Total Synthesis of (±)-Phytochromobilin Starting from Two Pyrrole Derivatives

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Dedicated to Professor Albert Eschenmoser in recognition of his fundamental contributions to organic chemistry

Abstract: (±)-Phytochromobilin was synthesized as an acid form by developing a convenient method for the preparation of A- and D-rings starting from a 2-tosylpyrrole derivative, followed by efficient construction of A/B- and C/D-ring components via Wittig-type coupling reaction of 5-tosylpyrrolinones with 2-formylpyrrole, and palladium catalyzed deprotection of allyl esters of propanoic acid side chains at C-8 and C-12.

Key words: phytochrome, tetrapyrrole, phytochromobilin, total synthesis, transformation of pyrroles

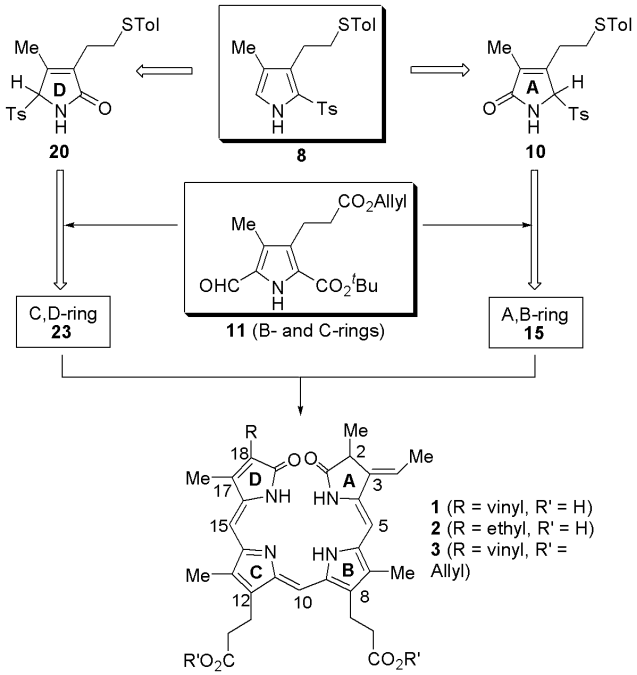
Phytochrome is a chromoprotein concerned in a variety of processes in higher plants such as growth, development, and morphogenesis etc. The chromophore named phytochromobilin (**1**) is a linear tetrapyrrole derivative and covalently bound to the apoprotein at A-ring. Recent developments in gene technology have made it possible to assemble the chromophores such as phytochromobilin (**1**) and phycocyanobilin (**2**), the latter of which is a chromophore of phycocyanin found in algal photosynthetic systems, with the apoproteins obtained by the over-expression of the corresponding cDNA in bacteria and yeast. The photophysical and photochemical properties of wild type phytochrome are quite similar to those of the reconstituted phytochromes.[1]

On the other hand, though the total syntheses of dimethyl ester derivatives of the chromophores **1** and **2** have been reported by Gossauer and his co-workers,[2] there had been no report regarding the syntheses of their acid forms applicable to assemble with the apoproteins.

In 1998, we reported the total synthesis of **2** and its derivatives bearing a photoreactive group at the D-ring in their acid form for the structure/function analysis of phytochrome.[3] Also Gärtner and his co-workers reported the syntheses of **1** and its isomer by the cleavage at C-10 of biliverdin IXα dimethyl ester with the thiobarbiturate anion.[4]

In this paper, we wish to describe the novel total synthesis of (±)-phytochromobilin (**1**) in its acid form starting from 4-methyl-3-[2-(tolythio)ethyl]-2-tosylpyrrole (**8**) as a common precursor of the A- and D-rings and 2-formylpyrrole **11** which is common to the B- and C-rings via our Wittig-type coupling reaction (Scheme 1).

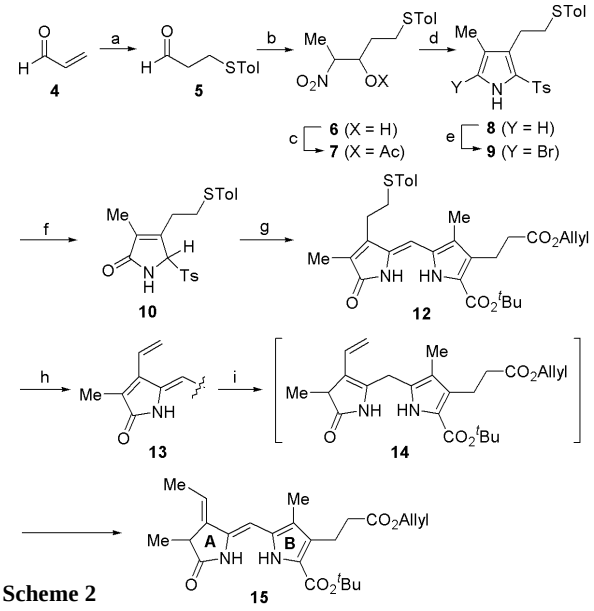
Recently we reported a new and efficient construction of the A/B-ring component for the syntheses of phycocyanobilin (**2**) and its derivatives using 4-(1-methoxyethyl)-3-methyl-5-tosyl-1,5-dihydro-2*H*-pyrrol-2-one as a precursor of the A-ring and 2-formylpyrrole **11**.[3c]



Scheme 1

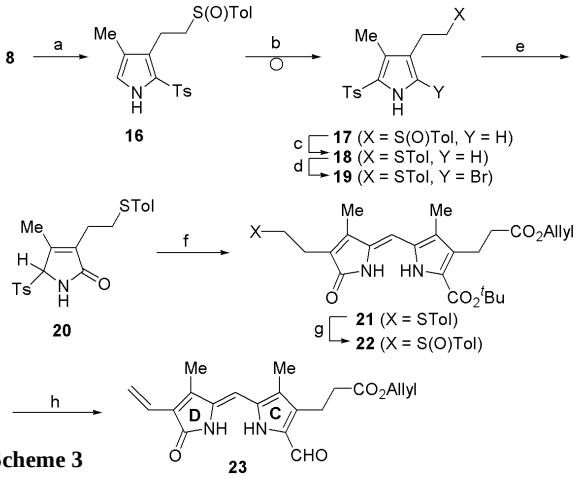
If it is possible to construct the A/B- and C/D-rings components of **1** starting from the same building blocks and the same coupling way, respectively, it will provide a much more efficient route for the total synthesis of **1**. Based on this strategy, we first prepared the A/B-ring component (Scheme 2).

The aldehyde **5**, readily available by addition of *p*-toluenethiol to acrolein (**4**), was reacted with nitroethane to give nitroalcohol **6**, which was acetylated and cyclized utilizing tosylmethyl isocyanide (TosMIC)[5] in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the pyrrole derivative **8** being regarded as a common precursor of the A- and D-rings.[6] The 5-tosylpyrrolinone **10** was obtained from **8** by bromination and subsequent acid hydrolysis, then coupled with **11** using DBU and PBu_3 to afford **12** according to our previous method.[7]



Scheme 2

[a] TolSH (0.83 eq) in THF/H_2O (2/1, v/v) at rt, 3 h; **5** 98% (based on TolSH). [b] 1 M KOH/MeOH (0.1 eq) in $EtNO_2$ (20 eq); **6** 93%. [c] Ac_2O (1.4 eq), DMAP (0.04 eq) in THF at rt, 10 min; **7** quant. [d] TosMIC (1.0 eq), DBU (2.2 eq) in CH_3CN at -40 °C - rt, overnight at rt; **8** 96%. [e] $PhMe_3N^+Br_3^-$ (1.1 eq) in CH_2Cl_2 at 0 °C, 10 min; **9** 98%. [f] TFA/H_2O (5/1, v/v) at rt, 4 h; **10** 63%. [g] (i) THF solution of DBU (1.5 eq) was added dropwise over a period of 30 min to the mixed solution of **11** (1.0 eq), 10 (1.2 eq), PBu_3 (2.0 eq) in THF at 0 °C - rt, 21 h at rt. (ii) cat. I_2 in CH_2Cl_2 at rt, 2 d; **12** 80%. [h] (i) mCPBA (1.0 eq) in CH_2Cl_2 at rt, 5 min. (ii) xylene, reflux, 2 h; **13** 95%. [i] (i) Al(Hg) (3.0 eq) in THF/H_2O (10/1, v/v) at rt, 1 h. (ii) TsOH·H_2O (1.0 eq) in CH_2Cl_2 at rt, 2 min; **15** 75%.

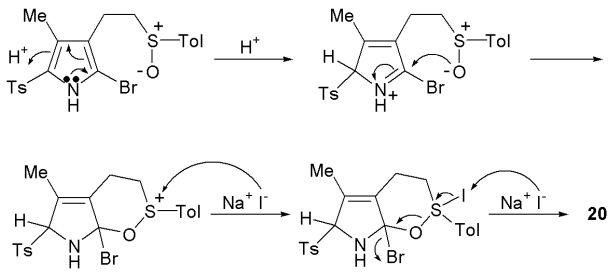


Scheme 3

[a] mCPBA (1.0 eq) in CH_2Cl_2 at 0 °C, 10 min; **16** 97%. [b] TsNa (0.1 eq) in $CHCl_3$/TFA (9/1, v/v) at 25 °C, 48 h; **17** 42% (34% of **16** was recovered). [c] $(COCl)_2$ (1.2 eq), NaI (2.5 eq) in CH_3CN at 0 °C, 10 min; **18** 92%. [d] $PhMe_3N^+Br_3^-$ (1.1 eq) in CH_2Cl_2 at 0 °C, 10 min; **19** quant. [e] (i) mCPBA (1.0 eq) in CH_2Cl_2 at rt, 5 min. (ii) NaI (5 eq) in TFA at rt, 10 min; **20** 90%. [f] (i) THF solution of DBU (1.5 eq) was added dropwise over a period of 30 min to the mixed solution of **11** (1.0 eq), **20** (1.2 eq), PBu_3 (2.0 eq) in THF at 0 °C - rt, 8 h at rt. (ii) cat. I_2 in CH_2Cl_2 at rt, overnight; **21** 77%. [g] mCPBA (1.0 eq) in CH_2Cl_2 at 0 °C, 1 min; **22** 95%. [h] (i) TFA at rt, 30 min, then $CH(OMe)_3$ at rt, 1 h. (ii) xylene, reflux, 1 h; **23** 62%.

The 2-(tolylthio)ethyl group of **12** was converted to a vinyl group by heating the corresponding sulfoxide obtained by oxidation with mCPBA to afford **13**. Compound **13** was then reduced with aluminum amalgam to **14**,[8] followed by acid treatment to result in the formation of A/B-ring component **15**.[9] The transformation of **13** to **15** through the reduced intermediate **14** is of interest in connection with the mostly speculative pathway for the transformation of biliverdin IXα to **1** through "dihydrobiliverdin."[10]

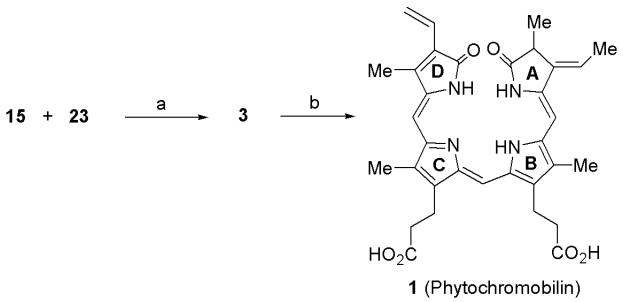
On the other hand, we previously reported a novel synthetic method of C/D-ring component of phytochromobilin dimethyl ester utilizing 4-methyl-5-tosyl-3-(2-tosylethyl)pyrrolinone.[7c] However, it required a strong base and high temperature at the stage to introduce a vinyl group to the D-ring by elimination of the tosyl group. Therefore, 2-tosylpyrrole **8** was at first transformed to **17** by tosyl rearrangement of the sulfoxide **16** obtained by oxidation of **8** (Scheme 3).[11] Then the sulfoxide **17** was reduced by $(COCl)_2$/NaI in CH_3CN to the sulfide **18**.[12] We then tried to hydrolyze the α-brominated pyrrole **19** to the 5-tosylpyrrolinone **20** under acidic conditions as previously reported,[7a] but the yield was disappointingly poor. Many attempts to improve the yield of the desired **20** by hydrolysis were unfruitful. Ultimately, it was found that **20** as a precursor of D-ring was available in high yield by treating the corresponding sulfoxide of **19** with NaI in TFA under anhydrous conditions for a short time. A probable reaction mechanism is shown in Scheme 4.



Scheme 4

The 5-tosylpyrrolinone **20** thus obtained was coupled with the 2-formylpyrrole **11** using PBu_3 in the presence of DBU to afford **21**, which was transformed to C/D-ring component **23**[13] via successive treatments, namely, oxidation to the sulfoxide **22**, formylation with methyl orthoformate under acidic conditions accompanied by decarboxylation, and elimination of *p*-toluenesulfinic acid to form a vinyl group.

Thus, the A/B- and C/D-ring components (**15** and **23**) were in hand. They were coupled in methanol using conc. sulfuric acid as a catalyst after removing the *t*-butoxycarbonyl group of **15** with TFA to afford the desired diallyl ester of (±)-phytochromobilin (**3**), whose structure was fully characterized by spectroscopic methods (Scheme 5).[14]



Scheme 5

[a] **15** (2.0 eq), TFA at rt, 20 min, then TFA was evaporated and MeOH solution of **23** (1.0 eq) was added, cat. conc. H_2SO_4 at rt, 1 h; **3** 80%. [b] cat. $Pd(PPh_3)_4$, morpholine (4.0 eq) in THF at rt, 1 h; **1** not yet optimized.[16]

The allyl ester groups could be deprotected[15] according to the method described in the previous paper to obtain the acid form of phytochromobilin (**1**).[16] However, **1** was extremely unstable compared to phycocyanobilin (**2**) prepared previously.[3,4]

As described above, phytochromobilin (**1**) was successfully prepared starting from two pyrrole derivatives, **8** and **11**, employing novel synthetic reactions, i.e., 1) rearrangement of the tosyl group of 2-tosylpyrroles to the 5-position under acidic conditions, 2) efficient transformation of α-bromopyrroles to the corresponding pyrrolinones under anhydrous acidic conditions, 3) Wittig-type coupling reaction between 5-tosylpyrrolinones and 2-formylpyrrole, followed by reductive transformation to the A/B-ring component, 4) protection and deprotection of propanoic acid side chains via allyl esters.

Investigation on the reconstituted chromoproteins using synthesized racemic **1** and other phycobilins prepared so far is in progress for the structure/function analysis of phytochrome.

References and Notes

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15: mp 112.0-113.5 °C (from cyclohexane/hexane); IR (KBr) 3369, 3169, 2979, 1732, 1699, 1681, 1640, 1442, 1364, 1318, 1278, 1254, 1180, 1156, 1127, 1053, 989, 934, 772, 713 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 1.39 (d, J = 7.32 Hz, 3H), 1.54 (s, 9H), 1.85 (d, J = 7.32 Hz, 3H), 1.98 (s, 3H), 2.54 (t, J = 8.17 Hz, 2H), 3.00 (t, J = 8.17 Hz, 2H), 3.24 (brq, J = 7.56 Hz, 1H), 4.59 (d, J = 5.85 Hz, 2H), 5.23 (dd, J = 1.22, 10.09 Hz, 1H), 5.30 (dd, J = 1.22, 17.20 Hz, 1H), 5.70 (s, 1H), 5.92 (ddt, J = 10.09, 17.20, 5.85 Hz, 1H), 6.18 (dq, J = 2.20, 7.32 Hz, 1H), 8.58 (s, 1H), 9.05 (s, 1H) ppm; Found: C, 67.13; H, 7.61; N, 6.36%. Calcd for $C_{24}H_{32}N_2O_5$: C, 67.27; H, 7.53; N, 6.54%.
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(13) **23**: mp 171.5-173.0 °C (from $CHCl_3$/AcOEt); IR (KBr) 3330, 3166, 3016, 2920, 2860, 1726, 1704, 1661, 1594, 1543, 1507, 1450, 1415, 1387, 1340, 1258, 1169, 1094, 986, 927, 793, 712 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 2.10 (s, 3H), 2.11 (s, 3H), 2.61 (t, J = 7.61 Hz, 2H), 3.06 (t, J = 7.61 Hz, 2H), 4.58 (d, J = 7.87 Hz, 2H), 5.21 (dd, J = 1.33, 10.36 Hz, 1H), 5.29 (dd, J = 1.33, 17.06 Hz, 1H), 5.42 (dd, J = 1.74, 11.60 Hz, 1H), 5.90 (ddt, J = 10.36, 17.06, 7.87 Hz, 1H), 5.93 (s, 1H), 6.16 (dd, J = 1.74, 17.43 Hz, 1H), 6.47 (dd, J = 11.60, 17.43 Hz, 1H), 9.66 (s, 1H), 10.67 (s, 1H), 11.01 (s, 1H) ppm; Found: C, 67.41; H, 6.37; N, 7.63%. Calcd for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26; N, 7.90%; HRMS (EI): (M^+), Found: m/z 354.1583. Calcd for $C_{20}H_{22}O_4N_2$:354.1580.
(14) **3**: mp 177.5-179.0 °C (decomp.) (from $CHCl_3$/hexane); IR (KBr) 3337, 3088, 3009, 2914, 1733, 1675, 1610, 1590, 1449, 1416, 1375, 1279, 1243, 1223, 1166, 1096, 1052, 981, 929, 803, 748, 697 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 1.33 (d, J = 7.44 Hz, 3H), 1.88 (d, J = 7.32 Hz, 3H), 2.05 (s, 3H), 2.14 (s, 3H), 2.21 (s, 3H), 2.58 (t, J = 7.56 Hz, 4H), 2.91 (t, J = 7.56 Hz, 2H), 2.95 (t, J = 7.56 Hz, 2H), 3.11 (brq, J = 7.44 Hz, 1H), 4.58 (m, 4H), 5.22 (dm, J = ca.10.3 Hz, 2H), 5.28 (dm, J = 17.08 Hz, 1H), 5.29 (dm, J = 17.08 Hz, 1H), 5.40 (dd, J = 2.20, 11.53 Hz, 1H), 5.83 (s, 1H), 5.84-5.95 (m, 1H), 6.09 (s, 1H), 6.22 (dd, J = 2.20, 17.63 Hz, 1H), 6.39 (dq, J = 2.19, 7.32 Hz, 1H), 6.52 (dd, J = 11.53, 17.63 Hz, 1H), 6.65 (s, 1H), 7.26 (s, ca.2H) ppm; UV/Vis (MeOH) λmax 372 (ε = 50,000), 631 (ε = 16,000) nm; HRMS (FAB): (M^++1), Found: m/z 665.3328. Calcd for $C_{39}H_{45}O_6N_4$:665.3339.
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(16) To a mixed solution of **3** (31 mg, 0.0467 mmol) and $Pd(PPh_3)_4$ (11 mg, 0.00952 mmol) in THF (5 ml) was added a 0.1 M THF solution (1.87 ml) of morpholine (0.187 mmol) in the dark under nitrogen atmosphere at room temperature. After stirring for 1 h at room temperature, the solvent was evaporated. The resulting residue was separated by a silica gel column chromatography ($CHCl_3$/MeOH/AcOH = 200/15/1). The last green fraction was evaporated and dissolved again in $CHCl_3$ containing 1% MeOH. After a repetition of the back-extraction with 1/15 M phosphate buffer solution (pH 7.8;

KH_2PO_4/Na_2HPO_4; 10 ml x 4), the combined aqueous extracts were acidified by addition of 1 M acetate buffer solution (pH 4.8; NaOAc/AcOH) and extracted with $CHCl_3$ containing 1% MeOH (10 ml x 3). The solid residue obtained by evaporation of organic solvent was recrystallized from $CHCl_3$ (containing 1% MeOH)/hexane.

1: 2 mg (a purple solid; 7%; not yet optimized. Though improvement of isolation and purification of unstable (especially during separation) **1** is now in progress, it seems to be rather stable after recrystallization upon storage at low temperature in the dark under nitrogen atmosphere). UV/Vis (MeOH) λmax 372 (ε; not determined), 636 (ε; not determined) nm, $\varepsilon_{372}/\varepsilon_{636} = 3.20$; HRMS (FAB) ($M^+$+1), Found: m/z 585.2725. Calcd for $C_{33}H_{37}O_6N_4$:585.2713.

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