Total Syntheses of Doubly Locked Biliverdin Derivatives toward Elucidation of the Stereochemistry of Phytochrome Chromophore

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(Received November 2, 2007; CL-071214; E-mail: inomata@cacheibm.s.kanazawa-u.ac.jp)

To elucidate the stereochemistry of phytochrome chromophore in far-red light absorbing form (Pfr), four different types of doubly locked biliverdin (BV) derivatives, 5*Zs*15*Ea*-BV, 5*Za*15*Ea*-BV, 5*Es*15*Ea*-BV, and 5*Ea*15*Ea*-BV, were synthesized for the first time in free acid forms.

Phytochromes are a wide spread family of red/far-red light responsive photoreceptors first discovered in plants.[1,2] They play critical roles in various light-regulated processes, ranging from phototaxis and pigmentation in bacteria to seed germination, chloroplast development, shade avoidance, and flowering in higher plants. All phytochromes have covalently attached linear tetrapyrrole (bilin) chromophore that absorbs light in red and far-red region. Three different bilins are used as chromophores: land plant uses phytochromobilin (PΦB), some algal species and cyanobacteria use phycocyanobilin (PCB), whereas other bacteria use biliverdin (BV).

We have been studying on the total syntheses of natural and unnatural bilin chromophores,[3] and have succeeded in synthesizing PΦB, PCB, the modified PCBs, BV, and its analogs including locked derivatives,[4] in free acid forms by developing efficient methods for the preparation of each of pyrrole rings, a new coupling reaction between them, and palladium-catalyzed deprotection of allyl propanoate side chains of the B- and C-rings under mild conditions.[3]

Assembly experiments of the synthesized chromophores with phytochrome apoproteins in vitro and in vivo have provided us insights into the structure and function of phytochromes, especially the stereochemistry at the C-15 position of the BV chromophore in red light absorbing Pr-form and far-red light absorbing Pfr-form of *Agrobacterium* phytochromes Agp1 and Agp2 was determined to be 15*Z*-*anti* and 15*E*-*anti* configuration and conformation, respectively, by using the chemically synthesized locked chromophores.[5] In a similar manner, the stereochemistry at the C-5 position was determined to be 5*Z*-*syn* in Pr-form.[6] Consequently based on these results, we proposed that the stereochemistry of the Agp1 and Agp2 chromophores is 5*Zs*/10*Zs*/15*Za* in the Pr-form and 5*Za*/10*Zs*/15*Ea* or 5*Ea*/10*Zs*/15*Ea* in the Pfr-form.[6] The former stereochemistry of the Pr-from was recently confirmed by the X-ray crystallographic analysis of the chromophore-biding domain of the two bacteriophytochromes,[7] while the stereochemistry of the Pfr-form is still open question. Therefore, the doubly locked BV derivatives, 5*Zs*15*Ea*-BV **1**, 5*Za*15*Ea*-BV **2**, 5*Es*15*Ea*-BV **3**, and 5*Ea*15*Ea*-BV **4** (Figure 1), were synthesized toward elucidation of the stereochemistry of phytochrome chromophore in Pfr-form. The formation of noncovalent photoactive adducts between the BV chromophore, which does not have a vinyl group at the A-ring, and Agp1 or Agp2 apoprotein was already confirmed.[8]

We have established the synthetic method of the formylated

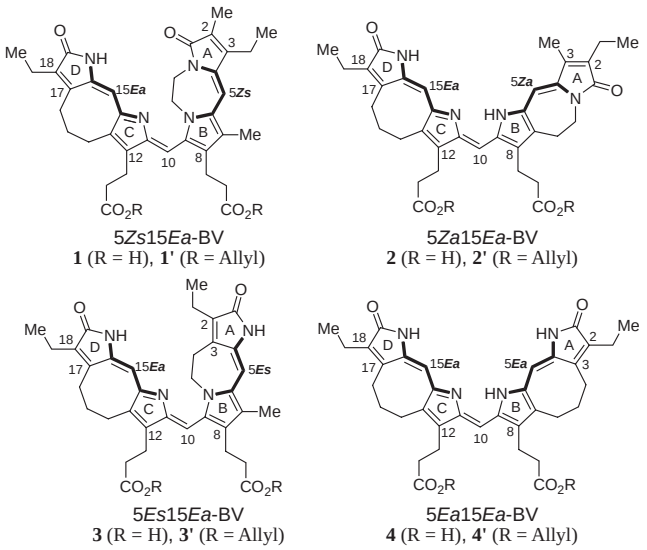
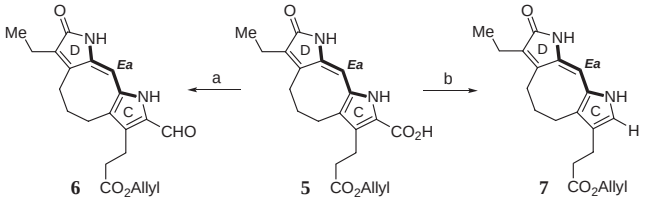


Figure 1.

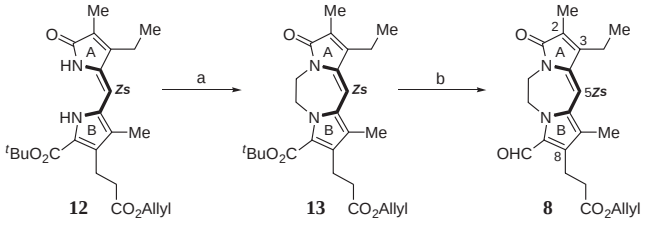


Scheme 1. a) TFA/$(MeO)_3CH$ (2/1, v/v), 0 °C–rt, 1 h, then H_2O. **6**, 40%. b) TFA (10 mL/mmol), 1 h, **7**, 70%.

CD-ring component **6** with *E*-*anti* stereochemistry via the pyrromethenone **5**.[9] Thus, it must be possible to synthesize the doubly locked BV derivatives **1**–**4** by coupling the formylated AB-ring components, *Z*-*syn* **8**, *Z*-*anti* **9**, *E*-*syn* **10**, and *E*-*anti* **11** with the CD-ring component **7**[10] available by treating **5** with trifluoroacetic acid (TFA) (Scheme 1).

The formylated *Z*-*syn* AB-ring components **8**[11] was prepared according to the modified procedure of the previously reported synthetic method of the locked *Z*-*syn* CD-ring component as shown in Scheme 2.[4] The use of catalytic amount of *n*-Bu_4NBr was found to be effective to get reproducible high yield of the intermediate **13**.

We employed the previously prepared locked *Z*-*anti* and *E*-*syn* CD-ring components[4] as the corresponding *Z*-*anti* and *E*-*syn* AB-ring components, **9** and **10**, since it was confirmed that the replacement of the methyl group at C-2 position with ethyl group does not influence to the assembly experiments.[12] Similarly the formylated *E*-*anti* CD-ring component **6** was used as the *E*-*anti* AB-ring component **11** (Figure 2).



Scheme 2. a) NaH (7 equiv.), *n*-Bu_4NBr (0.2 equiv.), $(BrCH_2)_2$ (10 mL/mmol), in THF, rt, overnight. **13**, 80%. b) (1) in TFA, rt, 0.5 h. (2) $(MeO)_3CH$ (1.1 equiv.), rt, 1 h, then H_2O. **8**, quant.

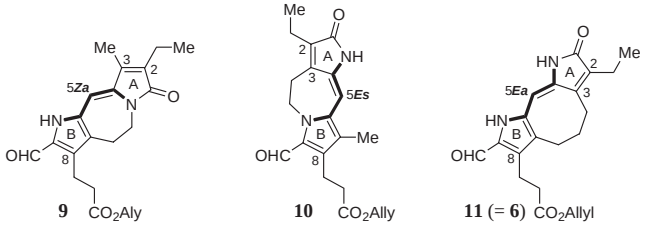


Figure 2.

$$\mathbf{7} + \mathbf{8} \xrightarrow{a} \mathbf{1'} \xrightarrow{b} \mathbf{1} \quad \text{(Eq. 1)} \qquad \mathbf{7} + \mathbf{9} \xrightarrow{a} \mathbf{2'} \xrightarrow{b} \mathbf{2} \quad \text{(Eq. 2)}$$

$$\mathbf{7} + \mathbf{10} \xrightarrow{a} \mathbf{3'} \xrightarrow{b} \mathbf{3} \quad \text{(Eq. 3)} \qquad \mathbf{7} + \mathbf{11} \xrightarrow{a} \mathbf{4'} \xrightarrow{b} \mathbf{4} \quad \text{(Eq. 4)}$$

Scheme 3. a) H_2SO_4 (2.0 equiv.) in MeOH, rt, 1 h, **1′**, 40%, **2′**, 62%, **3′**, 60%, **4′**, 67%. b) $[Pd(PPh_3)_4]$ (0.2 equiv.), TsNa (2 equiv.) under N_2, in THF/MeOH (1/1), 30 min, rt, **1**, 60%, **2**, 70%, **3**, 70%, **4**, 80%.

These AB-ring components **8–11** were then coupled with **7** under acidic conditions to afford the corresponding diallyl esters of the doubly locked BV-derivatives **1′–4′**,[13–16] followed by deprotection of allyl ester moieties by treating with sodium *p*-toluenesulfinate (NaTs) in the presence of Pd-catalyst to give the free acid forms **1–4**[13–16] (Scheme 3, eqs 1–4).

Though the diallyl esters **1′–4′** were readily isolated, the free acid forms **1–4** are rather unstable. ^{1}H NMR spectra suggested **1–4** exist as a mixture of conformers around the C9–C10 single bond and/or protonated and deprotonated species on the C-ring nitrogen in pyridine-d_5 solution.

As described above, we succeeded in preparing the doubly locked BV derivatives **1–4** for the first time. Preliminary assembly experiment of **1–4** with Agp1 and Agp2 apoproteins suggested that 5*Ea*15*Ea*-BV is the most likely structure of Pfr-form by observing absorption spectra of their adducts.[17]

The present work was financially supported in part by a Grant-in-Aid for Scientific Research (B) (No. 19350082) from Japan Society for the Promotion of Science (JSPS).

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10 **7**: Mp 202–203 °C (from $CHCl_3$/hexane). IR (KBr) 1727, 1658, 1627 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.12 (3H, t, $J = 7.6$ Hz), 1.98 (2H, quin, 6.5 Hz), 2.35 (2H, t, 6.3 Hz), 2.38 (2H, q, 7.6 Hz), 2.46 (2H, t, 6.3 Hz), 2.62 (2H, t, 7.7 Hz), 2.80 (2H, t, 7.7 Hz), 4.59 (2H, d, 5.9 Hz), 5.23 (1H, dd, 10.5, 1.4 Hz), 5.30 (1H, dd, 17.3, 1.4 Hz), 5.91 (1H, ddt, 17.3, 10.5, 5.9 Hz), 6.14 (1H, s), 6.67 (1H, d, 2.4 Hz), 7.92 (1H, s), 8.06 (1H, s). HRMS (FAB) $[M + 1]^+$, Found: m/z 341.18725. Calcd for $C_{20}H_{25}N_2O_3$: 341.18653.

11 **8**: Mp 80–81 °C (from $CHCl_3$/hexane). IR (KBr) 1735, 1690, 1647 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.19 (3H, t, $J = 7.5$ Hz), 1.94 (s, 3H), 2.10 (s, 3H), 2.55 (2H, q, 7.5 Hz), 2.58 (2H, t, 7.3 Hz), 3.03 (2H, t, 7.3 Hz), ca. 3.5–4.5 (4H, br), 4.58 (2H, d, 5.8 Hz), 5.23 (1H, dd, 10.5, 1.4 Hz), 5.29 (1H, dd, 17.3, 1.4 Hz), 5.91 (1H, ddt, 17.3, 10.5, 5.8 Hz), 5.98 (1H, s), 9.71 (1H, s). HRMS (FAB) $[M + 1]^+$, Found: m/z 383.19667. Calcd for $C_{22}H_{27}N_2O_4$: 383.19709.

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13 **1′**: Mp 95–96 °C (from $CHCl_3$/hexane). IR (KBr) 3262, 1734, 1675, 1623 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (3H, t, $J = 7.6$ Hz), 1.20 (3H, t, 7.6 Hz), 1.86 (2H, m), 1.95 (3H, s), 2.14 (3H, s), 2.36–2.47 (4H, m), 2.50–2.64 (8H, m), 2.96 (2H, t, 7.6 Hz), 2.98 (2H, t, 7.6 Hz), 4.23 (4H, br), 4.56 (4H, m), 5.20 (1H, dd, 10.5, 1.5 Hz), 5.21 (1H, dd, 10.5, 1.5 Hz), 5.28 (2H, dd, 17.3, 1.5 Hz), 5.88 (2H, m), 6.03 (1H, s), 6.31 (1H, s), 7.00 (1H, s), 7.41 (1H, s). UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 377 ($\varepsilon = 12200\,M^{-1}\,cm^{-1}$), 698 (27200) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 705.36435. Calcd for $C_{42}H_{49}N_4O_6$: 705.36521. **1**: Mp (decomposed) 260 °C (from $CHCl_3$/hexane). UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 381 ($\varepsilon = 31900\,M^{-1}\,cm^{-1}$), 692 (37700) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 625.30158. Calcd for $C_{36}H_{41}N_4O_6$: 625.30261.

14 **2′**: Mp 210–211 °C (from $CHCl_3$/hexane). IR (KBr) 3433, 1734, 1685, 1617 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.13 (3H, t, $J = 7.5$ Hz), 1.16 (3H, t, 7.5 Hz), 1.97 (2H, m), 2.17 (3H, s), 2.36–2.48 (6H, m), 2.52 (2H, t, 6.3 Hz), 2.59 (2H, t, 7.5 Hz), 2.61 (2H, t, 7.5 Hz), 2.84 (2H, m), 2.93 (2H, t, 7.5 Hz), 2.98 (2H, t, 7.5 Hz), 3.91 (2H, m), 4.57 (4H, m), 5.21 (2H, d, 10.4 HZ), 5.28 (2H, dd, 17.0, 1.5 Hz), 5.88 (2H, ddt, 17.0, 10.4, 5.8 Hz), 6.36 (1H, s), 6.40 (1H, s), 6.85 (1H, s). NH protons were not observed clearly. UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 366 ($\varepsilon = 12600\,M^{-1}\,cm^{-1}$), 626 (33500), 661 (65900) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 691.34839. Calcd for $C_{41}H_{47}N_4O_6$: 691.34956. **2**: Mp (decomposed) 280 °C (from $CHCl_3$/hexane). UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 366 ($\varepsilon = 12000\,M^{-1}\,cm^{-1}$), 619 (29600), 660 (61400) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 611.28824. Calcd for $C_{35}H_{39}N_4O_6$: 611.28696.

15 **3′**: Mp 152–153 °C (from $CHCl_3$/hexane). IR (KBr) 3418, 1733, 1676, 1622 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (3H, t, $J = 7.3$ Hz), 1.16 (3H, t, 7.3 Hz), 1.88 (2H, m), 2.11 (3H, s), 2.35–2.50 (8H, m), 2.55 (4H, t, 7.6 Hz), 2.62 (2H, t, 7.6 Hz), 2.95 (2H, t, 7.6 Hz), 2.98 (2H, t, 7.6 Hz), 3.21 (2H, br), 4.57 (4H, m), 5.18–5.32 (4H, m), 5.89 (2H, m), 6.25 (1H, s), 6.37 (1H, s), 6.97 (1H, s), 8.83 (1H, brs), 8.87 (1H, brs). UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 378 ($\varepsilon = 12900\,M^{-1}\,cm^{-1}$), 694 (34600) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 691.34909. Calcd for $C_{41}H_{47}N_4O_6$: 691.34956. **3**: Mp (decomposed) 290 °C (from $CHCl_3$/hexane). UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 377 ($\varepsilon = 7100\,M^{-1}\,cm^{-1}$), 688 (19100) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 611.28578. Calcd for $C_{35}H_{39}N_4O_6$: 611.28696.

16 **4′**: Mp 170–171 °C (from $CHCl_3$/hexane). IR (KBr) 3427, 1733, 1683, 1619 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.14 (6H, t, $J = 7.5$ Hz), 1.92 (4H, m), 2.40 (4H, q, 7.6 Hz), 2.39–2.47 (4H, m), 2.51 (4H, t, 6.1 Hz), 2.61 (4H, t, 7.6 Hz), 2.97 (4H, t, 7.6 Hz), 4.58 (4H, d, 5.7 Hz), 5.21 (2H, dd, 10.4, 1.5 Hz), 5.28 (2H, dd, 17.0, 1.5 Hz), 5.89 (2H, ddt, 17.0, 10.4, 5.7 Hz), 6.34 (2H, s), 6.91 (1H, s). NH protons were not observed clearly. UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 371 ($\varepsilon = 8400\,M^{-1}\,cm^{-1}$), 651 (27900) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 691.34988. Calcd for $C_{41}H_{47}N_4O_6$: 691.34956. **4**: Mp (decomposed) 249 °C (from $CHCl_3$/hexane). UV–vis (MeOH + a few drops of 0.5 M HCl) λ_{max} 369 ($\varepsilon = 9900\,M^{-1}\,cm^{-1}$), 648 (41500) nm. HRMS (FAB) $[M + 1]^+$, Found: m/z 611.28510. Calcd for $C_{35}H_{39}N_4O_6$: 611.28696.

17 Personal communication to K. I. from T. Lamparter and I. Malina, Universität Karlsruhe. The detailed investigation for the resulting holoprotein is in progress.

Published on the web (Advance View) January 26, 2008; doi:10.1246/cl.2008.198