Synthesis of Doubly Locked 5*Zs*15*Za*-Biliverdin Derivatives and Their Unique Spectral Behavior

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Doubly locked 5*Zs*15*Za*-biliverdin (BV) derivatives were synthesized toward the investigation of stereochemistry and function of the chromophore in bacteriophytochromes. The unique spectral behavior of the doubly locked 5*Zs*15*Za*-BV derivatives was observed by UV–vis spectroscopy.

Phytochromes are photoreceptive chromoproteins ubiquitously present in plants and have also been found in bacteria and fungi. They interchange reversibly by specific wavelengths of light between two isomers, red light absorbing Pr form and far-red light absorbing Pfr form, and it is known to cause the photoreaction referred to as "red/far-red photoreversible reaction."[1] This photoreaction plays an essential role in many biological processes, such as seed germination, growth, and flowering in plants; phototaxis, and pigmentation in bacteria. The open-chain tetrapyrroles serve as a chromophore of phytochromes, phytochromobilin (PΦB) for land plants, phycocyanobilin (PCB) for cyanobacteria, and biliverdin (BV) for some other bacteria. Recently, we revealed that BV covalently binds to the apoproteins of *Agrobacterium* phytochromes Agp 1 and Agp 2 via the A-ring vinyl side chain,[2,3] and that the absorption spectra of the locked 15*Za*-BV and 15*Ea*-BV adducts resemble the spectra of Pr and Pfr, respectively.[3]

Hildebrandt and his co-workers proposed that a chromophore in the plant phytochrome corresponding to Pr form has a 5Za15Za structure on the basis of DFT calculation of resonance Raman spectra.[4] On the other hand, we found that the chromophore in the Pr form of Agp 1 and Agp 2 has a 5Zs15Za structure,[3b] which was also confirmed by X-ray crystallography for other bacteriophytochromes.[5] To investigate the structure and function of the BV chromophores, we herein describe the synthesis of doubly locked BV derivatives, which have 5Zs15Za configuration and conformation corresponding to Pr form of Agp 1 and Agp 2. A retrosynthetic analysis of the doubly locked 5*Zs*15*Za*-BV (**1**) is shown in Figure 1.

Compound **6** as the precursor of the B-ring was previously prepared from allyl 4-oxobutanoate (**7**) obtained by the reaction of γ-butyrolactone (**8**) with sodium allyloxide and subsequent oxidation.[6] However, it was not easy to get reproducible high yield of the key intermediate, allyl 4-hydroxybutanoate (**9**), owing to the recyclization back to **8** under basic conditions. Therefore, **8** was hydrolyzed with an equimolar amount of DBU, followed by esterification with allyl bromide to avoid the recyclization to lactone **8**. The initial hydrolysis completed within 1 h at rt. Yield of the following allylation was strongly affected by stirring efficiency due to the heterogeneous reaction phase. Thus, THF, acetone, or DMF was added to improve the yield. Fortunately, the reaction mixture with a small amount of DMF was kept homogeneous throughout the reaction to afford **9** in

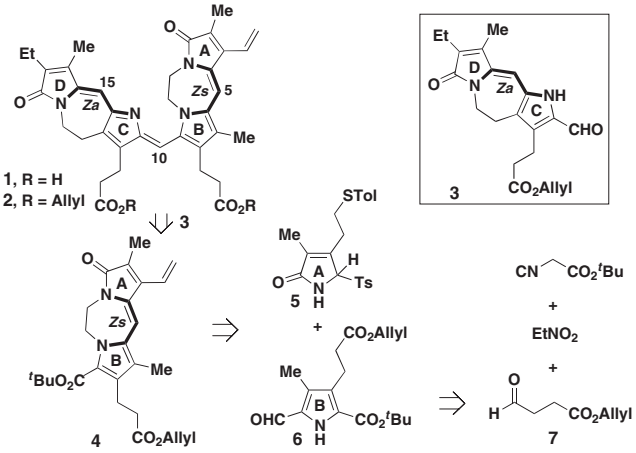
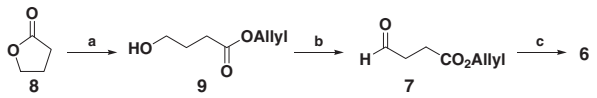


Figure 1. Retrosynthesis of doubly locked 5*Zs*15*Za*-BV (**1**).

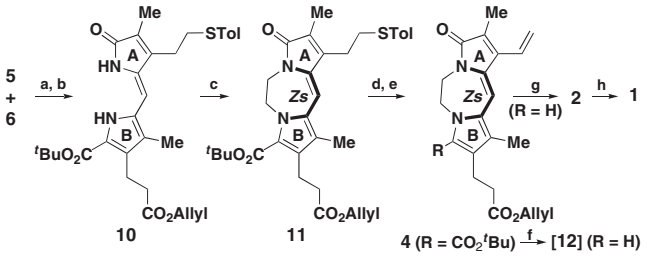
94% yield, while the reaction with THF or acetone was still heterogeneous in the allylation stage. Compound **9** was oxidized to the corresponding aldehyde **7**, from which the B-ring precursor **6** was prepared according to our previous method (Scheme 1).[6]

Applying our original Wittig-type reaction (Scheme 2), 5-tosylpyrrolinone **5** and formylpyrrole **6** were coupled to dipyrrole **10** as a mixture of E and Z isomers, then E isomer was converted to Z isomer with a catalytic amount of I_2.[7] (*Z*)-**10** was locked in Zs configuration and conformation with $BrCH_2CH_2Br$ in the presence of *n*-Bu_4NBr. The vinyl group of compound **4** was introduced by the elimination of the tolylthio group via oxidation from the locked product **11**. Compound **12**, which was produced by decarboxylation of **4** with TFA, was mixed with compound **3**[8] under acidic conditions to construct the doubly locked 5*Zs*15*Za*-BV diallyl ester **2**.[9] 5*Zs*15*Za*-BV (**1**)[10] in free acid form was furnished by treating **2** with sodium *p*-toluenesulfinate (TsNa) in the presence of Pd catalyst.

When the UV–vis spectrum of **2** was measured as usual, interestingly the color of the solution in methanol changed from blue to green within a few minutes. As such phenomenon has not been observed so far for other synthesized chromophores, the UV–vis spectra of 5*Zs*15*Za*-BV diallyl ester **2** were carefully investigated (Figure 2).



Scheme 1. [a]H_2O (13.0 equiv), DBU (1.0 equiv), DMF (0.28 mL/mmol), rt, 1 h; then allyl bromide (1.5 equiv), rt, 4 h. **9**, 94% from **8**. [b]$(COCl)_2$ (1.1 equiv), DMSO (2.0 equiv), Et_3N (5.0 equiv), CH_2Cl_2, −78 °C to rt, 1 h, **7**, 85%. [c]Ref. 6.



Scheme 2. [a]*n*-Bu_3P (2.2 equiv), DBU (1.5 equiv), THF, 0 °C to rt, 8 h. [b]I_2 (0.2 equiv), CH_2Cl_2, rt, 4 h, (*Z*)-**10**, 90% in two steps. [c]NaH (7.0 equiv), *n*-Bu_4NBr (0.2 equiv), $(BrCH_2)_2$ (10 mL/mmol), THF, rt, 15 h, **11**, 80%. [d]*m*CPBA (1.0 equiv), CH_2Cl_2, 0 °C, 0.5 h. [e]DBU (3.0 equiv), DMF, 100 °C, 1 h. **4**, 85% in two steps. [f]TFA (5 mL/mmol), rt, 40 min. [g]**3** (1.0 equiv), TFA (4.0 equiv), MeOH, rt, 1 h; then mixed with **12**, rt, 6 h. **2**, 90%. [h][$Pd(PPh_3)_4$] (0.2 equiv), TsNa (2.0 equiv), THF/MeOH (1/1), rt, 30 min, **1**, 70%.

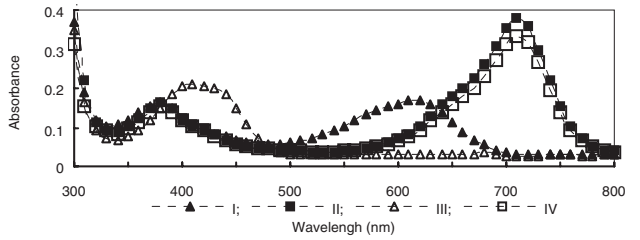
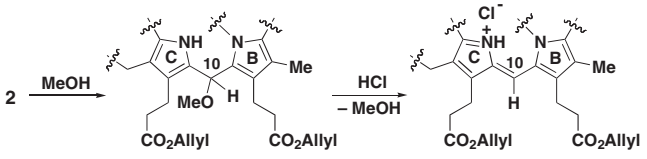


Figure 2. Absorption spectra of a solution of **2** in $CHCl_3$/MeOH (1/4, v/v). **I**, a freshly prepared solution of **2**; **II**, **I** + one drop of 1 M HCl; **III**, a solution of **2** kept for 24 h at rt; **IV**, **III** + one drop of 1 M HCl.



Scheme 3.

The freshly prepared solution of **2** in $CHCl_3$/MeOH (1/4, v/v) had absorptions at 387 and 621 nm, and they shifted to 385 and 718 nm by addition of one drop of 1 M HCl. On the other hand, when the solution of **2** in $CHCl_3$/MeOH (1/4, v/v) was kept at rt, a spectral change was observed. The absorption around 621 nm was diminished and a new peak appeared around 425 nm as the color turned green. After 24 h, the peak around 621 nm disappeared completely, and the solution changed to yellow. It was also observed that a methine proton at C10-position of **2** disappeared in the ^{1}H NMR spectrum of the residue obtained by evaporation of the solvent of solution **III** (Figure 2), although the spectrum became rather complicated. Very interestingly the absorption bands around 385 and 718 nm appeared again when one drop of 1 M HCl solution was added to the resulting yellow solution **III**. These facts suggest that the π-conjugation of tetrapyrrole was destroyed at the C10-position of **2** by the addition of methanol and recovered under the acidic conditions (Scheme 3).

Almost the same spectral behavior was observed for the free acid form **1**, while the addition of methanol proceeded much faster than in the case of **2** probably owing to the self-protonation by the free acids onto the nitrogen atom of the C-ring.

As described above, we successfully prepared the doubly locked 5*Zs*15*Za*-BV derivatives **1** and **2** corresponding to the chromophore in Pr form of Agp 1 and Agp 2, and found their unique spectral behavior by UV–vis spectroscopy, which is highly suggestive for our previous observation that the Agp 1 and Agp 2 adducts with the "15-syn chromophores" absorbed in the blue spectral region only,[3] probably due to the common syn conformation at the C5- or C15-position of the bilin chromophores.

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9 **2**: Mp 180–183 °C (from CH_2Cl_2/hexane). IR (KBr): 2964, 2924, 2873, 2855, 1735, 1682, 1618, 1580, 1455, 1424, 1385, 1353, 1275, 1151, 1065, 985, 951, 933, 892, 845, 755 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.11 (t, J = 7.6 Hz, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.12 (s, 3H), 2.39 (q, J = 7.6 Hz, 2H), 2.53 (t, J = 7.6 Hz, 2H), 2.58 (t, J = 7.6 Hz, 2H), 2.82 (br, 2H), 2.94 (t, J = 7.4 Hz, 4H), 3.89 (br, 2H), 4.54 (d, J = 5.5 Hz, 4H), 5.18 (dt, J = 10.5, 1.4 Hz, 1H), 5.19 (dt, J = 10.5, 1.4 Hz, 1H), 5.25 (dt, J = 17.0, 1.4 Hz, 2H), 5.64 (dd, J = 17.6, 1.4 Hz, 1H), 5.67 (dd, J = 11.5, 1.4 Hz, 1H), 5.81–5.91 (m, 2H), 6.18 (s, 1H), 6.28 (s, 1H), 6.66 (dd, J = 17.6, 11.7 Hz, 1H), 6.94 (s, 1H). The protons of the ethylene bridge between the nitrogens of A- and B-rings appeared around 4.30 ppm as a very broad signal. UV–vis (0.1 M HCl in MeOH): λ_{max} = 377 (ε = 28540 M^{-1} cm^{-1}), 709 (82210) nm. HRMS (FAB) [M + 1]$^+$, Found: m/z 703.35061. Calcd for $C_{42}H_{47}N_4O_6$: 703.34956.
10 **1** was isolated as HCl salt, a dark blue solid. Decomposed above 270 °C. IR (KBr): 3450, 2969, 2931, 2865, 2556, 1696, 1615, 1590, 1459, 1418, 1397, 1355, 1305, 1281, 1179, 1132, 1062, 987, 954, 883, 695 cm^{-1}. ^{1}H NMR (C_5D_5N, 400 MHz): δ 1.01 (t, J = 7.5 Hz, 3H), 1.83 (s, 3H), 1.93 (s, 3H), 2.03 (s, 3H), 2.28 (q, J = 7.5 Hz, 2H), 2.83–2.90 (m, 6H), 3.16 (t, J = 7.1 Hz, 2H), 3.23 (t, J = 7.6 Hz, 2H), 3.98 (br, 2H), 5.58 (d, J = 11.7 Hz, 1H), 5.59 (d, J = 17.8 Hz, 1H), 6.25 (s, 1H), 6.54 (s, 1H), 6.75 (dd, J = 17.8, 11.5 Hz, 1H), 7.56 (s, 1H). The protons of the ethylene bridge between the nitrogens of A- and B-rings appeared around 4.48 ppm as a very broad signal and CO_2H protons were not observed clearly. UV–vis (0.1 M HCl in MeOH): λ_{max} = 379 (ε = 28020 M^{-1} cm^{-1}), 710 (83400) nm. HRMS (FAB) [M + 1]$^+$ (observed as a free chromophore without HCl), Found: m/z 623.28643. Calcd for $C_{36}H_{39}N_4O_6$: 623.28696.

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