doi:10.1246/cl.2011.632

Published on the web May 21, 2011

Total Synthesis of Doubly Locked 5*Za*15*Ea*-Biliverdin Derivative: A Convergent Synthesis of the *E-anti* Dipyrrole Component Locked with a 7-Membered Ring

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Doubly locked 5*Za*15*Ea*-biliverdin (BV) derivative was synthesized toward the investigation of stereochemistry and function of the Pfr-form chromophore in bacteriophytochromes. A convergent synthetic method was established for the locked *E-anti* dipyrrole component with a 7-membered ring.

Phytochromes, photoreceptive chromoproteins, carry a covalently attached linear tetrapyrrole (bilin): land plants use phytochromobilin (PΦB), cyanobacteria use phycocyanobilin (PCB), and other bacteria use biliverdin (BV). Phytochromes mediate various developmental processes of plants and bacteria, through the photoconversion between the red light-absorbing (Pr) and the far-red-light-absorbing (Pfr) forms. The data of photointerchange were not unambiguous and have been interpreted in different ways.[1,2]

Hildebrandt and his co-workers proposed that chromophore in the plant phytochrome corresponding to Pr-form has 5*Za*15*Za* structure and Pfr-form has 5*Zs*15*Ea* on the basis of DFT calculated Raman spectra.[2] On the other hand, we found that the chromophore in Pr-form of Agp 1 and Agp 2 has the 5*Zs*15*Za* structure,[3] which was confirmed by X-ray crystallography for other bacteriophytochromes.[4] The absorption spectra of doubly locked 5*Zs*15*Za*-BV adducts resembled the spectrum of natural BV-binding phytochrome in Pr-form.[3b,5] Furthermore, we found that the Pfr-form of the phytochrome has a structure most like 5*Za*15*Ea* or 5*Ea*15*Ea* based on the investigation using doubly locked BV derivatives that have no vinyl group on the A-ring required for the coupling with apoprotein.[3b,6] To investigate the structure and function of the BV chromophores in Pfr-form, we herein describe the synthesis of doubly locked BV derivative, which has 5*Za*15*Ea* configuration and conformation corresponding to Pfr-form.[6] A retrosynthetic analysis of the doubly locked 5*Za*15*Ea*-BV **1** is shown in Figure 1. The locked *Z-anti* AB-ring component **2** was synthesized in a similar method reported previously by us.[7] On the other hand, we established a new convergent synthetic method for the locked *E-anti* CD-ring component **3** in the present work.

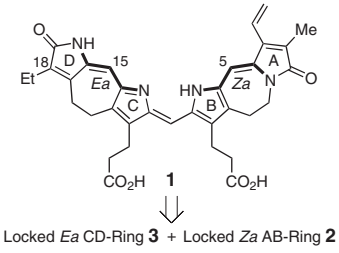
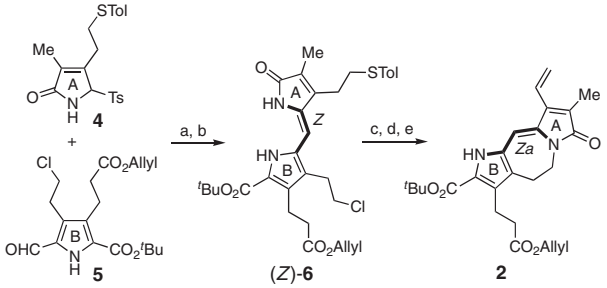


Figure 1. Retrosynthesis of doubly locked 5*Za*15*Ea*-BV **1**.

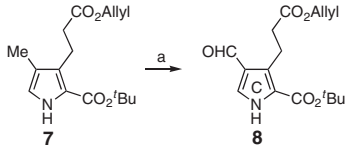
Tosylpyrrolinone **4** and formylpyrrole **5** were prepared according to the previous method.[7,8] Applying our original Wittig-type reaction, tosylpyrrolinone **4** as the A-ring precursor and formylpyrrole **5** as the B-ring precursor were coupled to dipyrrole **6** as a mixture of *E*- and *Z*-isomers, then *E*-isomer was converted to *Z*-isomer with a catalytic amount of I_2 (Scheme 1). (*Z*)-**6** thus obtained was readily cyclized at 50 °C in the presence of DBU in THF affording the desired cyclized *Za* product.[7] After oxidation of tolylthio group on the A-ring, a vinyl group was introduced by refluxing the resulting sulfoxide in DMF under basic conditions to afford the locked *Za* AB-ring component **2**.[5a]



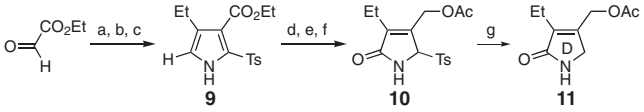
Scheme 1. a) $^{n}Bu_3P$ (2.2 equiv), DBU (1.5 equiv), THF, 0 °C to rt, 4 h. b) I_2 (0.3 equiv), CH_2Cl_2, rt, 4 h. (*Z*)-**6**, 85% in 2 steps. c) DBU (3.0 equiv), THF, 50 °C, overnight, 80%. d) *m*CPBA (1.0 equiv), CH_2Cl_2, rt, 4 h. e) pyridine (10 equiv), DMF, reflux, 2 h. **2**, 80% in 2 steps.

Though we have synthesized the *E-anti* dipyrrole component locked with an 8-membered ring,[9] this method still remained unsatisfying due to the linear synthetic path. On the other hand, all other sterically fixed chromophores except the *E-anti* dipyrrole have been so far locked with a 7-membered ring. Therefore, a new convergent method was established for the synthesis of the *E-anti* CD-ring component locked with a 7-membered ring to unify the ring size for fixation of the stereochemistry.

The synthesis of 4-formylpyrrole **8** as the C-ring precursor was developed in our lab recently, via selective oxidation of pyrrole **7** with DDQ in the presence of MeOH (Scheme 2).[10]



Scheme 2. a) DDQ (2.2 equiv), MeOH (20 equiv), CH_2Cl_2, rt, 8 h. **8**, 70%.



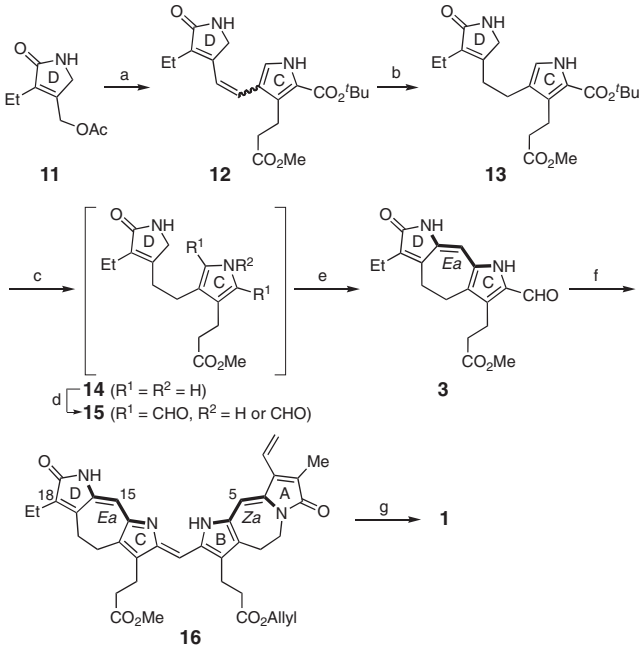
Scheme 3. a) Ethyl glyoxalate (50% in toluene) (1.0 equiv), 1-nitropropane (1.2 equiv), Et_3N (0.2 equiv), reflux to depolymerize ethyl glyoxalate in toluene, 5 min, then rt, 3 h. b) Ac_2O (1.5 equiv), DMAP (0.2 equiv), 0 °C to rt, 4 h. c) TosMIC (1.0 equiv), DBU (2.0 equiv), MeCN, −40 °C to rt, 4 h. **9**, 50% in three steps. d) LAH (1.8 equiv), 0 °C to rt, 15 h, 80%. e) Ac_2O (1.0 equiv), DMAP (0.2 equiv), 0 °C to rt, 4 h. 95%. f) *m*CPBA (1.5 equiv), CH_2Cl_2, rt, 5 h. **10**, 70%. g) $NaBH_4$ (1.0 equiv), EtOH/THF (1/1, v/v), 0 °C, 0.5 h. **11**, 70%.

4-(Acetoxymethyl)pyrrolinone **11** as the D-ring precursor was prepared starting from ethyl glyoxalate, which was coupled with 1-nitropropane via the Henry reaction (Scheme 3). After acetylation of the resulting nitro-alcohol, the obtained mixture of nitro-acetate and nitro-olefin was converted to the corresponding pyrrole-3-carboxylate **9** by applying Barton's method[11] with tosylmethyl isocyanide (TosMIC). Pyrrole-3-carboxylate **9** was reduced to the corresponding alcohol by treating with lithium aluminum hydride (LAH), followed by acetylation with acetic anhydride in the presence of a catalytic amount of DMAP. After bromination, conversion of the bromopyrrole to the corresponding tosylpyrrolinone by our previous acidic redox protocol failed using TFA, DMSO, and Zn metal.[7b,12] Therefore, we developed a new procedure using *m*CPBA to directly oxidize the pyrrole at the α-free position to the corresponding pyrrolinone. 3-(Acetoxymethyl)pyrrole was oxidized to 5-tosylpyrrolinone **10** in 70% yield, followed by reductive elimination of the tosyl group with $NaBH_4$ to afford the 5-unsubstituted pyrrolinone **11** as the D-ring precursor.

The D-ring precursor **11** bearing an acetoxy group at the allylic position was activated by a catalytic amount of Pd[13] to react with ^{n}Bu$_3$P generating the phosphonium intermediate, which was further converted to the corresponding ylide by treatment with ^{n}BuLi at low temperature to couple with formylpyrrole **8** via the Wittig olefination affording the coupled product **12** as a mixture of *E*- and *Z*-isomers (Scheme 4). At this stage, the allyl ester side chain of compound **8** was converted to methyl ester in the product **12** due to the basic conditions using methanol. The resulting olefin mixture **12** was reduced by hydrogenation to afford the product **13** connected with an ethylene carbon chain between pyrrole and pyrrolinone. After successive treatment with TFA to afford **14** by decarboxylation and with $(MeO)_3CH$, the resulting product **15** bearing the α-diformylated C-ring was cyclized with DBU to *Ea* CD-ring component **3** with a 7-membered ring.[14]

Locked *Za* AB-ring **2** and *Ea* CD-ring **3** were coupled with TFA–MeOH in one-pot to construct the doubly locked 5*Za*15*Ea*-BV allyl methyl diester **16**, which was further hydrolyzed under acidic conditions to give the doubly locked 5*Za*15*Ea*-BV **1** in free acid form for the assembly experiments with phytochrome apoprotein.[15]

As described above, we succeeded to prepare the doubly locked 5*Za*15*Ea*-BV **1** corresponding to the chromophore in Pfr-form of Agp 1.[6] Development of a convenient method for the synthesis of the locked *E-anti* dipyrrole component is essential for the synthesis of the chromophore corresponding to the Pfr-form.



Scheme 4. a) i) ^{n}Bu$_3$P (10 equiv), [Pd(PPh$_3$)$_4$] (0.05 equiv), NaBr (2.0 equiv), MeOH/THF (1/1, v/v), reflux, overnight. ii) ^{n}BuLi in hexane (1.0 equiv), −40 to −10 °C, 0.5 h. iii) **8** (1.0 equiv), −10 °C to rt, overnight. **12**, 50% (*E*/*Z*, 1/2). b) 5% Pd/C (0.1 equiv), H_2, MeOH, rt, 3 h. **13**, 90%. c) TFA (5 mL mmol^{-1}), rt, 2 h. d) $(MeO)_3CH$ (5 mL mmol^{-1}) was then added, rt, 2 h. e) DBU (3.0 equiv), THF, 50 °C, overnight. **3**, 50% in 3 steps. f) **2** (1.0 equiv), TFA (5 mL mmol^{-1}), rt, 1 h, then MeOH (5 mL mmol^{-1}), rt, 1 h. **16**, 80%. g) 12 M aq. HCl (5 mL mmol^{-1}) in Et_2O (5 mL mmol^{-1}), rt, overnight. **1**, 80%.

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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13 Y. Tsukahara, H. Kinoshita, K. Inomata, H. Kotake, *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3013.
14 *Ea* CD-ring component **3**: Mp 120 °C (from $CHCl_3$). IR (KBr): 3222, 2961, 2931, 2867, 1734, 1699, 1674, 1610, 1446, 1366, 1270, 1203, 1173, 1130, 1056, 1034 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.11 (t, 3H, $J = 7.8$ Hz), 2.40 (q, 2H, $J = 7.8$ Hz), 2.58 (t, 2H, $J = 7.8$ Hz), 2.72 (m, 2H), 2.77 (m, 2H), 3.06 (t, 2H, $J = 7.8$ Hz), 3.67 (s, 3H), 6.26 (s, 1H), 7.99 (br, 1H), 9.55 (s, 1H), 10.04 (br, 1H). HRMS (FAB) m/z: $[M + H]^+$ found: 329.15008; calcd for $C_{18}H_{21}N_2O_4$: 329.15014.
15 Doubly locked 5*Za*15*Ea*-BV **1**: isolated as HCl salt, a dark blue solid. Decomposed above 270 °C. IR (KBr): 3391, 2925, 2853, 1697, 1599, 1517, 1458, 1415, 1341, 1280, 1208, 1127, 992, 947, 916 cm^{-1}. ^1H NMR (C_5D_5N, 400 MHz): δ 1.05 (t, 3H, $J = 7.8$ Hz), 1.95 (s, 3H), 2.36 (q, 2H, $J = 7.8$ Hz), 2.61 (m, 2H), 2.70 (m, 2H), 2.82 (m, 2H), 2.85 (t, 2H, $J = 6.9$ Hz), 2.90 (t, 2H, $J = 6.9$ Hz), 3.14 (t, 2H, $J = 6.9$ Hz), 3.20 (t, 2H, $J = 6.9$ Hz), 3.96 (br, 2H), 5.50 (d, 1H, $J = 11.9$ Hz), 5.75 (d, 1H, $J = 17.9$ Hz), 6.70 (dd, 1H, $J = 11.9$, 17.9 Hz), 7.48 (s, 1H), 7.61 (s, 1H), 7.69 (s, 1H). The NH and CO_2H protons were not observed clearly. The structure was also confirmed by 2D NMR (COSY and NOESY). UV–vis (0.1 M HCl in MeOH): $\lambda_{max} = 377.5$ ($\varepsilon = 11744\ M^{-1}\ cm^{-1}$), 673.5 (45052) nm. HRMS (FAB) m/z: $[M + H]^+$ found: 595.25513; calcd for $C_{34}H_{35}N_4O_6$: 595.25566.

