followed by treating with a mixture of formic acid and trifluoroacetic acid (TFA) at 5 °C to afford the pyrromethenone derivative **8a** and/or its decarboxylated form **8b**. Subsequent reflux in DMF in the presence of pyridine afforded the desired A/B-ring component **3** in 57% yield in three steps. This compound **3** is also available by treating BV diallyl ester with thiobarbituric acid.[6]

The C/D-ring component **4** was prepared from D-ring **5**, which was already available according to our previous method,[7]

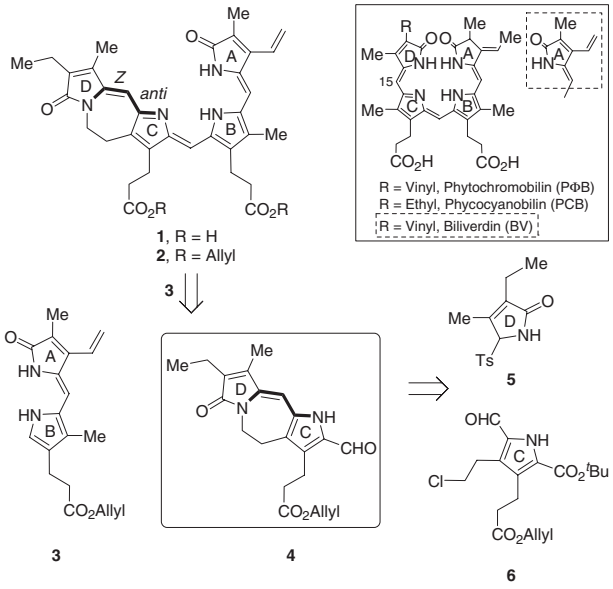
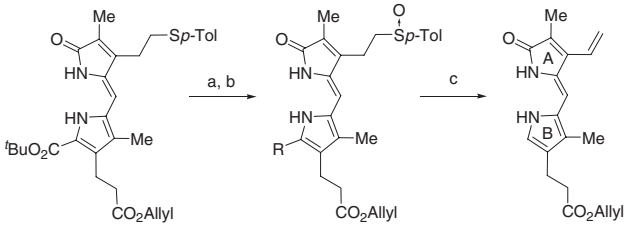


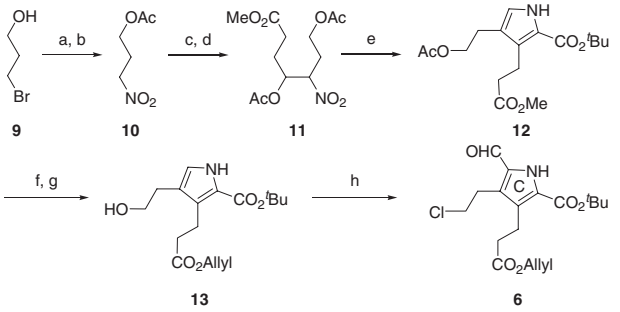
Figure 1.



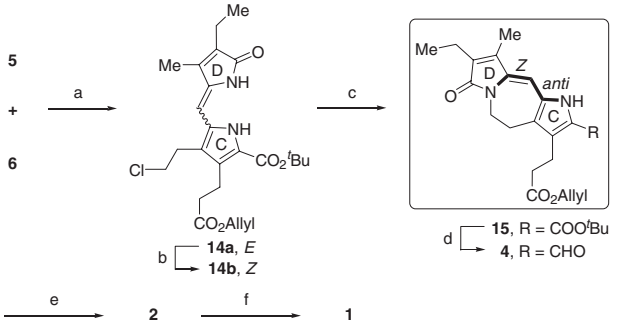
Scheme 1. a) *m*CPBA (1.0 equiv.) in CH_2Cl_2, 0 °C, 15 min. b) Formic acid/TFA (2/1, v/v), 5 °C, 1 h. Crude mixture of **8a**, **8b** was used for the next step without isolation. c) Pyridine (10.0 equiv.) in DMF, reflux, 2 h. **3**, 57% from **7**.

and the C-ring **6** carrying a good leaving group to afford the cyclized product. Commercially available 3-bromo-1-propanol (**9**) was first acetylated followed by nitration reaction using sodium nitrite in the presence of phloroglucinol in DMF to give 3-nitropropyl acetate (**10**) in 60% yield. Compound **10** was coupled with an oxo-ester in a similar manner for our preparation of the B- and C-rings,[2a,e] followed by acetylation of the resulting alcohol to give the nitro acetate **11** in 66% yield. When compound **11** was treated with *t*-butyl isocyanoacetate and DBU according to Barton's method[8] in acetonitrile, the pyrrole derivative **12** was obtained in 55% yield. Saponification of **12** with KOH, followed by allyl esterification using allyl bromide in

the presence of DBU in THF/DMF gave the pyrrole **13** in 60% yield. When formylation of **13** by the Vilsmeier reaction was attempted to give the formylated product in situ, chlorination of the hydroxy group proceeded simultaneously to afford the formylpyrrole **6** bearing a good leaving group in 94% yield.



Scheme 2. a) Ac_2O (1.1 equiv.), DMAP (0.2 equiv.) in THF, 0 °C–rt, 3 h. b) $NaNO_2$ (2.0 equiv.), phloroglucinol dihydrate (1.1 equiv.) in DMF, rt, overnight. **10**, 60% from **9**. c) $CH_3O_2CCH_2CH_2CHO$ (1.0 equiv.), KOH (0.2 equiv.) in MeOH, 0 °C–rt, overnight. d) Ac_2O (1.1 equiv.), DMAP (0.2 equiv.) in THF, 0 °C–rt, 4 h. **11**, 66% from **10**. e) $CNCH_2CO_2t$-Bu (1.0 equiv.), DBU (2.2 equiv.) in MeCN, −40 °C–rt, 6 h. **12**, 55%. f) KOH (5.0 equiv.) in MeOH, 0 °C, 2 h. g) AllylBr (1.1 equiv.), DBU (1.0 equiv.) in THF/DMF (2/1, v/v), 0 °C–rt, 1.5 h. **13**, 60% from **12**. h) $POCl_3$ (2.5 equiv.) in DMF, 80 °C, 2 h, then aq 10% NaOAc. **6**, 94%.



Scheme 3. a) *n*-Bu_3P (2.5 equiv.), DBU (1.5 equiv.) in THF, 0 °C–rt, 4 h. **14a**, 84%; **14b**, 14%. b) cat. I_2 in CH_2Cl_2, rt, 24 h. **14b**, 80%. c) DBU (3.0 equiv.) in THF, 50 °C, overnight. **15**, 76% from **14b**. d) TFA/$(MeO)_3CH$ (2/1, v/v), 0 °C–rt, 1 h, then H_2O. **4**, quant. e) **3** (1.0 equiv.), H_2SO_4 (2.0 equiv.) in MeOH, rt, 1 h. **2**, 87%. f) $[Pd(PPh_3)_4]$ (0.2 equiv.), TsNa (2.1 equiv.) in THF/MeOH (1/1, v/v), rt, 10 min. **1**, 90%.

Our original Wittig-type coupling reaction between tosyl pyrrolinone **5** and formylpyrrole **6** proceeded satisfactorily using tri(*n*-butyl)phosphine in the presence of DBU in THF at 0 °C–rt to afford the C/D-ring component **14a**, **14b** in 98% yield. We found that the *E*-isomer of C/D-ring component **14a** should be converted to the *Z*-isomer **14b** prior to the cyclization by treating with a catalytic amount of iodine in methylene chloride. The *Z*-form **14b** was easily cyclized in the presence of DBU in THF at 50 °C affording the desired cyclized product **15** in 76% yield. Subsequent formylation was accomplished by treating with trimethyl orthoformate in TFA at 0 °C–rt for 1 h to give the formylated C/D-ring component **4** in quantitative yield.[9]

The coupling reaction between the C/D- and A/B-ring components, **4** and **3**, was carried out under acidic conditions to afford the sterically fixed BV diallyl ester derivative **2** in 87% yield.

Finally, the deprotection of the allyl ester was achieved by Pd(0)-catalyzed reaction[2b,d] using sodium *p*-toluenesulfinate as a nucleophile instead of morpholine, which has been used in our previous method, in THF/MeOH to give the desired chromophore **1** in 90% yield.[10]

It can be expected that such sterically fixed chromophores will open the new avenues for investigation of the stereochemistry and function of phytochrome chromophores both in vitro and in vivo in near future.[11]

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

References and Notes

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9 ^{1}H NMR of compound **4**: ($CDCl_3$) δ 1.12 (t (J = 7.5 Hz), 3H), 2.10 (s, 3H), 2.40 (q (7.5), 2H), 2.62 (t (7.3), 2H), 2.86 (brt, 2H), 3.07 (t (7.3), 2H), 3.94 (br, 2H), 4.56 (d (5.7), 2H), 5.22 (dd (1.4, 10.4), 1H), 5.28 (dd (1.4, 17.2), 1H), 5.88 (ddt (17.2, 10.4, 5.7), 1H), 6.30 (s, 1H), 9.59 (s, 1H), 11.07 (s, 1H).
10 To a mixed solution of **2** (50 mg, 0.074 mmol) and $[Pd(PPh_3)_4]$ (17 mg, 0.015 mmol) in THF (1.9 mL) was added a solution of TsNa (27 mg, 0.155 mmol) in MeOH (1.9 mL) under nitrogen atmosphere at room temperature. After stirring for 10 min, the solvent was evaporated and the residue was separated by silica gel column chromatography ($CHCl_3$/MeOH/AcOH = 200/15/1). The blue fraction was evaporated and the resulting solid residue was recrystallized from $CHCl_3$/hexane. **1**: 40 mg (a blue solid, 90% yield), Mp above 300 °C. ^{1}H NMR (C_5D_5N) δ 1.11 (t (J = 7.5 Hz), 3H), 2.10 (s, 3H), 2.13 (s, 3H), 2.35 (s, 3H), 2.40 (q (7.5), 2H), 2.83 (t (7.1), 2H), 2.85–2.90 (m, 4H), 3.14 (t (7.1), 2H), 3.20 (t (7.1), 2H), 4.01 (br, 2H), 5.61 (dd (1.2, 11.7), 1H), 5.70 (dd (1.2, 17.8), 1H), 6.31 (s, 1H), 6.61 (s, 1H), 6.76 (dd (17.8, 11.5), 1H), 7.57 (s, 1H). NH and CO_2H protons were not observed clearly. UV/Vis (MeOH) λ_{max} 385 (ε = 23,355), 645 (ε = 17,729) nm; HRMS (FAB) ($M^+ + 1$), Found: m/z 597.2706. Calcd for $C_{34}H_{37}N_4O_6$: 597.2713.
11 The sterically fixed BV derivative **1** could be assembled with Agp1 forming covalent bond (personal communication from T. Lamparter, Freie Universitaet, Berlin). The detailed investigation for photochemical function of the resulting holoprotein is in progress.

Published on the web (Advance View) September 4, 2004; DOI 10.1246/cl.2004.1258