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

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(Received February 28, 2005; CL-050259)

Biliverdin (BV) derivative bearing the *E-anti* C/D-ring component, which is sterically fixed by 8-membered ring, was synthesized toward investigation of the structure and function of the chromophore in phytochrome.

Light is one of the major environmental signals that influence plant growth and development. Plants can detect almost all facets of light, e.g. wavelength and quantity, by using photoreceptors such as phytochromes. 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 responds to red/far-red light which interchanges between *Z*- and *E*-forms at C-15 position of the chromophore. Some bacterial phytochromes carry biliverdin (BV) as natural chromophore, and it was found that the BV covalently binds to the apoprotein of *Agrobacterium* phytochrome Agp1 via its A-ring vinyl side chain.[1] The interchange between the physiologically inactive red-light absorbing P_r (*Z*-form) and the active far red-light absorbing P_{fr} (*E*-form) is the most essential for the light absorbing and biological processes in the phytochrome chromophore function (Figure 1).[2]

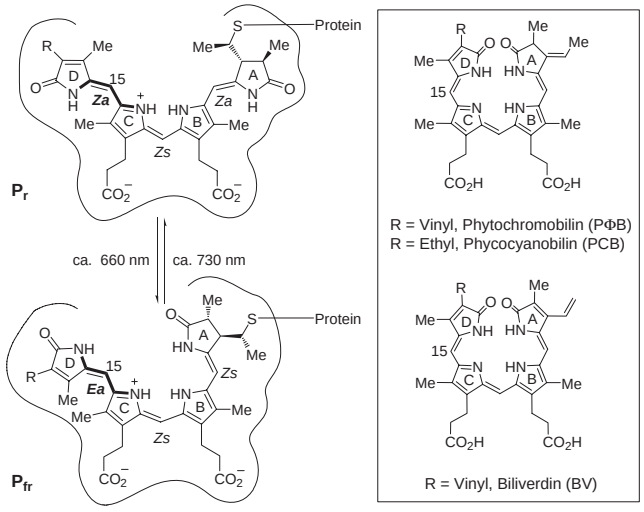


Figure 1.

In the course of our studies on the phytochrome chromophores, we synthesized natural and unnatural chromophores to analyze the structure and function of the chromophores in the reconstituted phytochromes.[3–5] Furthermore, we have recently succeeded for the first time in synthesizing the sterically locked *Z-anti* BV derivative corresponding to P_r form,[6] which made it possible to directly confirm the stereochemistry around C-15 position.[2]

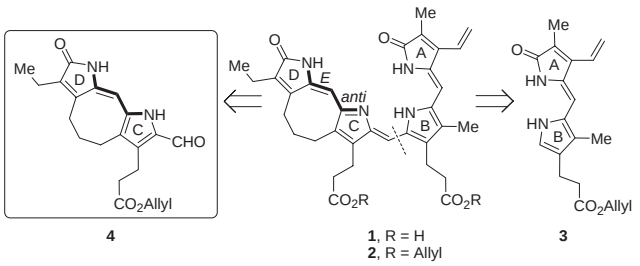


Figure 2.

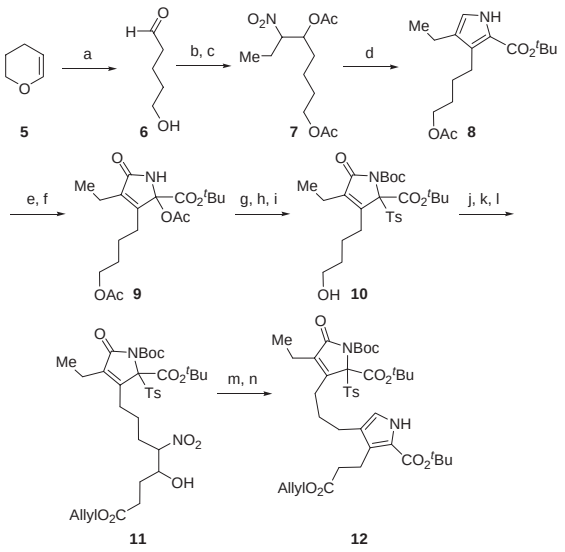
In the present work, we developed an efficient method for the synthesis of the sterically locked *E-anti* BV derivative corresponding to P_{fr} form (Figure 2).

The A/B-ring component **3** common to locked BV derivatives was prepared according to our previous method.[6]

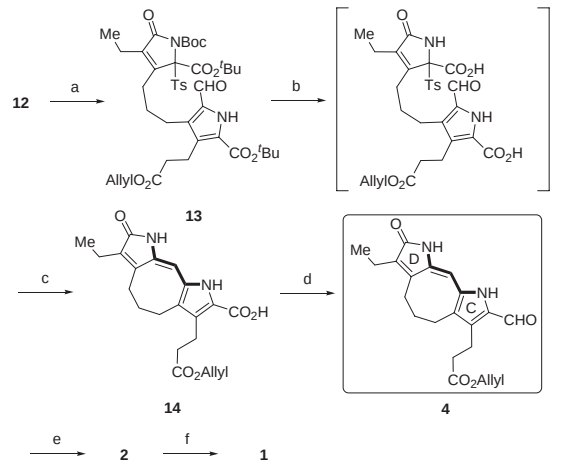
On the other hand, the C/D-ring component **4** was prepared starting from 5-hydroxypentanal (**6**), which can be obtained from the commercially available 3,4-dihydro-2*H*-pyran (**5**) as shown in Scheme 1. The Henry reaction with 1-nitropropane followed by acetylation in the presence of 4-(dimethylamino)pyridine (DMAP) gave the nitro-diacetate compound **7**, which was allowed to react with *t*-butyl isocyanoacetate in the presence of DBU in THF applying Barton's method[7] to give the pyrrole derivative **8** in 67% yield.

Iodination of α-position of the pyrrole **8** with *N*-iodosuccinimide (NIS) followed by oxidation utilizing $Pb(OAc)_4$ in toluene gave the pyrrolinone derivative **9** in 90% yield from **8** in two steps. The α-acetoxy group of the pyrrolinone derivative **9** was replaced with Ts group using anhydrous sodium *p*-toluenesulfinate (TsNa) in refluxing THF, followed by protection of the pyrrolinone-NH using di-*t*-butyl dicarbonate in MeCN. Then, hydrolysis of the acetate group in 0.5 M methanolic HCl afforded the N-protected tosylpyrrolinone derivative **10** in 92% yield from **9** in three steps. Iodination of the resulting alcohol using iodine and triphenylphosphine in the presence of imidazole in MeCN followed by nitration using sodium nitrite in the presence of phloroglucinol in DMF gave the tosylpyrrolinone derivative bearing a nitro group in its side chain in 38% yield. The resulting nitro compound was allowed to react with allyl 4-oxobutanoate according to the Henry reaction to construct the nitro-alcohol side chain of the tosylpyrrolinone derivative **11** in 64% yield. Acetylation of the resulting alcohol **11** followed by reaction with *t*-butyl isocyanoacetate gave the dipyrrole derivative **12** in 82% yield from **11** in two steps.

Compound **12** was subjected to the Vilsmeier reaction to afford the formylated dipyrrole derivative **13** in 68% yield as shown in Scheme 2. Compound **13** was then treated with 99% formic acid to cleave the Boc and *t*-butyl esters giving the dicarboxylic acid derivative, which was subjected to our original Wittig-type coupling reaction using tri(*n*-butyl)phosphine in the



Scheme 1. a) In 0.1 M aq HCl, rt, 30 min. b) 1-Nitropropane (2.0 equiv.), KOH (0.2 equiv.) in MeOH, 0 °C–rt, 3.5 h. c) Ac_2O (2.2 equiv.), DMAP (0.2 equiv.) in THF, 0 °C–rt, 1 h. **7**, 38% from **5**. d) $CNCH_2CO_2$-*t*-Bu (1.0 equiv.), DBU (2.2 equiv.) in THF, 0 °C–rt, overnight, **8**, 67%. e) NIS (1.2 equiv.) in acetone, rt, 1 h. f) $Pb(OAc)_4$ (1.5 equiv.) in toluene, rt, 2 d. **9**, 90% from **8**. g) TsNa (2.1equiv.) in THF, reflux, 1.5 h. h) Boc_2O (1.5 equiv.), DMAP (0.2 equiv.) in MeCN, −40 °C–rt, 1 h. i) In 0.5 M methanolic HCl (prepared by diluting 12 M HCl with MeOH), rt, overnight, **10**, 92% from **9**. j) I_2 (1.2 equiv.), Ph_3P (1.2 equiv.), imidazole (2.5 equiv.) in MeCN, rt, 1 h. k) $NaNO_2$ (2.0 equiv.), phloroglucinol dihydrate (1.1 equiv.) in DMF, rt, 7 h. l) $OHCCH_2$-CH_2CO_2-Allyl (2.0 equiv.), KOH (0.3 equiv.) in THF, 0 °C–rt, overnight, **11**, 24% from **10**. m) Ac_2O (1.1 equiv.), DMAP (0.2 equiv.) in THF, 0 °C, 2 h. n) $CNCH_2CO_2$-*t*-Bu (4.0 equiv.), DBU (5.0 equiv.) in MeCN, 0 °C–rt, 4 h. **12**, 82% from **11**.



Scheme 2. a) $POCl_3$ (2.0 equiv.) in DMF, 65 °C, 2 h, then aq 10% NaOAc. **13**, 68%. b) 99% HCO_2H, rt, overnight. c) *n*-Bu_3P (2.5 equiv.), DBU (1.5 equiv.) in THF/DMF, −75 °C–rt, overnight. **14**, 74%. d) TFA/$(MeO)_3CH$ (2/1, v/v), 0 °C–rt, 1 h, then H_2O. **4**, 40%. e) **3**, H_2SO_4 (2.0 equiv.) in MeOH, rt, 1 h. **2**, 71%. f) $[Pd(PPh_3)_4]$ (0.2 equiv.), TsNa (2.1 equiv.) in THF/MeOH (1/1, v/v), rt, 10 min. **1**, 70%.

presence of DBU in THF/DMF at −75 °C–rt to give the pyrromethenone derivative **14** in 74% yield. Compound **14** was formylated by treating with trimethyl orthoformate in TFA to give the desired formylated *E-anti* C/D-ring component **4** in 40% yield.[8]

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 71% yield.

Finally, the deprotection of the allyl esters was achieved by Pd(0)-catalyzed reaction in the presence of sodium *p*-toluenesulfinate as nucleophile in THF/MeOH to give the desired BV derivative **1** bearing the *E-anti* C/D-ring component in 70% yield.[9]

The chromophore **1** thus prepared was assembled with Agp1 apoprotein and found to form covalent bond showing the absorption spectrum corresponding to P_{fr} form.[10]

The present work was financially supported in part by Grant-in-Aid for Scientific Research (B) (No. 15350021) from Japan Society for the Promotion of Science (JSPS). One of the authors (K. I.) gratefully acknowledges Dr. Tilman Lamparter of Freie Universität Berlin for giving us helpful information.

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8 ^{1}H NMR spectrum of compound **4**: ($CDCl_3$, 400 MHz) δ 1.13 (t J = 7.6 Hz, 3H), 1.96 (p 6.4, 2H), 2.36–2.42 (m, 4H), 2.51 (t 6.6, 2H), 2.64 (t 7.7, 2H), 3.08 (t 7.7, 2H), 4.57 (d 5.9, 2H), 5.23 (dd 10.5, 1.2, 1H), 5.28 (dd 17.3, 1.5, 1H), 5.88 (ddt 17.2, 10.5, 5.9, 1H), 6.19 (s, 1H), 8.31 (brs, 1H), 9.63 (s, 1H), 9.98 (brs, 1H).
9 ^{1}H NMR spectrum of compound **1**: (C_5D_5N, 400 MHz) δ 1.17 (t J = 7.5 Hz, 3H), 1.94 (m, 2H), 2.06 (s, 3H), 2.12 (s, 3H), 2.32 (t 6.1, 2H), 2.44 (q 7.5, 2H), 2.53 (t 6.1, 2H), 2.88 (t 7.3, 4H), 3.20 (t 7.1, 2H), 3.21 (t 7.3, 2H), 5.60 (d 11.7, 1H), 6.74 (d 17.8, 1H), 6.27 (s, 1H), 6.72 (dd 11.4, 17.8, 1H), 6.72 (s, 1H), 7.59 (s, 1H), 8.42 (s, 1–2H), 11.76 (s, 1H). $CO_2\underline{H}$ protons were not observed clearly. UV–vis (MeOH) λ_{max} 381 (ε = 40,533), 630 (ε = 29,260) nm; HRMS (FAB) $[M + 1]^+$, Found: m/z 597.2741. Calcd for $C_{34}H_{37}N_4O_6$: 597.2713.
10 Personal communication to K. I. from T. Lamparter and S. Noack, Freie Universität Berlin. The detailed investigation for the resulting holoprotein is in progress.

Published on the web (Advance View) May 14, 2005; DOI 10.1246/cl.2005.800